Role of Propolis in Attenuating Arsenic Toxicity in Rat Testes

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Abstract- *Arsenic trioxide has been implicated in the pathogenesis of several disorders. The present study aimed to investigate the effect of arsenic trioxide on testes of male SD rats and the ameliorative potential of honey bee propolis against the toxicity. A significant increase in the level of lipid per oxidation and decrease in the levels of antioxidant activities were observed in arsenic treated rats. Arsenic generates ROS by decreasing the activation of antioxidant enzymes thereby causing stress in the testis of rats. Co-administration of propolis with arsenic reversed the oxidative stress induced in testis of rats by arsenic alone.*

Keywords- Arsenic, Propolis, Testes, Antioxidants

I. INTRODUCTION

Honeybee products, such as honey, bee pollen, propolis, royal jelly, beeswax, and bee venom, have long been used in traditional medicine. Reference to healing properties of honey and other bee products is found in many religious texts including the Veda, Bible, and Quran. Honey and propolis are bee products that have been used for centuries in folk medicine. Propolis is a complex resinous mixture collected by bees from plant exudates and mixed with hypo-pharyngeal secretions, beeswax and pollen (Burdock et al., 1998). It has been shown to have a broad spectrum of biological activities which are principally attributed to the presence of flavonoids and caffeic acid phenethyl ester (CAPE) (Isla et al., 2001). In the present study, ameliorative potential of propolis, on some of the parameters indicative of oxidative stress in testes, blood was investigated in arsenic exposed rats. Arsenic was chosen because arsenic and many of its compounds are especially potent poisons. The high affinity of arsenic oxides for thiols is usually assigned as the cause of the high toxicity. Arsenic disrupts ATP production through several mechanisms.

II. MATERIALS AND METHODS

1. Experimental animals

Normal Sprague-Dawley male rats weighing 200gm-290gm, bred at Central Animal House of Panjab University, Chandigarh, were used for the study. They were fed ad libitum on pellet diet and water period of throughout the treatment. The animals were weighed before and after experiments.

2. Preparation of propolis extract

The propolis extract was prepared by following the method of Mani et al. (2006). Propolis was collected from bee hive. It was ground into fine pieces. Placed the proper amount of propolis and alcohol (30gm propolis and 70% ethanol to make 100ml volume) in a container and sealed the top, kept it protected from light. It was shaken moderately at intervals for two weeks at room temperature. The propolis extract was filtered twice, dried and stored in sealed bottles at 4°C.

Doses and organization of experimental groups

Arsenic trioxide was administered to rats at concentration of 3mg/kg b.wt through oral gavage. The dose of propolis was 250mg/kg b.wt. Saline was used as a carrier. The animals were divided into 3 groups with each group containing six rats.

Group I: - This group served as control.

Group II: - The animals in this group received arsenic trioxide (3mg/kg body weight) for period of 14 days.

Group III: - The animals in this group were administered with arsenic trioxide (3mg/kg body weight) and propolis extract (250mg/kg body weight) for period of 14 days.

Preparation of samples for biochemical analysis:-

(a) Preparation of post mitochondrial supernatant (PMS):-

Testes and blood samples were collected from each animal for the biochemical analysis and chilled immediately at 4°C. Small pieces of testes were carefully removed from each

rat and was washed with ice-cold saline and prepared for PMS. Testes were homogenized at 3000 rpm for 2 minute in ice followed by centrifugation at 4°C at 10,000 g to obtain PMS.

(b) Serum:-

Blood was collected through cardiac puncture from all the groups of rats separately and serum was prepared by centrifugation at 5000 rpm for 20 min.

Oxidative stress parameters such as lipid per oxidation, reduced glutathione and activity of GST were assayed in the testes, in this study. Serum SGPT, SGOT and bilirubin were tested in the serum of experimental rats. Values are expressed as means ± SD; n=6 for each treatment group. *Represents p<0.05; **Represents p < 0.01; ***Represents p< 0.001. (+) Indicate % increase and (-) indicate % decrease.

