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In - Vitro Analysis of Antiallergic Activity of Neem (*Azadirachta Indica*) for Reduction of Wheat Allergens

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Abstract: Plants are the origin of life on earth and essential for people's livelihood since ancient times, plants have been an excellent source of medicine. Survival of mankind is unfeasible without plant kingdom. The name of the Neem tree in Sanskrit is 'Arishtha' which means that reliever of 'Sickness'. According to healing system of Ayueda the Neem tree will strengthen an individual resistance to disease, Neem efficiently regulate immune system and other function. Neem is used for a wide range of disorder incorporate diarrhea, inflammatory diseases, antimicrobials, skin diseases, urinary disorders, antiseptics, bronchitis, cough, asthma, fever, hypertension, and infected burns skin diseases, malaria and many more ailments. In the present study characterization of wheat protein have been done and found the possible allergens having molecular weight 7 kDa, 9 kDa, 15 kDa, 12 kDa, 17 kDa, 36 kDa 45 kDa and 77 kDa. But after the treatment of wheat protein with Neem (*Azadirachta indica*) extract showed the high reduction of allergenic proteins. There are no allergic bands have been seen after treatment with Neem.

Keywords: *Azadirachta indica*, allergenic proteins, characterization

1. INTRODUCTION

Plants plays a vital role in prolong the life on earth. Herbs are constantly an ideal source of drugs in ancient time. In the olden times, traditional drugs of plant derivation were used for the treatment of different diseases. Different experiments and investigation shows that a number of Ayurvedic herbs have that help in preventing asthmatic problem without any side effects and frequently helps in recovering from asthma, The WHO identify that asthma is a main public health problem caused by the genetic and environmental factors. Various factors that trigger the immune system such as, fumes, dust and pollen grains along with genetic factors have been investigated which include immensely assist in managing the disease. The medicinal use of Neem in India since ancient time, in Sanskrit medical verified that Neem seeds, roots, fruits, leaves and oil are beneficial for many types of diseases.

In India the tree is still considered as 'village dispensary'. Different parts of Neem are beneficial for the therapeutic used, over 135 compounds have been extracted as of different parts of neem (Ganguli, 2002). The medicines properties of Neem are controversial because Neem is used for manufacturing the number of medicine and other products such as pharmaceuticals, cosmetics and toiletries. All parts of Neem are used for different conditions like Neem seeds, fruits, roots, oil, bark, and leaves generally are used in the treatment of diarrhea, inflammatory diseases, antimicrobials, skin diseases, urinary disorders, antiseptics, bronchitis, cough, asthma, fever, hypertension, and infected burns. Nichols *et al*., (1995) reported that numerous herbs were used in many traditional systems such as Unani, Ayurvedic and Siddha in India for therapeutic treatment of asthma. In India, Neem (*Azadirachta indica* A. Juss) considered as very useful traditional medicinal plant. All parts of the Neem tree have a number of medicinal properties, therefore commercially utilizable, and hence considered as an important source for the unique natural products for development of industrial products and for the development of medicines against different diseases. (Lagachu *et al*., 2011). Thakur *et al*., (1981) described the medicinal utilities of the neem tree, especially neem leaves, neem bark and fruit of the neem. Chopra *et al*., (1956) described the importance of neem oil; it has been used for the treatment of various skin infections. Upadhyaya, (1997) reported the activity of neem, it stimulates the INF-γ , IL-1,TNF-α production, worked as the anti-eczema response of spleen cells and enhances proliferative. Neem (Twig) provides the reliefs from many diseases such as asthma, diabetes, cough, intestinal worms, obstinate urinary disorder piles, spermatorrhoea, tumor and many other diseases.

2. MATERIALS AND METHODS

Different varieties of wheat were collected from Nuclear Institute of Agriculture Tandojam (NIA) and one unknown variety was purchased form local market. The names of the varieties are GP-205, Marvi-2000, Soghat-90, Sindh-90, Sarsabz, Kiran-95, Amber, Khirman, Anmol-91, TJ-83, GP-256, jauhar-18, Mehran-89, Local. The samples were stored and labeled in air tight glass bottles to ensure safety. The fresh Neem fruit were collected from Sindh University. The analysis was carried out at Institute of

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Biochemistry University of Sindh, Jamshoro. All the chemicals were used in this research supplied by Merck electrophoretic grade.

