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PROTECTIVE ROLE OF HIGH DIETARY PROTEIN SUPPLEMENTATION AGAINST ARSENIC INDUCED REPRODUCTIVE TOXICITY IN MALE ALBINO RAT MODEL

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ABSTRACT

The present study has been undertaken to evaluate the efficacy of high dietary protein supplementation as protective measure against arsenic induced impairment of male reproductive parameters in albino rat model. Arsenic treated animals showed significantly impaired reproductive parameters compared to the control animals. The animals supplemented with dietary protein (Pea and Egg albumin) have showed significantly better status of reproductive parameters than arsenic treated animals. Finally, the group of animals supplemented both arsenic and high protein diet have shown improved status of sperm count, sperm motility, testicular and epididymal weight and also histo architecture of testis.

KEY WORDS: Sperm Count, Sperm Motility, Epididymis, Albumin, Histoarchitecture, Arsenic Toxicity.

INTRODUCTION

The use of metals has been critical to the progress and human civilization. It would be difficult to imagine and advance society without extensive utilization of metallic compounds. Metals are unique environmental toxicants in that there all naturally existing and in many cases are ubiquitous within the human environment. In addition, all life has evolved in the presence of metals and organism have been forced to deal with this potential toxic, yet even present elements.[1] Among these metals, arsenic exhibits complex metabolism and is possibly the most abundant and potential carcinogen.[2] Arsenic is present in the nature in stable form as inorganic trivalent or pentavalent. Arsenic is a metalloid compound having

properties intermediate between a metal and non- metal however it is frequently referred as a sources including drinking water and food. Significant exposure to arsenic occurs through both anthropogenic and natural sources. Occupational exposure to arsenic is common in smelting industry, micro electronics industry and in occupational setting where arsenic is used to manufacture pesticides wood preservatives and superfund sites where industrial waste are disposed. However human exposure to arsenic occurring mainly through drinking water has become an important global public health concern. An analysis of 25000 tube wells in West Bengal reveals that the arsenic concentration reaches nearly to the concentration of permissible limit for drinking water as per WHO.[3]

Several nations in the world such as Argentina, Australia, Bangladesh, Chile, China, Hungary, India, Mexico, Peru, Thailand, and the U.S. have shown concentrations higher than the guideline value recommended by WHO. Adverse health effects from arsenic exposure have been documented in China, Bangladesh, India (West Bengal), and the USA. Currently, the worst problem exists in Bangladesh and West Bengal (India) where millions are being exposed to unhealthy amounts of arsenic through drinking water.

In process of arsenic metabolism, inorganic arsenic is methylated to monomethyl arsenic acid (MMA) and finally to dimethyl arsenic acid (DMA) followed by a renal excretion. In this process of biomethylation, constant depletion of methyl causes DNA hypomethylation and thus generates mutation followed by carcinogenesis.[2] Arsenic affects the mitochondrial enzymes impair the cellular respiration and causes cellular toxicity. It can also substitute phosphate intermediate which could theoretically slow down the rate of metabolism and interrupt the production of energy.[4] Male infertility is reflected by low sperm count, low sperm motility and bad quality of sperms. Sodium arsenate has been found to have an inhibitory effect on the activity of testicular steroidogenic enzyme Δ^5 3β hydroxysteroid dehydrogenase & 17β hydroxysteroid dehydrogenase and to reduce the weight of testis and accessory sex glands in rats. High arsenic level may suppress the sensitivity of gonadotroph cells to GnRH as well as gonadotropin secretion by elevating plasma levels of gluco corticoids. These ultimately lead to the development of gonadal toxicity.