III. RESULTS

Body weight

There was a steady decrease in body weight of rats administered arsenic trioxide while the animals in the control group showed a steady gain in body weight during the period of treatment. When propolis was co-administered with arsenic it was observed that the body weight declined initially for a period of 8 days after which there was steady increase up to day 14.

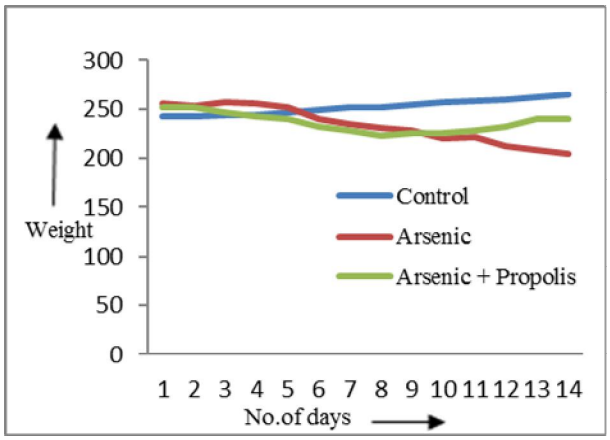

Figure 1. Changes in the body weight of rats.

Effect of treatment on lipid per oxidation

Malondialdehyde (MDA) is used as an indirect index of lipid per oxidation. Results recorded that animals administered with arsenic showed highly significant increase (p <0.001) in the level of MDA in the testes as compared to the control group. On the other hand lipid per oxidation was attenuated after co-administration of propolis as evidenced by a significant decrease (p<0.05) in measured MDA compared with arsenic treated rats.

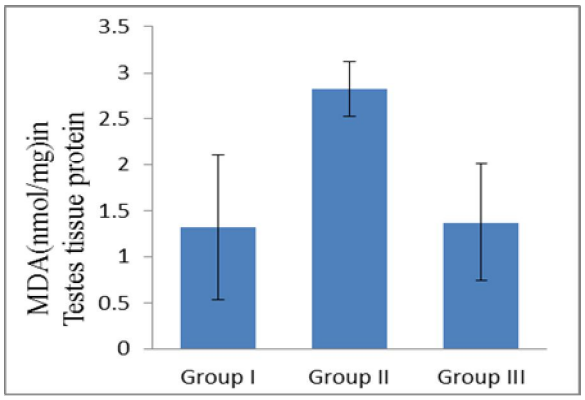

Figure 2. MDA level in testes

Effect of treatment on reduced glutathione:

The testicular content of GSH (reduced glutathione) was significantly decreased (p <0.01) after arsenic treatment (Group II) compared with the respective control. Co-administration of arsenic and propolis (Group III) significantly (p<0.05) increased the GSH levels compared with the arsenic treated rats (Fig. 3)

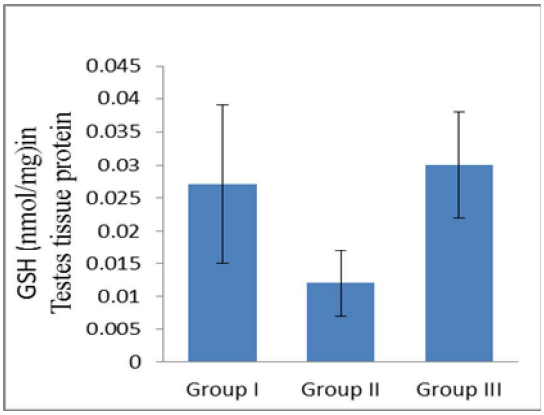

Figure 3. GSH level in testes.

