UNIVERSITY OF SINDH
JOURNAL OF ANIMAL SCIENCES

(USJAS)

HEC Recognized

(USJAS)

ISSN Print: 2521-8328
ISSN Online: 2523-6067

Volume: 06
Number: 04
December 2022

Website: http://sujo.usindh.edu.pk/index.php/USJAS
Email: editor.usjas@usindh.edu.pk

UNIVERSITY OF SINDH
JOURNAL OF ANIMAL SCIENCES

VOLUME 6 **DECEMBER 2022** **NUMBER 4**

Journal Issue DOI: https://doi.org/10.57038/usjas.v6i04

Quarterly publications
Dean, Faculty of Natural Sciences & Department of Zoology,
University of Sindh, Jamshoro, Sindh-Pakistan

ISSN (E): 2523-6067
ISSN (P): 2521-8328

Sindh University Press.

UNIVERSITY OF SINDH JOURNAL OF ANIMAL SCIENCES
Website: https://sujo.usindh.edu.pk/index.php/USJAS
Uni. Sindh. J. Anim. Sci., 6(4), December, 2022
Email: editors.usjas@usindh.edu.pk
ISSN (P): 2521-8328
ISSN (E): 2523-6067
Published by University of Sindh, Jamshoro.

Editorial Team

UNIVERSITY OF SINDH JOURNAL OF ANIMAL SCIENCES
Website: https://sujo.usindh.edu.pk/index.php/USJAS
Uni. Sindh. J. Anim. Sci., 6(4), December, 2022
Email: editors.usjas@usindh.edu.pk
ISSN (P): 2521-8328
ISSN (E): 2523-6067
Published by University of Sindh, Jamshoro.

International Editorial Advisory Board

UNIVERSITY OF SINDH JOURNAL OF ANIMAL SCIENCES
Website: https://sujo.usindh.edu.pk/index.php/USJAS
Uni. Sindh. J. Anim. Sci., 6(4), December, 2022
Email: editors.usjas@usindh.edu.pk
ISSN (P): 2521-8328
ISSN (E): 2523-6067
Published by University of Sindh, Jamshoro.

National Editorial Advisory Board

Technical Advisory Member

UNIVERSITY OF SINDH JOURNAL OF ANIMAL SCIENCES
Website: https://sujo.usindh.edu.pk/index.php/USJAS
Uni. Sindh. J. Anim. Sci., 6(4), December, 2022
Email: editors.usjas@usindh.edu.pk
ISSN (P): 2521-8328
ISSN (E): 2523-6067
Published by University of Sindh, Jamshoro.

With the increase in population, third world countries today are facing many problems, supply of sufficient food being one of them. In animal sciences we have to understand and preserve the vast diversity of species on our planet. Losing them would be a huge shame and almost a crime of humanity. We have caused a continuous trouble that leads to species extinction. Just because we are the "dominant" species on Earth, it doesn't mean that we can do whatever we want without suffering consequences. We do not have to protect endangered species only, but we also have to protect species essential for the continuation of Earth's life. Believe it or not, without animals, humans would die out pretty quickly. First of all, there would be no more meat. But we can't all become vegetarians either if there are no insects to pollinate the plants. From animals, we can also learn about our anatomy and can understand the function of our bodies in a better way, which help us combat human diseases. In termination, animal's science is an important field that applies to many real-world situations.

University of Sindh Journal of Animal Sciences (USJAS) will promote and involve the study of various disciplines in Zoological Sciences i-e Entomology, Endocrinology, Molecular biology, Parasitology, Wildlife management and Conservation, animal's diversity and systematic etc. This journal will be ideal platform for anyone working in Animals Sciences. In addition, the published data to provide additional opportunity for access to advanced standing in existing tertiary level education programs. Researcher will be exposed to the main aspect of animal science including, safety management planning strategies, food and fiber, systematic of individual and making a means to preserve a rapidly declining global ecosystem.

UNIVERSITY OF SINDH JOURNAL OF ANIMAL SCIENCES
Website: https://sujo.usindh.edu.pk/index.php/USJAS
Uni. Sindh. J. Anim. Sci., 6(4), December, 2022
Email: editors.usjas@usindh.edu.pk
ISSN (P): 2521-8328
ISSN (E): 2523-6067
Published by University of Sindh, Jamshoro.

MISSION

University of Sindh Journal of Animal Sciences (USJAS) will introduce a viable model of research linkages together with scholarly experience sharing and transferring of inspired evidence. It also promotes the latest emerging trends in zoological sciences along with provision of an awareness of the ethical aspects of animals. Besides its fundamental importance in maintaining the sustainable agricultural, protection and conservation of animals in country it will also educate the public about the need to protect and preserve the environment as a long-range goal for the welfare of future generations. The core focus of (USJAS) is concentrated on promoting and propagating novel and innovative research amongst the readers of this journal.

AIM OF JOURNAL

The primary aim of University of Sindh Journal of Animal Sciences (USJAS) is to gain hands on experience in order to acquire the knowledge necessary for the critical analysis of the results and make appropriate recommendations in all fields of Animal sciences. The aim of this journal is to encourage researchers, investigators and scientists to publish their research findings allowing wider dissemination of their intellectual knowledge, with the aim of applying those for the benefit of the society. The newly launched journal would cover full spectrum of the specialties in Animal's sciences. It would include original research articles, review articles, case reports, short commendation, and scientific findings from within specified domain areas of Zoology. The journal strictly follows the guidelines proposed by Higher Education Commission (HEC) Pakistan. The most important criterion for acceptance/rejection is originality of the material presented in the manuscript.

UNIVERSITY OF SINDH JOURNAL OF ANIMAL SCIENCES
Website: https://sujo.usindh.edu.pk/index.php/USJAS
Uni. Sindh. J. Anim. Sci., 6(4), December, 2022
Email: editors.usjas@usindh.edu.pk
ISSN (P): 2521-8328
ISSN (E): 2523-6067
Published by University of Sindh, Jamshoro.

What is need for publishing this Journal?

The University of Sindh Journal of Animal Sciences (USJAS) with modernized and cost effectiveness will light the tools for numerous directions and problems related to improve identification of pest species, conservation of wild animals, diversity of animals including animal breeding, environmental impact of animal, agriculture, diseases, nutrition and animal products. When animals grow well and stay healthy, farmers can produce more meat, milk or eggs for our consumption. They check meat quality or screen milk for pathogens. Advances in food safety keep humans healthy and increase the world's supply of nutritious food. Beside this, articles regarding entomological science contribute to the betterment of humanity by detecting the role of insects in the spread of disease and discovering ways of protecting food and fiber crops, and livestock from being damaged. Journal provides the way how beneficial insects contribute to the well-being of humans, animals, and plants. This journal will also defend and assess the application of well proven research activities in natural science particularly, Zoology, Physiology, Fresh Water Biology & Fisheries, Biochemistry and Biotechnology of host universities; neighboring and sister universities which are performing research activities on any area of animal's sciences. They have necessity of proper platform for their research exposure around the country as well as in world.

UNIVERSITY OF SINDH JOURNAL OF ANIMAL SCIENCES
Website: https://sujo.usindh.edu.pk/index.php/USJAS
Uni. Sindh. J. Anim. Sci., 6(4), December, 2022
Email: editors.usjas@usindh.edu.pk
ISSN (P): 2521-8328
ISSN (E): 2523-6067
Published by University of Sindh, Jamshoro.

Who will contribute?

Articles published in (USJAS) masses of all age and income groups. Moreover, this journal will make substantial contribution to the researchers and students of Zoology, Entomology, Pest Management, Plant Protection, Agriculture Sciences and Medical Sciences; further, wild life department and extension wings will also be benefited. The objectives set at promotion of research, the food and fiber crops, livestock, animal's diversity, diseases, obesity and to establish breeding programs, nutrition programs and help preserve exotic wildlife. Beside this, Parasitological section gives the reader a broad, in-depth coverage of medically important parasites. Such coverage is essential to give students the awareness and understanding necessary for proper diagnosis, treatment, and prevention of the parasitic infections. This journal will be valuable in skill enhancement, and knowledge refreshment of the working staff of academia and research.

The Specific Thematic Areas are under:

- ✓ Taxonomic/faunistic data on any taxon of any geological age.
- ✓ New descriptions of taxa, if they are accomplished with proper diagnoses, keys and/or revision of at least at species group level.
- ✓ Taxonomic revisions of extant (or ''recent'') and fossil animal groups.
- ✓ Checklists and catalogues, phylogenetic and evolutionary analyses.
- ✓ Breeding and ecology of animals.
- ✓ Integrate pest management tactics.
- ✓ Economic thresholds and optimization.
- ✓ Genetic manipulation of pest population.
- ✓ Utilization of precision agriculture as an (IPM) tool.
- ✓ Speciation, adaptive radiation and extinction of species.
- ✓ Genetics and Reproduction behavior.
- ✓ Gene & DNA replication and recombination.
- ✓ Environmental planning and rational use allocation.
- ✓ A global program for the protection of genetic resource areas.
- ✓ Deserts and areas subject to desertification.
- ✓ Fossil records of animals from any region, etc.

UNIVERSITY OF SINDH JOURNAL OF ANIMAL SCIENCES
Website: https://sujo.usindh.edu.pk/index.php/USJAS
Uni. Sindh. J. Anim. Sci., 6(4), December, 2022
Email: editors.usjas@usindh.edu.pk
ISSN (P): 2521-8328
ISSN (E): 2523-6067
Published by University of Sindh, Jamshoro.

PUBLICATION

University of Sindh Journal of Animal Sciences (USJAS) is published quarterly i.e., 4 times a year: March, June, September and December, by the Office of Dean Faculty of Natural Sciences and Department of Zoology, University of Sindh, Jamshoro.

COPYRIGHT

Printed at: Sindh University Press.

OPEN ACCESS POLICY

University of Sindh Journal of Animal Sciences (USJAS) provides an immediate open access to its content on the principle that making research freely available to the public supports a greater global exchange of knowledge. To enable unrestricted usage our Journal is following **CC-BY-NC** license. Researchers can copy and redistribute the material in any medium or format, for research purpose, condition to formal reference to the original work. Authors can self-archive publisher's version of the accepted article in digital repositories and archives.

CONTACT INFORMATION

Dr. Riffat Sultana
Editor,
Journal office, Department of Zoology, University of Sindh, Jamshoro Sindh, Pakistan.
Journal Website: http://sujo.usindh.edu.pk/index.php/USJAS
E-mail: editor.usjas@usindh.edu.pk, riffat.sultana@usindh.edu.pk
Contact: +92-333-2776771

UNIVERSITY OF SINDH JOURNAL OF ANIMAL SCIENCES
Website: https://sujo.usindh.edu.pk/index.php/USJAS
Uni. Sindh. J. Anim. Sci., 6(4), December, 2022
Email: editors.usjas@usindh.edu.pk
ISSN (P): 2521-8328
ISSN (E): 2523-6067
Published by University of Sindh, Jamshoro.

REVIEW POLICY AND PROCESS

University of Sindh Journal of Animal Sciences (USJAS) follows a full double-blind peer review process, comprising the following steps:

- ✓ All papers submitted to the (USJAS) are first examined by an Editor for the initial review. The editor may assign to experts to review the quality and scope of the submitted manuscript. If the article fails to meet the journal criteria, the paper is rejected immediately and authors are notified.

- ✓ Once the paper is submitted, the editor ensures that the paper follows the **double-blind review policy**. If the author names are found, then the author's name(s) and affiliation(s) are removed from the paper and updated version is uploaded on the system by the editor.

- ✓ Every submitted article is checked for the Similarity report by the Editor, before forwarding it to the reviewers. **Turnitin website** is used by the journal as prescribed by Higher Education Commission (HEC) Pakistan, to check the similarity of paper. As per HEC policy, in case manuscript has been found to have a similarity index of more than 19% it will be immediately either returned back to the author for correction and resubmission, or will be rejected and archived. This decision is made by the editor, based on the similarity ratio. (Please Note that the parameters for similarity check involve, add to No Repository, Exclude Bibliography, Exclude Quoted Text).

- ✓ If the paper satisfies the criteria, then the editor will send the article to at least (02) reviewers for review. Each article will be reviewed by at least 01 National and 01 International reviewers with a double-blind, peer review policy.

- ✓ Reviewers are recruited from the national and international universities having a PhD with reputed research profile (checked through Google Scholar, Research Gate and Web of Science) for the given area/ field of submitted article.

- ✓ Each reviewer is initially invited to review the article showing them the article title and abstract only. Upon acceptance of the review request the full paper is presented and a review form is used to record their feedback and suggestions through online management system.

UNIVERSITY OF SINDH JOURNAL OF ANIMAL SCIENCES
Website: https://sujo.usindh.edu.pk/index.php/USJAS
Uni. Sindh. J. Anim. Sci., 6(4), December, 2022
Email: editors.usjas@usindh.edu.pk
ISSN (P): 2521-8328
ISSN (E): 2523-6067
Published by University of Sindh, Jamshoro.

- ✓ The reviewers' recommendations will determine whether to “Accept Submission”, “Revision required”, “Resubmit for Review” or “Decline Submission”. The final decision if made by the Editor based on the reviewers’ report.

- ✓ Once the decision has been made, the review response - including the review form and suggestion, are sent to all authors of the article with editorial decision.

- ✓ For the papers which require only revisions, the editor will re-review the paper for ensuring that the reviewer’s suggestions have been incorporated or not.

- ✓ For papers that require resubmission for review, will undergo second Round of review by the same or different reviewers to ensure that the quality of the revised paper is acceptable.

- ✓ After final Acceptance, the Author/Corresponding author will be notified and paper will be forwarded to Publication Manager/Editorial Assistant for copy-editing, layout editing and proof reading.

UNIVERSITY OF SINDH JOURNAL OF ANIMAL SCIENCES
Website: https://sujo.usindh.edu.pk/index.php/USJAS
Uni. Sindh. J. Anim. Sci., 6(4), December, 2022
Email: editors.usjas@usindh.edu.pk
ISSN (P): 2521-8328
ISSN (E): 2523-6067
Published by University of Sindh, Jamshoro.

TABLE OF CONTENTS

UNIVERSITY OF SINDH JOURNAL OF ANIMAL SCIENCES
DOI: https://doi.org/10.57038/usjas.v6i04.5834
Uni. Sindh. J. Anim. Sci., vol. 6(4); 1-6, 2022
Email: editors.usjas@usindh.edu.pk
ISSN (P): 2521-8328
ISSN (E): 2523-6067
Published by University of Sindh, Jamshoro.

PLANT-BASED TREATMENT OF HELMINTHS IN CALVES

OYERONKE ADENIKE ADEKOLA[1], AKINWALE DAMI OLABIMISI[2], GBOLAGADE BENJAMIN ADESIJI[3], SOLA EMMANUEL KOMOLAFE[*3]

[1]Department of Agricultural Extension and Rural Development, Federal University of Agriculture, Abeokuta, Ogun State, Nigeria
[2]Department of Agricultural Technology, Oyo State college of Agriculture and Technology, Igboora, Oyo State, Nigeria
[3]Department of Agricultural Extension and Rural Development, University of Ilorin, Ilorin, Kwara State, Nigeria

ARTICLE INFORMATION

Article History:
Received: 23rd September 2022
Accepted: 15th December 2022
Published online: 31st December 2022

Author's contribution
OAA, designed the study, MAR and GBA performed the experiments, SEK complied the data.

Key words:
Efficacy, Indigenous, Plants, Helminth, Cattle

ABSTRACT

This study examines the indigenous plants practices for the treatment of helminths in cattle among settled agro-pastoralist in Ogun and Oyo State, Nigeria. The study was a pretest-posttest design. Three sets of helminth infected cattle were each exposed to herbal treatment (Indigenous plant concoction (Group A, n=6), alverticine drug (convectional helminthic drug (Group B, n=6) orally for 1-14 days while the control group (Group C, n=6) were untreated. Faecal egg count was obtained using the Modified Mac Master and saturated salt solution technique and data was analysed using ANOVA for repeated measures. The significant effect of treatment on FEC count across the four days of treatment ($p < 0.05$) as the FEC count significantly decline across from day 1 - 4 for both the herbal than the conventional treatment groups while the control group remains chronically infected with the helminths till 12th week. FEC count significantly decline faster for convectional drug group (FEC count cleared on 2nd day) compared to the herbal group (FEC count cleared on 4th day) ($p < 0.05$). Cows exposed to herbal drug significantly (90.36±4.780) had better weight gain than those exposed to conventional treatment (85.65±7.405) while there was significant weight loss observed in the control group (76.68 ±6.435) at the 12th week. The study concludes that herbal concoctions based traditional knowledge of medicinal plants were efficacious in the treatment of helminths infestation. It is therefore recommended that regular control measure should be practiced and agro-pastoralist needed to be educated in proper usage of anti-helminths drugs and its administration.

1. INTRODUCTION

There is increased awareness of the enormous healing potentials of indigenous plants in Africa (Adesiji et al., 2014). The low investment in scientific veterinary implies that indigenous veterinary practices must be harnessed and improved upon. These indigenous veterinary practices have been in operation in bits and pieces from generation to generation, but have to become the driver for bottom-up development in cattle production for the sake of sustainability. Sustaining the aroused interest of agro-pastoralists in the use of indigenous technologies, therefore, becomes imperative.

This research is based on the hypothesis which fosters the use of collective action towards achieving a sustainable and environmental friendliness which allows stakeholders to share ideas and work together. Helminthiasis is a disease condition caused by internal parasitic worms that invade the internal organs of livestock while Helminths are endo-parasites comprising of a large and varying group of invasive parasites. According to National Animal Disease Information Service (NADIS, 2010) who ascertained that livestock industry in Nigeria is endowed with 13.8 million cattle out of which 97 percent are Zebu breeds, 34.8 million goats, 22 million sheep, 72.4 million local chicken, 11.8 million ducks, 4.8 million guinea fowls, and 3.1 million pigs. According to the study

*Corresponding Author: kemmas04@yahoo.com

conducted by Mapiye and Sibanda (2005) showed that large flock sizes were raised by livestock farmers that utilized indigenous plants/technologies compared with those that used contemporary veterinary treatment. This indicated that indigenous plants/technologies could be a more efficacious alternative when used properly on livestock. This corroborates the need for researchers to take an inventory of indigenous plants used by agro-pastoralists in treating cattle with helminths infestation along with their dosages and application for them not to be lost. The success stories of indigenous technologies used in livestock management in Nigeria are corroborated by Kolawole et al. (2007); Okwoche, (2013); and Kubkomawa et al. (2013). These suggest the efficacy of these technologies on livestock rearing. Nevertheless, the innovativeness of agro-pastoralists goes a long way in determining their practice of indigenous plants.

Agro-pastoralists are expected to be persuaded enough to develop and relate with researchers on indigenous plants/technologies being used for necessary documentation because of its reported relative advantage over orthodox medicine, but their demographic, economic, social and psychological characteristics can refuse them from this opportunity. Prevalent helminths in Africa according to Keyyu et al. (2005) are Nematode; *Haemonchus, Cooperia, Bunostomum, Trichuris, Oesophagostomum, Trichuris*, and *Strongyloides. Cestodes* include; *Moneziawhile* Trematodes consists of; *Paramphistomum, Fasciola, Dicrocoelium* which are usually more pronounced in the intestine of ruminant animals. One approach common among the indigenous group for the control of helminths over diseases is the use of indigenous technology. The term 'Indigenous technology' is otherwise used when this phenomenon is based on the traditional knowledge system that is handed down orally and improved from generation to generation (Tabuti, 2003).

Essentially all herds/flocks in a grass-based production system are affected. Parasitological indices for measuring resistance include fecal egg counts and worm burden at necropsy which have been extensively used to determine the intensity of parasitic gastrointestinal helminth infections (Sreter et al., 1994 and Bisset et al., 1996). However, Fakae et al., (2004) found individual variability in West African Dwarf goats which showed outstanding positive responsiveness in FEC following experimental *H. contortus* infections. This confirmed earlier nematode resistance characteristics of some breeds of sheep measured by reduced FEC, which was found to be heritable between 0.23-0.41 (Woolaston and Piper, 1996). The practical value of the use of FEC as a measure of resistance has been demonstrated in several successful breeding programs (Gray, 1991). The study objectives are to: ascertain the efficacy of indigenous plants for the treatment of helminths compared to conventional methods of treating helminths among agro-pastoralist in the study area contamination (Sabullah et al., 2015). Nickel (Ni) is a transition metal, and considered as a significant micronutrient, always present in small quantity in animal tissues and seems to be well regulated. Nickel may cause negative impacts on fish health when present in deficiency or excess amount (Eisler, 1998; Hayat, 2007). According to Clark and Keasling (2002) Ni causes some morphological transformations in cell and chromosomal aberrations. However, Vieira et al. (2009) reported that he accumulation of Ni in aquatic organism creates acute and chronic toxicity.

Elevated level of heavy metals in aquatic ecosystem can prompt oxidative stress in aquatic life (Almeida et al., 2002). The inequality between the antioxidants activity and the creation of reactive oxygen species (ROS) result in oxidative stress (Lushchak, 2011). According to Sayeed et al. (2003) ROS can cause damage at cellular level resulted in oxidative damages enhance the diseases and also cause deaths (Iwama et al., 2011; Herrera et al., 2009). Animals have antioxidants defense system to cope with harmful effects of ROS which includes enzymes viz. superoxide dismutase, catalase and peroxidase (Jia et al., 2013). Catalase present in peroxisomes, plays a significant role in conversion of harmful free radical hydrogen peroxide to oxygen and water molecule (Dellali et al., 2001). Stress response in fish against toxicant exposure can be detected by evaluating the biochemical parameters. Now, use of these oxidative stress biomarkers has been greatly increased in ecotoxicology field. Therefore, this study was designed to relate the organs- and duration- dependent changes in catalase activity of *L. rohita* due to nickel toxicity.

2. MATERIALS AND METHODS

Experimental Site

The experiment was conducted at the Teaching and Research Farm of the Federal University of Agriculture, Abeokuta in the Derived Savannah Zone of Nigeria.

Experimental Research Design Stage

Experimental materials used in experiments to determine the survivors of helminths infection include: disposable hand gloves, acuvetsam-ple bottle, sample bottle rack, cattle fecal samples, faeces sieve and spatula, ordinary water, pasture pipette, Mac-Master slide, Light microscope, glass slide and cover slide, conic flask and saturated solution of sodium chloride. Antihelminth like Ivermectin injection was administered on experimental cattle detected with helminths as a conventional method of treating helminths. All experimental calves to be treated were tagged with numbers individually and administered with a compendium of indigenous plants as

recommended by agro-pastoralists within the axis of the University premises; the indigenous plants identified were Ficuscapensis (*Moraenae*), *Anthocleista djalonensis* (*longaniaceae*) and *Parinapari polyandra (*Chrysobalanaceae*)* as shown in Table 1. This three plants species were used outside the listed plants species that agro-pastoralist have been familiar with in the study area.

Sample examination

Faecal egg count was obtained using the Modified Mac Master and saturated salt solution technique according to Zajac et al. (2006). This produced a quantitative estimate of egg output for nematodes and cestodes and assessed faecal egg output in experimental animals. The following procedures were used to determine the faecal egg count: Flotation method and Faecal Egg Count through Modified Mac Master Technique.

Statistical analysis

The data were analyzed by Analysis of Variance (ANOVA) used to compare treatment means.

3. RESULTS AND DISCUSSION

Effect of treatment on faecal egg count

ANOVA result in Table 2 revealed the significant effect of treatment on FEC across the four days of treatment ($p < 0.05$). The FEC was also demonstrated to significantly decline across from day1 to day 4 due to treatment. Results show that conventional drug caused a significant decline between Day 1 (4300.00±788.811) to Day 3 (0.00±0.00). The herbal drug significantly cleared the worm loads between Day1 (4500.00±623.61) to Day 4 (0.00±0.00) while the no significant changes were observed in the control group. However, while the FEC increased faster after the third month for the animals in the herbal treatment group than the conventional the control remains chronically infected with the helminths.

Effect of treatment on mean weight of Cattle after 12 weeks

ANOVA result in Table 3 revealed that there is no significant effect of treatment on mean weight across the 12 weeks of treatment ($p<0.05$). It was demonstrated there was significant weight gain from week 0 to week 12 due to exposure to treatment for the cows in the herbal and conventional groups. Results show that the mean weight for cows exposed to herbal drug significantly change between week 0 (84.20 ±3.293) and week 12 (90.36±4.780). The conventional treatment significantly improves the weight gain between week 0 (83.50±7.472) and week 12 (85.65±7.405) while there was significant weight loss observed in the control group from week 0 (82.80±3.736) and week 12 (76.68 ±6.435). This indicate the efficacy of the three plants species used to reduce the FEC of helminths in the cattle among agro-pastoralist.

4. CONCLUSION

Based on the findings of the study, it could be concluded that the agro-pastoralists and resource-poor traditional farmers in Africa and many parts of the developing world widely accept the use of herbal remedies based on local plant preparations which offer an alternative to the expensive and often inaccessible or adulterated commercial anthelminthic. In essence, traditional control methods of cattle helminthiasis were found to be well established and utilized by the respondents. The study has indicated that southwest communities are rich in traditional knowledge on medicinal plants diversity and have used them to treat livestock helminthiasis and has, therefore, become a success story. Hence, indigenous plants have become the most preferred and treasured asset of the community host although their conservation is seriously threatened to local extinction.

This study therefore recommend that agro-pastoralist can come together to find ways or work-out plans to conserve and protect the loss of some useful indigenous plants identified, improving farm management system and routine deworming of farm animals is of great importance to animal production as this will reduce the rate of helminths infestation on cattle rearing husbandry, the high prevalence rate of helminthiases in livestock needed to be checked periodically to discontinue infestation of the animals with helminths, regular control measure should be practiced and agro-pastoralist on the mode of administration in order to ensure proper usage of indigenous plants/herbs and anti-helminths drugs and its administration.