Few research works have been carried out previously showing the protection against arsenic toxicity in various systems on different animal models. Protective effect of Mentha piperita against arsenic induced toxicity in liver of Swiss albino mice were performed in 2007.[5] Study has been undertaken on arsenic toxicity in male gonads of rats and its protection was

also proved by high dietary protein supplementation.[6] Effect of Zingiber officinale (Ginger) has been shown on sodium arsenite induced reproductive toxicity in male rats.[7] Adverse health effects have been found due to arsenic exposure and modification was also found by dietary supplementation of jaggery in mice.[8] Protective effect of *a*-Lipoic acid was observed against arsenic trioxide induced acute cardiac toxicity in rats.[9] A National toxicology program workshop review was undertaken to evaluate the association between arsenic and diabetes.[10] Effect of vitamin E was observed on sperm parameters and DNA integrity in sodium arsenite-treated rats.[11] According to few researchers tetra hydro curcumin potentially attenuates arsenic induced oxidative hepatic dysfunction in rats.[12] Effects of arsenic on osteoblast differentiation on bone mineral density and microstructure in ratswas performed in vitro.[13] Protective effect of Emblica officinalis in arsenic induced biochemical alteration and inflammation in mice was observed previously.[14] Biochanin A ameliorates arsenic induced hepato and hematotoxicity in rats.[15] Protective effects of Vernonia amygdalina against sodium arsenite induced genotoxicity in rat was observed.[16] It was observed in research work before that there was protective effects of zinc and vitamin E for arsenic induced mitochondrial oxidative damage in rat brain.[17] A short review was undertaken on medicinal plants and natural products in amelioration of arsenic toxicity.[18]

Association between nutritional status and arsenic toxicity is well established. In recent studies dietary protein have been found to have an antioxidant activities.[19-21] Pea (*Pisum sativum*) is a good source of dietary plant proteins, while egg albumin is an animal protein. The antioxidant activities of pea and egg albumin has been studied using different liposomal models and the result show a minimization in lipid peroxidation, thus preventing the damage produced by the free radicals.[19] Therefore in this study the antioxidant properties of pea and an animal protein egg albumin were explored if they have any protective role on the arsenic induced gonadal toxicity of male rats.

METHODS AND MATE RIALS

Drug Preparation: 0.7 mg of sodium arsenite is dissolved in 100 ml of distilled water and this is the stock solution.

Preventive drug preparation: 37 g of pea paste and albumin of 1 egg was added in 30 ml of distilled water and thus high protein diet was prepared.

Animals Used and Maintenance: 24 Swiss albino male rats, weighting between 90- 180 g, were used for the experiments. They were housed in cleaned room under normal natural conditions of room temperature (27-28^{o} C), photoperiod which was around 12-14 hrs , constant humidity (60%) and were acclimatized for 7 days to the laboratory conditions, prior to commencement of the experiment. All the rats were maintained on balanced laboratory food and drinking water. Animals were randomly divided equally into four groups of six animals each, as follows.

Group A: control.

Group B: arsenic treated.

Group C: Pea and albumin diet supplemented and

Group D: arsenic with Pea and albumin diet supplemented group.

For chronic oral exposure to arsenic, a dose was selected (3 mg / kg body wt / day), which is within the range of LD of a 70 kg body wt. human (1 – 4 mg / kg) and lesser than one thirteenth ofLD_{50} value of rats (40 mg / kg body wt.). Accordingly, animals of group B and group D were orally treated with aqueous solution ofsodium arsenite, 3 mg / kg body wt. / day for 28 days. The animals of group C and group D, in addition, were supplemented with Pea (37 g) which contributed 8.5% protein and albuminthatis approximalelyhigh protein in the formulation of a high protein diet. The initial body weights of the rats were recorded and maintained in the laboratory throughout the experimental period.

Animal sacrifice and measurement of parameters

Body weight and weight of testis & epididymis measurement: After completion of 28 days of treatment, final body weight of all the rats were taken. After sacrificing the animal, blood was collected directly from hepatic vein and allowed to coagulate, clear serum was collected and stored. Testis and epididymis from each rat were dissected out and treamed off adipose tissues and weights were taken. One testis from each rat was processed for histology.

Counting of sperm

Immediately after the sacrifice the cauda portion was cut from epididymis. The cauda was kept in 5ml diluents (Phosphate buffer solution). This was kept for 5mins at 37^{o}c, it was then taken out, and an incision was given through the cauda and the sperms were dispersed in the fluid. From the dispersed spermatozoal suspension 25 µl was charged on a Neubauer Haemocytometer and the numbers of sperms were counted and calculated using WBC chambers.[22] The composition of phosphate buffer solution are given below.