Effect of treatment on Glutathione –S-transferase (GST):

GSTs are present in eukaryotes and in prokaryotes, where they catalyse a variety of reactions. Arsenic trioxide (Group II) was found to inhibit glutathione-S-transferase activity significantly (p<0.001) in testes. However, propolis (Group III) co-treatment considerably increased enzyme activity in testes male rats (Fig.4).

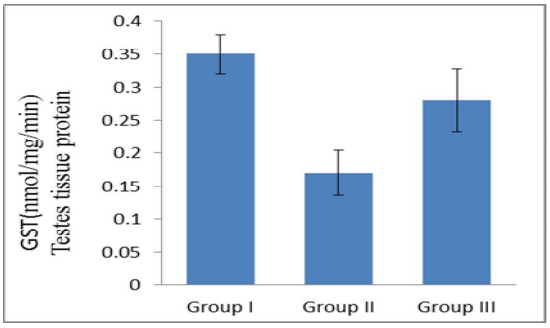

Figure 4. GST level in testes

Effect of treatment on Serum Glutamate Pyruvate Transaminase (SGPT/ALT):

Serum was prepared from blood of rats as described in methodology. The arsenic exposure (Group II) significantly increased ALT activities as compared to control group. But co-treatment with propolis reduced the toxic effect of arsenic by a decrease in ALT as compared to arsenic group (Fig.5).

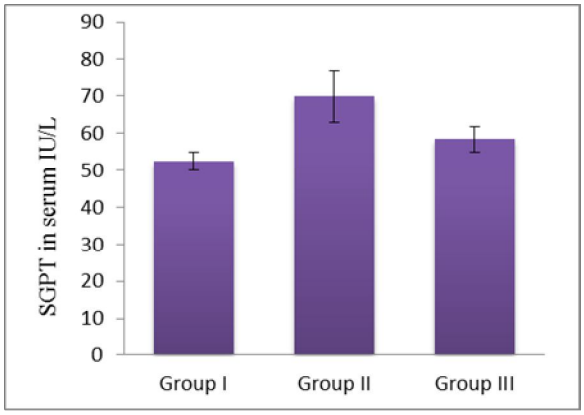

Figure 5. SGPT level in serum.

Effect of treatment on Serum Glutamate Oxaloacetic Transaminases (SGOT/AST):

Serum Glutamate Oxaloacetic Transaminase also called AST (Aspartate transaminase) is a pyridoxal phosphate (PLP)- dependent transaminase enzyme. AST catalyses the reversible transfer of an α-amino group between aspartate and glutamate and is an important enzyme in amino acid metabolism. Administration of arsenic (Group II) significantly ($p<0.001$) increased AST activities as compared to control group .But co-treatment of propolis reduced the toxic effect of arsenic by a significant decrease in AST activity as compared to arsenic group (Fig.6).

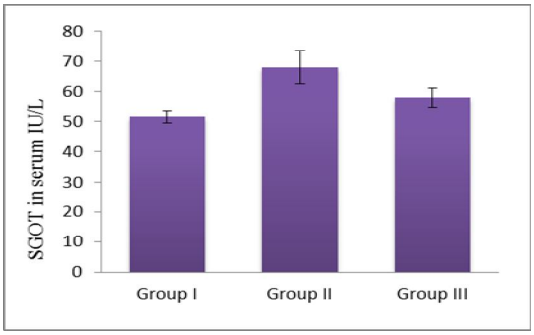

Figure 6. SGOT level in serum.

Effect of treatment on bilirubin level in serum

Bilirubin (formerly called hematoidin) is the yellow breakdown product of normal heme catabolism. It is excreted in bile and urine, and elevated levels may indicate certain diseases. Results showed a significant increase ($p<0.001$) in total bilirubin level after treatment with arsenic trioxide (Group II) when compared to control group. Meanwhile, there was a decrease in the level of bilirubin in arsenic + propolis treated rats (Group III) when compared to the arsenic treated group (Fig.7).