5. CONFLICT OF INTEREST

All authors have declared that there is no conflict of interests regarding the publication of this article.

REFRENCES

Adesiji, G.B., Komolafe, S.E., and Ibrahim, M. (2014). Sources of Indigenous Knowledge on Healing Practices among farmers in Kwara State, Nigeria. *Sarhad Journal of Agriculture* 30, 272–275.

Akhtar, M.S., Zafar, I., Khan, M.H., and Lateef, M. (2000). Anthelmintic activity of medicinal plant with particular references to their use in animals in the Indo-Pakistan Subcontinent. *Small Ruminant Research* 38, 99–107.

Bisset, S.A, Vlassoff, A., Douch, P.G.C., Jonas, W.E., West, C.J., and Green, R.S. (1996). Nematode burdens and immunological responses following natural challenge in Romney Lambs selectively

bred for low and high faecal egg count. *Veterinary Pathology* 61, 249-263.

Fabusoro, E. (2009). Use of collective Action for land accessibility among settled Fulani agro-pastoralist in southwest Nigeria. *Sustainability Science* 4, 199-213.

Fabusoro, E., Omotayo, A.M., Apantaku, S.O., and Okuneye, P.A. (2010). Forms and Determinants of Rural Livelihoods Diversification in Ogun State, Nigeria. *Journal of Sustainable Agriculture* 34, 417–438.

Fakae, B.B., Musongong, G.A., Chiejina, S.N., Behnke, J.M., Ngongeh, L.A., and Wakelin, D. (2004). Variability in the resistance of the Nigeria West African Dwarf goats to abbreviated escalating trickle and challenge infections with *Haemonchus contortus. Veterinary Parasitology* 122, 51-65.

Gray, G.D. (1991). Breeding for resistance to *Trichostrongyle* nematodes in sheep'. In: *Breeding for Disease Resistance in Farm Animals. J.B. Owen and R.F.E. Oxford, P.P. Walling ford Efc.* CAB International Pp. 139-161.

Keyyu, J.D, Monrad, J., Kyvsgaard, Kassuky, A.A. (2005). Epidemiology of Fasciolagigantica and Amphistomes in cattle on traditional, small-scale dairy and large dairy farms in southern highlands of Tanzanian. *Trop Animal Health production*. 37, 303-314.

Kolawole, O.D., Okorie, V.O., Ogidiowa, M.T., and Adeogun, M.O. (2007). Ethno-veterinary practices amongst smallholder farmers in Ekiti State, Nigeria. *African Journal of Traditional, Complementary and Alternative Medicines* 4, 434-442.

Kubkomawa, H.I., Nafarnda, D.W., Adamu, S.M., Tizhe, M.A., Daniel, T.K., Shua, N.J., Ugwu, C.C., Opara, M.N., Neils, J.S. and Okoli I.C. (2013). Ethno-veterinary health management practices amongst livestock producers in Africa – A review. *World Journal of Agricultural Sciences* 1, 252-257.

Mapiye, C., and Sibanda S. (2005). Constraints and opportunities of village chicken production systems in the smallholder sector of Rushinga district of Zimbabwe. *Livestock Research for Rural Development*, 17, 1-7.

National Animal Disease Information Service (NADIS) (2010). Health Bulletin: Respiratory Disease in Cattle. UK, NADIS, pp 1-3.

Okwoche, V.A. (2013). Indigenous poultry management practices in Zone C Area of Benue State Agricultural Development Project, Nigeria. *The Social Sciences*, 8, 13-18.

Sreter, T., Kassai, T., and Takacs, E. (1994). The heritability and specificity of responsiveness to infection with *Haemonchus contortus*in sheep. *International Journal of parasitology* 24, 871-876.

Tabuti, J.R.S., Dhillion, S.S., and Lye, K.A. (2003). Ethnoveterinary medicines for cattle (*Bosindicus*) in Bulamogi county, Uganda: Plant species and mode of use. *Journal of Ethnopharmacology* 88, 279-286.

Woolaston, R.R., and Piper, L.R. (1996). Selection of Menrio sheep for resistance to *Haemonchus contortus:* Genetic variation, *Animal Science* 62, 451-460.

Zajac, M.A., and Garya, C. (2006). Veterinary Clinical Parasitology. 7th edition, *Black well Publishing Company, United Kingdom.*156.

Table 1. Medicinal Plants used by agro-pastoralists for the treatment of helminths

Name of the plant species	Habit	Local name	Herbarium number	Plant part(s) used	Mode of Administration
Ficuscapensis (*Moraenae*)	Stem barks	Epo-Obo	110607	bark, leaves, seeds	Bark, leaves and seed were grinded till powder form and added with salt for easy palatability, given orally a day for at least 5 days for relief of helminthes
Anthocleista djalonensis (*longaniaceae*)	Tree	Ewe Saapo	110630	bark, leaves, seeds	Bark, leaves and seed are grinded till powder and added with salt for easy palatability, given orally a day for at least 5 days for relief of helminthes
*Parinapari polyandra (*Chrysobalanaceae*)* (leaves, bark, seed)	Stem bark	Ewe Opoto	110629	Leaves with fruits	Same procedure as above

Table 2: Descriptive and mean differences showing the effect of treatment on FEC EGGS

Treatment Days	Herbal	Conventional	Control
	Mean ± SD	Mean ± SD	Mean ± SD
Day 1	4500.00 ± 623.61^{a}	4300.00 ± 788.811^{a}	4750.00 ± 1086.53^{a}
Day 2	1010.00 ± 521.643^{b}	680.00 ± 345.768^{a}	5200.00 ± 918.94^{c}
Day 3	410.00 ± 357.305^{a}	0.00 ± 0.00^{a}	5250.00 ± 824.958^{b}
Day 4	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}	4990.00 ± 2247.196^{b}
1st month after	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}	6700.00 ± 1159.502^{b}
2nd month after	1810.00 ± 1384.397^{b}	450.00 ± 772.082^{a}	6100.00 ± 1197.219^{c}
3rd month	2700.00 ± 1358.103^{a}	2370.00 ± 1531.920^{a}	6800.00 ± 1229.273^{b}

*Means with different subscript are significantly different from each other at 0.05 level of significance.

Table 3: Descriptive and mean differences showing the effect of treatment on the mean weight of cattle after 12 weeks

Treatment	Herbal	Conventional	Control
Weeks	Mean ± SD	Mean ± SD	Mean ± SD
0 week	84.20 ± 3.293a	83.50 ± 7.472a	82.80 ± 3.736[a]
2 weeks	86.68 ± 5.347[c]	83.00 ± 7.777[b]	80.54 ± 7.637[a]
4 weeks	87.17 ± 5.166[c]	83.65 ± 7.587[b]	78.05 ± 8.120[a]
6 weeks	87.99 ± 5.193 [c]	84.51 ± 7.359[b]	76.37 ± 7.534[a]
8 weeks	90.80 ± 3.810 [c]	85.25 ± 7.144[b]	76.55 ± 7.162[a]
10 weeks	89.81 ± 5.130 [c]	86.23 ± 7.014[b]	77.59 ± 6.224[a]
12 weeks	90.36 ±4.780 [c]	85.65 ±7.405[b]	76.68 ±6.435[a]

*Means with different subscript are significantly different from each other at 0.05 level of significant

UNIVERSITY OF SINDH JOURNAL OF ANIMAL SCIENCES
DOI: https://doi.org/10.57038/usjas.v6i04.5835
Uni. Sindh. J. Anim. Sci., vol. 6(4); 7-12, 2022
Email: *editors.usjas@usindh.edu.pk*
ISSN (P): 2521-8328
ISSN (E): 2523-6067
Published by University of Sindh, Jamshoro.

EFFECT OF HIGH DOSE (200MG/KG) OF ZnTe (ZINC TELLURIDE) ON SEROLOGY OF MALE ALBINO MICE (*MUS MUSCULUS*)

SAIRA BANO[1], AHMAD ALI*[2], SHEIKH MUHAMMAD AZAM[3], MATEEN TAHIR[4], ZEENAT ZAFAR[5], KHUZEEMA TANVEER[6], MUHAMMAD FAIZAN[7], SHOAIB HASSAN[1]

[1]Institute of Zoology, Bahaudin Zakariya University Multan, Pakistan
[2]Department of Zoology, Islamia University, Bahawalpur, Pakistan
[3]Department of Zoology, Division of Science & Technology, University of Education Lahore, Pakistan
[4]School of Life Sciences, Northeast Normal University, Renmin St. 5268, Changchun, 130024, China
[5]Nishter Medical University Multan, Punjab, Pakistan
[6]Institute of Microbiology, Faculty of Veterinary and animal Sciences, University of Agriculture Faisalabad, Pakistan
[7]Department of Zoology, University of Agriculture, Faisalabad, Pakistan

ARTICLE INFORMATION

Article History:
Received: 28th September 2022
Accepted: 23rd December 2022
Published online: 31st December 2022

Author's contribution
SB & AA Conceptualization, designed the experiments, editing, data analysis, formal Analysis, methodology, SMA, SH & MF visualization, writing-original draft, KH, ZZ & MT writing draft, Reviewed the paper.

Key words:
Mus musculus, Zinc Telluride, Serology, Nanoparticles

ABSTRACT

For this experiment, oral suspension of ZnTe was given to male albino mice for fifteen days. Two groups were devised as Treated and Control. Each group consisting of three treatment organisms. It is observed that the level of cholesterol, Triglycerides, and protein increased significantly (p = 0.21, 0.37, and 0.18 respectively) in treated animals as compared to control group. Moreover, the level of Albumin increased non-significantly (p = 0.75) in treated animals as compared to control group. Results of the study also showed significantly increase in level of Globulin and SGPT (ALT) (p = 0.14, and 0.026) in treated animals in contrast to control group, while significantly decrease in creatinine (p = 0.026) in treated animals as compared to control group.

1. INTRODUCTION

Among the necessary macro and microminerals, zinc (Zn) is important for cell division, the production of protein and DNA, and the metabolism activity of roughly 300 metabolic enzymes (Bhowmik et al., 2010). In addition, the body cannot store much zinc, therefore it needs a constant supply from the food (Rink & Gabriel 2001). Researchers have made progress in nanotechnology during the past decade. As this new field grows, scientists are getting better at making nanoparticles with amazing properties that can be used in many different ways (Zhao et al., 2016). Accumulation of nanoparticles in various tissues including brain and testis tissue in laboratory rats which were subjected in different ways. These nanoparticles have the ability to cross testes and blood-brain barrier (Kreuter, 2007).

Nanotechnology offers creative answers in the health, materials science, optical, and electronics fields. Zn nanoparticles have been researched as anti-cancer medicines (Anjum et al., 2021). According to Ersching et al. (2010) zinc telluride is a Group II to VI compound semi-conductor with a directly band gap of 2.26 eV at ambient temperature. According to Promnopas et al. (2014) ZnTe typically has a cubic structure (zinc blende or sphalerite), but it may also be manufactured as hexagon crystal (wurtzite form) (Dwivedi et al., 2009). Highly efficient multi-junction solar cells (Jioa et al., 2015), terahertz (TH) equipment (Loffler et al. 2005), light emitting diodes (LEDs), photodetectors (Liu et al., 2013), light emitting diodes (LEDs), solar panels (Promnopas et al., 2014), light emitting diodes (Shaygan et al., 2014), optoe (Lincheneau et al., 2014).

*Corresponding Author: ahmad.ali@iub.edu.pk

The crystalline structure and particle density affect each of those. For the past few decades, many scientists have been fascinated by studying nanoparticles. Because they have a wider range of qualities than materials. Actually, several techniques have been used to create various types of nanoparticles, such as Cd-chalcogenide, and they all have qualities that rely on their size (Orii et al. 2007). For the synthesis of ZnTe nanoparticles, several researchers have used a variety of methods, including electrodeposition (Xia et al. 2003), chemical synthesis (Dwevdi et al. 2009), thermal evaporation (Sharma et al. 2013), microwave - assisted extraction (Mohd et al. 2012), vaporisation technique (Feng et al. 2013), spray gasification (Kim et al., 2011).

In order to assess the potential effects of ZnTe NP on the Serology of albino mice and to calculate the changes posed by the high dose (200ml/Kg) of these nanoparticles, an experiment was conducted on male albino mice (Mus musculus) to compare the effect of higher doses of ZnTe nanoparticles on specific serum biochemicals that may serve as general indicators of optimum physiological functions.

2. MATERIALS AND METHODS

ZnTe nanoparticles

A wet chemical synthesis technique was used to synthesize the ZnTe nanoparticles, which were then examined using TEM, XRD, and SAED to determine their composition. Debye Scherrer's equation was used to calculate the crystallite sizes of the synthesized nanoparticles, and it revealed that they were 6 nm in size (Dhungana et al., 2016).

Animals

We purchased 4-week-old adult male albino mice (*Mus musculus*) from the institute of pharmacy and pharmacology, Bahuddin Zakariya University, Multan, Pakistan. The rats were randomly assigned to woody cages (one animal per cage) and acclimatized for 15 days at a temperature of 22°C±1°C and 50%±1% humidity with a 10-hour artificial cycle and access ad libitum to fresh tap water and a mouse diet for 15 days before the experiment. The experiments were accomplished following the research protocols recognized by the ethical committee of the Islamia University of Bahawalpur, Pakistan.

Control group and Treated group

*Mus musculus*were randomly divided into two groups, each group contain 3 animals.

1. Control group: *Mus musculus* of this group were administered 0.9 % Nacl saline solution orally for two weeks. Control group didn't receive any other treatment throughout the whole period of the experiment.
2. Treated Group (High-dose ZnTe): *Mus musculus* of this group received 200mg/kg body weight of Zinc Telluride nanoparticles orally by a gastric tube once per day for two weeks

Zinc Telluride Nanoparticles Solution

A zinc telluride nanoparticles solution was made by dissolving 12mg into 100ml and 200ml of distilled water to make a stock solution (Dunpall, 2016). The dosage was determined by the relative sizes of the mice. For a 30g mouse, a dose of 0.036µl will be applied from the stock solution (Ghosh et al., 2011). A similar process was repeated for the control group, which got the standard saline solution.

Methods

After 15 days (24 hours from the last dose) mice from control and treated group were subjected to;

Determination of Serological Parameters

Male albino mice used as controls and recipients of the treatment had blood samples collected in Eppendorf tubes and centrifuged at 14000 RPM for 8 minutes. To use diagnostic kits to measure cholesterol, creatinine, low density lipoprotein (LDL), high density lipoprotein (HDL), and triglycerides, the separated serum was put into fresh, labelled tubes.

Cholesterol

Using O.R.I. Reinbeker-75, the volume of the sample of cholesterol was done (Hamburg, Germany). The amount of cholesterol in blood samples was determined using BIOMED diagnostic tools made by Egy Chem (Egypt). A prepared reagent and a reference solution (R1) were included in the cholesterol kit (R2). Each serum sample's 10 ml received 1 ml of R2. After mixing the samples, they were left to incubate for fifteen minutes at room temperature. The material's wavelength was set at 340 nm. A blank solution was used to reset the instrument to zero (cuvette containing prepared reagent and distilled water). A glass vial was filled with 1 ml of the stock solutions, and the absorption of that sample was compared to the blank samples. The standard solution's values was 0.402 mg/dl. The cholesterol quantity (mg/dl) for each serum sample was evaluated in comparison to the blank sample (Table 1).

Following formula was used for the calculation of cholesterol:

$$\text{Cholesterol mg/dl} = \frac{\text{Absorbance of sample}}{\text{Absorbance of standard}} \times 200$$

Table 1. Recipe for cholesterol determination

Reagent / Sample	Standard sample	Blank sample	Test sample
Reagent (R2)	1000 µl	1000 µl	1000 µl
Standard (R1)	10 µl	Nill	Nill
Test sample	Nill	Nill	10 µl
Distilled water	Nill	10µl	Nill

High density lipoprotein (HDL)

Using O.R.I., Reinbeker 75, the quantitative measurement of high density lipoprotein was completed (Hamburg, Germany). The BIOMED kit is made by Egy chem (Egypt). Each serum sample's absorption was determined at 546 nm. The precipitant reagent was part of the HDL kit (R). 500 l of precipitant were combined with 200 l of blood serum. The samples were then blended and kept for at room temperature for ten minutes. After that, the mixture was centrifuged at 12000 RPM for 2 minutes. 10 l of the supernatant from the centrifugation process was added to 1 ml of the BioMed saturated fat reagent. With a blank cuvette filled with distilled water and cholesterol reagent, the instrument was set to zero. After combining 10 l of standard R1 with 1 ml of cholesterol reagent, absorbance was calculated in comparison to a blank sample.

The HDL cholesterol was measured by following formula;

$$\text{HDLCholesterolmg/dl} = \frac{\text{Absorbance of sample}}{\text{absorbanceofstandard}} \times 50$$

Triglyceride

Utilizing O.R.I., Reinbeker -75, and BIOMED kits made by Egy Chem, triglyceride was identified. The object's wavelength was selected at 550 nm. The triglyceride package included a ready-to-use R2 and a typical (R1). Each serum sample was added in 10l increments to 1ml of R2. The materials were combined and incubated at room temperature for approximately 15 minutes. A blank sample was used to reset the instrument to zero. Prior to reading the serum samples, 10 l of R1 and 1 ml of R2 were combined in a glass cuvette, and their absorbance was compared to a blank sample. The standard solution yielded a result of 0.205 mg/dl. The serum samples were compared to a blank sample for analysis, and absorbance/concentration values (mg/dL) for each sample were noted.

Creatinine

Utilizing the diagnostic kit made by Egy chem and the O.R.I Reinbeker 4000, the quantitative assessment of creatinine in each blood sample was performed. Two reagents, R2, R3, and a reference reagent were included in the creatinine kit (R1). 1 ml of the working solution, which was made by combining 500 l of R2 and 500 l of R3 in a 1:1 ratio, was then added to 100 l of the blood serum. After mixing the samples, the standard's or specimen A1 absorbance was measured after 30 seconds. Second absorbance A2 of the standards or samples was measured at 500 nm after two minutes.

Protective measures

Before treating mice with stock solution, every time we used hand gloves, hand towels and laboratory mask as a protective measure. We used micropipette with labelled amount of stock solution to treat mice so to attain accuracy in doses. After every treatment we washed hands with antiseptic hand wash.

3. RESULTS AND DISCUSSION

Two sample t-test was applied to evaluate the ZnTe treated group with high dose (200mg/Kg) in male albino mice (Mus musculus) as compared to control group. It is observed that the level of cholesterol increased significantly ($p = 0.21$) in treated animals as compared to control group. It is observed in our results that the level of Triglycerides increased significantly ($p = 0.37$) in treated animals as compared to control group.Our results showed that the level of protein increased significantly ($p = 0.18$) in treated animals as contrast to control group.

The results revealed that the level of Albumin increased non-significantly ($p = 0.75$) in treated animals as compared to control group.The results showed that the level of Globulin increased significantly ($p = 0.14$) in treated animals in contrast to control group. When the results were observed that the level of Creatinine decreased significantly ($p = 0.026$) in treated animals as compared to control group. The results when examined showed that the level of SGPT(ALT) increased significantly ($p = 0.24$) in treated animals in comparison control group (Table 2) (Figure 1).

Metals include zinc with extremely little doses of zinc are important for maintaining human health, it is referred to as a "essential trace element". All animal species, including humans, need zinc for healthy development and development, and a lack of it slows down growth (Prasad, 1995). It is present in many biological systems and processes and is essential for many biological processes, including the immune system, wound healing, blood coagulation, thyroid function, and many more.

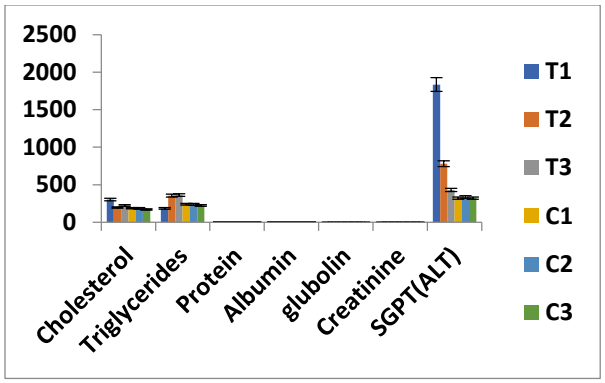

Figure 1: Serum Biochemistry

Zinc levels are comparatively high in meats, fish, milk products, nuts, beans, and whole grains. Zinc insufficiency is prevalent around the world but rare in the world. Slow development, low insulin levels, appetite loss, irritability, widespread hair loss, rough and dry skin, slowly healing wounds, a poor sense of taste and smell, diarrhoea, and other symptoms are some of the manifestations. Alcoholism, chronic renal failure, common chronic illnesses, and gastrointestinal problems that interfere the food intake (malabsorption syndromes) are all linked to moderate micronutrient deficiencies. Zinc deficiency is often brought on by inadequate food consumption. However, it could also be brought on by malabsorption and long-term conditions such diabetic, lymphoma, liver problems, and sickle cell anaemia. Our results revealed that the level of SGPT (ALT) significantly increased (P=0.24) when high doses (200mg/kg) of zinc telluride nanoparticles (ZnTe) were introduced in albino mice as compared to the controlled group. Our results are in contrast to those of (Andriollo-Sanchez et al,. 2008) who reported that the level of ALT remains stable, this is due to the reason that hepatic and cardiac functions were not damaged which was caused due to the intake of zinc gluconate. Our results are related to those of (Sharma et al., 2012) who reported that the level of ALT increases by giving zinc oxide. Our results also showed difference with those of (Navarro et al., 1993) who showed that the increase in ALT activities in plasma is mostly caused by the enzymes (LDH) leaking into the bloodstream from the liver cytosol as a result of liver injury and disturbance of normal liver function (Shakoori et al., 1994).

Our results revealed that the level of creatinine significantly decreased when high doses (200mg/kg) of zinc telluride nanoparticles (ZnTe) were introduced in albino mice as compared to the controlled group. Our results showed divergence to those (Andriollo-Sanchez et al., 2008) who reported that the level of creatinine didn't fall off due to which no renal dysfunctions occur. This is also due to the inoculation of zinc gluconate.

Our results revealed that the level of cholestrol gradually increased when high doses (200mg/kg) of zinc telluride nanoparticles (ZnTe) were introduced in albino mice as compared to the controlled group. Our results as compared to those of (Esmaeillou *et al.*, 2013) studied that no significant changes takes place in total protein cholesterol level. Slight swelling in the renal glomerulas was observed which is due to irregular array of the veins, hydrophobic degeneration with the fatty liver and loss of sinusoid.

Our results revealed that the level of protein gradually increased with the DF=3 when high doses (200mg/kg) of zinc telluride nanoparticles (ZnTe) were introduced in albino mice as compared to the controlled group. As compare to those of Eltohamy and Younis (1991) showed a significant reduction (P<0.05) in protein, compared to control, when treated with low dose of zinc. (Prasad, 1996) found that Zinc deficiencies that influence synthesis of DNA, protein synthesis and cell division.

Our results revealed that the level of Serum albumin non-significantly increased while globulin increased significantly when high doses (200mg/kg) of zinc telluride nanoparticles (ZnTe) were introduced in albino mice as compared to the controlled group. Our findings showed that rats fed half as much zinc as controls had lower immunoglobulin and serum albumin levels (Prasad, 1998). This is a result of the diet's inadequate zinc intake. Levels of GST were increased as a result of a Zn deficit (Jagadeesan, 1989).

4. CONCLUSION

In conclusion, when high dose of ZnTe was orally treated to albino mice then the serum biochemistry (cholesterol, triglycerides, protein, globulin and SGPT(ALT) was increased significantly While the level of albumin increased non-significantly. On the other hand, the level of creatinine decreased significantly and showed variation.

5. CONFLICT OF INTEREST

All authors declared no conflict of interests.

REFRENCES

Andriollo-Sanchez, M., Hininger-Favier, I., Meunier, N., Venneria, E., M. O'Connor, J., Maiani, G., Polito, G., Bord, S., Ferry, M., Coudray, C. & Roussel, A. (2008) No Antioxidant Beneficial Effect of Zinc Supplementation on Oxidative Stress Markers and Antioxidant Defenses in Middle-Aged and Elderly

Subjects: The Zenith Study, *Journal of the American College of Nutrition*, 27:4, 463-469, DOI: 10.1080/07315724.2008.10719726

Bhowmik, D., Chiranjib, K.P. & Sampath, K. (2010). A potential medicinal importance of zinc in human health and chronic disease. *International Journal of Pharma Biomedical Research*, 1:05–11.

Dwivedi, D. K., Dayashankar, & Dubey, M. (2009). Synthesis, characterization and electrical properties of ZnTe nanoparticles. *Journal of Ovonic Research*, 5(2):35-41.

Eltohamy, M.M. & Younis, M. (1991). Response of testes, epi-didymis, and seminal vesicle of rabbits to zinc deficiency. Archieve of Experimental Veterinary medicine, 45, 155–160.

Ersching, K., F. L. Faita, C. E. M. Campos, T. A. Grandi, & Pizani, P.S. (2010). Ageing effect on mechanically alloyed ZnTe nanocrystals. *Journal of Alloys and Compounds*, 493:294-298.

Esmaeillou, M., Moharamnejad, M. & Hsankhani R. (2013) Toxicity of ZnO nanoparticles in healthy adult mice. *Environmental Toxicology and Pharmacology*, 35(1): 67–71.