Salt	Concentration(g/L)
NaCl	8.0
KCl	0.2
Na_2HPO_4	1.42
KH_2PO_4	0.24

Observations of sperm motility: For sperm motility count the cauda epididymis from all four groups was obtained as mentioned earlier and each cauda was kept in 5 ml PBS 0.2 M, pH 7.4 at 37^{o}c. 25 µl of this was taken on a clean slide covered with a cover slip and was observed under a microscope. Total numbers of motile sperms per 100 sperms were counted.[22]

Histology of testis: Histological slide was prepared with haematoxylene-eosin procedure.

Statistical analysis: The data were expressed as mean±SEM and were analysed statistically followed by multiple comparison 't' test, which was used for statistical evaluation of the data. In addition to this, two-tailed Student's 't' test was performed to determine the level of significance between the means.[23]

RESULTS

Effect of arsenic on body weight: Twenty eight days after a single dose of sodium arsenite treatment there wassignificant decrease in body weight (P<0.01) when the values compared with the control. But When compared normal to drug with highly protein diet supplemented groupthen there was significant change in body weight(P<0.005).

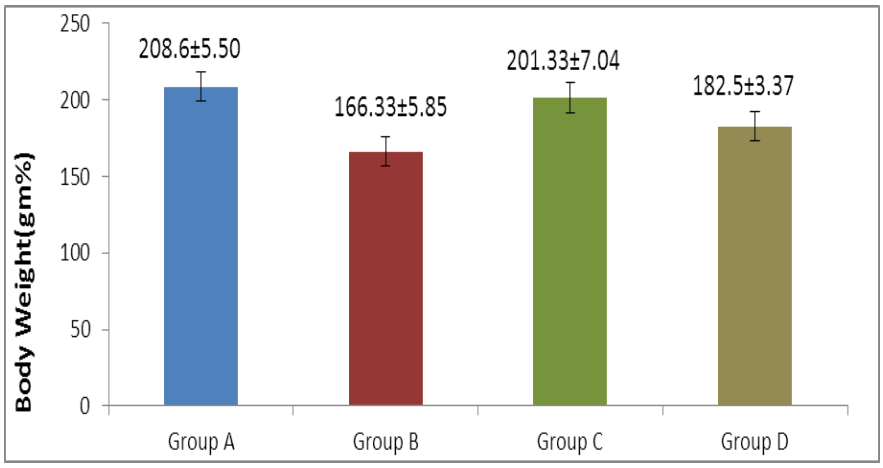

Fig. 1: Comparison of average body weight(gm.) of rats of control: Group A; drug treated: GroupB; high protein supplement : Group C; & drug treated with high protein supplemented: GroupD.

Effect of arsenic on testicular and epididymal weight: Twenty eight days after a single dose of arsenic trioxide treatment there was highly significant decrease in testicular weight (P<0.025)(Fig :2) and epididymis weight(P<0.005) when the values compared with the control. When compared normal to drug withhighly protein diet supplemented group there was significant increase in testicular weight (P<0.05) & epididymis weight (P<0.005).

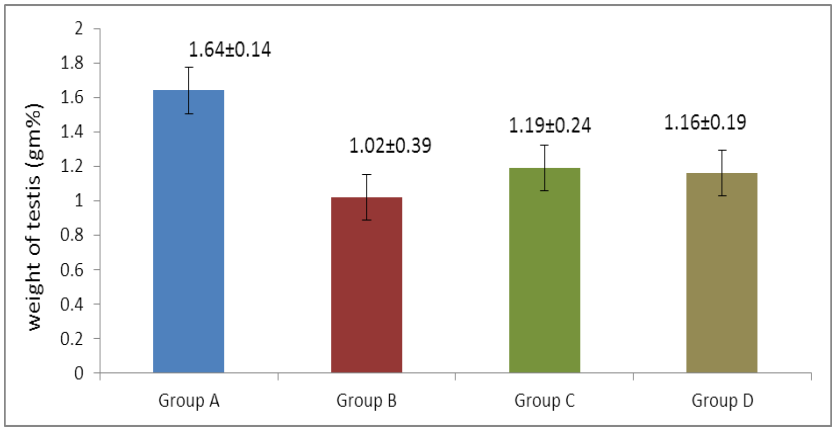

Fig. 2: Comparison of averageweight of testis (gm.) of rats of control: Group A; drug treated: GroupB; high protein supplement : Group C; & drug treated with high protein supplemented: Group D.