Neem Extraction

The remedial utilities have been described, mainly for leaf, fruit and bark (Thakur *et al*., 1981). The aqueous extract of fruits of Neem has been used (Sen, *et al*., 1992).

Sodium Dodecyl Sulphate Polyacrylamide Gel Electrophoresis (SDS-PAGE)

SDS-PAGE electrophoresis is much effected technique for characterization of proteins. First we extracted the wheat protein in phosphate buffer (pH 7.5) than incubated this extracted protein in Neem extract over night. Took 40 µl of this mixture and 10 µl sample buffer (30% glycerol, 5% 2-Mercaptoethanol, 5% SDS, 0.06% BPB and 0.5M Tris HCl, pH 8) for 5 min than cooled in ice for 5 min and centrifuge at 14000 rpm for 5 min at -40 ^{0}C. 30% of acrylamide solution were used for preparation of gel, 10% Resolving gel buffer (PH 8.8) and 4% Stacking gel buffer (PH 6.8) were used for the separation of protein fractions. After completion of movement of protein on gel, the gel was stained with 0.25% of Coomassie brilliant Blue R 250(CBB) and destined with destining solution (30 mL methanol and 10mL acetic acid were added in 60 mL distilled water). After destining the blue color protein bands were appeared on gel, the bands were calculated on gel documentation system (BioRad).

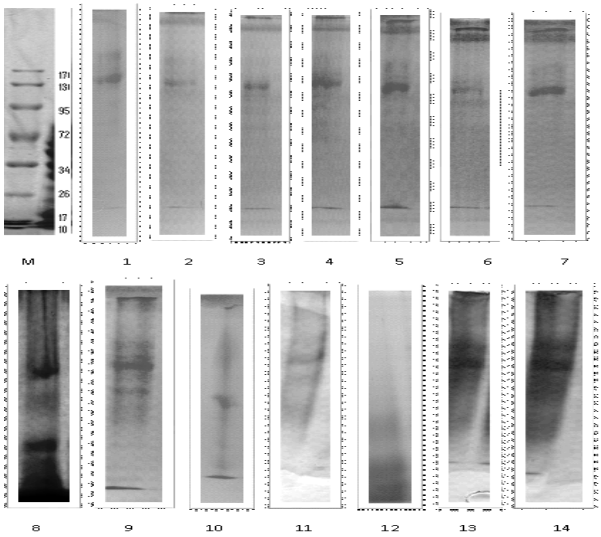
3. RESULTS ANS DISCUSSION

Wheat contains a number of proteins but few proteins are allergenic to some individuals. Wheat is not common allergenic food in the Pakistan however wheat considers to be numbers of major allergen to some individual. Result regarding a characterization of selected wheat varieties cultivated in Sindh indicated that the possible allergens are present having molecular weight 7 kDa, 9 kDa, 15 kDa, 12 kDa, 17 kDa, 36 kDa 45 kDa and 77 kDa. Table.1 shows that the world literature values of wheat allergen. Pfeil *et al*., (1990) reported that protein having a molecular weight 15kDa, 17kDa and 47 kDa is the major allergens of wheat while James *et al*., (1997) demonstrated that 15 kDa wheat proteins identified as alpha amylase inhibitor have allergenic activity.

Shaista *et al* (2015) investigated 55 allergic patients which were sensitive to wheat allergy, had a various allergic symptoms e.g. Eczema, Wheezing, Urticaria, Vomiting, abdominal pain, gastrointestinal symptoms, Respiratory symptoms and asthma.

Different studies suggested that different plants have various antiallergic, anti inflammatory, antiasthamic, antifungal, anticarcinogenic, antioxidative and immunomodulatory effects.