Feng, X., K. Singh, S. Bhavanam, V. Palekis, L. D. Morel, & Ferekides, C. (2013). Preparation and characterization of ZnTe as an interlayer for CdS/CdTe substrate thin film solar cells on flexible substrates. *Thin Solid Films*, 535:202-205.

Hossain, M. S., R. Islam, & Khan, K. A. (2008). Structural, elemental compositions and optical properties of ZnTe:V thin films. *Chalcogenides Letters*, 5(1):1-9.

Jagadeesan V. (1989). Study of activating and conjugating enzymes of drug metabolism in zinc deficiency. *Indian journal of experimental biology*, 27(9), 799–801.

Jiao, S., Q. Shen, I. Mora-Sero, J. Wang, Z. Pan, K. Zhao, Y. Kuga, X. Zhong, & Bisquert, J. (2015). Band engineering in core/shell ZnTe/CdSe for photovoltage and efficiency enhancement in exciplex quantum dot sensitized solar cells. *ACSNANO*, 9(1):908- 915.

Kim, J. D., J. W. Kim, J. E. Kim, & Koo, K. K. (2011). Formation of 1-D ZnTe nanocrystals by aerosol-assisted spray pyrolysis. *Korean Journal of Chemical Engineering*, 28(4):1120-1125

Lincheneau, C., M. Amelia, M. Oszajca, A. Boccia, F. D'Orazi, M. Madrigale, R. Zanoni, R. Mazzaro, L. Ortolani, V. Morandi, S. Silvi, K. Szacilowski, & Credi, A. (2014). Synthesis and properties of ZnTe and ZnTe/ZnS core/shell semiconductor nanocrystals. *Journal of Materials Chemistry C*, 2:2877-2886.

Loffler, T., T. Hahn, M. Thomson, F. Jacob, & Roskos, H. G. (2005). Large–area electro-optic ZnTe terahertz emitters. *Optic Express*, 13(14):5353-5362.

Mohd, S., S. Aarya, R. Singh, M. Arora, G. Bhagavannarayana, & Senguttuvan, T. D. (2012). Synthesis of ZnTe nanoparticles by microwave irradiation technique and their characterization. *Nanoscience Nanotechnology Letter*, 4:405–408.

Navarro, C., Wu, L.F. & Mandrand-Berthelot, M.A. (1993). The nik operon of Escherichia coli encodes a periplasmic binding-protein-dependent transport system for nickel. *Molecular Microbiology*, 9:1181-1191.

Orii, T., M. Hirasawa, & Seto, T. (2007). Effect of in situ annealing on structure and optical properties of ZnTe nanoparticles produced by pulsed laser ablation. *Journal of Physics*, 59:716-719

Prasad, A.S. (1995). *Zinc: an overview. Nutrition*, 11: 93-9.

Prasad, A.S. (1998). Zinc and immunity. In: Pierce, G.N., Izumi, T., Rupp, H., Grynberg, A. (eds) Molecular and Cellular Effects of Nutrition on Disease Processes. *Developments in Molecular and Cellular Biochemistry*, 26. Springer, Boston, MA. https://doi.org/10.1007/978-1-4615-5763-0_8

Promnopas, W., T. Thongtem, & Thongtem, S. (2014). ZnTe Semiconductor-polymer gel composited electrolyte for conversion of solar energy. *Journal of Nanomaterials* (http:// dx.doi.org/10.1155/2014/529629).

Rink, L. & Gabriel, P. (2001). Extracellular and immunological actions of zinc. *Bimetals*, 14:367–383.10.1023/A:1012986225203

Shakoori, A.R., Yasmin, G. Ahmed, M. & Shakoori, A.R. (1994). Effect of sumicidin super on blood serum proteins and acetylcholine esterase activity of broiler chicks of *Gallus domesticus*. Proceedings of Pakistan Congress Zoology, PCZ'1994 Zoological Society of Pakistan, Government College, Lahore, pp: 73-79.

Sharma, D. C., S. Srivastava, Y. K. Vijay. & Sharma, Y. K. (2013). Preparation and optical properties of ZnTe/ZnTe:Cr. *Advanced Materials Letters,* 4(1):68-70.

Shaygan, M., T. Gemming, V. Bezugly, G. Cuniberti, J.-S. Lee, & Meyyappan, M. (2014). In situ observation of melting behavior of ZnTe nanowires. *Journal of Physical Chemistry*, 118:15061-15067.

Xia, Y., P. Yang, Y. Sun, W. Yiying, B. Mayers, B. Gates, Y. Yin, F. Kim, & Yan, H. (2003). One-Dimensional nanostructures: synthesis, characterization and applications. *Advanced Materials,* 15(5):353- 389.

Table 2: Effect of high dose 200mg/kg of Zinc Telluride nanoparticles on male Albino mice 200mg/kg/body weight

Parameters	T1	T2	T3	C1	C2	C3
Cholesterol	303.2	196.1	223.8	188.9	186.4	170.2
Triglycerides	185.3	355.9	361.7	241.9	238.2	225.5
Protein	6.6	5.3	6	5.8	4.8	4.5
Albumin	5.8	4.9	5.3	5.5	5.5	5.3
Glubolin	0.8	0.4	0.7	0.3	0.38	0.33
Creatinine	0.6	0.2	0.26	1.12	1.2	1.1
SGPT(ALT)	1833.2	779.2	431	321.8	335.8	322.9

UNIVERSITY OF SINDH JOURNAL OF ANIMAL SCIENCES
DOI: https://doi.org/10.57038/usjas.v6i04.5837
Uni. Sindh. J. Anim. Sci., vol. 6(4); 13-17, 2022
Email: *editors.usjas@usindh.edu.pk*
ISSN (P): 2521-8328
ISSN (E): 2523-6067
Published by University of Sindh, Jamshoro.

SOME RECORDS OF ANT (HYMENOPTERA: FORMICIDAE) FROM DISTRICT POONCH, AZAD JAMMU AND KASHMIR, PAKISTAN

FARZANA AFSAR[1], MUHAMMAD RAFIQUE KHAN[1], ZAHID MAHMOOD SARWAR[3], AHMAD ZIA[2], QUDRAT ULLAH[4], MUHAMMAD QASIM[5], NAZEER AHMED[6], SUMARA ASLAM[2], MUHAMMAD ATHER RAFI[2], MUHAMMAD KAMAL SHEIKH[7], ADNAN SAMEI[8]

[1]Department of Entomology, University of the Poonch, Rawalakot, Azad Jammu & Kashmir.
[2]National Insect Museum, National Agricultural Research Center, Islamabad.
[3]Department of Entomology, Bahauddin Univesity Zakariya University, Multan, Punjab.
[4]Department of Zoology, University of Peshawar, Peshawar.
[5]Department of Zoology, Kohsar University Murree, 47150 Punjab, Pakistan.
[6]Department of Entomology, University of Swabi, Khyber Pakhtunkhwa, Pakistan.
[7]Planning and Development Division, Pakistan Agricultural Research Council, Islamabad,
[8]Department of Zoology, Islamia College University, Peshawar.

ARTICLE INFORMATION

Article History:
Received: 3rd November 2022
Accepted: 21st December 2022
Published online: 31st December 2022

Author's contribution
F.A, M.R.K., A.Z. & M.A.R designed the study, F. A. collected the specimens. Q.U., Z. M. S & M.Q identified the specimens. N.A., S.A. M.K.S 7 A.S. helped the literature and finalized the manuscript.

Key words:
Ant, Hymenoptera, Formicidae, Poonch, Azad Jammu and Kashmir, Pakistan

ABSTRACT

In the present study 12 species belonging to 10 genera of three subfamilies namely; Formicinae, Myrmicinae and Ponerinae under family Formicidae are reported from district Poonch, Azad Jammu and Kashmir. Among the recorded species, six species belonging to Formicinae, four in Myrmicinae, and two species in Ponerinae. All the 12 species are reported first time for district Poonch, Azad Jammu and Kashmir. however, only one species namely, *Odontoponera transversa* (Smith, 1857) of subfamily Ponerinae is new record from Pakistan.

1. INTRODUCTION

The family Formicidae of the order Hymenoptera contains a group of social insects known as ants. Millions of individuals make up the colonies in which they dwell. In the symbiotic relationship, they also play an important role between plants, arthropods, fungi, and microbes (Hölldobler & Wilson, 1990; Clarke & Kitching, 1995; Jolivet, 1996; Schultz & Mcglynn, 2000). In trophic soil, ants perform more crucial roles than earthworms such as nutrient recycling, biotic interactions, scavenging, pollination, and soil aeration (Carroll & Janzen, 1973; Hölldobler & Wilson, 1994; Folgarait, 1998; Risch & Jurgensen, 2008;Bahrti, 2011).

Ants act as bio-control agents (Hölldobler & Wilson, 1990; Latifian et al., 2018) and also known as the largest group of predators for termite and arthropod in World (El Keroumi et al., 2010; Symondson,2002; Sanders & Veen 2011; Fernandes et al., 2010). Ants also act as a pest in many crops. Some ants injure the agricultural as well as horticultural crops and acquire the phloem from injured crop plants to obtain carbohydrates (Stewart & Vinson, 1991). In addition to being a host for tiny mammals and birds, they pose a threat to humanoid health by spreading illnesses like typhoid fever, dysentery, and tuberculosis. The primary causes of these illnesses are ants' crawling and feeding habits over mucus coughed up and faeces (Brown, 1965). Monomorium ants also irritated the Humans and domestic animals (Vander Meer, 1990;Solis et al., 2010;Vinson, 1986). Some ants, such as bullet and fire ants include pepridine alkaloids in their poison sacs, which are harmful to those who are overly sensitive (Bharti, 2011; Clarke, 1986;Stafford, 1996).

*Corresponding Author: a_rafiam@yahoo.com

Family Formicidae holds approximately 1500 species under 22 subfamilies (Hölldobler & Wilson, 1990; Guénard, 2013). Hosoishi and Ogata reported 145 species and 61 subspecies under a single genus *Crematogaster* from Asia (Hsoishi & Ogata, 2009). Sharaf et al. (2018) recorded 123 species under 24 genera belonging to four subfamilies included two new to science species namely *Aphaenogaster sarae* and *Aphaenogaster asmaae* from Oman (Rasheed et al., 2019). Rasheed et al. reported 103 species of ant fauna, belonging to 35 genera under seven subfamilies from Pakistan. Recently Khudadad et al. (2021) reported 28 species, under 18 genera of family Formicidae from district Mansehra, Pakistan from which six species reported first time from Pakistan Usman et al. (2017) reported 17 species of ants from District Karak, Khyber Pakhtunkhwa Pakistan. Bodlah et al. (2016) recorded two species namely *Tetraponera allaborans* and *T. nigra* from district Rawalpindi and Islamabad, Pakistan. Ahmed et al. (2013) reported seven species from Quetta, Baluchistan, Pakistan. Umair et al. (2012) reported 21 species of the family Formicidae from Potohar Plateau of Pakistan, eight of these were economically important pests.

Azad Jammu and Kashmir, Pakistan has an importance in biogeography position but the taxonomic studies of ant's fauna of Azad Jammu and Kashmir, have been badly neglected. Keeping in view the importance of ants the present study was planned for exploring ant species of district Poonch, Azad Jammu and Kashmir, Pakistan.

2. MATERIALS AND METHODS

Collection of ants was made by conducting many surveys during 2013 in agronomic crops, woodlands, vegetables, blooming plants, nurseries, and open fields from the different localities of district Poonch. Manually and Pitfall traps were used to collect the ants' specimens. After being properly stretched and pinned, collected specimens were deposited in wooden boxes along with tags. These specimens were identified up to species level by using the available literature, taxonomic keys and books. The samples were further verified by using an identified collection kept at the National Insect Museum (NIM), National Agricultural Research Center (NARC), Islamabad Pakistan. Identified specimens with complete detail were deposited in Department of Entomology, University of Poonch Rawalakot (UPR), Azad Jammu and Kashmir. The topography of district Poonch is primarily mountainous and hilly, with plains, valleys, freshwater reservoirs, stunning lakes, rivers. Rainfall occurs in this area in both winter and summer seasons and recorded on average 1400 mm.

3. RESULTS AND DISCUSSION

Family Formicidae Latreille, 1809
Subfamily Formicinae Latreille, 1809

Genus *Camponotus* Mayr, 1861

1. *Camponotus compressus* Fabricius, 1787

Material Examined: AZAD JAMMU AND KASHMIR: Poonch: Khaigala, 2 workers, 11.vi.2013, 3 workers, 28.vii.2013, 2 workers, 2.vi.2013; Alisojal, 2 workers, 28.vii.2013; Rawalakot, 1 worker, 6.vi.2013; Chaprian, 3 workers, 4.vi.2013; Hajira, 1 worker, 10.vi.2013, leg. Farzana; ex UPR.

Remarks: Earlier Umair et al. and Usman et al. reported this species from different localities of Pakistan e.g., Khyber Pakhtunkhwa: Karak; Punjab: Rawalpindi, Taxila, Gujar Khan, Kahuta, Islamabad (Usman et al., 2017; Umair et al., 2012).

2. *Camponotus oblongus* Smith, 1858

Material Examined: AZAD JAMMU AND KASHMIR: Poonch: Khaigala, 2 workers, 11.vi.2013; Topa, 2 workers, 16.iii.2013, leg. Farzana; ex UPR.

Remarks: Umair et al. and Rasheed et al. already reported this species from Punjab: Rawalpindi (Rasheed et al., 2019; Umair et al., 2012).

Genus *Formica* Linnaeus, 1758

3. *Formica fusca* Linnaeus, 1758

Material Examined: AZAD JAMMU AND KASHMIR: Poonch: Paniola, 3 workers, 2.iv.2013; Alisojal, 2 workers, 28.vii.2013; Rawalakot, 1 worker, 6.vi.2013; Hajira, 1 worker, 27.vii.2013, leg. Farzana; ex UPR.

Remarks: Earlier Rasheed et al. reported this spices from Gilgit-Baltistan **(**Karakorum) (Rasheed et al., 2019).

Genus *Lepisiota* Santschi, 1926

4. ***Lepisiota frauenfeldi* (Mayr, 1855)**

Material Examined: AZAD JAMMU AND KASHMIR: Poonch: Paniola, 6 workers, 15.iii.2013; 3 workers, 17. v.2013; Alisojal, 4 workers, 11.vi.2013; Rawalakot, 4 workers, 15.iii.2013; Chaprian, 2 workers, 4.vi.2013; Mandol, 3 workers, 27.vii.2013; Thorar, 2 workers, 16.vi.2013; Banjosa, 4 workers, 17.iii.2013; 4 workers, 8.vi.2013, leg. Farzana; ex UPR.

Remarks: Earlier Umair et al. described this species from Potohar Plateau of Pakistan (Umair et al., 2012).

Genus *Polyrhachis* Smith, 1857

5. *Polyrhachis hodgsoni*, Forel, 1902

Material Examined: AZAD JAMMU AND KASHMIR: Poonch: Hajira, 2 workers, 10.vi.2013; 1 worker, 27.vii.2013; Alisojal, 3 workers, 28.vii.2013; Rawalakot, 4 workers, 2 workers, 10.vi.2013; Banjosa, 2

workers, 17.vi.2013; 3 workers, 19.iv.2013; Topa, 3 workers, 16.iii.2013, leg. Farzana; ex UPR.
Remarks: Earlier Umair et al. reported this species from Pakistan (Islamabad) (Umair et al., 2012).

6. *Polyrhachis grisescens* Emery, 1895
Material Examined: AZAD JAMMU AND KASHMIR: Poonch: Khaigala, 3 workers, 11.vi.2013, leg. Farzana ex UPR.
Remarks: This species already reported by Rasheed et al. from Kohat, Pakistan (Rasheed et al., 2019).

Subfamily Myrmicinae
Genus *Crematogaster* Lund, 1831
7. *Crematogaster rothneyi* Mayr, 1878
Material Examined: AZAD JAMMU AND KASHMIR: Poonch: Khaigala, 3 workers, 11.vi.2013; 1 worker, 3.ix.2013; Paniola, 5 workers, 15.iii.2013; Alisojal, 1 worker, 11.vi.2013; Banjosa, 3 workers, 11.iii.2013; 2 workers, 19. v.2013; Hajira, 2 workers, 10.vi.2013; Thorar, 2 workers, 16.iii.2013; 3workers, 18.v.2013, leg. Farzana; ex UPR.
Remarks: This species was already reported from Potohar Plateau of Pakistan [31]. Most recently, Usman et al. recorded this species from Karak district Khyber Pakhtunkhwa province of Pakistan (Usman et al., 2017).

Genus *Holcomyrmex* Mayr, 1879
8. ***Holcomyrmex scabriceps* Mayr, 1878**
Material Examined: AZAD JAMMU AND KASHMIR: Poonch: Khaigala, 3 workers, 11.vi.2013; 5 workers, 20.vii.2013; Hajira, 5 workers, 10.vi.2013; Thorar, 2 workers, 16.iii.2013; 6 workers, 3.iv.2013; Mandol, 3 workers, 10.vi.2013; 2 workers, 27.vii.2013; 3 workers, 16. viii.2013; Banjosa, 2 workers, 17.iii.2013; 5 workers, 4.iv.2013, leg. Farzana; ex UPR.
Remarks: This species was previously reported by Umair et al. from Potohar Plateau and Usman et al. from Karak district, Khyber Pakhtunkhwa province, Pakistan (Usman et al., 2017;Umair et al., 2012).

Genus *Monomorium* Mayr, 1855
9. *Monomorium fossulatum* Emery, 1894
Material Examined: AZAD JAMMU AND KASHMIR: Poonch: Khaigala, 2 workers, 11.vi.2013; 3 workers, 18. viii.2013; Alisojal, 5 workers, 28. viii.2013; 2 workers, 3.ix.2013, Chaprian, 3 workers, 17.iii.2013; 02 workers, 4.iv.2013; 3 workers, 8.v.2013, Hajira, 5 workers, 27.vii.2013; Thorar, 1 worker, 16.iii.2013; 2 workers, 10.vi.2013, leg. Farzana; ex UPR.
Remarks: Most recently, Usman et al. reported this species from Karak district, Khyber Pakhtunkhwa province, Pakistan (Usman et al., 2017).

Genus *Pheidole* Westwood, 1839
10. ***Pheidole latinoda* Roger, 1863**
Material Examined: AZAD JAMMU AND KASHMIR: Poonch: Paniola, 3 workers, 15.iii.2013; 1 worker, 17. v.2013; 2 workers, 6.vi.2013; Alisojal, 1 worker, 11.vi.2013; Thorar, 02 workers, 3.iv.2013, leg. Farzana; ex UPR.
Remarks: Recently, Rasheed et al. reported from Khyber Pakhtunkhwa: Charsadda, Umerzai (Rasheed et al., 2020).

Subfamily Ponerinae
Genus *Myopopone* Roger, 1861
11. *Myopopane moelleri* Bingh, 1860
Material Examined: AZAD JAMMU AND KASHMIR: Poonch: Khaigala, 2 workers, 11.vi.2013; 5 workers, 28. viii.2013; Paniola, 4 workers, 15.iii.2013; 5 workers, 6.vi.2013; Hajira, 1 worker, 10.vi.2013; Mandol, 2 workers, 27.vii.2013; Banjosa, 2 workers, 17.iii.2013; Topa, 2 workers, 3.iv.2013, leg. Farzana; ex UPR.
Remarks: Recently Khudadad et al. reported this species from district Mansehra, Khyber Pakhtunkhwa, Pakistan (Khudadad et al., 2021).

Genus *Odontoponera,* Mayr, 1862
12. ***Odontoponera transversa* (Smith, 1857)**
Material Examined: AZAD JAMMU AND KASHMIR: Poonch: Hajira, 1 worker, 2.ix.2013, leg. Farzana; ex UPR.
Remarks: New record for Pakistan as well as district Poonch of Azad Jammu and Kashmir.

4. CONCLUSION

Ants (Hymenoptera: Formicidae) are advantageous and damaging insects. Ants damage yields, seeds, leaves, and grassland. They are good pollinators, decomposers, and scroungers. Ants perform symbiotic relationships with mealy bugs, aphids, fungi and Lepidoptera insects. In Pakistan small work has been done on its taxonomy. The present study reported 12 species under 10 genera in three subfamilies: six species under four genera of subfamily Formicinae, four species belong to four genera of subfamily Myrmicinae, and two species under two genera of subfamily Ponerinae, from District Poonch, Azad Jammu and Kashmir. Out of the 12 species, one species *Odontoponera transversa* (Smith, 1857) of subfamily Ponerinae is new record for Pakistan.

5. CONFLICT OF INTEREST

All authors have declared that there is no conflict of interests regarding the publication of this article.

REFRENCES

Ahmed, H. A., Noor, S., Sani, I. A., Kanwal, S., Khudaidad, S., & Khawar, M. (2013). Ant fauna (Hymenoptera: Formicidae) of Quetta, Balochistan, Pakistan. *Serranga*, *18*(2), 47-59.

Bharti, H. (2011). List of Indian Ants (Hymenoptera: Formicidae). *Halteres*, *3*, 79-87.

Bodlah, I., Rasheed, M. T., Fareen, A. G., Ajmal, M. S., & Bodlah, M. A. (2016). First record of two new species of genus Tetraponera (Hymenoptera: Pseudomyrmecinae: Formicidae) from Pakistan. *Journal of Entomology and Zoology Studies*, *4*(4), 1028-1030.

Brown Jr, W. L. (1965). Contributions to a reclassification of the Formicidae. IV. Tribe Typhlomyrmecini Hymenoptera). *Psyche*, *72*(1), 65-78.

Carroll, C. R., & Janzen, D. H. (1973). Ecology of foraging by ants. *Annual Review of Ecology and systematics*, 4, 231-257.

Clarke, C. M., & Kitching, R. L. (1995). Swimming ants and pitcher plants: a unique ant-plant interaction from Borneo. *Journal of Tropical Ecology*, *11*(4), 589-602.

Clarke, P. S. (1986). The natural history of sensitivity to jack jumper ants (Hymenoptera formicidae Myrmecia pilosula) in Tasmania. *Medical Journal of Australia*, *145*(11-12), 564-566.

El Keroumi, A., Naamani, K., Dahbi, A., Luque, I., Carvajal, A., Cerda, X., & Boulay, R. (2010). Effect of ant predation and abiotic factors on the mortality of medfly larvae, Ceratitis capitata, in the Argan forest of Western Morocco. *Biocontrol Science and Technology*, *20*(7), 751-762.

Fernandes, F. L., Picanço, M. C., FERNANDES, M. D. S., Xavier, V. M., Martins, J. C., & Salva, V. F. (2010). Controle biológico natural de pragas e interações ecológicas com predadores e parasitóides em feijoeiro. *Biosci. J*, *26*, 6-14.

Folgarait, P. J. (1998). Ant biodiversity and its relationship to ecosystem functioning: a review. *Biodiversity & Conservation*, *7*(9), 1221-1244.

Guénard, B. (2013). *An overview of the species and Ecological Diversity of Ants*. eLS. John Wiley and Sons, Ltd: Chichester.

Hölldobler, B., & Wilson, E. O. (1990). The ants Belknap Press of Harvard University Press. *Cambridge, Massachusetts*.

Hölldobler, B., & Wilson, E. O. (1990). *The ants*. Harvard University Press.

Hosoishi, S., & Ogata, K. (2009). A check list of the ant genus *Crematogaster* in Asia (Hymenoptera: Formicidae). *Bulletin of the Institute of Tropical Agriculture, Kyushu University*, *32*(1), 43-83.

Jolivet, P. (1996). Ants and plants: an example of coevolution. Enlarged ed. *Backhuys, Leiden, 303pp*.

Khudadad, S., Rafi, M. A., Zia, A., Khan, M. S., Parveen, G., Sheikh, M. K. & Shah, S. W. (2021). Ant fauna (Hymenoptera: Formicidae) of district Mansehra, Pakistan. *Pakistan Journal of Agricultural Research*, *34*(3), 664-671.Pakistan Journal of Agricultural Research, 34(3): 664-671.

Latifian, M., Habibpour, B., & Kard, B. (2018). Predator ants of the date palm termite Microcerotermes diversus Silvestri and effects of ant morphometric characteristics on ant functional response. *American Journal of Entomology*, *2*(2), 16-22.

Rasheed, M. T., Bodlah, I., e Fareen, A. G., Wachkoo, A. A., Huang, X., & Akbar, S. A. (2019). A checklist of ants (Hymenoptera: Formicidae) in Pakistan. *Sociobiology*, *66*(3), 426-439.

Rasheed, M. T., Bodlah, I., Gull-e-Fareen, A., Ali, H., & Raja, M. B. M. (2020). Distribution and Trophic Associations of Ant Genus Monomorium Mayr, 1855 (Hymenoptera: Formicidae: Myrmicinae) With Aphids In Pothwar Region of Pakistan. *Journal of Animal and Plant Sciences*, *30*(2), 391-397.

Risch, A. C., & Jurgensen, M. F. (2008). Ants in the soil system-a hydrological, chemical and biological approach. *Journal of Applied Entomology*, *132*(4), 265.

Sanders, D., & van Veen, F. F. (2011). Ecosystem engineering and predation: the multi-trophic impact of two ant species. *Journal of Animal Ecology*, *80*(3), 569-576.

Schultz, T. R., & McGlynn, T. P. (2000). *The interactions of Ants with other organisms. Di dalam: Agosti D, Majer JD, Alonso LE*. Schultz TR, editor. Ants: Standard Methods for Measuring and Monitoring Biodiversity. Washington: Smithsonian Institution Press.

Sharaf, M. R., Fisher, B. L., Al-Dhafer, H. M., Polaszek, A., & Aldawood, A. S. (2018). Additions to the ant fauna (Hymenoptera: Formicidae) of Oman: an updated list, new records and a description of two new species. *Asian Myrmecology*, 10, 1-38.