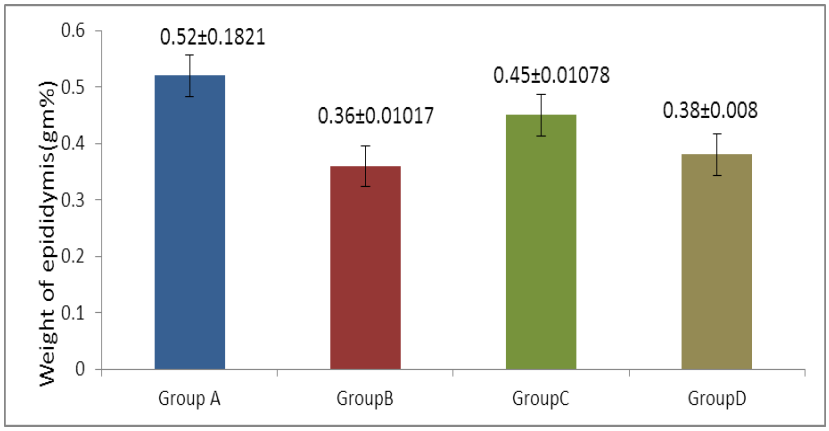

Fig. 3: Comparison of average weight of epididymis (gm) of rats of control: Group A; drug treated: GroupB; high protein supplement: Group C; & drug treated with high protein supplemented: GroupD.

Analysis of spermatozoal status

Effect of sodium arsenite on spermcount: The following figureshows that there is a reduction in the number of matured spermatozoa in case of drug treated group as compared to that of control. But drug with high protein supplemented group an increased in (P<0.05 level) sperm count towards normal was observed.

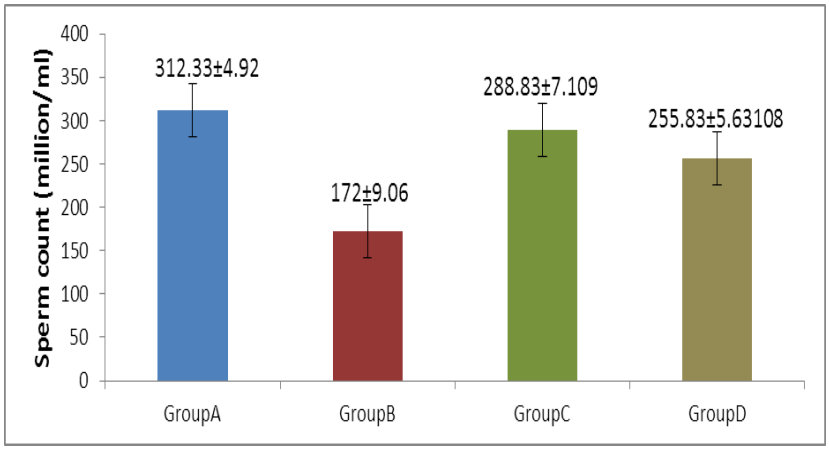

Fig. 4: Comparison of average of sperm count (million/ml.) of rats of control: Group A; drug treated: GroupB; high protein supplement : Group C; & drug treated with high protein supplemented: Group D.

Effect of arsenic in sperm motility: A decrease in sperm motility was observed in arsenic treated group as compared to control group whereas administration of drug with high protein diet minimized the decrease in motility as reflected in the values nearer to that of control (Fig.5).