Characterization of wheat proteins after treatment with antiallergic plant (Neem) in different varieties of Sindh has been shown in **(Table 1) (Fig. 1) and (Table 2)** .The result of banding pattern of wheat proteins of different varieties treatment with the Neem Show 1 or 2 bands with molecular weight 34 kDa, 70 kDa, 72 kDa, 95 kDa, 130 kDa, 170 kDa, 171 kDa and 180 kDa, 170kDa. Kiran-91 and khirman contain two bands with molecular weight (135 kDa, 180 kDa) and (135kDa, 171 kDa) respectively. Amber, Sindh-90 and Sarsabz have only 170 kDa protein after the proceeding with Neem, while Jauhar-18 variety contain 171 kDa protein although Mehran, Anmol, TJ 83, GP-205, Soghat -90 and local varieties contain 2 bands . In the present study we characterized the hypo- allergic activity of Neem, our result shows that the complex of wheat proteins and extract of anti-allergic plant (Neem) reduce the allergenic proteins which are mainly cause of asthma and other allergenic symptoms. The reduction may be due to the treatment of wheat proteins with anti-allergic plant converted the protein into amino acid and peptides. The anti-allergic plant reduce the protein fraction specially those specific proteins (e.g. 12kDa, 15 kDa, 17 kDa, 20 kDa, 31 kDa, 36 kDa, 47 kDa and 77kDa) which are involved in allergic reactions. Makio Kobyash (2005) demonstrated that the anti-allergic activity reduce allergenic protein may be the due to the degradation or hydrolyzed into amino acid and peptides. Jinu Lagachu *et al*., (2011) reported that Neem is used as medicinal plant, Chopra *et al*., (1956) described that Neem is used as various skin infection. Katiyar CK *et al*., (1997) reported that Neem have Immuno modulatory activity. Ray, A *et al*., (1996) stated that the aqueous extract of Neem leaf possesses effective immune stimulant activity and is used for cell-mediated responses and humoral immunity. Varma *et al*., (1976) reported that the neem (Twig) provides the reliefs from many diseases such as asthma, diabetes, cough, intestinal worms, obstinate urinary disorder piles, spermatorrhoea, phantom tumor and many other diseases. Our result shows that allergenic protein could not be detected after treatment with Neem.



M-Marker 1-Kiran, 2- Amber, 3- Sindh, 4- Sarsabz, 5-Khirman, 6-Jauher, 7-Mehran, 8-Anmol, 9- TJ.83, 10-GP.256, 11-GP.205, 12- Marvi, 13-Soghat, 14- Local.

Fig. 1: Characterization of selected varieties of wheat protein after treatment with Neem(Azadirachta indica)

Table 1: World Reported Allergens

Molecular weight of allergens in kDa	World reported Literature
7kDa, 9kDa	Battais F *et al*., (2004)
12-kDa	(Amano M,Kimoto M *et al*., 1998)
15-kDa,36-kDa,47-kDa	(James JM, Sixbey Jp *et al*., 1997)
17-kDa and 20-kDa	(Kimoto M *et al*., 1998)
31-kDa	(Sanchez-Monge R *et al*., 1992)
77-kDa	(Lethto *et al*., 2003)
Tetrameric a-amylase inhibitor CM17	(Kimoto M *et al*., 1998)
Tetrameric a-amylase inhibitor CM16	(Armentia A, *et al*., 1993) (Amano M, *et al*., 1998) (Kimoto M, *et al*., 1998)

Table 2: Banding pattern of Wheat protein proceeded with Neem (*Azadirachta indica*)

Wheat varieties	Banding pattern of wheat proteins (kDa) proceeded with Neem									
Kiran-95	---	---	---	---	---	135	---	---	---	180
Amber	---	---	---	---	---	---	170	---	---	---
Sindh-90	---	---	---	---	---	---	170	---	---	---
Sarsabz	---	---	---	---	---	---	170	---	---	---
Khirman	---	---	---	---	---	135	---	171	---	---
Jauher-18	---	---	---	---	---	---	---	171	---	---
Mehran-89	---	---	---	---	---	---	170	---	---	180
Anmol-91	34	---	---	---	---	---	170	---	---	---
TJ-83	---	---	---	95	---	---	170	---	---	---
GP-256	---	70	---	---	---	---	---	---	---	---
GP-205	---	---	---	---	---	---	170	---	175	---
Marvi	---	---	72	---	---	---	---	---	---	---
Soghat	---	---	---	---	130	---	---	171	---	---
Local	---	---	---	---	130	---	170	---	---	---