Solis, D. R., Fox, E. G. P., Kato, L. M., Jesus, C. M. vD., Yabuki, A. T., Campos, A. E. D. C., & Bueno, O. C. (2010). Morphological description of the immatures of the ant,

Monomorium floricola. *Journal of Insect Science*, 10(1).

Stafford, C. T. (1996). Hypersensitivity to fire ant venom. *Annals of Allergy, Asthma & Immunology*, *77*(2), 87-99.

Stewart, J. W., & Vinson, S. B. (1991). Red imported fire ant damage to commercial cucumber and sunflower plants. *Southwestern Entomologist*, *16*(2), 168-1760.

Symondson, W. O. C., Sunderland, K. D., & Greenstone, M. H. (2002). Can generalist predators be effective biocontrol agents. Annu. Rev. Entomol., 47, 561–594.

Umair, M., Zia, A., Naeem, M., & Chaudhry, M. T. (2012). Species composition of ants (Hymenoptera: Formicidae) in Potohar plateau of Punjab province, Pakistan. *Pakistan Journal of Zoology*, *44*(3), 699-705.

Usman, K., Gul, S., Rehman, H. U., Pervaiz, K., Khan, H., Aslam, S., ... & Gul, S. (2017). Field observations on the incidence of Ants fauna (Hymenoptera) of Karak Khyber Pakhtunkhwa, Pakistan. *Journal of Entomology and Zoology Studies*, *5*(4): 390-393.

Vander Meer, R.K. (1990). Applied Myrmecology: A World Perspective (1st ed.). CRC Press. https://doi.org/10.1201/9780429043109

Vinson, S. B. (1986). *Economic Impact and Control of Social insects*. New York; Praeger, 421 pp.

Table 1. STATUS OF ANTS FAUNA FROM DISTRICT POONCH, AZAD JAMMU AND KASHMIR, PAKISTAN

Family	Sub-family	Genus	Species	Occurrence Status
Formicidae	Formicinae	*Camponotus*	*Camponotus compressus*	New - Poonch
			Camponotus oblongus	New – Poonch
		Formica	*Formica fusca*	New - Poonch
		Lepisiota	*Lepisiota frauenfeldi*	New – Poonch
		Polyrhachis	*Polyrhachis hodgsoni*	New – Poonch
			Polyrhachis grisescens	New - Poonch
	Myrmicinae	*Crematogaster*	*Crematogaster rothneyi*	New – Poonch
		Holcomyrmex	*Holcomyrmex scabriceps*	New – Poonch
		Monomorium	*Monomorium fossulatum*	New – Poonch
		Pheidole	*Pheidole latinoda*	New - Poonch
	Ponerinae	*Myopopone*	*Myopopane moelleri*	New – Poonch
		Odontoponera	*Odontoponera transversa*	1st – Pakistan New – Poonch
1	**3**	**10**	**12**	**01–New to Pakistan, 12–New to Poonch, Azad Jammu and Kashmir**

UNIVERSITY OF SINDH JOURNAL OF ANIMAL SCIENCES
DOI: https://doi.org/10.57038/usjas.v6i04.5838
Uni. Sindh. J. Anim. Sci., vol. 6(4); 18-27, 2022
Email: *editors.usjas@usindh.edu.pk*
ISSN (P): 2521-8328
ISSN (E): 2523-6067
Published by University of Sindh, Jamshoro.

PHENOTYPIC CHARACTERIZATION OF INDIGENOUS KACHHI SHEEP BREED

SHAKEEL AHMED TUNIO[1], MUHAMMAD NAEEM[1*], ATIQUE AHMED BEHAN[1], ASMATULLAH KAKA[2], HUMA RIZWANA[1], NASIR RAJPUT[3], NOOR UN NISA MARI[1]

[1]Department of Livestock Management, Faculty of Animal Husbandry and Veterinary Sciences, Sindh Agriculture University, Tandojam
[2]Department of Animal Reproduction, Faculty of Animal Husbandry and Veterinary Sciences, Sindh Agriculture University, Tandojam
[3]Department of Poultry Husbandry, Faculty of Animal Husbandry and Veterinary Sciences, Sindh Agriculture University, Tandojam

ARTICLE INFORMATION

Article History:
Received: 1st October 2022
Accepted: 21st December 2022
Published online: 31st December 2022

Author's contribution
All authors contributed equally.

Key words:
Phenotypic characteristics, Quantitative traits, Kachhi sheep

ABSTRACT

For the evaluation of the phenotypic and morphometric characteristics of Kachhi sheep, a total of 300 adult Kachhi sheep (female240 and male 60) were selected from different study areas (Hyderabad, Matiary, Mirpurkahs, Sanghar, Umerkot and Thaparkar) of Sindh province. Among all the study areas majority of male and female Kachhi sheep physically possessed plain white coat with black and brown (tan) color, compact body shape, coarse type wool, convex face profile, roman nose, small pendulous ears, sloppy rump, dark colored hooves, and small cylindrical tail, compact udder. Moreover, majority of male and female sheep were noted with wattles, remarkably few cases of female and male Kachhi sheep were detected with deviated Morphometric characters. Average live body weight and linear body measurements (chest girth, body length, height at withers, facial length, ear length, head width and tail length) of both sexes, at Matiary area were observed comparatively ($P<0.05$) higher, followed by Hyderabad, Sanghar, Mirpurkhas, Umerkot and Tharparkar vicinities of Sindh. However, statistical ($P<0.05$) differences were recorded in the morphometric traits between male and female Kachhi sheep among all the selected vicinities of Sindh province.

1. INTRODUCTION

Pakistan is naturally gifted with the huge wealth of sheep genetic resources. According to Pakistan economic survey sheep population estimates are 31.9 million (GOP, 2021-22). In Pakistan there are about of 31 sheep breeds, which may be distributed on agro-ecological zones and provincial basis in Pakistan (Isani and Baloch, 1996). On the basis of morphological characteristics sheep breeds could be classified into two categories thin-tailed (Baltistani, Buchi, Cholistani, Damani, Hissardale, Kaghani, Kail, Kajli, Kali, Kooka, Lohi, Pahari, Poonchi, Sipli&Thalli) or fat-tailed (Balkhi, Balochi, Bibrik, Dumbi, Gojal, Harnai, Hashtnagri, Khijloo, Kohai Ghizar, Latti, Michni, Rakhshani, Tirahi&Waziri) and relatively used to produce milk, mutton, and coarse type wool (Wahid, 1982).

Conferring to (FAO) food and agriculture organization, phenotypic and genetic classifications are vital tools for categorizing animal breed, which should be initial phase towards the advance approaches for their ecological use, management, and protection. Among populations phenotypic evaluation a reasonable interpretation of genetic variations is based on morphological characters and the evaluation of morphologic characters have been verified to be appropriate in evaluating genetic difference within and between populations when all morphologic variations are considered at the same time (Lix & Sojobi, 2010).

The breed performance has been assessed with the help of phenotypic and genotypic categorization. For documentation of breed Phenotype is considered as a result of genotype and environment. The phenotype of breed can be distributed into three main classes' i-e physical description or measurement, productive traits,

*Corresponding Author: mnrajput@sau.edu.pk

and environmental adaptation. Physical attributes description includes (skin color, chest girth, body length, height, tail length and type, wool type) and presence or absence of horns. For the animal strain or breed definitions physical features are possibly the mostly used norms. For this purpose, several efforts have been performed to study these qualities to characterize the population and the categorization of sheep population on the basis of multivariate evaluation of physical traits is its example (Henson, 1992). In Sindh province more than a dozen breeds of sheep are prevailing and choice of breed is climatic reliant. Kachhi sheep breed is frequently found in district Tharparkar and adjoining desert areas of Sindh. It is mutton, milk and wool type sheep breed, they are medium sized breed comprising of white body coat and with a black or brownish stumpy head and black or brown markings on legs, also comprised of small ears, Roman nose, and a well-developed compact udder (Tahir, 2005). The main object of this study was to assess the morphological description of Kachhi sheep by using qualitative and quantitative characteristics reared at various vicinities of Sindh province. The hypothesis of this study was the assumption that phenotypic assessments of indigenous sheep breeds might be comfort and initiate the categorization of specific breed criteria and official breed authentication process, and, in return, more local business opportunities for smallholders may be created.

2. MATERIALS AND METHODS

Phenotypic or morphological characterization of Kachhi Sheep

The study areas were chosen based on comprehensive survey conducted on the socioeconomic status of Kachhi Sheep at identified vicinities of Sindh province, a total of 300 adult Kachhi Sheep apiece of 50 from each identified area were selected for the morphological characteristics and body measurements characteristics determination. Total Six agro based vicinities of Sindh; Hyderabad, Matiary, Mirpurkhas, Sanghar, Umerkot and Tharparkar districts were selected on the basis of population of Kachhi breed. All these chosen vicinities are well identified concentrated (livestock populous) regions of the Sindh.

Sampling procedures and sample size determination

For this purpose of study, a multi-stage sampling process was selected for the assortment of household samples and Kachhi Sheep. For sampling sheep population, gravid ewes, neutered sheep, offspring, lambs (male and female) were not involved in the trial sheep populace to upsurge the exactness of quantifiable characters and to signify the mature sheep populace. Afterward, simple random sampling method was applied for sample sheep collection.

Sample size fortitude for Kachhi sheep breed

Kachhi Sheep sample size at each study area of Sindh province was observed by using the Cochran's formula. Collectively 300 Kachhi Sheep were selected, each of 50 sheep samples from the locality of Hyderabad, Matiary, Mirpurkhas, Sanghar, Umerkot and Tharparkar vicinities of Sindh, Pakistan were collected for evaluating the phenotypic (qualitative) and morphometric (quantitative) traits with the 10:40 ratios of mature male and mature female sheep, respectively, according to procedure of (FAO, 2012).

Phenotypic/Morphometric characterization of indigenous sheep

To study the qualitative and quantitative characteristics of Kachhi Sheep, a breed standard description list designed by (FAO, 2012) was accurately followed for Kachhi sheep breed. Data collected for qualitative characters of both sexes (male and female) in respect of skin color type, coat color pattern, wool type, head and face profile, wattles, ears positioning, rump outline, color of hooves, and tail profile were noted by pictorial and eye observations of the Kachhi breed. All the straight-lined body sizes of both sexes of Kachhi Sheep noted in early before noon, with the standup on a smooth surface and animals in held up head position. In regard of quantitative traits (body length, chest girth, wither height, head width, facial Length, ear length and tail length) were measured by using plastic measuring tape identical in cm and body weight of animal was estimated by using spring weighing balance.

All the animals were categorized by their gender, location, and age groups. Sheep's age classification was made using dentition. All the male and female sheep were classified into three age groups: A (12-18 months), B (19-24months) and C (25-30 months), respectively.

Data collection

Data in respect of morphological characteristics and linear body measurements was collected through observations and field measurement. Data collected during the study was interpreted in the Microsoft Excel (2010).

Data analysis

The data was analyzed by using SAS version 9.3, 2014 and SPSS version 20 regarding both qualitative and quantitative characteristics for each individual variable. Chi-square test was used to estimate the numerical

consequence between categorical variables using locality as a fixed influence (SPSS, version16.0) with gender and vicinities as fixed effects were subjected to factorial analysis of variance of live body weight and linear body measurements. For separation of significance of means the Tukey's simultaneous test was used.

3. RESULTS AND DISCUSSION

Phenotypic characterization of Kachhi Sheep

Phenotypic characteristics of female and male with the ratio of 40:10 Kachhi Sheep from different study areas of Sindh province were estimated and consequences are presented in Table-1. Results revealed that majority of non-spotted skin coat pattern of female and male Kachhi sheep was recorded at (Hyderabad, Matiary, Mirpurkhas, Sanghar, Umerkot and Tharparkar) vicinities as compared to few spotted female and male sheep at above mentioned study areas. In respect of coat color patterns non-significant (P>0.05) difference was recorded in both sexes of Kachhi sheep at various vicinities of Sindh. Findings of present study are agreeing with the observations of (Hailemariam, et al., 2018) reported that the major coat colors were plain black (34%), red (19.6%) and black dominant (11.8%).

The dominant of black coat color is obvious in the cold region. Conflicting to current findings (Whannou et al., 2021) reported sheep with predominated coat color (spotted white and brown) patterns were closely related with that plateau and oueme valley zones and plain or uniform white or composite coat color with a predominantly spotted black, brown, or white patterns sheep from probe, coastal and bassila zones. Relatively higher percentage of Kachhi Sheep skin coat type was noted ash white with black in both sexes of Kachhi breed at Hyderabad, Matiary, Mirpurkhas, Sanghar, Umerkot and Tharparkar contrast to both sexes having white with brown (tan) skin color at various vicinities of Sindh province. These findings are in resemblance with (Goran et al., 2019) stated in Nigeria sheep breeds on the basis of main qualitative characters commonly used for breed description with a supremacy of spotted white and some individuals from Sahelian sheep breed of Nigeria presented in front being black or brown and white ears (bicolor coat). In regard of skin color type present study revealed that non-significant difference (P>0.05) was recorded in both male and female Kachhi sheep at various vicinities (Hyderabad, Matiary, Mirpurkhas, Sanghar, Umerkot and Tharparkar) of Sindh. Moreover (Florezc et al., 2020) reported that majority of Sudan Bayo sheep were observed yellow bay followed by clear bay and waxed bay, respectively. More than half of the Blanco sheep (Sudan) were detected brown in color, with brown and white spots, but frequently white with brown spots and remaining percentage was distinguished by entirely white coat color pattern.

Furthermore, the coat color difference was also detected in vizayangaran sheep breed (Gangaraju, 2010) and Vembur sheep breed (Selvakumar, 2016). The Pantaneiro sheep hair color was observed typically white 74.6 percent, yellow hair color 13.2 percent and spotted hair color types 11.1percent had intermediate occurrence and black hair was unusual 1.1 percent and 77.6 percent color of wool was typically white (Aranda et al., 2021). Amongst all the study areas both sexes of Kachhi sheep were observed with coarse type wool with high to medium length which agree with the findings of (Amelmal, 2011) reported that the majority (81.96%) of study area sheep population were observed with medium and smooth (coarse) type wool tracked by short and smooth (14.6%), long and (3.41%) smooth hair. Moreover, majority (98.12%) of sheep reported with hairy coat and very few (1.88%) were noted with wooly type coat (Reddy et al., 2020). In current investigation at different vicinities of Sindh beneath the facial profile of both genders of Kachhi sheep 100 percent convex face profile was observed that are in resemblance with the conclusions of (Reddy et al., 2020) reported that head profile was predominantly convex in majority of sheep while few sheep were noted with slightly convex heads.

In all the study areas both female and male Kachhi sheep were observed with small and pendulous ears as reported by (Chaudhary, 2013), (Yadav, 2011), (Reddy et al., 2020) that ear orientations frequently pendulous in Macherla and Munjal sheep breed. In all the study areas in both sexes (female and male Kachhi sheep) majority of observations of largely sloppy back rump profile was noted, at the different study parts only some observations of flat (smooth) rump profile were observed in both sexes which are in connection with findings of (Tilahun, et al., 2019) stated that larger hind rump shapes by straight, inclines up towards the rump and curved hind rump profile were observed. In most of observations rump profiles were enclosed and sloping by slight flat. Majority of male and female Kachhi sheep in all the study areas were noted with wattles, while few number of male and female Sheep were observed with absence of wattles at the selected study areas, these observations are in settlement with findings of few authors (FAO, 1982), (Devendra et al., 2009), (Chaudhary, 2013) who reported the presence of wattles in south Indian breeds.

The hooves of both sexes (female and male) Kachhi sheep were noted darker in color at all the study areas as reported by (Nazeer & Shah, 2018) that in Pakistan the indigenous small ruminants with good body linear measurements and growth traits, coat color and hooves darkerin color. In another study conducted by (Tsegaye

et al., 2013) reported marketing value of domestic animal Significantly affected by phenotypic traits like color of skin, compacted body structure and darker hooves of the small ruminants. Moreover, during present study in respect of tail profile of both sex of Kachhi sheep significant (P<0.05) difference was recorded. At all the selected areas of study the predominant thin and cylindrical tail trend was seen in both sexes of Kachhi sheep breed. These consequences of current investigation are in similarity with the findings of (Chaudhary, 2013), (Reddy et al., 2020) reported that majority of macherla sheep had slender type thin tail, and these findings also agree with (Singh et al., 2007) observed that majority of Nali sheep of India were noted with thin and medium sized tail. In all the study areas (Hyderabad, Matiary, Mirpurkhas, Sanghar, Umerkot and Tharparkar) majority of observations in the female Kachhi sheep with predominant compact udder profile were recorded, while only in few observations among all the study areas female sheep were seen with loose udder. Similarly, to current concerns (Monau, et al., 2018) revealed during classification of Tswana female goat's compact udder profile was recorded. In backing (Vlad et al., 2014) in the South region of Romania, evaluated the morphometric uniqueness of small ruminant noted with compact udder. The availability of feed, management conditions and natural grazing could be attributable to the differences for these traits of interest of small ruminants (Cam et al., 2010).

Quantitative and linear traits of female Kachhi Sheep

For the identification of sheep breed population growth and body conformation considered to be most valuable characters. The average body weight and linear body measurements of female Kachhi Sheep in the study area are showed in Table-4.2 (A) & (B).

Morphometric (Measureable) characters: (Body weight and linear body measurements) are the most significant characteristics, which are very supportive for classification of breeds in population. During the current study significant (P>0.05) difference observed in average body weight, chest girth, body length, body height, ear length, facial length, tail length, and width of head in both sexes of Kachhi sheep at various study areas and noted relatively (P<0.05) higher at Matiary as compared to Hyderabad, Sanghar, Mirpurkhas, Umerkot and Tharparkar. Same consequences were achieved by (Pervage et al., 2009) stated that average (body weight, chest girth, body height, body length, ear length, facial length, tail length, and width of head) were significantly influenced by locality and environmental conditions. Moreover, among this chest girth (major linear body measurement) is broadly utilized to categorize the bodily traits of small ruminant pedigree and variety (Solomon, 2009). In resemblance with present results (Gebreyowhens and Kumar, 2017), (Getahun et al., 2008), (Bekalu, 2014) stated that higher chest girth recorded in male of small ruminants associated to female ones.

In present study the chest girth was the most exceptional predictor variable of both (male and female) Kachhi sheep, chest girth might be utilized as a sole prognosticator of body weight as reported by several authors in same type of studies (Taye, et al., 2010). In concurrence with present observations various authors have established that the chest girth is considered as the first and vital tool for the estimation of live body weight and secondly body length of domestic animals (Ahmed, 2013), (Hulunim, 2014).

4. CONCLUSION

Based on present findings it was concluded that the plain white body coat with brown and black color at neck and legs in both sexes of Kachhi sheep was recorded higher at all the study areas with Roman nose**,** small pendulous ears, wattles, compact udder, dark colored hooves and thin and cylindrical tail. Moreover, average live body weight and linear body measurements of both sexes of Kachhi sheep at Matiary were observed significantly higher as compared to other study areas.

5. CONFLICT OF INTEREST

All authors have declared that there is no conflict of interests regarding the publication of this article.

REFRENCES

Ahmed, S. (2013). On-farm phenotypic and production system Characterization of indigenous goats in Horro Guduru Wollega Zone, Western Ethiopia *M.sc. thesis Haramaya, university*, 112.

Amelmal, A. (2011). Phenotypic characterization of indigenous sheep types of Daeuro zone and konta special woreda of SNPPR, Ethopia. *M.Sc. Thesis Haramaya University, Dire Dawa, Ethopia.*

Aranda, A.N., Silva, M.C.D., Crispim, B.A., Ledesma, L.M., Lenis, P.R., Silva, A.L.A., Leonardo, A.P., Fernando, Junior, M.V & Barufatti, A. (2021). Qualitative Characters of Indigenous Sheep in Central Brazil: Putting Phenotype into Perspective. *Academic Editor: Luc Legal. Diversity*, 13(11), 51

Bekalu, M. (2014). Phenotypic characterization of indigenous goat types and their production system in West Gojam Zone of Amhara region,

Ethiopia. *MSc. Thesis Haramaya University*, 109.

Cam, M.A., Olfaz, M., & Soydan, E. (2010). Possibilities of using morphometric characteristics as a tool for body weight prediction in Turkish hair goats (Kilkeci). *Asian Journal of Animal and Veterinary Advances*. 5 (1), 52-59.

Choudhury, M. P., Sarkar, A., Islam, S. C. F., Ali, A., Bhuiyan, A., Ibrahim, M., & Okeyo, A. (2012). Morphometry and performance of Black Bengal Sheep at the rural community level in Bangladesh. *Journal of Animal Science*, 41 (9), 83-89.

Devendran, P., Kandasamy, N., Panneerselvam, S., & Thiruvenkadan, A.K. (2009). Rearing Environment and husbandry practices of Coimbatore sheep. *Indain Journal of Animal Science,* 80, 470-472.

FAO. (1982). Sheep and Goat breeds of India by Acharya R.M.

FAO. (2012). Phenotypic characterization of Animal Genetic Resources. FAO Animal production and health guidelines. *FAO. Rome, Italy*.

Florez M., Julie, Hernandez P., Bustamante, M., Vergara, Y., Oscar, G. (2020). Morphological and phenoroptic characterization of Sudan female Colombian Creole Hair Sheep "OPC". *Revista MVZ Cordoba, Univerdae de Coridoba, Colombia,* (25), 1.

Ganguraju, G. (2010). Studies on characterization and performance of Vizianagaram sheep of north coastal Andhra Pardesh. *Ph.D. Thesis. Sri. Venkateswara Veterinary University, Tirupatti.*

Gebreyowhens, W., & Kumar, R. (2017). Phenotypic characterization of indigenous Maefur goat population in Tigray, Northern Ethiopia. *International Journal of Biodiversity Conservation, 9*(5), 130-145.

Getahun, L. (2008). Productive and Economic performance of Small Ruminant production in production system of the Highlands of Ethiopia. *Ph.D. dissertation. University of Hohenheim, Stuttgart-Hohenheim, Germany*.

GOP, (2022). Pakistan Economic Survey (2021-22). *Economic Advisor's Wing, Finance Division, Government of Pakistan, Islamabad.*

Goran, K.E., Kouadja, G. S., Kouassi, N. C., Loukou, N.E., Eka, J. Y., & Dayo, G. K. C. (2019). Primary morphological characterization of west Africa dwarf (Djallonke) ewes from cot d" lvoire based on qualitative and quantitative traits. *International Journal of Genetics and Molecular Biology*, 11(2), 16-28.

Hailemariam, F., Gebremicheal, D., & Hadgu, H. (2018). Phenotypic characterization of sheep breeds in Gamogofa zone. *Agriculture & Food Security*, 7: 27

Henson, E.L. (1992). In-situ conservation of livestock and poultry. *FAO Animal Production and Health*, 99

Hulunim, G. (2014). On-farm phenotypic characterization and performance evaluation of Bati, Borena and short eared Somali goat populations of Ethiopia. *MSc. thesis submitted to the School of Graduate Studies of Haramaya University, Ethiopia*, 140.

Isani, G.B., & Baloch, M. N. (1996). Sheep and goat breeds in Pakistan. *Press Corporation, Karachi, Pakistan*, 23-29.

Lix, L. M., & Sojobi T. T. (2010) Discriminant analysis for repeated measured data: *A Review of Psychology*1: 146.

Monau, P. I., Visser, C., Nsoso, S.J., & Marle-Koster, E. (2018). Phenotypic and genetic characterization of indigenous Tswana goats. *South African Journal of Animal Sciences*, *48*(5), 925-934.

Nazeer, M., & Shah, S.H. (2018). Morphological characterization of indigenous goat breeds of Khyber Pakhtunkhwa, Pakistan. *Sarhad Journal of Agriculture, 34*(2), 258-267.

Pervage, S., Ershaduzzaman, M., Talukder, M. A. I., Hasan, M. N., & Khandoke, M. A. M. Y. (2009). Phenotypic Characteristics of Indigenous Sheep of Bangladesh. *Bangladesh Journal of Animal Science*, 38(1&2), 1-6

Reddy,P. P., Vinoo, R., Muralidhar, M., Venkatasesaiah, Ch., AswaniKumar, K. & Sudhakar, K. (2020). Socio-economic status, sheep husbandry practices and morphological patterns of Macherla sheep, as lesser-known sheep breed of Andhra Pardesh. *Journal of Animal Research*, 827-835.

Selvakkumar. R., Sivakumar, T., MeenakshiSundaram, S., Jawahar, K.T.P. & Vanan T.T. (2016). Phenotypic Characterization of Vembur sheep in its inbreeding tract. *Indian veterinary Journal*, 93 (11), 36-38.

Singh, G., Jain, & Yadav, D. K. (2007). Evaluation of Nali Sheep under field conditions. *Indian Journal Animal Science,* 77(11), 1158-1160.

Solomon, G. (2009). *Goat breeds of Ethiopia*: Technical Bulleting No.27. A guide for identification and utilization. Editors: Alemu Y, Kassahun, T.A. Gipson and R.C. Merkel. *Sheep and Goat Productivity Improvement Program* (ESGPIP).

Tahir M., (2005). Breeds of sheep in Pakistan. Animal Breeding and Genetics, *University of Agriculture, Faisalabad, Pakistan, Wikipedia,* 1-1.

Taye, M., Abebe, G., Gizwa, S., Lema, S., Mekoya, A. (2010). Traditional Management system and

linear body measurements of washera sheep in the western highland of the Amhara National Regional State, *Ethopia Livestock Research and Rural Development, 22*, 169.

Tilahun, H., Haile, A., &Seid, A. (2019). Phenotypic characterization of indigenous goats in North Shewa Zone, Amhara region, Ethiopia. *International Journal of Research Studies in Agricultural Sciences*, *5*(7), 44-55.