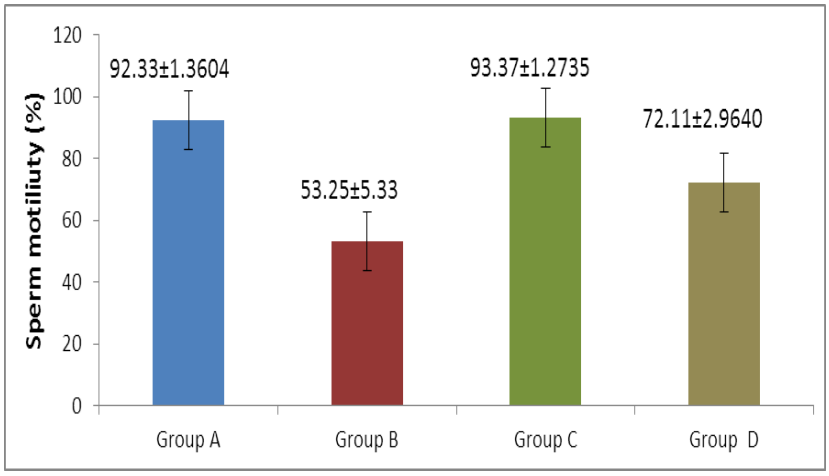

Fig. 5: Comparison of average of sperm motility (%) of rats of control: Group A; drug treated: GroupB; high protein supplement : Group C; & drug treated with high protein supplemented: Group D.

Histological analysis

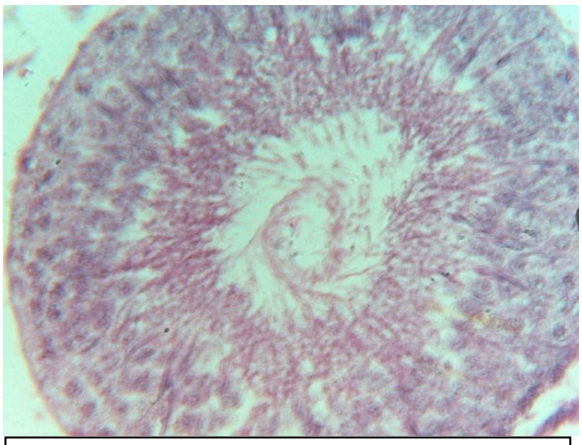

Fig. 6(A): H/E stained section of control testis showing 10X40x.

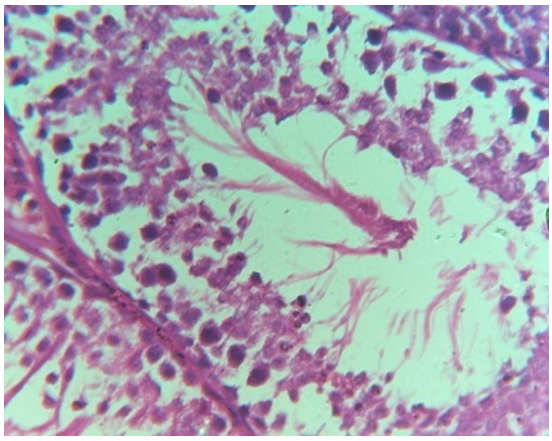

Fig. 6(B): H/E stained section of arsenic treated testis in 10X40x magnification.

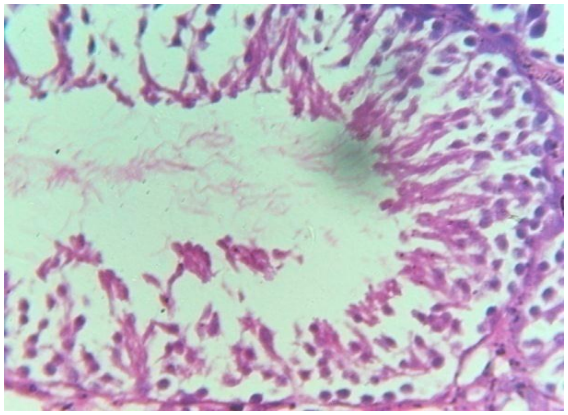

Fig. 6(C): H/E stained section ofarsenic treated with dietary protein supplemented testis in

