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

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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).

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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.

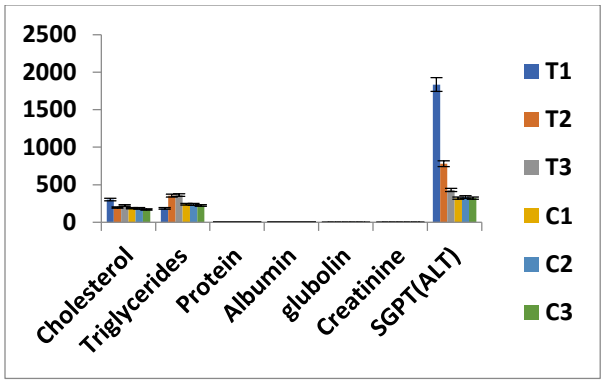

Figure 1: Serum Biochemistry

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.

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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