Tsegaye, D., Belay B., & Haile, A. (2013). Morphological Characterization of Indigenous Hararghe Highland Goat Breed in Their Native Environment, West Hararghe, Ethiopia. *American-Eurasian Journal of Scientific Research*, (8), 72-79.

Vlad, I., Maftei, M., Ianiţki, D., Stanciu M., &Fiţa, A. (2014). Morpho-productive characteristics of Saanen goats in the south of Romania. *Animal Science and Biotechnology*, *47*(1), 300-305.

Wahid A. (1982). General pattern of production, breeds, management, disease, nutrition performance and problems. Pakistan agriculture research council, Islamabad. *In. proc. international seminar on sheep and wool,* 14-38.

Whannou, H.R.V., Afatondji, C.U., Ahozonlin, M.C., Spanoghe, M., Demblon, D.LD. Romuald M. (2021). Morphological variability within the indigenous sheep population of Benin. *PloS ONE,* 16 (10), 0258761.

Yadav, D.K., Arora, R., Bhatia, S., and Singh, G. (2011). Morphological Characterization, production and reproduction status of Munjal-a threatened sheep population of Northwest India. *Indian Journal of Animal Sci*ence, 81, 943-945

Table-1 Morphologic/Phenotypic traits of Kachhi sheep in different vicinities of Sindh, Pakista2

Phenotypic characteristics		Vicinities of Sindh Province					
		Hyd. N (%)	**Matiary N (%)**	**MPK N (%)**	**Sangher N (%)**	**Umerkot N (%)**	**Tharparkar N (%)**
Coat color Pattern	Female sheep spotted/patchy	4(10)	6(15)	5(12)	4(10)	3(7)	2(5)
	Female sheep plain	36(90)	34(85)	35(88)	36(90)	37(93)	38(95)
	Male sheep spotted/patchy	2(20)	1(10)	2(20)	2(20)	1(10)	1(10)
	Male sheep plain	8(80)	9(90)	8(80)	8(80)	9(90)	9(90)
X^2value =	**8.61 NS**					**P -value (0.05) = 0.9870**	
Skin color type	Female sheep white and brown	6(15)	4(10)	6(15)	7(18)	8(20)	6(15)
	Female sheep white and black	34(85)	36(90)	34(85)	33(82)	32(80)	34(85)
	Male sheep white and brown	2(20)	1(10)	1(10)	2(20)	2(20)	2(20)
	Male sheep white and black	8(80)	9(90)	9(90)	8(80)	8(80)	8(80)
X^2value =	**6.94(NS)**					**P- value (0.05) = 0.9969**	
Wool type	Female sheep curly	0(00)	0(00)	0(00)	0(00)	0(25)	0(00)
	Female sheep coarse	40(100)	40(100)	40(100)	40(100)	40(75)	40(100)
	Male sheep curly	0(00)	0(00)	0(00)	0(00)	0(00)	0(00)
	Male sheep coarse	10(100)	10(100)	10(100)	10(100)	10(100)	10(100)
X^2value =	**4.68(NS)**					**P -value (0.05) = 0.9114**	
Nose profile	Female sheep straight	0(00)	0(00)	0(00)	0(00)	0(00)	0(00)
	Female sheep roman type	40(100)	40(100)	40(100)	40(100)	40(100)	40(100)
	Male sheep straight	0(00)	0(00)	0(00)	0(00)	0(00)	0(00)
	Male sheep roman type	10(100)	10(100)	10(100)	10(100)	10(100)	10(100)

X^2value =	**4.68(NS)**						**P- value (0.05) = 0.9114**
Phenotypic characteristics		**Vicinities of Sindh Province**					
		Hyderabad N (%)	**Matiary N (%)**	**Mirpurkhas N (%)**	**Sanghar N (%)**	**Umerkot N (%)**	**Tharparkar N (%)**
Head/facial profile	Female sheep Straight	0(00)	0(00)	0(00)	0(00)	0(00)	0(00)
	Female sheep Convex	40(100)	40(100)	40(100)	40(100)	40(100)	40(100)
	Male sheep Straight	0(00)	0(00)	0(00)	0(00)	0(00)	0(00)
	Male sheep Convex	10(100)	10(100)	10(100)	10(100)	10(100)	10(100)
X^2value =	**4.68(NS)**						**P-value (0.05) = 0.9114**
Ear orientation	**Female sheep Erect**	**0(00)**	**0(00)**	**0(00)**	**0(00)**	**0(00)**	**0(00)**
	Female sheep Pendulous	40(100)	40(100)	40(100)	40(100)	40(100)	40(100)
	Male Erect	0(00)	0(00)	0(00)	0(00)	0(00)	0(00)
	Male sheep Pendulous	10(100)	10(100)	10(100)	10(100)	10(100)	10(100)
X^2value =	**4.68(NS)**						**P-value (0.05) = 0.9114**
Rump profile	Female sheep Flat	4(10)	2(5)	1(3)	0(0)	2(5)	1(3)
	Female sheep Sloping	36(90)	38(95)	39(97)	40(100)	38(95)	39(97)
	Male sheep Flat	2(10)	2(20)	1(10)	1(10)	0(0)	0(10)
	Male sheep Sloping	8(80)	8(80)	9(90)	9(90)	10(100)	10(100)
X^2value =	**15.12(NS)**						**P-value (0.05) = 0.7697**
Wattles	Female sheep with wattles	36(90)	36(90)	38(95)	35(88)	37(93)	38(95)
	Female sheep without wattles	4(10)	4(10)	2(5)	5(12)	3(7)	2(5)
	Male sheep with wattles	9(90)	8(80)	9(90)	8(80)	9(90)	9(90)
	Male sheep without wattles	1(10)	2(20)	1(10)	2(20)	1(10)	1(10)
X^2value =	**8.22(NS)**						**P-value (0.05) = 0.9903**
Hooves	Female sheep Dark color	40(100)	40(100)	40(100)	40(100)	40(100)	40(100)
	Male sheep	10(100)	10(100)	10(100)	10(100)	10(100)	10(100)

	Dark color						
X^2value =	**4.68$^{(NS)}$**						**P-value (0.05) = 0.9114**
Tail	Female sheep Thin and cylindrical	40(100)	40(100)	40(100)	40(100)	40(100)	40(100)
	Female sheep fat	0(00)	0(00)	0(00)	0(00)	0(00)	0(00)
	Male sheep Thin and cylindrical	10(100)	10(100)	10(100)	10(100)	10(100)	10(100)
	Male sheep Fat	0(00)	0(00)	0(00)	0(00)	0(00)	0(00)
X^2value =	**4.68$^{(NS)}$**						**P-value (0.05) = 0.9114**
Udder	Female Compact	38(94)	36(90)	39(97)	35(88)	37(92)	39(97)
	Female Loose	2(6)	4(10)	1(3)	5(12)	3(8)	1(3)
X^2value =	**9.93($^{(NS)}$**						**P-value (0.05) = 0.4467**

(N) = Number of sheep showing a specific qualitative character, (%) = Percent, X^2 = (Pearson chi-square), *Significant difference ($P < 0.05$), (NS) = non-significant difference.

Table 4.2 (A) Quantitative traits of Female Kachhi sheep at different vicinities of Sindh province.

Vicinities of Sindh	Body weight (Kg)	Chest girth (Cm)	Body length (Cm)	Body Height (Cm)	Ear length (Cm)	Face length (Cm)	Tail length (Cm)	Head width (Cm)
Matiary	35.16^{a}	76.40^{a}	73.45^{a}	73.68^{a}	12.05	21.11^{a}	15.53^{a}	11.84^{a}
Hyderabad	33.96^{a}	74.23^{ab}	71.58^{b}	72.43^{a}	11.98	20.15^{b}	14.15^{b}	10.80^{a}
Sanghar	32.73^{a}	73.51^{b}	70.35^{b}	71.95^{a}	11.88	19.66^{b}	13.65^{b}	9.95^{ab}
Mirpurkhas	32.43^{ab}	72.70^{b}	69.95^{b}	71.10^{ab}	11.78	18.10^{c}	12.88^{bc}	9.46^{b}
Umerkot	31.88^{ab}	71.66^{bc}	68.61^{bc}	70.38^{b}	11.70	18.70^{c}	12.35^{c}	8.95^{bc}
Tharparkar	30.95^{b}	70.74^{c}	67.26^{c}	69.65^{b}	11.60	17.98^{c}	11.70^{c}	8.70^{c}
P value	0.043	0.037	0.032	0.044	0.311*^{NS}	0.041	0.038	0.036
SE	0.357	0.308	0.310	0.208	0.121	0.147	0.170	0.114

*NS= non-significant

*(abc) = Superscripts with different letters in same column varied significantly from one another.

Table 4.2. (B) Quantitative traits of Male Kachhi sheep at different vicinities of Sindh province.

Vicinities of Sindh	Body weight (Kg)	Chest girth (Cm)	Body length (Cm)	Body Height (Cm)	Ear length (Cm)	Facial length (Cm)	Tail length (Cm)	Head width (Cm)
Matiary	44.30 [a]	86.10 [a]	78.80 [a]	81.77 [a]	14.33 [a]	22.70 [a]	15.90 [a]	12.00 [a]
Hyderabad	42.85 [b]	84.00 [b]	76.60 [b]	79.20 [b]	13.20 [ab]	21.20 [b]	15.10 [a]	11.30 [a]
Sanghar	41.40 [b]	83.60 [b]	75.00 [b]	78.10 [b]	12.50 [b]	20.45 [b]	14.80 [a]	10.90 [a]
Mirpurkhas	40.90 [b]	82.00[bc]	74.70 [b]	76.50 [c]	11.85 [b]	19.00[bc]	13.20 [ab]	9.90 [b]
Umerkot	39.10 [b]	81.00 [c]	73.20 [c]	75.10 [c]	11.45 [b]	18.05 [c]	12.70 [b]	9.70 [b]
Tharparkar	37.50 [c]	79.15 [c]	72.50 [c]	73.30 [c]	10.90 [b]	17.85 [c]	11.95 [b]	9.20 [b]
P value	0.039	0.035	0.041	0.032	0.043	0.027	0.037	0.044
SE	0.818	0.788	0.467	0.382	0.291	0.228	0.251	0.228

*(abc) = Superscripts with different letters in same column varied significantly from one another.

UNIVERSITY OF SINDH JOURNAL OF ANIMAL SCIENCES
DOI: https://doi.org/10.57038/usjas.v6i04.5839
Uni. Sindh. J. Anim. Sci., vol. 6(4); 28-34, 2022
Email: editors.usjas@usindh.edu.pk
ISSN (P): 2521-8328
ISSN (E): 2523-6067
Published by University of Sindh, Jamshoro.

EVALUATING THE SERUM MARKERS FOR EARLY DIAGNOSIS OF HCV INDUCED HEPATOCELLULAR CARCINOMA

AASMA SIDDIQUI, SARFRAZ ALI TUNIO, SHAISTA BANO, NAVISH LODHI

Institute of Microbiology, University of Sindh- Jamshoro, Pakistan

ARTICLE INFORMATION

Article History:
Received: 26th October 2022
Accepted: 18th December 2022
Published online: 31st December 2022

Author's contribution
AS, SB, SAT conceived and designed research. AS conducted experiments. NL contributed to analytical tools. SB and SAT analyzed data. AS, SB, and SAT wrote the manuscript. SB and SAT reviewed and edited the manuscript critically

Key words:
HCV, HCC, Cirrhosis, Chronic liver disease, serum markers

ABSTRACT

Hepatocellular carcinoma is a global public health problem which is intensified at developing countries due to lack of early diagnosis. Since Hepatitis C virus related morbidity accounts five million people in Pakistan, rate of morbidity and mortality of HCV related Hepatocellular carcinoma infections is high in Pakistan, and it is attributed to its diagnosis after its development. The public has the lack of awareness about the importance and benefits of liver biopsy testing; therefore, improved, and non-invasive methods of HCC diagnosis are urgently needed. The aim of the present study was to evaluate the importance of serum markers in early diagnosis of HCV induced Hepatocellular carcinoma and to develop a robust set of serological markers for their early detection and diagnosis. A total of 60 HCV positive patients including Chronic Liver disease (n=20), Cirrhosis (n=20), HCC patients (n=20) and 20 healthy volunteers (n=20) were enrolled in this study after an extensive screening process. Blood samples were collected from all the subjects and processed for serum separation followed by immunological, molecular, and biochemical analysis using Enzyme Linked Immunosorbant Assay, Real-Time PCR, complete blood picture, and a range of biochemical tests. Results showed gender wise variability among the subjects from each of the study groups where in the male subjects remained dominant with male and female ratio as 61.75:38.75. The average age of male subjects was observed between 42y, 43y, 49y and 37 years in comparison of female subjects who were 46y, 53y, 50y and 47y in CLD, cirrhosis, Hepatocellular carcinoma, and control groups, respectively. The data has also demonstrated that Hepatocellular carcinoma patients having Alpha Fetoprotein level >1000 showed the increased level of ALP and GGT with decreased HB and HCT. However, the Hepatocellular carcinoma patients having AFP level in the range of 100-500 had total bilirubin level > 3.0, while those having AFP level <100 has all serological markers in normal range. In addition, the patients of HCC showed increased PT with decreased albumin level. The increases level of albumin level (>3-<4.0) was observed from the serum samples of cirrhosis and Chronic liver disease patients. The increase in albumin up to 4.0 accompanied with the increased level of ALP and PT while decreased HB. In conclusion, different biochemical parameters may be used to distinguish the patients having Hepatocellular carcinoma from those suffering from cirrhosis and chronic liver disease induced by HCV infection. The findings of present study may help to enhance the outcome for patients with HCC by enabling the diagnosis to be made at an earlier stage of the disease.

1. INTRODUCTION

Hepatocellular carcinoma (HCC) has been placed as sixth common cancer type and categorized in first three cancer causing disease that is fatal and leads to death in a short time span.

*Corresponding Author: sarfraz.tunio@usindh.edu.pk

The estimated mortality rate associated with hepatitis is 0.5 million persons per year (Graham & Swan, 2015; Lozano et al., 2012). The reason of such high mortality and morbidity is that HCV infection does not show prominent signs and symptoms at an early stage (Blackard, Shata, Shire, & Sherman, 2008; Chung, 2005). Chronic hepatitis results in wound healing process that causes fibrosis in liver and

ultimately the development of cirrhosis. The main cause of cirrhosis due to liver fibrosis is hepatitis C virus (HCV), that may develop into hepatocellular carcinoma (HCC), which is called as HCV induced HCC and that occurs with only 7% survival rate. HCV is a main cause of hepatic malignancy that leads to many deaths worldwide. HCV is categorized into six genotypes which help in therapeutically and epidemiological information (Afridi et al., 2009). Reports show that HCV induced non cirrhotic liver may develop into HCC in up to 20% of cases, but the process of hepato-carcinogenesis still remains a question (Alkofer, Lepennec, & Chiche, 2011; Bralet et al., 2000). Prolonged infection with HCV results in CLD, liver cirrhosis, necrosis, that finally terminated in hepatic failure (Yilma, Saxena, & Mehta, 2022).

HCC is reported as the most common type of cancer, worldwide (Looi et al., 2008). HCC has been placed as sixth common cancer type and categorized in first three cancer causing disease that is fatal and leads to death in a short time span (Chidambaranathan-Reghupaty, Fisher, & Sarkar, 2021; Looi et al., 2008). Unhygienic conditions, improper screening of tests, poor knowledge are the factors that aids in the development of HCC at advanced stage that results in poor prognosis of disease and ultimately high mortality. Many studies have shown that in HCV and HBV infection, the presence of aflatoxin in dietary products and alcohol consumption were the main causative factors for liver cancer (Thorgeirsson & Grisham, 2002). In Pakistan, HCV was found as the main factor responsible for the development of liver cancer (Khan et al., 2009), and about 70% patients of HCC were found infected with HCV whereas HBV co infection has been found in 20% of HCC positive cases (Butt et al., 2013). In Pakistan, the prevalence of hepatic inflammation is alarming showing 4.8% positivity for anti HCV antibodies and for 2.5% HBV surface antigen with (Abbas, Jafri, & Hamid, 2010; Bahadar, Khan, Israr, & Ahmad, 2016). The prolonged untreated infection leads to the development of chronic liver disease that gradually progresses to fibrotic stage, cirrhosis, hepatic damage and finally HCC (Hajarizadeh, Grebely, & Dore, 2013).

The most common serum protein in human is albumin with normal range of 3.1 – 6.0 gm/dl of serum. The overall picture of serum proteins can be drawn by the assessment of the total serum protein which should be in range 5.1 – 9.5 (g/dl) in the serum of a healthy person. Regarding assessment of various clinical illnesses, it is observed that the serum proteins electrophoresis plays a role of initial medical diagnosis. On the basis of biochemical properties, the proteins are differentiated by protein electrophoresis. The SDS-PAGE assessment shows that the albumin as well as globulins dominates the serum protein electrophoresis. The globulins make a minimum portion of proteins in serum with normal range of 1.5 – 4.8 gms/dl. The expression of serum proteins remains variable in electrophoresis based on the disease condition as well as physiological disorders of the patient (Ravel, 1995). Albumin and globulins are two known dominated serum proteins manufactured by the liver. Consequently, the accurate running of kidneys as well liver can be indicated by the values of Albumin protein. The greater levels of globulin and/or albumin can be the signs of different body functioning disorders and infections including leukemia, myeloma, hemolytic anemia, hepatitis, tuberculosis, lymphoma and alcoholism and macroglobulinemia while the values less than normal values may be signs of immune diseases, malnutrition and kidney diseases (Ravel, 1995).

The diagnosis of HCV is usually done by the serological and molecular techniques via the detection of the virus antibodies and nucleic acid in the serum of human, respectively. Serological testing is cost effective and is used to detect antibodies made by body against the infection of virus. It is less sensitive and reliable because the titer of antibodies in human serum changes from time to time, therefore, the accuracy of the testing may be challenged (J.-M. Pawlotsky et al., 2000). The serological testing for HCV involves Enzyme linked immunosorbent assay (ELISA) which is an immunological assay. ELISA antibodies from human serum after four to ten weeks of the infection. The limitations with respect to diagnosis of HCV by the immunological assays (e.g ELISA) occur in case of co-infection in which the immune system of the patient is usually suppressed.

Therefore, false negative results may occur (J. M. Pawlotsky, 2002) Therefore, in addition to serological tests, the molecular tests, such as PCR is required as confirmatory test for HCV in human patients including immunosuppressive individuals, co-infected with HIV, and/or alcoholics. qPCR test is a quantitative test and determine the viral load of HCV in human serum (Dienstag, 2002). However, the detection of viral genotypes is done using qualitative PCR. HCV genotypes may be detected by using ELISA technique but the reliability is still a question (Heydtmann, Shields, McCaughan, & Adams, 2001). Due to lack of sensitive and specific diagnostics for the detection of HCC at an early stage, there is growing interest and need to develop novel HCC serum markers with greater sensitivity and specificity. The present study was carried out to investigate HCV induced HCC by molecular, biochemical and immunological methods to make a consensus about the reliability and sensitivity of these diagnostics test for the patients of Sindh, Pakistan and the efforts were made to identify the protein markers that could be used for early detection and diagnosis of HCV induced HCC. Therefore, the aim of present study was to investigate the serum markers for their role in early diagnosis of HCV induced HCC at Hyderabad, Sindh.

2. MATERIALS AND METHODS

Study design, location and population

This was a prospective cross-sectional study carried out from February 2017 to January 2018 at Hyderabad, Sindh Pakistan. The present study included the samples collected across various cities of Sindh. The subjects from different cities across the Sindh, who were attending the Civil Hospital Hyderabad and Asian Institute of Medical Sciences were enrolled. Information regarding age, gender, ethnic group, birthplace, medication, and history of illness were from each participating patient, with their consent.

A total of eighty blood samples belonging to four groups i.e., CLD (*n*=20), Cirrhosis patients (*n*=20), HCC patients (*n*=20) confirmed known cases and healthy volunteers (*n*=20) were enrolled in this study. All blood samples were subjected for serum separation followed by immunological, molecular, and biochemical analysis using ELISA, Real-Time PCR, and range of biochemical tests. Samples were collected in three types of tubes i.e. Gel tube, EDTA and sodium citrate containing tubes. Gel tubes were used for biochemical testing, EDTA containing blood samples were used for HB/HCT whereas the Sodium containing blood samples were used for Prothrombin time. Samples obtained from healthy volunteers were initially tested for anti-HCV antibodies by ELISA method. Samples collected from CLD, Cirrhosis and HCC were subjected to real time PCR analysis for quantitative detection of HCV. Blood samples were used for separation of serum using BD Vacutainer tubes followed by centrifugation. at 4000 rpm for 10 minutes. the separated serum was collected in fresh eppendorff tube. Samples were either used immediately or stored at -20ºC for future use.

Enzyme linked Immunosorbant Assay

Anti-HCV antibodies were detected using 3rd generation ELISA by using Bio Elisa HCV 4.0 kit as per manufacturer's guidelines. Briefly, the micro plates were coated with recombinant antigens representing epitopes of HCV: Core, NS3, NS4 and NS5.The test serum samples obtained from patients were added to the wells of micro plate. After incubation, the micro plate wells were washed twice with buffer and added the secondary antibody i.e. rabbit anti-human IgG conjugated with peroxidase. Following incubation, the micro plate was washed three time to remove any unbound antibodies or non-specifically bound to the plate. The wells were then added with chromogenic enzyme substrate solution for development of a colour. In case of appearance of blue colour, the result was considered positive while colourless reaction indicated the negative result of the tested sample.

Molecular detection of HCV using Real Time PCR

QIAamp Viral RNA Mini Kit (Qiagen) was used as per protocol supplied by manufacturer, for Viral RNA preparation and subsequent detection of HCV from human serum samples. The kit comprises of the selective binding properties of a silica- based membrane with the speed of microspin or vacuum technology and is highly useful for simultaneous processing of multiple samples. The sample was first lysed under highly denaturing conditions to inactivate RNases and to ensure isolation of intact viral RNA. Buffering conditions were then adjusted to provide optimum binding of the RNA to the QIAamp membrane, and the sample was loaded onto the QIAamp Mini spin column. The RNA attached to the membrane, and contaminants were washed away in two steps using two different wash buffers. High-quality RNA (free of proteins, nucleases) was then eluted in a RNase-free buffer, which was either used directly or stored.

Investigation of biochemical markers

The blood samples of all four different categories were subjected to various biochemical tests in order to determine the variation in patient's serum profile. All samples were tested for total bilirubin (TBil), Alanine transaminase (ALT), Gama glutamyle transaminase (GGT), Alkaline phosphatise (ALP), Creatinine, HB/HCT, Albumin, Alfa feto protein, prothrombin time (PT) as per the manufacturer's instructions. Clinical chemistry tests were applied by an automatic biochemical analyzer (AU5400, Olympus, Japan). HB/ HCT was detected using Hematology Analyzer (XE-1800, SYSMEX, Japan). PT was measured in automated coagulation instrument (CA-7000, SYSMEX, Japan). AFP was measured by Automated Immunoassay Analyzer (COBAS6000, ROCHE, Switzerland).

3. RESULTS AND DISCUSSION

The present study focused on investigation of serum markers for early detection of HCV induced HCC. Four groups of individuals belonging to CLD, Cirrhosis, HCC patients and healthy individuals' categories were analyzed for variation in serological markers to assist in diagnosis of HCC at early stage of development. Among all the samples collected from different categories of patients, 63.16 % were males and 36.84% females. Comparative analysis of gender-wise study of all the four groups showed dominancy of male participant. The highest ratio of males was seen in CLD group. Average age of CLD, Cirrhotic, HCC patients and healthy individuals was 42, 48, 49 and 41 years respectively, while the male and female ratio was 72:28%, 55:45%, 60:40% and 64:36% respectively. The ratio of non-symptomatic v/s symptomatic 55: 45%, 33:67%, 20:80%. With no symptoms in healthy individuals respectively. The true prevalence data for HCV could not be ascertained because the most studies are

hospital based with small sample size. However, the highest prevalence was recorded in Hyderabad whereas the lowest prevalence was recorded in Mirpur Khas.

Gender wise distribution of all participants of this study

Data analysis showed that 61.25% (n=49) of the subjects were male individuals while 38.75% (n=31) were females individuals. The gender wise variability was observed among the subjects from each of the four groups of the present study. The male subjects remained dominant in CLD group with 16.25%, Cirrhosis group with 13.75%, HCC group with 15 % and control group with 16.25% with average age 42y, 43y, 49y and 37y respectively in comparison of female subjects who were 8.75% in CLD, 11.25% in Cirrhosis group 10% in HCC while 8.75% in control group with average age 46y, 53y, 50y and 47y respectively (Fig. 1).

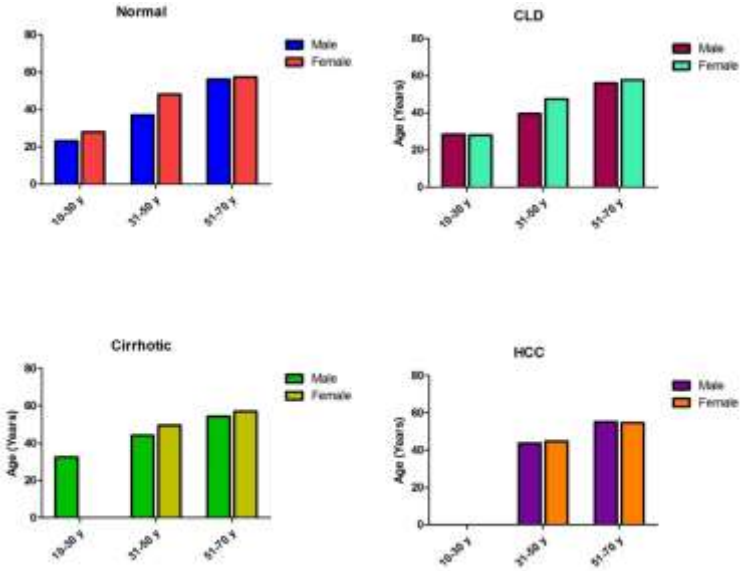

Figure 1. Bar graph showing the gender wise distribution of subjects of all groups of the present study (the mean age of male and female in each group is presented in the graph).