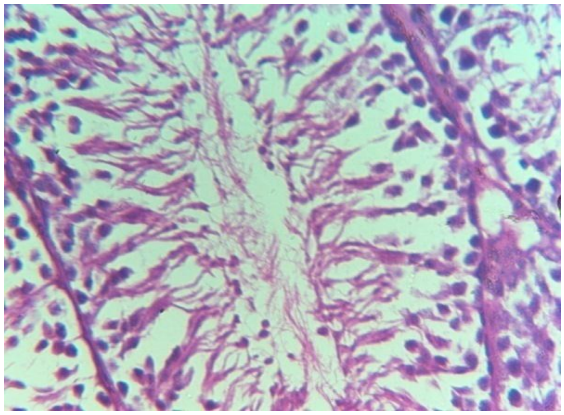

Fig. 6(D): H/E stained section of dietaryprotein supplemented testis in 10X40x magnification.

The H/E stained histological sections of arsenic treated testis showed increase in luminal areas associated with reduced accumulation of spermatozoa, signs of necrotic changes with disarray in cellular organization (Fig:6:B) compared to that of control (Fig:6:A).Protein supplemented rat testis (Fig:6:C) showed the partial recovery compared to the treated group (Fig:6:B). Recovery includes an accumulation of increased spermatozoa in the luminal areas along with partial amelioration of arsenic induced changes.

DISCUSSION

Increase in the luminal areas of the seminiferous tubules associated with decreased spermatozoal mass might be due to low levels of gonadotropins in arsenic treated rats, and these low levels are responsible for the decreased production of steroidogenic enzymes.[4] It

has been established that arsenic administration leads to decrease in steroidogenic enzymes synthesis[4]. Thus the low levels of gonadotropins and possibly testosterone might be responsible for the decrease in the spermatozoal mass in the lumen. Decrease in epididymal spermatozoal number provides support towards this histological observation and weight of epididymis. Arsenic causes lipid peroxidation by generation of reactive oxygen species (ROS).[24] This peroxidation may cause rupture of cell as well as nuclear membrane. This might be responsible for the observed necrosis and disarray in cellular organization in histological section (Fig:6:B). Evidence suggests that arsenic induces free radical formation and thus the generated reactive oxygen species (ROS) react with the polyunsaturated fatty acid (PUFA) rich spermatozoa, specially the mid spermatozoa and results in peroxidation which finally leads to destruction in spermatozoa causing reduced motility.[4] Pea plus egg albumin diet supplementation along with arsenic treatment reveals that the decrease in sperm count and motility due to toxic effects of arsenic is minimized. As a possible mechanism it could be stated that either pea or egg albumin or both have a recovery role on sodium arsenite mediated toxicity by inducing an antioxidant effect against the oxidative stress .The pea or pea and egg albumin supplement is effective in reducing the production of nitric oxide and malondialdehyde (lipid peroxidation marker) which could markedly increase the activity of the antioxidant enzymes. This could not only overcome the oxidative stress caused by arsenic but also suppresses the ROS generation from other sources.[19] Studies on sodium arsenite induced toxicity on male gonad reveal a good deal of changes in histology of seminiferous tubule, associated with decreased spermatozoal mass. Sperm count, and motility are seen to be affected and the possible mechanisms behind these changes have been discussed. Supplementation of specific proteins with the normal diet causes significant recovery from all these toxic effects.

CONCLUSION

From the present study it may be concluded that egg albumin and pea protein enriched diets may effectively attenuate the arsenic induced array of male reproductive dysfunctions. The protective effects of the referred proteins possibly involve their antioxidative and methyl transferase potential that inhibit enzymatic and non-enzymatic lipid peroxidation and reduce oxidative stress. Co-administration of arsenic with pea and egg albumin markedly reduced the effect of arsenic mediated toxicity on male reproductive system of rat. Contamination of food products with rising concentration of arsenic in environment, high dietary protein like

pea and egg albumin may be recommended to those people who have threats from the increased arsenic concentration.

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