REFERENCES:

Amano, M., H. Ogawa K. Kojima T. Kamidaira S, Suetsugu M. Yoshihama T. Satoh T Samejima I. Matsumoto (1998) Identification of the major allergens in wheat flour responsible for baker's asthma. Biochem J 330:1229-34

Armentia A, R, S.´nchez-Monge L. Gomez D. .Barber G. Sa´lcedo (1993). In vivo allergenic activities of eleven purified members of a major allergen family from wheat barley flour. Clin Exp Allergy,23:410–415

Battais F, J. P. Douliez D, Marion (2004). Involvement of Lipid Transfer Proteins in food allergy to wheat. In Gluten proteins, The Royal Society of Chemistry: Cambridge, UK.

Chopra, R. N., S. L. Nayer, and I. C. Chopra, (1956). Glossary of Indian Medicinal Plants, CSIR, New Delhi.

Ganguli S .(2002). Neem: A therapeutic for all seasons. Current Science 82(11):1304-1308.

James J. M, J. P. Sixbey R. M. Helm, G. A. Bannon, A.W. Burks (1997). Wheat a-amylase inhibitor: A second route of allergic sensitization. J Allergy Clin Immunol; 99:239–44.

Jinu L., J. C. Kalita, G. C. Sarma. (2011). Anti-allergic plants traditionally used by the Mishing tribe of Assam, India, International Journal of Science and Advanced Technology; Vol. 1(7) :160-168.

James J. M, J. P, Sixbey R. M. Helm . (1997). Wheat alpha-amylase inhibitor: a second route of allergic sensitization. J Allergy Clin Immunol; 99 (2): 239-44.

Katiyar C. K, N. B, Brindavanam P, Tiwari D.B.A. Narayana (1997). Immunomodulator products from Ayurveda: current status and future prospectives. In: SN Upadhyay Ed. Immunomodulation, Narosa Publishing House, New Delhi; 163-87.

Kimoto M, M, K.Yoshikawa N. Takahashi M. B. Okita H. Tsuji (1998). Identification of allergen in cereals and their hypoallergenization. I. Screening of allergens in wheat and identification of an allergen, Tri a Bd 17K. Ann Report Interdisipl Res Inst Environ Sci; 17:53– 60.

Lehto M., K. Palosuo E. Varjonen M. L. Majuri U. Anderson T. Reunala H. Alenius (2003). Huumoral and cellular responses to gliadin in wheat deprndant exercise induce anaphlyaxis. Clin. Exp. Allergy. 33;90-95.

Makio K. .(2005). Immunological functions of soy sauce: hypoallergenicity and antiallergic activity of soy sauce.Journal of Bioscience and Bioengineering.100(2); 144–151.

Nichols D. J, F. G. Longsworth (1995). Prevalence of exercise-induced asthma in school children in Kingston, St. Andrew and St. Catherine, Jamaica. West Indian Med J; 44:16-9.

Pfeil T,, U. Schwa W T. Ulmer W. König (1990). Western blot analysis of water-soluble wheat flour (Triticum vulgaris) allergens. Int Arch Allergy Appl Immunol; 91: 224- 231.

Ray A., B D. Banerjee, P. Sen (1996). Modulation of humoral and cell mediated immune responses. Indian J. Exp. Biol., 34: 698-701.

Sen P., P. K, Medinata A. Ray (1992). Neem – an immunostimulant. Ind. J. Exp. Biol., 12: 1170 – 1175.

Sanchez-Monge R., L Gomez D. Barber C. Lopez-Otın A. Armentia G. Salcedo (1992). Wheat and barley allergens associated with baker's asthma. Glycosylated subunits of the a-amylase inhibitor family have enhanced IgE-binding capacity. Biochem J.281:401–5.

Thakur R., S. Singh S. B. Goswami A.(1981). Aromatic plants. Curr Res Med ; 3: 135-140.

Upadhyaya S. N. (1997). Immunomodulation, Narosa Publishing House: New Delhi; 149- 297.

Varma, G. S., (1976). Miracles of Neem Tree, Rasayan Pharmacy, New Delhi.