Analysis of the biochemical serological markers

Serum samples from the subjects of the four groups comprising three groups of patients (CLD, Cirrhosis, HCC) and one control group (healthy volunteers) were analyzed for the variations in their serological parameters (Hematological, biochemical, molecular), in order to assist in the diagnosis of HCC at the early stage of the development of deadly disease, HCC. Further analysis showed that in CLD group male and female ratio was 65%:35% while in cirrhosis group, male and female ratio was 55%:45% which different from that of observed from CLD group. Similarly, the analysis of HCC patients showed male and female ratio 60%:40%. The gender wise analysis of control group showed male and female ratio 65%:35%. The comparative analysis of gender wise distribution of all the four groups included in the present study showed dominancy of male participant with the highest ratio seen in CLD group. However, in CLD patients and healthy volunteers the ratio of male was increased up to the age of 50 years as compared to females. All three groups except healthy volunteers (control) shows highest ratio of male at age group more than 30 years as described in next section.

The average HB value obtained from control group was 12.91 ± 1.13 g/dl. However, the subjects from CLD showed 11.96 ± 1.73 g/dl average value of HB which seems lower than the values obtained from the subjects of control group. Similarly, average HB level was much lower in the blood samples of cirrhotic and HCC patients with average values of 10.86 ± 1.44 g/dl and 9.89 ± 2.15 g/dl, respectively (Fig. 2A). The Hematocrit analysis of the four groups, revealed that the average value obtained from normal group was 38.8 ± 4.72 %. However, CLD patients showed 34.88 ±5.86 % which seems lower than the value obtained from normal group of this study. Similarly, Hematocrit level was much lower in cirrhotic and HCC patients with average values of 32.62 ± 5.93 % and 29.13 ± 4.50 %, respectively and it does not fall into the standard normal percentage for this parameter (Fig. 2B). Total bilirubin is a parameter which has got standard range 0.4-0.9 mg/dl. In present study, among the four groups, average value obtained from normal group was 0.6 ± 0.3 mg/dl. However, CLD patients showed 1.40± 0.61 mg/dl which seems higher than the value obtained from normal group of the present study. Cirrhotic group showed 2.36 ± 1.71 mg/dl which was relatively much higher than the values obtained from the CLD group and also does not fall into the standard normal range for this parameter. Similarly, total bilirubin was also higher in HCC group of the present study with average values of 2.81 ± 2.14 mg /dl (Fig. 2C). SGPT is a parameter which has standard value up to 45 U/l. Data showed that the average value obtained from the serum samples taken from the subjects of control (normal) group was 32.15 ± 10.61 U/l whereas 45.85 ± 14.67 U/l from CLD which seems higher than the former. Similarly, cirrhosis patients showed average 52.2 ± 20.43 U/l was observed from cirrhosis group which are also much higher than the values obtained from the normal group and does not fall into the standard normal range for this parameter. In the same manner, SGPT level was much higher in HCC group with average value of 57.25 ± 29.90 U/l (Fig. 2D).

Alkaline phosphatase is a parameter which has standard range 100-290 U/l. In the present study, among the four groups, average value obtained from normal group was 124.20 ± 13.87 U/l. However, CLD patients showed 136.75 ± 26.76 U/l which seems higher than the value obtained from normal group of this study (Fig. 3A). Similarly, GGT level was much higher in cirrhotic and HCC patients with average values of 139.2 ± 31.37 U/l and 148.5 ± 30.44 U/l, respectively and does not fall into the standard normal range

for this parameter (Fig. 3B). GGT is a parameter which has standard value up to 55 U/l. In the present study, among the four groups, average value obtained from normal group was 32.8 ± 9.7 U/l. However, CLD patients showed 41.35 ± 13.9 U/l which seems higher than the value obtained from normal group of this study.

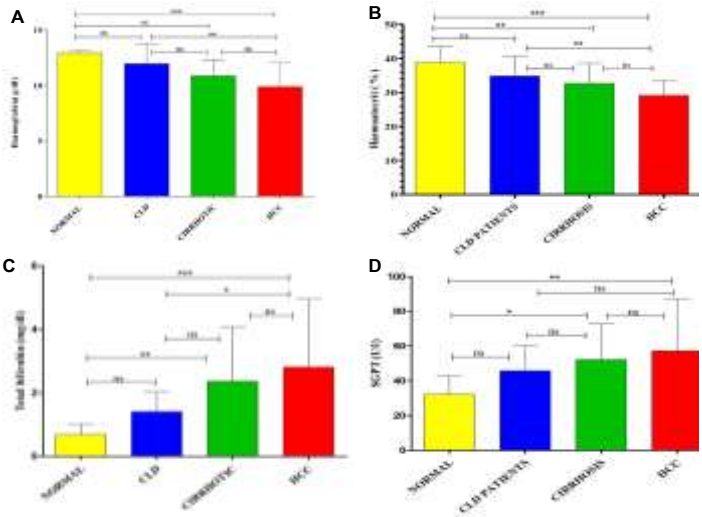

Figure 2. Graph showing the analysis of A) hemoglobin level B) hematocrit level C) serum total bilirubin level D) serum SGPT level in normal, chronic liver disease, cirrhosis, and hepatocellular carcinoma groups. The data is representative of 20 independent individuals of each group (*p< 0.05, ** p <0.01 and *** p< 0.001), ns= non-significant.

Similarly, GGT level was much higher in cirrhotic and HCC patients with average values of 67.35 ± 13.07 U/l and 106.2 ± 11.81 U/l respectively and also does not fall into the standard normal percentage for this parameter (Fig. 3B). Albumin is a parameter which has standard range 3.5-5.5 g/dl. In the present study, among the four groups, average value obtained from normal group was 4.550 ± 0.68 g/dl. However, CLD patients showed 4.52 ± 0.85 g/dl which seems lower than the value obtained from normal group of this study. Similarly, albumin level was much lower in cirrhosis and HCC group that showed average value of 3.63 ± 1.14 g/dl and 3.43 ±1.01 g/dl respectively, which was much lower than the values obtained from the normal group and also does not fall into the standard normal range for this parameter (Fig. 3C). Creatinine is a parameter which has got standard range 0.3-1.0 mg/dl. In present study, the average value obtained from the normal group was 0.63 ± 0.26 mg/dl. However, higher average value of creatinine was observed from CLD, cirrhosis, and HCC groups with 0.87 ± 0.49 mg/dl, 1.31 ± 1.01 mg/dl and 1.62 ± 0.73 mg/dl respectively. All these values do not fall into the standard normal range for this parameter (Fig. 3D).

Prothrombin time is a parameter which has got standard range of 11-13 seconds. In present study, among the four groups, average time obtained from normal group was 12.75 ± 1.51 seconds. However, CLD patients showed 15.5 ± 3.76 seconds which seems higher than the value obtained from normal group of this study. Similarly, Prothrombin time was much higher in cirrhosis and HCC patients with average values of 18.0 ± 6.20 seconds and 20.20 ± 9.63 seconds respectively and also does not fall into the standard normal percentage for this parameter (Fig 4A). Alfa feto protein is a parameter which has got standard value <10ng/ml (Saini, Bhagat, Sharma, Duseja, & Chawla, 2006).

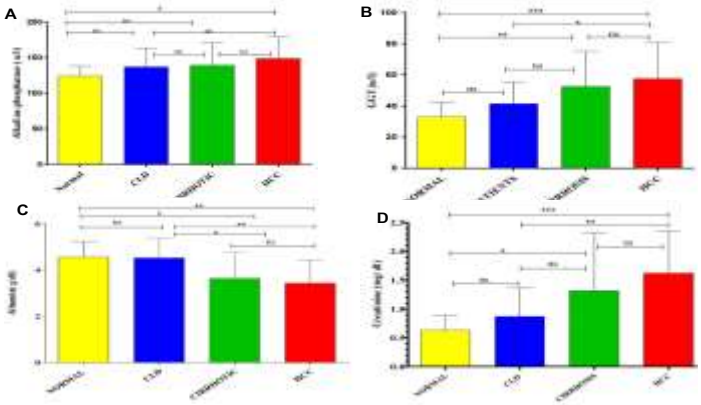

Figure 3. Graph showing the analysis of A) serum alkaline phosphatase level B) serum GGT level C) serum albumin level D) serum Creatinine level in normal, chronic liver disease, cirrhosis and hepatocellular carcinoma groups. The data is representative of 20 independent individuals of each group (*p< 0.05, ** p <0.01 and *** p< 0.001), ns= non-significant.

In present study, average value obtained from normal group was 3.65 ± 1.34ng/ml. However, CLD patients showed 4.70 ± 1.38ng /ml which seems fall in the value obtained from normal group of present study. Conversely, cirrhosis patients showed 308.13± 111.53ng/ml which was much higher than the values obtained from the normal group and does not fall into the standard normal range for this parameter. Similarly, Alfa feto protein level was much higher in HCC patients with average values of 395.51 ± 160ng/ml (Fig 4.14). Furthermore, analysis of serum alfa feto protein level showed statistically significant (P <0.001) in HCC and cirrhotic groups, whereas CLD group was statistically non-significant as compared with healthy group. Among all the groups, except healthy, the highest level of alfa feto protein was observed in HCC group that was statistically highly significant (P <0.001) with that of CLD group of the study. On the contrary, the HCC group showed statistically significant (P <0.05) with Cirrhotic Group The level of alfa feto protein observed in CLD group was statistically highly significant (P <0.001) with cirrhotic group of the present study (Fig 4B). The present study instigates the role of serological marker for diagnosis of HCV induced HCC at earlier stages. Four groups of individuals including patients

of chronic liver disease (CLD, Cirrhosis, hepatocellular carcinoma (HCC) and healthy volunteers (controls) were selected and enrolled in the present study. Except healthy volunteers, all other three groups of individuals were HCV positive. Serum markers were investigated by a wide range of biochemical tests including hemoglobin, hematocrit, Albumin, ALP, PT and AFP.

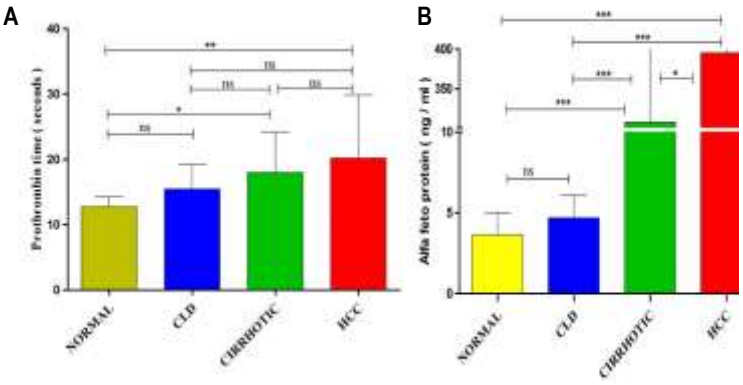

Figure 4. Graph showing the analysis of A) serum Prothrombin level B) Alfa feto proteins in normal, chronic liver disease, Cirrhosis, and hepatocellular carcinoma groups. The data is representative of 20 independent individuals of each group (*p< 0.05, ** p <0.01 and *** p< 0.001), ns= non-significant.

The data demonstrated that in all CLD patients, albumin level was >3.0 and the CLD patients with albumin <4.0 had increased level of ALP than normal mid limit. Increase in ALP was observed with increased PT and decreased HB. In cirrhotic group all patients showed albumin level >2.6. Cirrhotic patients with positive AFP level possessed Total bilirubin level >1.0 and was in decompensated stage. The HB value do not fall into the standard normal range. Among all groups except control (healthy), the highest level of HB was observed. The difference between the HB in CLD and cirrhosis patients was non-significant whereas it was significant between CLD and HCC patients (P <0.01). However, no significant variation in HB was observed between cirrhosis and HCC group of the study.

The analysis of hematocrit showed statistically significant variation in HCC, in cirrhotic group whereas, statistically non-significant in CLD group as compared with the healthy group. Among all groups except healthy, the highest level of hematocrit was observed in CLD group that was statistically non-significant with cirrhotic group and with HCC group. However, no significance in hematocrit level was observed between cirrhotic and HCC group of the study. However, HCC group showed lower mean hematocrit than cirrhotic group of the study. Serum Total bilirubin level analysis revealed that there was marked difference in HCC, (<0.01) in Cirrhotic group in comparison with control group. However, CLD group was statistically non-significant as compared with control group. Among all groups except healthy, the highest level of Total bilirubin was observed in HCC group that was statistically significant (P <0.05) with that of CLD and non-significant with cirrhotic group. Analysis of serum SGPT level showed significant variation in HCC (P<0.01), and in cirrhosis (P <0.05) groups non-significant in CLD group in comparison with control group. However, the average value of the CLD group was higher than the control group of this study. Among all the groups, except control, the highest level of SGPT level was observed. No significant correlation was found between CLD and cirrhosis, between cirrhosis and HCC, between CLD and HCC. However, the mean value was higher in cirrhotic group of the study. Furthermore, analysis of serum ALP level showed that in all the groups, except healthy, no significance was seen among all the three groups of the present study. However, the highest level of ALP was observed in HCC group.

The data analysis of serum GGT level showed that among all groups except healthy, the highest level of GGT was observed in HCC that was statistically significant (P <0.05) with that of CLD and non-significant with cirrhotic group of the study. The level of serum GGT in the CLD group showed non-significant with cirrhotic group of the study however the mean value was higher in cirrhotic group as compared to the CLD group of the study. The serum creatinine level was significantly different in HCC group, (P <0.05) in Cirrhotic as compared with control group. However, CLD group showed non-significant difference as compared with healthy group. Among all groups except healthy, the highest level of creatinine was observed in HCC that was statistically significant (P <0.01) with that of CLD group. However, statistically no significance was observed between CLD Vs cirrhosis and cirrhosis Vs HCC groups of the present study. Furthermore, the analysis of prothrombin time was statistically significant in HCC group (P <0.01), in cirrhotic (P <0.05) group whereas CLD groups was statistically non-significant as compared with healthy group of the present study. Among all the groups, except healthy, no significance was seen among all the three diseased groups of the present study. However, the highest prothrombin time was observed in HCC group of the present study.

4. CONCLUSION

The differentiating characters observed in cirrhotic group with that of CLD was elevation of AFP, Total bilirubin and PT with decrease in albumin and the formation of ascetic fluid in patients with AFP level increased. HB/HCT and SGPT were approximately equal in CLD and cirrhotic patients. Finally, the biochemical data of healthy controls was within normal range as compared with standard range. Overall male ratio was higher as compared to female. Males are more prone to disease due to high risk factors. There is

need to locate the disease at chronic stage so that it cannot progress to the symptomatic stage.

5. CONFLICT OF INTEREST

All authors have declared that there is no conflict of interests regarding the publication of this article.

6. ACKNOWLEDGEMENT

Authors are thankful to the Staff and management of Asian Institute of Medical Sciences for cooperation in collecting samples and data of patients.

REFRENCES

Abbas, Z., Jafri, W., & Hamid, S. (2010). Management of hepatitis B: Pakistan Society for the Study of Liver Diseases (PSSLD) practice guidelines. *J Coll Physicians Surg Pak, 20*, 198-201.

Afridi, S., Naeem, M., Hussain, A., Kakar, N., Babar, M. E., & Ahmad, J. (2009). Prevalence of hepatitis C virus (HCV) genotypes in Balochistan. *Molecular biology reports, 36*(6), 1511-1514.

Alkofer, B., Lepennec, V., & Chiche, L. (2011). Hepatocellular cancer in the non-cirrhotic liver. *Journal of visceral surgery, 148*(1), 3-11.

Bahadar, N., Khan, F., Israr, M., & Ahmad, S. (2016). The correlation between RT-PCR and ELISA assay on hepatitis C positive serum samples. Pure and Applied Biology. In.

Blackard, J. T., Shata, M. T., Shire, N. J., & Sherman, K. E. (2008). Acute hepatitis C virus infection: a chronic problem. *Hepatology, 47*(1), 321-331.

Bralet, M. P., Régimbeau, J. M., Pineau, P., Dubois, S., Loas, G., Degos, F., . . . Terris, B. (2000). Hepatocellular carcinoma occurring in nonfibrotic liver: epidemiologic and histopathologic analysis of 80 French cases. *Hepatology, 32*(2), 200-204.

Butt, A. S., Hamid, S., Wadalawala, A. A., Ghufran, M., Javed, A. A., Farooq, O., . . . Jafri, W. (2013). Hepatocellular carcinoma in Native South Asian Pakistani population; trends, clinico-pathological characteristics & differences in viral marker negative & viral-hepatocellular carcinoma. *BMC research notes, 6*(1), 137.

Chidambaranathan-Reghupaty, S., Fisher, P. B., & Sarkar, D. (2021). Hepatocellular carcinoma (HCC): epidemiology, etiology and molecular classification. *Advances in cancer research, 149*, 1-61.

Chung, R. T. (2005). Acute hepatitis C virus infection. *Clinical infectious diseases, 41*(Supplement_1), S14-S17.

Dienstag, J. L. (2002). The role of liver biopsy in chronic hepatitis C. *Hepatology, 36*(S1), S152-S160.

Graham, C. S., & Swan, T. (2015). A path to eradication of hepatitis C in low-and middle-income countries. *Antiviral research, 119*, 89-96.

Hajarizadeh, B., Grebely, J., & Dore, G. J. (2013). Epidemiology and natural history of HCV infection. *Nature reviews Gastroenterology & hepatology, 10*(9), 553.

Heydtmann, M., Shields, P., McCaughan, G., & Adams, D. (2001). Cytokines and chemokines in the immune response to hepatitis C infection. *Current opinion in infectious diseases, 14*(3), 279-287.

Khan, A., Tanaka, Y., Azam, Z., Abbas, Z., Kurbanov, F., Saleem, U., . . . Mizokami, M. (2009). Epidemic spread of hepatitis C virus genotype 3a and relation to high incidence of hepatocellular carcinoma in Pakistan. *Journal of medical virology, 81*(7), 1189-1197.

Looi, K. S., Nakayasu, E. S., Diaz, R. A. d., Tan, E. M., Almeida, I. C., & Zhang, J.-Y. (2008). Using proteomic approach to identify tumor-associated antigens as markers in hepatocellular carcinoma. *Journal of proteome research, 7*(9), 4004-4012.

Lozano, R., Naghavi, M., Foreman, K., Lim, S., Shibuya, K., Aboyans, V., . . . Ahn, S. Y. (2012). Global and regional mortality from 235 causes of death for 20 age groups in 1990 and 2010: a systematic analysis for the Global Burden of Disease Study 2010. *The lancet, 380*(9859), 2095-2128.

Pawlotsky, J.-M., Bouvier-Alias, M., Hezode, C., Darthuy, F., Remire, J., & Dhumeaux, D. (2000). Standardization of hepatitis C virus RNA quantification. *Hepatology, 32*(3), 654-659.

Pawlotsky, J. M. (2002). Use and interpretation of virological tests for hepatitis C. *Hepatology, 36*(5B), s65-s73.

Ravel, R. (1995). Clinical Application of Laboratory Data. *Clinical Laboratory Medicine*, 309-330.

Saini, N., Bhagat, A., Sharma, S., Duseja, A., & Chawla, Y. (2006). Evaluation of clinical and biochemical parameters in hepatocellular carcinoma: experience from an Indian center. *Clinica chimica acta, 371*(1-2), 183-186.

Thorgeirsson, S. S., & Grisham, J. W. (2002). Molecular pathogenesis of human hepatocellular carcinoma. *Nature genetics, 31*(4), 339-346.

Yilma, M., Saxena, V., & Mehta, N. (2022). Models to Predict Development or Recurence of Hepatocellular Carcinoma (HCC) in Patients with Advanced Hepatic Fibrosis. *Current Gastroenterology Reports*, 1-9

UNIVERSITY OF SINDH JOURNAL OF ANIMAL SCIENCES
DOI: https://doi.org/10.57038/usjas.v6i04.5840
Uni. Sindh. J. Anim. Sci., vol. 6(4); 35-40, 2022
Email: editors.usjas@usindh.edu.pk
ISSN (P): 2521-8328
ISSN (E): 2523-6067
Published by University of Sindh, Jamshoro.

FIELD AND LABORATORY STUDIES ON THE TOXICITY OF LAMBDA-CYHALOTHRIN FOR CONTROLLING DESERT LOCUST (*SCHISTOCERCA GREGARIA*)

ZAHID SALEEM[1], HUSSAIN ALI[2]*, MUHAMMAD NAVEED[3], MUHAMMAD ANWAR KHAN[4] MUHAMMAD YOUNUS [2], MUHAMMAD AFZAAL[2], HABIB UR REHMAN[5]

[1]Entomology Section, Agriculture Research Station Jarma, Kohat, Pakistan
[2]Entomology Research Program, Agricultural Research Institute Tarnab Peshawar, Pakistan
[3] Directorate of Plant Protection, Department of Agriculture Extension Peshawar, Pakistan
[4] Office of the District Director Agriculture Extension South Waziristan, Pakistan
[5]Entomology Section, Agricultural Research Institute D.I. Khan, Pakistan

ARTICLE INFORMATION

Article History:
Received: 12th November 2022
Accepted: 26th December 2022
Published online: 31st December 2022

Author's contribution
ZS, HA, MN, MA conducted the trials. MY, MA, HUR and HA analyzed the data and wrote the paper.

Key words:
Hoppers, Mortality, *Schistocerca gregaria*, Lambda-cyhalothrin

ABSTRACT

Desert locust has negative impact on world vegetation, severely affected Africa and Asia in 2020-21. The pest persists in both gregarious and solitary forms. In Pakistan, the majority of the nation experienced a desert locust attack in 2020. Seven to ten districts in Khyber Pakhtunkhwa were seriously impacted. The study was carried out to test the efficacy of different concentrations of Lambda-cyhalothrin 2.5 EC against adult and hoppers/nymph in field and laboratory conditions. The applied concentration was 4%,3%, 2%, 1% and 0.5 % for adults in field and lab conditions, while 2%, 1.5%, 1%, 0.5% and 0.25% for nymph's trials. Under field condition against adults all the applied concentrations were at par with each other except 0.5%, which showed significantly lower mortality rate (60%). A similar trend of toxicity was also recorded for the laboratory trials. More than 85% mortality was recorded in all treatment except 0.5%, which was 65%. Against hopper all concentration showed significant higher mortality (above 80%) except the nymphs sprayed with 0.25% concentration under field conditions. Similar results were also obtained for vitro trials against nymphal stage. It is concluded an average concentration (3%) should be applied for adult to avoid resistance and pest escape. For hoppers/nymph the recommended concentration should be 1%. Further studies should be carried out regarding the resistance to different types of insecticides under field and laboratory conditions.

1. INTRODUCTION

The common species of desert locust *Schistocerca gregaria* (Orthoptera: Acrididae) is widely distributed in the world. It has short horned and is considered as devastating pest in Africa and Asia (Song, 2004; Lovejoy et al., 2006; Cheseto et al., 2015). Worldwide a total of 700 disturbed and among them 50 belong to genus *Schistocerca.* Only four species of this genius has swarming formation habitat and considered as trans boundary pests (Song 2004; Song et al., 2017). *S. gregaria* has phase changing ability and can be shifted very quickly from solitary to gregarious. In gregarious form they made swarm and travel across Africa and

*Corresponding Author: hussaintanha@yahoo.com

In 2019-2020 Pakistan face sever desert locust plague across three provinces. The species were later identified as *S. gregaria gregaria* travelled all the way from Africa to Asia via Middle *East* (Sultana et al., 2021 Ahmad et al., 2020). This species goes under incomplete metamorphosis. Nymph has further five instars. Environmental conditions greatly affect the life stage and development period. The desert locust takes weeks to months to became mature. In solitary the losses are less and it remains calm, while it causes huge losses to crops in gregarious form (Meinzingen, 1993). Color also different in both forms, solitary phase has greenish to brownish color while gregarious has yellowish to blackish color (Pener, 1991; Ayali, 2019).

Desert locust usually travel from one country to another, having 50 km/day speed. Different control methods were used for the control of desert locust. Among them mainly pesticides are used in emergency situations. Extra use of Pesticides causes environmental pollution as well having adverse effect on non-target organisms as well (Lecoq, 2010). Biocontrol can also be used but they are mostly effective against early stages of the pests (Ali et al., 2021). Bio-pesticides such as *Metarhizium anisopliae* Var. *acridum* were widely used against the pests in Africa and Australia and best results were obtained against nymphs (Abdelatef and Hartbauer 2020; Abdelatef, 2005; Ali et al 2021).

Plant based extracts also good for the control of desert locust, as they are non-toxic to other animals but cannot be apply on large scale in emergency situations (Koul et al. 2008; Abdelatef and Hartbauer 2020). Biocontrol and Bio pesticides require time, optimum temperature and humidity for controlling the desert locust and cannot be applied in emergency situations. Pesticides such as Lambda-Cyhalothrin 2.5 EC, Chlorpyrifos 40 EC, and Deltamethrin 2.5 EC can be sued in emergency situation to obtained immediate results to tackle the situation effectively (Ahmad et al., 2020).

The present studie was carried out to evaluate the efficacy of different chemicals and concentrations against adults of desert locust and hoppers/nymphs under field and laboratory conditions in southern districts of Khyber Pakhtunkhwa.

2. MATERIALS AND METHODS

Location of the study

The experiments were carried out during February to May 2020 at desert locust affected districts of Khyber Pakhtunkhwa, Pakistan. The laboratory trials were carried out in Agriculture Research Institute, Dera Ismail Khan, while the field experiments were carried out at Dera Ismail Khan, Tank and Lakki Marwat districts.

Adult Trials

Adults of desert locust were selected from Dera Ismail Khan and Tank for the study. For each treatment three different trees were randomly selected and were sprayed. Lambda-Cyhalothrin 2.5 EC, were applied. at 4%, 3%, 2%, 1% and 0.5% solution/concentration. Control was also kept for comparison, having no chemical treatment. For laboratory trials a total of 100 adults were selected for each concentration. Control cage having no treatment was also kept for comparison. Data were recorded on mortality after 3 hours and 24 hours.

Hoppers/Nymph Trials

The 2nd instar nymphs/hoppers were selected for the experiment from the fields and brought to the laboratory for cage experiments to the infield one-meter area was caged with field cages. The cages were sprayed with 2%, 1.5%, 1%, 0.5% and 0.25% concentration of Lambda-Cyhalothrin for nymph's trials. A total of 100 nymphs were selected for each concentration in both field and laboratory trials. For comparison control cage was also placed.

Data Recording

The data was collected at 3 hrs. and 24 hrs. intervals. The number of dead and alive adults and hoppers/nymphs were counted in field and laboratory and the number were recorded. The average temperature during the field experiment ranged from 28-33°C with average humidity range from 30-40%. The weather conditions were generally sunny during the trial period. In laboratory experiment the temperature were kept constant (26-27°C) having 30% humidity.

Statistical Analysis

The data was statistically analyzed by using computer program Statistix. Least significant difference (LSD) was calculated at <0.05.

3. RESULTS AND DISCUSSION

Table-1 shows the efficacy of different concentrations of Lambda-Cyhalothrin 2.5 EC against adults of desert locust during field conditions. All the concentration showed significantly ($P<0.05$) higher mortality than 0.5%, which showed significantly lower mortality after 3 and 24 hours of treatment. Lowest mortality was recorded in control. The data in table-2 shows the efficacy under the laboratory conditions. A similar trend like field were recorded and all the concentration was with par with each other regarding mortality except 0.5%, which was significantly lower in percent mortality (65%) than the other concentrations. In control 3% mortality was recorded after 24 hours under laboratory conditions.

All the tested concentration showed significantly ($P<0.05$) higher mortality (above 80% after 3 hours and 90% after 24 hours in both locations against hoppers in field trials except 0.25%, which showed significantly lower percent mortality (Table-3). In laborator trails after 3 hours above 80% mortality was recorded in cages treated with 2%, 1.5%, 1%, 0.5% of Lambda-Cyhalothrin 2.5 EC. The recorded mortality 53% and 62 % after 3 and 24 hours respectively in 0.25% concentration was significantly lower compared to the remaining concentrations. In control 3% mortality was recorded in laboratory trials (Table-4).

In the current study all the concentration of lambda was effective against adult desert locusts in the field except 0.5%. A lower concentration may be used for the management of desert locust in the emergency to save the environment from contamination. Earlier studies conducted showed good results in chemical treatment against desert locust but it should be judicial application (FAO, 1998). Lambda- Cyhalothrin 2.5EC is very effective under both field and laboratory conditions in the trials and high mortality was recorded in both trials. Our results in similarity with those obtained by Khawar et al, 2020). They tested different pesticides with different concentrations and among all the tested insecticides they found Lambda- Cyhalothrin 2.5 EC more potent and recorded more than 80% mortality against the adult. Furthermore, they also recorded more than 90% mortality in against nymphal stage in both field and laboratory conditions. They found Buprofezin 25 WP least effective against desert locust. In another study conducted by Metaweh and Ali (1999) and Mamadou and Sarr (2009) used different insecticides and among them they found that Lambda-Cyhalothrin is extra effective to adult locusts and pest respond more under control conditions as compared to field conditions. In the current studies against adult's different dose were used and all of them were effective. The 3% and 2% concentration of the Lambda cyhalothrin 2.5 EC showed great mortality against adults in in field and laboratory conditions and can be used. Against hoppers/nymphs lower concentrations were used and they were effective. Similar results were obtained in an earlier studied carried out by Holt and Copper (2006). They used different insecticides against the desert locust hoppers different instar and found that lower concentration can also gave good results. Abdel-Fatah et al.,2012 also observed that Lambda- Cyhalothrin 2.5 EC is more toxic to the hoppers compare to Chlorpyrifos 40 EC and can give more than 80% mortality in field conditions.

4. CONCLUSION

It is concluded from the trials that Lambda-Cyhalothrin is an effective insecticide against desert locust and can be used in emergency situations depending on its availability in Pakistan. The recommended dose for the adult is 3% (3 liters/100 liter of water) for field application against the nymphal stage 1% (1 liter/ 100 liters of water) is good for getting above 80% mortality.

5. CONFLICT OF INTEREST

All authors have declared that there is no conflict of interests regarding the publication of this article.

REFRENCES

Abdelatti, Z. A. S., and Hartbauer, M. 2020. Plant oil mixtures as a novel botanical pesticide to control gregarious locusts. *Journal of Pest Science*. 93(1), 341-353.

Abdelatef G. M.2005. Effect of green muscle on locust and grasshoppers. Final report of project (FAO) CRC/EMPRES (PR27209). pp:53.

Abdel-Fatah T.A., Mohamed, G. A, Abdel-Lattef, G.M. 2012.Effectiveness of some insecticides against the desert locust and barsem grasshopper in the field. *Egyptian Journal of Applied Science* 27(7):89-96.

Ali., H., Bitanni, S., Haya,t Z., Asad, A., Fazal, S., Toheed I. , Zahid S. , Irfan U. K., Kiran S., and Obaid U. S.2021. Efficacy of different concentrations of Green-Guard (Metarhizium anisopliae Var. Acridum) against desert Locust (*Schistocerca gregaria*) under lab and field conditions. *Bioscience Research*. 18(3): 2362-2367.

Ayali, A., 2019. The puzzle of locust density-dependent phase polyphenism. *Current Opinion in Insect Science*. 35: 41- 47. https://doi.org/10.1016/j.cois.2019.06.008

Ahmad, J. N., Ahmad, S. J., Malik, M. A., Abid, A., Ali, M., Ahmad, E., ... & Ashraf, M. 2020. Molecular Evidence for the Association of Swarm Forming Desert Locust, *Schistocerca gregaria gregaria* (Forskål) in Pakistan with Highly Prevalent Subspecies in Sahara Desert of Africa. *Pakistan Journal of Zoology*, *52*(6), 2233.

Chapuis, M.P., Foucart, A., Plantamp, C., Blondin, L., Leménager, N., Benoit, L., Gay, P.E. and Bazelet,C.S., 2017. Genetic and morphological variation in non-polyphenic Southern African populations of the desert locust. *African*

Entomologist, 25: 13-23. https:// doi.org/ 10.4001/003.025.0013

Cheseto, X., Kuate, S.P., Tchouassi, D.P., Ndungu, M., Teal, P.E. and Torto, B., 2015. Potential of the desert locust *Schistocerca gregaria* (Arthoptera: Acrididae) as an unconventional source of dietary and therapeutic sterols. *PLoS One*, 10: 1-13. https://doi.org/10.1371/journal.pone.0127171

FAO. 2014. Evaluation of field trial data on the efficacy and selectivity of insecticides on locusts and grasshoppers. Report by the FAO Locust Pesticide Referee Group. Locust Pest. Referee Group Meet., 10th, Gammarth, pp 63-66.

Holt, J., & Cooper, J. F. 2006. A model to compare the suitability of locust hopper targets for control by insecticide barriers. *Ecological modelling*, 195(3-4), 273-280.

Koul O., S. Walia and G. S. Dhaliwal. 2008. Essential Oils as Green Pesticides: Potential and Constraints. *Biopesticides International*. 4(1): 63–84.

Khawar J.A. , Asad A., Muhammad M., Qurban A., Dilbar H., Humaira M., Faisal H, Imran N., Muhammad J. S., Muhammad F. A. , Muhammad Z., Muhammad K. M. Toxicological Impact of different Insecticides on the Desert Locust **(***Schistocerca gregaria (*Forsk) (Acrididae). *Life Science Journal.* 2020;17(8):6-11.

Lovejoy, N. R., Mullen, S. P., Sword, G. A., Chapman, R. F., & Harrison, R. G. 2006. Ancient trans-Atlantic flight explains locust biogeography: Molecular Phylogenetic of Schistocerca. *Proceedings of the Royal Society B: Biological Sciences*, *273*(1588), 767-774.

Mamadou, A. and Sarr M. 2009. Impact of tow insecticides used in the control of the desert locust on *Psammotermeshy bostoma* (Desneus). *Nigerian African Entomology*, 17(2):147-153.

Meinzingen, W.F., 1993. A guide to migrant pest management in Africa. Food and Agriculture Organization of United Nation, FAO, pp. 184.

Metaweh HH, and Ali IA. 1999. Toxicity and inducing knock down of some insecticides to fifth instar nymphs of the desert locust collected from the desert or reared in the laboratory. *Egyptian Journal of Applied Science*, 14(3): 276-286.

Pener, M.P., 1991. Locust phase polymorphism and its endocrine relations. *Advances in Insect Physiology*., 23: 1-79. https://doi.org/10.1016/S0065-2806(08)60091-0

Song, H. 2004. On the origin of the desert locust *Schistocerca gregaria* (Forskål)(Orthoptera: Acrididae: Cyrtacanthacridinae). *Proceedings of the Royal Society of London. Series B: Biological Sciences*, *271*(1548), 1641-1648.

Song, H., Foquet, B., Mariño-Pérez, R., & Woller, D. A. 2017. Phylogeny of locusts and grasshoppers reveals complex evolution of density-dependent phenotypic plasticity. *Scientific reports*, *7*(1), 1-13.

Sultana R, Kumar S, Samejo AA, Soomro S, Lecoq M. 2021. The 2019–2020 upsurge of the desert locust and its impact in Pakistan. *Journal of Orthoptera Research* 30(2): 145–154. https://doi.org/10.3897/jor.30.65971.

Table-1: Toxicity of Lambda-cyhalothrin concentrations against Desert locust adults under field conditions.

Dose/Concentration	% mortality at Dera Ismail Khan		% mortality at Tank	
	3 hours	24 hours	3 hours	24 hours
4 liter/100 liter of water (4%)	75 c	85 c	72 c	87 c
3 liter/100 liter of water (3%)	75 c	83 c	73 c	82 c
2 liter/100 liter of water (2%)	74 c	83 c	72 c	81 c
1 liter/100 liter of water (1%)	70 c	81 c	71 c	82 c
500 ml /100 liter of water (0.5%)	50 b	60 b	55 b	62 b
Control	02 a	02 a	03 a	03 a

Table-2: Toxicity of Lambda-cyhalothrin concentrations against Desert locust adults under laboratory conditions.

Dose/Concentration	% mortality	
	3 hours	24 hours
4 liter/100 liter of water (4%)	78 c	89 c
3 liter/100 liter of water (3%)	78 c	89 c
2 liter/100 liter of water (2%)	77c	87 c
1 liter/100 liter of water (1%)	73 c	85 c
500 ml /100 liter of water (0.5%)	54 b	65 b
Control	01 a	03 a

Table-3: Toxicity of Lambda-cyhalothrin concentrations against Desert locust nymphs under field conditions.

Dose/Concentration	% mortality at Dera Ismail Khan		% mortality at lakki Marwat	
	3 hours	24 hours	3 hours	24 hours
2 liter/100 liter of water (2%)	84 c	93 c	84 c	95 c
1.5 liter/100 liter of water (1.5%)	83 c	93 c	82 c	95 c
1 liter/100 liter of water (1%)	82 c	92 c	83 c	92 c
500 ml/100 liter of water (0.5%)	80 c	89 c	81 c	90 c
250 ml /100 liter of water (0.25%)	55 b	65 b	53 b	66 b
Control	03 a	05 a	04 a	05 a

Table-4: Toxicity of Lambda-cyhalothrin concentrations against Desert locust nymphs under laboratory conditions.

Dose/Concentration	% mortality	
	3 hours	24 hours
2 liter/100 liter of water (2%)	85 c	93 c
1.5 liter/100 liter of water (1.5%)	86 c	92 c
1 liter/100 liter of water (1%)	85c	93 c
500 ml/100 liter of water (0.5%)	80 c	88 c
250 ml /100 liter of water (0.25%)	53 b	62 b
Control	01 a	03 a

UNIVERSITY OF SINDH JOURNAL OF ANIMAL SCIENCES
DOI: https://doi.org/10.57038/usjas.v6i04.5841
Uni. Sindh. J. Anim. Sci., vol. 6(4); 41-47, 2022
Email: *editors.usjas@usindh.edu.pk*
ISSN (P): 2521-8328
ISSN (E): 2523-6067
Published by University of Sindh, Jamshoro.

COMPARATIVE IMMUNOGENIC RESPONSE OF LIVE VACCINE OF LASOTA AND MUKTESWAR STRAINS OF NEWCASTLE DISEASE VIRUS BY DIFFERENT ROUTES OF INOCULATION IN LAYER CHICKEN

ABDUL WHAB MANZOOR[1*], SAJJAD HUSSAIN[1], HAFIZ MUHAMMAD WAQAR[1], SADIA SARFARAZ[1], SHERAZ SHAHID[1], SAMIULLAH[1], AFEEFA SHAFIQ[1], SAJJAD ALI[1], RAIS AHMED[2], HALEEMA SADIA[3], MAHEEN MURTAZA[4], TASLEEM KAUSAR[5]

[1]Veterinary Research Institute, Zarrar Shaheed Road, 54810, Lahore Cantt.
[2]Department of Microbiology, University of Veterinary and Animal Sciences, Lahore.
[3]Department of Biotechnology, Balochistan University of Information Technology Engineering and Management Sciences, Quetta, Pakistan.
[4]Department of Zoology, The Islamia University of Bahawalpur, Bahawalpur.
[5]Department of Zoology, Government Sadiq College University, 63100, Bahawalpur

ARTICLE INFORMATION

Article History:
Received: 31st August 2022
Accepted: 24th December 2022
Published online: 31st December 2022

Author's contribution
AWM, SH, and HMW conceived idea and did research. S.S, SS, S, AS did analysis and prepared initial draft. SA, RA, HS, MM, TK revised manuscript, data analysis and prepared final draft

Key words:
ND-LaSota, ND-Mukteswar, Ocular, Drinking Water, Injection, Haemagglutination inhibition, Immune Response.

ABSTRACT

Newcastle disease (ND) has been one of the great challenges to Poultry in Pakistan and dealt with vaccination. Mortality and morbidity that are associated with it are economically significant. Two vaccines produced against Newcastle disease virus are Live ND-LaSota and ND Mukteswar vaccines and their immunogenic effects in backyard poultry. For that purpose, a total 130 of 6-8 weeks old layer cockerels were inoculated with both vaccines by different routes including Ocular, drinking water and Injection and serum samples were collected at 14, 21 and 35 days' post inoculation were analyzed by Heamaglutination inhibition test for antibody titers. The best route of administration for ND-LaSota was observed by ocular drops and ND- Mukteswar through injection. So it was concluded that both the vaccines needed booster doses in 4th to 5th week of primary inoculation. ND-LaSota vaccine was found to be best for primary induction.

1. INTRODUCTION

Poultry is one of the most well developed and advanced sectors of the economy of Pakistan employing almost 1.5 million citizens through direct or indirect employment. According to the Economic Survey of Pakistan 2018-19, poultry is a source of proteins for 220 million people of the country and contributes 1.4% to the national GDP (GOP, 2019). The emerging and prevalent infectious diseases pose great economic losses along with serious threat to the growth of rural as well as commercial poultry sector (Aalexander, 2003). These infectious diseases like Avian Infectious bronchitis (IB), Avian Influenza (AI), Newcastle disease (ND), Infectious Bursal disease (IBD) etc., are responsible for huge economic losses in the form of mortality, therapeutic expenses, cost of preventive measures and restrictions in exports of animal and poultry (Absalón et al., 2019).

One of the most notorious infectious diseases is Newcastle disease caused by Avian Paramyxovirus1 (APMV1) and characterized by various forms. Its viscerotropic form causes sudden and high mortality with intestinal hemorrhages while neurotropic form is associated with severe respiratory and nervous signs (El-Masry et al., 2019). The most important method of controlling ND is vaccination, accompanied by strict biosecurity measures and quarantine policy (Bello et al., 2018). Vaccine is prepared in different forms from lentogenic and mesogenic strains of ND virus e.g LaSota, Mukteswer, B1, V4, Komarov. Among these strains LaSota and Mukteswer are mostly used for vaccination development against ND in Pakistan (Rasool et al., 2021). LaSota is a lentogenic strain of the virus used for chicks less than 8 weeks of age in Pakistan. According to some studies, LaSota strain causes vaccination reaction, hence not recommended for naïve birds as well as mixed population of different aged flocks (Abel, 2018). Mesogenic strains (Mukteswer) are not recommended in

*Corresponding Author: whab2010@gmail.com

the chickens less than 8 weeks of age as they may cause respiratory distress and mild enteric disease and may cause mortality in immuno-compromised chickens. Mesogenic strains provide good immune response long lasting immunity and are usually used to boost the primary titer produced by Lentogenic strains (Zhao et al., 2012). ND viruses are constantly evolving but all types are considered to be same serological type and antibody produced by one vaccinal or pathogenic strain provides cross protection against all other strains (Bello et al., 2018).

In Pakistan, ND vaccines are produced from two strains (ND La Sota and ND Mukteswer) at veterinary Research Institute (VRI), Lahore which is a public sector institution. Vaccines produced are usually used for backyard poultry and small-scale farmers. The commercial poultry companies import ND vaccines from other countries. There was a need to compare the immunogenic efficacy of these two vaccines, inoculated by different routes including ocular drops, injection and drinking water, through serology. The immunogenic response following ocular drops of ND-LaSota and booster by injecting ND-Mukteswer vaccine was compared to determine the boosting effect of the vaccine. Therefore, this project was aimed to study the comparative efficacy of live vaccine of both strains with respect to immunogenic response followed by recommendations to the industry and backyard farmers in Pakistan about the comparative suitability of both vaccines for onward vaccine production in Pakistan.

2. MATERIALS AND METHODS

ND Vaccines (ND-LaSota & ND-Mukteswar) produced at VRI, Lahore were used for the current study. These vaccines were produced on 9-11 days old embryonated chicken eggs, received from poultry unit at VRI, Lahore, disinfected and incubated at 37ºC. The eggs were inoculated at 9th to10th day of incubation with 0.1 ml of 105-106 dilution of virus and were incubated for another 96 hours for La Sota and till the death of embryo in case of Mukteswar strain. Then eggs were chilled and harvested. The allantoic fluid was harvested in case of LaSota strain but both fluid and embryo were collected in case of Mukteswer strain. Vaccines were lyophilized by freezing at –35ºC, primary drying by gradually raising the temperature from -25ºC to Zero degree in 18 hours at 170 micro bar and secondary drying at +35ºC for 10 hours. The vials were then sealed and tested for presence of vacuum. Each vial contained 200 doses and quality was assured by safety, sterility, EID50 and ELD50.

The experiment was conducted on 130, day-old layer chicks which were purchased from the market and were reared at VRI, Lahore up to 06-08 weeks and were divided into three groups (01, 02, 03). The group 01 and 02 contained forty birds, each group was further divided into four subgroups (A, B, C, D) containing 10 birds each. The group 03 consisted of 50 birds and further divided into three subgroups (A, B and C) with group A and B having 20 birds each and group C with 10 birds as control. All the birds were kept under normal conditions in cages in experimental sheds of Veterinary Research Institute Lahore, Pakistan and study was conducted under legal and ethical rules of Government of the Punjab (Banu, 2009). All the birds were screened for antibody titer by haemagglutination inhibition (HI) assay. The birds with high antibody titer were replaced (Anon, 1971).

Group-1

The birds of subgroup A, B and C were inoculated with ND-LaSota Live vaccine @) 0.1ml/ bird by ocular drops, drinking water and intramuscular injection, respectively while the birds of group D were kept as control. The doses were calculated as107.85 EID50/dose. The antibody titer of all the birds in all sampling units of group 01 were checked by HI test after 14, 21 and 35days post-inoculation.

Group-2

Protocol adopted for group 01 was repeated using ND-Mukteswar vaccine. The doses were calculated as 107.20 ELD50/dose.

Group-3

This group of 50 birds was vaccinated with ND Live LaSota and ND Mukteswar live vaccine. In Subgroup A, 20 birds were vaccinated with ND-LaSota live Vaccine at day 01 by ocular route at dose rate of 0.1ml /bird while the birds of subgroup B were vaccinated with ND- LaSota live Vaccine at day 01 by Drinking water. Both groups were given a booster dose using 0.1ml intramuscular injection of ND- Mukteswar live vaccine 14 days after the first injection. The antibody titers of both sub-groups were measured with HI test after 14, 21 and 35 days after 2nd injection. Subgroup C with 10 birds was kept as control (Table 1).

Data was analyzed by ANOVA using SPSS (V 20.0). Statistical significance was defined as a value of $p<0.05$. Paired‘t’ test were performed to determine the significant differences in HI titres of chickens of a group after primary and secondary vaccination (Beri, 2005).

3. RESULTS AND DISCUSSION

ND-LaSota Vaccine

The titers of Sub-group A, inoculated by ocular drops of ND-LaSota (SGA-L) were considerably higher than other subgroups. Drinking water proved to be second best route of administration of LaSota vaccine. The titer of Subgroup B, administered through drinking water (SGB-

L) and subgroup C, inoculated through injection (SGC-L) remained higher than SGA-L at the end of experiment. The antibody response on inoculation by injection was poor when compared to other routes. Moreover, the antibody titers of all groups decreased in the 4th to 5th week post inoculation suggesting the booster dose in 5th week of first inoculation (Figure 1).

Table 1. Vaccine Inoculation Layout in Different Groups.

Group 01 (ND-LaSota)	Subgroup A	ND-LaSota live vaccine ocular drops 0.1 ml/bird
	Subgroup B	ND-LaSota live vaccine drinking water 0.1 ml/bird
	Subgroup C	ND-LaSota live vaccine Injection Intramuscular (I/M) 0.1 ml/bird
	Subgroup D	Control group
Group 02 (ND-Mukteswar)	Subgroup A	ND-Mukteswar live vaccine ocular drops 0.1 ml/bird
	Subgroup B	ND-Mukteswar live vaccine drinking water 0.1 ml/bird
	Subgroup C	ND-Mukteswar live vaccine Injection (I/M) 0.1 ml/bird
	Subgroup D	Control group
Group 03 (ND-LaSota + ND-Mukteswar)	Subgroup A	ND-LaSota live vaccine ocular drops and booster by ND-Mukteswar live vaccine Injection
	Subgroup B	ND-LaSota live vaccine Drinking water and booster by ND- Mukteswar live vaccine Injection
	Subgroup C	Control group

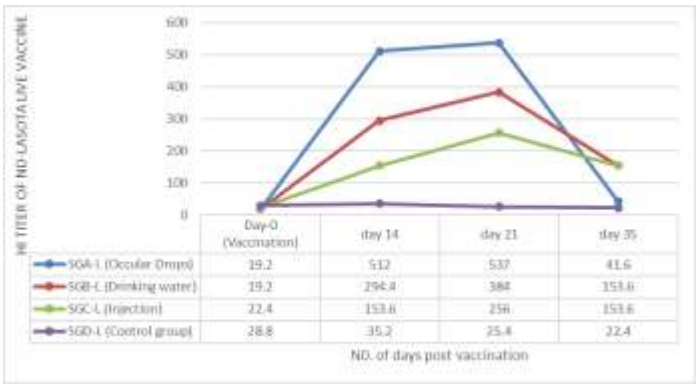

Figure 1: Antibody titre of ND-LASOTA live vaccine by different routes of inoculation

ND-Mukteswar Vaccine

The titer of all the groups were higher than control. Subgroup A, inoculated with ND- Mukteswar vaccine by ocular drops (SGA-M) showed poor titers as compared to other groups. Also, eye drops of Mukteswar vaccine caused redness of eyes in birds. The titers appeared late in SGA-M and remained same even after 35th day of inoculation. The titer of subgroup C, inoculated by injection (SGC-M) showed best results in group 02, suggesting the injection as best route of administration for ND- Mukteswar Vaccine. Inoculation by drinking water (SGB-M) showed intermediate response. Also, the titers of SGB-M and SGC-M decreased in the 5th week of initial inoculation suggesting the need of booster dose in the 5th week (Figure 2).

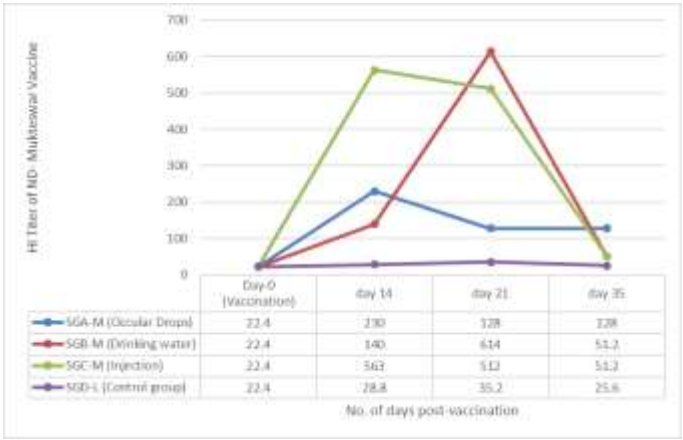

Figure 2: Antibody titre of ND- MUKTESWAR live vaccine by different routes of inoculation

Combination of ND-LaSota and ND-Mukteswar Vaccine

The inoculation of ND- Mukteswar vaccine as a booster following the ND-LaSota vaccine (SGA-LM and SGB-LM) showed considerable high titers even after 35 days of injection suggesting that ND-LaSota primary inoculation should be boosted with ND-Mukteswar vaccine by injection in 3rd to 4th week of primary inoculation (Figure 3).

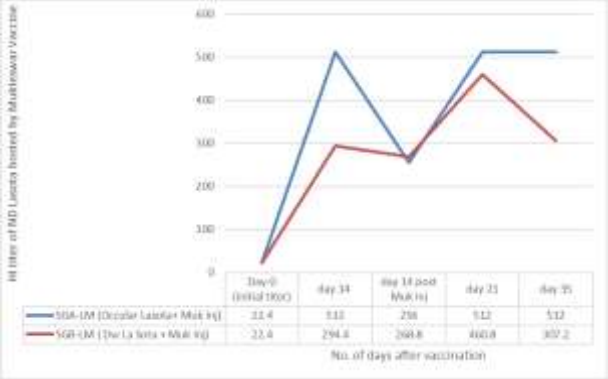

Figure 3: Antibody titre of ND LASOTA vaccine boosted by ND MUKTESWAR injection.

Comparative analysis of ND LaSota and Mukteswer Vaccines

All the routes of ND- LaSota and Muktesawer vaccines were compared on the basis of experimental data. The results showed that the inoculation by ocular drops proved to be best route of LaSota vaccine and worst for Mukteswar vaccine (Figure 4). The drinking water showed intermediate response as compared to other routes for both vaccines. Mukteswar vaccine performed better in drinking water as compared to Lasota (Figure 5). The injection proved to be best route of administration for ND-Mukteswar vaccine and worst for ND-LaSota (Figure 6). Both vaccines performed almost equally well when administered by their best route of inoculation i.e Occular drops for ND-LaSota and injection by ND-Mukteswar in 6-8 week old layer chicken (Figure 7).

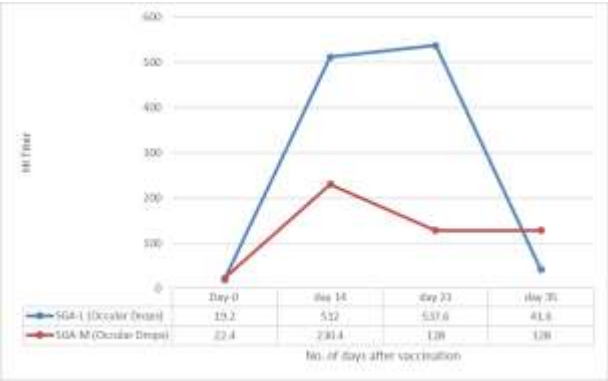

Figure 4: Comparison of ND-LASOTA and MUKTESWAR live vaccines by occular route

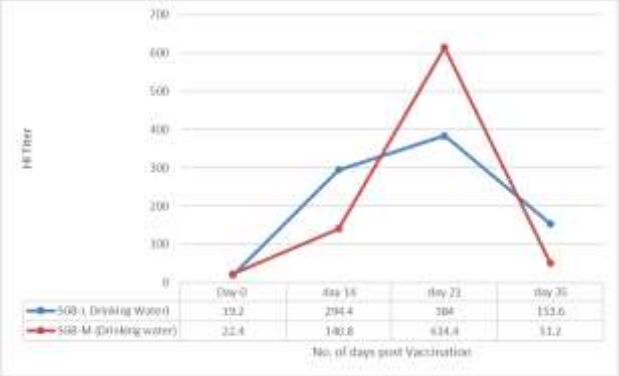

Figure 5: Comparison of ND-LASOTA and MUKTESWAR live vaccines by drinking water

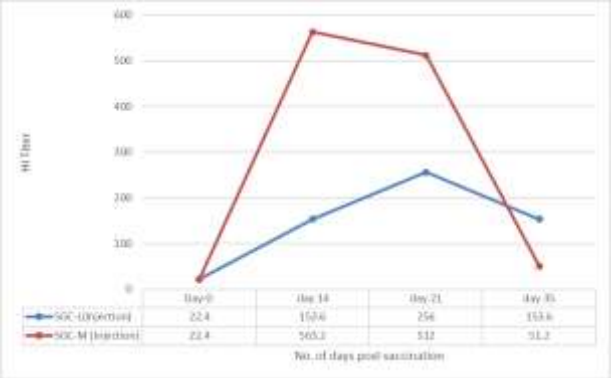

Figure 6: Comparison of ND-LASOTA and MUKTESWAR live vaccines by injection

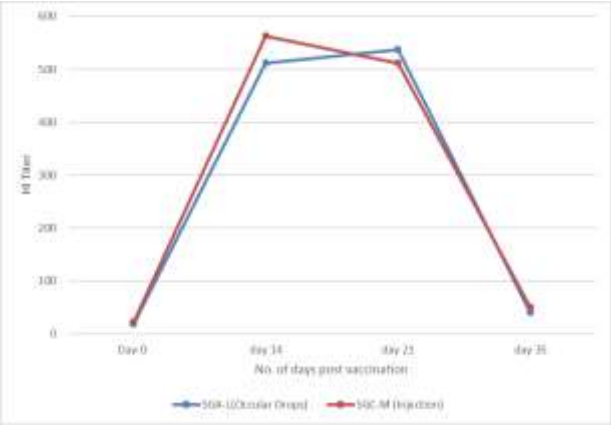

Figure 7: Comparison of ND-LASOTA (Occular Drops) and ND- MUKTESWAR (INJECTION)

Data was analyzed by ANOVA using SPSS (V 20.0). ND Lasota given to birds in three different routes (ocular, oral and injection) showed a significant difference ($P<0.05$, df=3, $F=5.759$). Analysis through post hoc test (LSD)

showed more significance in ocular route (P<0.05) (Table 2). Mukteswar strain given to birds in three different routes (ocular, drinking and injection) showed a significant difference (P<0.05, df=3, F=5.874). Analysis through post hoc test (LSD) showed more significance in injection route (P<0.05) (Table 2).

Data was analyzed by paired sample t- test using SPSS (v 20.0). Vaccine type (Lasota & Mukteswar) have statistically significant difference t (49) = -11.068, p=0.001. R value (0.949) indicates strong positive correlation between two vaccine types and also indicates strong significance for selection of vaccine route (Mean= -1.000, Std. Error= 0.090). Route of vaccination (Oral, Drinking & Injection)) and antibody titer have statistically significant difference t (49) = -9.431, p=0.001. R value (0.334) indicates strong positive correlation between Route of vaccination and antibody titer. This association also indicates strong significance for selection of vaccine route for good antibody titer in birds and very high significance of antibody titer than vaccine route (Mean= -304.84000, Std. Error= 32.324). Data was analyzed by paired sample t- test using SPSS (v 20.0). Route of vaccination (Oral, Drinking & Injection) and antibody titer have statistically significant difference t (49) = -9.431, p=0.001. R value (0.334) indicates strong positive correlation between Route of vaccination and antibody titer. This association also indicates strong significance for selection of vaccine route for good antibody titer in birds and very high significance of antibody titer than vaccine route (Mean= -304.84000, Std. Error= 32.324).

Newcastle disease has caused valuable damage to the poultry industry in Pakistan. For effective control of the disease, vaccination of the birds plays an important role. The indigenous vaccine production in Pakistan is minimal and most of the vaccines are imported. The vaccines produced against Newcastle disease virus at Veterinary Research Institute, Lahore are Live ND-LaSota and ND Mukteswar vaccines. The route of administration of the vaccine is equally important to protect the birds from the disease. A study was needed to determine the best route of administration of these vaccines.

The current study was aimed to discover the route of administration of the ND vaccines to get higher titers for effective control of the disease. Different routes of inoculation including ocular drops, drinking water and intramuscular injection were applied for ND-LaSota and ND-Mukteswar vaccine. The higher titer was observed with ND-LaSota vaccine inoculated by ocular drops. Rehmani (1996) reported similar findings as ND-LaSota vaccine administered intraocularly ranked the best. Similar findings were observed by Wegdan et al. (2015) and Hassanzadeh et al. (2020) where they found higher titers of ND vaccine when administered through intraocular route.

Low antibody titer was observed in case of vaccine inoculation through intramuscular injection for ND-LaSota vaccine while high HI titer was observed by intramuscular injection of ND-Mukteswar vaccine. The antibody response of Mukteswar vaccine through drinking water was found to be lower as compared to intramuscular injection route. Rehmani (1996) also reported low titer of Mukteswar vaccine when administered through drinking water.

HI Titer of both the vaccines dropped in the 4th to 5th week post inoculation. Similar findings were recorded by Sanda et al. (2015) where immunity was found low below the protective level in the 5th week post vaccination. Hence booster dose may be administered in 4th to 5th week post vaccination. A more recent developments in vaccines have been initiated against a wider range of viruses affecting different animals. These include lyophilized live Newcastle Disease (LaSota) vaccine (Khatoon et al., 2022) bovine Herpesvirus 1 gE Deleted Vaccine (Rehman et al., 2022) against newly isolated strains of herpesvirus in cattle from Pakistan (Rehman et al., 2021), and Multiepitope-based Subunit Vaccine (MESV) against foot and mouth disease virus. The efforts to save livestock and poultry from emerging and mutating pathogens in the study area through vaccine is need of the hour (Riaz et al., 2021).

4. CONCLUSION

Inoculation by ocular drops proved to be best route of LaSota vaccine and worst for Mukteswar vaccine. The drinking water showed intermediate response. Mukteswar vaccine performed better in drinking water. The injection proved to be best route of administration for ND-Mukteswar vaccine and worst for ND-LaSota. Both vaccines performed almost equally well when administered by their best route of inoculation i.e. Occular drops for ND-LaSota and injection by ND-Mukteswar in 6-8 week old layer chicken and both need booster injection in 4th to 5th week post vaccination.

5. CONFLICT OF INTEREST

All authors have declared that there is no conflict of interests regarding the publication of this article.

REFRENCES

Abdoshah, M., Hassanzadeh, M., Masoudi, S., & Yousefi, A. R. (2020). Comparison of the impact of different administration routes on the efficacy of a thermoresistant Newcastle disease vaccine in chickens. *Viral Immunology*, *33*(5), 361-366.

Abel, SG. (2018). Evaluation of the immune response of Newcastle disease virus vaccines in layer chickens, College of Veterinary Medicine and Agriculture, Addis Ababa University. Bishoftu, Ethiopia.

Absalón, A. E., Cortés-Espinosa, D. V., Lucio, E., Miller, P. J., & Afonso, C. L. (2019). Epidemiology, control, and prevention of Newcastle disease in endemic regions: Latin America. *Tropical animal health and production*, *51*(5), 1033-1048.

Alexander, D. J., & Senne, D. A. (2003). Newcastle disease. *Diseases of poultry*, *11*(1), 64-87.

Almatroudi, A., Khurshid, M., Mehmood, A., Nisar, M. A., Rasool, M. H., & Saqalein, M. (2021). Development, Biological Characterization, and Immunological Evaluation of Virosome Vaccine against Newcastle Disease in Pakistan. *BioMed Research International*, 2021.

Anene, B. M., Ezeibe, & M. C. O., Sanda, M. E. Effect of Route of Administration on Immune Response of Broiler Chicks to Newcastle Disease Vaccine (LaSota).

Anon, (1971). Methods for examining poultry biologies and for identification and quantifying avian pathogens. Newcastle disease, p.66. National Academy of sciences, Wasinghton, D.C.

Banu, N. A., Islam, M. S., Chowdhury, M. M. H., & Islam, M. A. (2009). Determination of immune response of Newcastle disease virus vaccines in layer chickens. *Journal of the Bangladesh Agricultural University*, *7*(2), 329-334.

Bello, MB., Ideris, A., & Yusoff, K. (2018). Diagnostic and vaccination approaches for Newcastle disease virus in poultry: the current and emerging perspectives. *Bio Med Res Inter* 7278459. doi:10.1155/2018/7278459.

Beri, G.C. (2005). Business Statistics, 2th edition, Tata Mc Graw-Hill publishing company limited, New Delhi, India

Chen, G., Gao, T. T., Li, W., Shi, X. M., Wang, Y. F., Zhao, Y., & Zhao, K. (2012). Preparation and efficacy of a live newcastle disease virus vaccine encapsulated in chitosan nanoparticles. *PloS one*, *7*(12), e53314.

El-Masry, S. S., Faiesal, A. A., Nasr-Eldin, M. A., & Othman, B. A. (2019). Comparative study of different vaccination routes against Newcastle disease in layer chickens. Arab Universities *Journal of Agricultural Sciences*, *27*(1), 231-238.

GOP (Government of Pakistan) Economic Survey of Pakistan, Ministry of finance, Government of Pakistan. 2019.

Khalafalla, A. I., Mahasin, E., & Wegdan, H. (2015). The effect of route of administration and dose on the immunogenicity and protective efficacy of Newcastle disease Thermostable vaccine I2 strain. *Int J Prev Med Res*, 5, 276-281.

Khatoon, A., Abidin, Z. U., Gul, S. T., Khan, A. R., Naeem, M., Qureshi, M. A., Ali, A., & Nawaz, M. Y. (2022). Lyophilization as a possible way to enhance the viability of live Newcastle disease (LaSota) vaccine: Suggesting the optimized method by comparing five different protocols. *Pak Vet J*, *42*(3): 404-408. http://dx.doi.org/10.29261/pakvetj/2020.047.

Rehman, H. U., Ghafoor, A., Rabbani, M., Riaz, A., Awan, F. N., & Raza, S. (2022). Locally prepared bovine herpesvirus 1 gE deleted vaccine induced immunogenicity in rabbits. *Pak Vet J*, *42*(1): 127-130. http://dx.doi.org/10.29261/pakvetj/2021.049.

Rehman, H. U., Rabbani, M., Ghafoor, A., Riaz, A., Awan, F. N., & Raza, S. (2021). First isolation and genetic characterization of bovine herpesvirus 1 from cattle in pakistan. *Pak Vet J*, *41*(1): 163-165. http://dx.doi.org/10.29261/pakvetj/2020.092

Rehmani, S. F. (1996). Newcastle disease vaccination: a comparison of vaccines and routes of administration in Pakistan. *Preventive Veterinary Medicine*, *25*(3-4), 241-248.

Riaz, R., Khan, M. S., Joyia, F. A., & Zia, M. A. (2021). Designing of Multiepitope-based Subunit Vaccine (MESV) against prevalent serotype of Foot and Mouth Disease Virus (FMDV) using immunoinformatics approach. *Pak Vet J*, *41*(3): 400-408. http://dx.doi.org/10.29261/pakvetj/2021.033.

Table 2 Antibody titre of Lasota vaccine and Mukteswar vaccine at different route of inoculation

Routes	Lasota given to group 1 (SGA-SGD) in three different routes	Mukteswar given to group 2 (SGA-SGD) in three different routes
	Mean± Std. Deviation	**Mean± Std. Deviation**
SGA (Ocular)	277.60 ± 293.21^{a}	127.20 ± 107.58^{ac}
SGB (Drinking)	212.80 ± 251.06^{ab}	207.20 ± 269.56^{ab}
SGC (Injection)	146.40 ± 92.90^{bc}	287.20 ± 288.26^{b}
SGD (Control)	28.00 ± 11.46^{cd}	28.00 ± 11.46^{C}

Different superscript within column indicate significant difference ($p<0.05$).
SGA= sub group A, SGB= sub group B, SGC= sub group C, SGD= sub group D.

UNIVERSITY OF SINDH JOURNAL OF ANIMAL SCIENCES
Website: https://sujo.usindh.edu.pk/index.php/USJAS
Uni. Sindh. J. Anim. Sci., 6(4), December, 2022
Email: editors.usjas@usindh.edu.pk
ISSN (P): 2521-8328
ISSN (E): 2523-6067
Published by University of Sindh, Jamshoro.

ETHICAL GUIDELINES AND GENERAL INSTRUCTION FOR CONTRIBUTOR

ETHICAL APPROVALS

In cases where animals are used in the submitted manuscript, the methods section must clearly indicate approval from the ethics committee of the institute or organization and should state that all efforts were taken to minimize pain and discomfort to the animal while conducting these experiments. Moreover, biomedical research involving human subjects shall follow international ethical guidelines.

DOUBLE BLINDED REVIEW

University of Sindh Journal of Animal Sciences (USJAS) believes in a fair and rigorous review process. Therefore, all submitted manuscripts will be reviewed by at least two experts in the appropriate field, determined by the Editor of the journal. The names of the reviewers will not be displayed on the manuscript and will not be disclosed to any authors.

PERMISSION

In case any part (e.g. Table or Figure) of the submitted manuscript has been taken from previously published work, it is the responsibility of the authors to obtain permission either from the publishers or from the authors depending on the copyright ownership. University of Sindh Journal of Animal Sciences (USJAS) can demand this permission anytime, pre- or post-publication of the study.

LIST OF POTENTIAL REFEREES

Authors are required to provide **at least four experts (2 foreign, 2 local)** from western/industrially developed countries. Only experts without prior connections or collaborations will be accepted. Full name, affiliation and official email address would be required during the online submission process. The reviewers should also not belong to the institution/university from where the manuscript has been submitted.

SUBMISSION

Submit your manuscript through the **eSubmit.** For any technical issues during submission please contact us at editor.usjas@usindh.edu.pk/riffat.sultana@usindh.edu.pk or The Editor, University of Sindh Journal of Animal Sciences (USJAS), Department of Zoology, University of Sindh, Jamshoro.

UNIVERSITY OF SINDH JOURNAL OF ANIMAL SCIENCES
Website: https://sujo.usindh.edu.pk/index.php/USJAS
Uni. Sindh. J. Anim. Sci., 6(4), December, 2022
Email: editors.usjas@usindh.edu.pk
ISSN (P): 2521-8328
ISSN (E): 2523-6067
Published by University of Sindh, Jamshoro.

TYPES OF MANUSCRIPT

RESEARCH ARTICLE

1. Title Page
2. Abstract
3. Introduction
4. Material and Methods
5. Results and Discussion
6. Conclusion
7. Acknowledgements
8. Author's Contributions
9. Conflict of interest
10. References
11. Tables and Figures

SHORT COMMUNICATION

Authors are advised that a short commendation reports on a significant piece of complete research that may be either a coherent component of an on-going research project that merits a special mention, or a publication a publication of choice that is conveniently published in a short format (2 pages). The basic style and submission should follow the same guideline as far full publication.

FORMATTING OF THE MANUSCRIPT

Title and Authorship Information Page (first page of the manuscript)
• Title of the manuscript
• Full names of author(s)
• Full institutional mailing addresses
• Email address(es), telephone of corresponding author(s)

ABSTRACT (150 Words Maximum)

The manuscript should contain an abstract. Abstracts shall not contain any references and should clearly and accurately summarize the main focus of the study. The word count should be under 150 words.

INTRODUCTION

This section should summarize existing literature sufficiently so that the readers understand the background of the findings. At the end, the introduction should contain the objectives of the study. Authors may use subheadings, if necessary.

UNIVERSITY OF SINDH JOURNAL OF ANIMAL SCIENCES
Website: https://sujo.usindh.edu.pk/index.php/USJAS
Uni. Sindh. J. Anim. Sci., 6(4), December, 2022
Email: editors.usjas@usindh.edu.pk
ISSN (P): 2521-8328
ISSN (E): 2523-6067
Published by University of Sindh, Jamshoro.

MATERIAL AND METHODS

This part should contain sufficient experimental and analytical details so that any readers can repeat the procedures without consulting the author of the study. To have better understanding of the methodologies, it is recommended that this section be divided under different sub-headings.

RESULTS AND DISCUSSION

This section may be divided by sub-headings or may be combined depending upon the nature of manuscript and the type of study.

CONCLUSION

This section should clearly explain the main conclusions of the work. It should not be more than one paragraph and should not repeat the text presented earlier in the text. Efforts should be made to highlight the importance and relevance of the study.

ACKNOWLEDGMENT

All acknowledgments (if any) should be included in the manuscript before the Reference section and may include supporting grants etc.

AUTHOR'S CONTRIBUTIONS

The manuscript must clearly state the contribution of each author and should convince editors that each author has contributed significantly to the study.

CONFLICT OF INTEREST

In cases where no conflicts of interests exist, authors should state that "The author(s) declare(s) that there is no conflict of interests regarding the publication of this article". Otherwise, they should mention any conflict of interest in this section of the manuscript.

UNIVERSITY OF SINDH JOURNAL OF ANIMAL SCIENCES
Website: https://sujo.usindh.edu.pk/index.php/USJAS
Uni. Sindh. J. Anim. Sci., 6(4), December, 2022
Email: editors.usjas@usindh.edu.pk
ISSN (P): 2521-8328
ISSN (E): 2523-6067
Published by University of Sindh, Jamshoro.

REFERENCES:

APA (7th Edition) Referencing Style should be followed.

Formatting of References

Reference entry (One author**):**

Sultana, R. (2019). A new genus Schizocomicus (Schizodactyloidea: Ensifera) from Sindh Pakistan. *Pakistan Journal of Zoology*, *51*(5), 1693-1697.

In-text citation:

Parenthetical: (Sultana, 2019)
Narrative: Sultana (2019)

Reference entry (Two authors**):**

Sultana, R., & Wagan, M.S. (2011). Test of few insecticides against the various developmental stages of *Hieroglyphus* Species (Hemiacridinae: Acrididae: Orthoptera), *Pakistan Journal of Zoology*, *43*(5), 941-946.

In-text citation:

Parenthetical: (Sultana & Wagan, 2011)
Narrative: Sultana and Wagan (2011)

Reference entry (More than two authors**):**

Sultana, R., Kumar, S., Samejo, A. A., Soomro, S., & Lecoq, M. (2021). The 2019–2020 upsurge of the desert locust and its impact in Pakistan. *Journal of Orthoptera Research*, *30*(2), 145–154.

In-text citation:

Parenthetical: (Sultana et al., 2021)
Narrative: Sultana et al. (2021)

Reference entry (Book**):**

Sultana, R., & Wagan, M. S. (2015). *Grasshopper and Locust of Pakistan.* Higher Education Commission, Islamabad.

In-text citation:

Parenthetical: (Sultana & Wagan, 2015)
Narrative: Sultana and Wagan (2015)

For Example:

- Parenthetical citation: According to new research … (Sultana, 2020).
- Narrative citation: Sultana (2020) notes that …

Preparation of Figures

Figures should be supplied in jpg format.

Preparation of Tables

Tables should be cited consecutively in the text and should not be abbreviated.

Returning of Galley Proof

The formatted galley proof and author's query form will be sent to corresponding author only and should be returned within 72 hrs. of receipt. After this, any editing will not be accepted.

UNIVERSITY OF SINDH JOURNAL OF ANIMAL SCIENCES
Website: https://sujo.usindh.edu.pk/index.php/USJAS
Uni. Sindh. J. Anim. Sci., 6(3), September, 2022
Email: editors.usjas@usindh.edu.pk
ISSN (P): 2521-8328
ISSN (E): 2523-6067
Published by University of Sindh, Jamshoro.

CALL FOR PAPERS

Dear Researchers

University of Sindh Journal of Animal Sciences (USJAS) is an open-access, double blind peer reviewed research journal, published quarterly by University of Sindh, Jamshoro. The journal covers a full spectrum of specialized domains in Entomology, Endocrinology, Pest & Pest control, Molecular Biology, Parasitology, Wildlife Management and Conservation Animal's diversity and Systematic. It includes original research articles, review articles, case reports and scientific findings. The journal strictly follows the guidelines proposed by Higher Education Commission (HEC) Islamabad, Pakistan.

The Editorial team of (USJAS) invites you to submit your quality paper in its next issue (Volume 7) in March 2023.

Last date for Submission for March Issue: 15th February 2023

Salient Features

- International Quality and Standards
- Open Access – Free to All
- Quick Review Process
- Double-Blind Peer Reviewed policy for high quality and transparency
- Publication within a Reasonable Short Period
- Effective Editorial Standards
- Both Online and Print Version
- Prompt Email Notification
- No APC and Publication Charges
- Online Submission and processing
- Officially published by University of Sindh, Jamshoro, Pakistan.

Visit the official website for furthers detail regarding scope and submission guidelines.

http://sujo.usindh.edu.pk/index.php/USJAS

ISSN (E)	**:**	2531-6067
ISSN (P)	**:**	2521-8328
Forthcoming Issue	**:**	Volume 7, Number 1, 2023
Submission by	**:**	15th February, 2023

UNIVERSITY OF SINDH JOURNAL OF ANIMAL SCIENCES
Website: *https://sujo.usindh.edu.pk/index.php/USJAS*
Uni. Sindh. J. Anim. Sci., 6(4), December, 2022
Email: *editors.usjas@usindh.edu.pk*
ISSN (P): 2521-8328
ISSN (E): 2523-6067
Published by University of Sindh, Jamshoro.

CALL FOR REVIEWERS

Interested to join Journal Reviewer Boards, Kindly Email Your CV or Scholars Profile to editor.usjas@usindh.edu.pk (Ph.D holder will be considered.)

Looking forward to receiving your feedback and research manuscript.

With Best Wishes,

Dr. Riffat Sultana
Editor of USJAS
Department of Zoology,
University of Sindh, Jamshoro, Pakistan
URL: http://sujo.usindh.edu.pk/index.php/USJAS
Email: editor.usjas@usindh.edu.pk or riffat.sultana@usindh.edu.pk