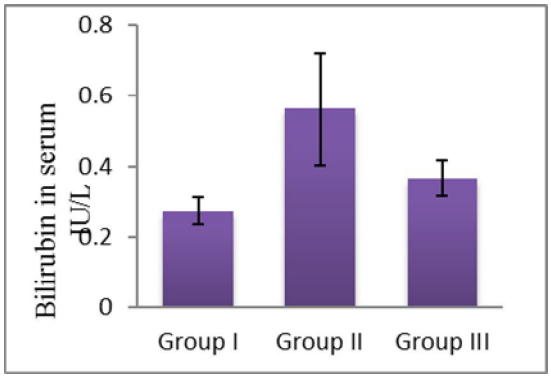

Figure 7. Level of Bilirubin in serum.

IV. DISCUSSION

Mammalian testes are highly susceptible to oxidative stress. Like all cells living under aerobic conditions, spermatozoa produce ROS, mostly originating from normal metabolic activity. High concentrations of ROS play an important role in the pathophysiology of damage to human spermatozoa. Increased generation of ROS and enhanced lipid peroxidation are considered responsible for the toxicity of a wide range of compounds. The present study was done to see the ameliorating effect of propolis on the arsenic induced toxicity in the testes of rat. It was seen that there was steady decrease in the body weight of rat administered with arsenic trioxide while the animals in the control group showed a steady gain in body weight during the period of treatment. The lower body weight suggested that arsenic treatment caused

derangements in digestion and absorption of diet. When propolis was co-administered with arsenic it was observed that the body weight declined initially after a period of 9 days after which a steady increase was recorded up to day 14.LPO is an autocatalytic free radical process whereby polyunsaturated fatty acids in cell membranes undergo degradation by a chain reaction to yield lipid hyper oxides which subsequently decompose to form a variety of toxic products including malondialdehyde. Malondialdehyde (MDA) is one of the final products of lipid per oxidation. The concentration of MDA shows the toxicity in the organ caused by free radicals. Free radicals such as ROS disturb cell membrane integrity through lipid per oxidation which causes cell damage. In a similar study Mahran et al., (2011) aimed to investigate the protective effects of propolis against reproductive toxicity of aluminium chloride in male rats. MDA level increased on treatment with aluminium chloride whereas co-treatment of propolis and aluminium chloride suppressed the lipid oxidation and level of MDA decreased near to control group. The results showed that propolis was effective in the protection against the reproductive toxicity of AlCl3 in male rats as observed in the present case. Lipid per oxidation reaction causes membrane damage which leads to a decrease in sperm motility, presumably by a rapid loss of intracellular ATP, and an increase in sperm morphology defects (Hsu et al., 1998).Reduced glutathione (GSH) is an antioxidant, preventing damage to important cellular components caused by reactive oxygen species such as free radicals and peroxides (Pompella et al., 2003). Glutathione is considered to be the most abundant redox scavenging molecule in the cell protecting it against free radicals, peroxides and other toxic compounds. The testicular content of GSH (reduced glutathione) was significantly decreased ($p<0.01$) in the present study after arsenic treatment when compared with the respective control. Co-administration of arsenic and propolis increased the GSH levels compared with the arsenic treated rats, although the level was below the control for GSH .Decrease in the GSH level adversely affects cellular thiol redox balance and makes the cells susceptible to a number of internal and environmental stresses. Attia et al., (2012) also studied the antioxidant role of propolis extract against oxidative damage of testicular tissue induced by insecticide chlorpyrifos (CPF) in rats. These results clearly indicated that treatment with CPF resulted in a significant decrease in the level of testes GSH as compared to control animals. While, male rats treated with propolis showed significant increase in testes GSH content as compared to control rats, the combination group showed that propolis extract was capable of restoring the level of GSH to the normal values.

GST on the other hand involves a large and complex family of proteins that catalyse the conjugation of reduced glutathione via the sulfhydryl group to electrophilic centres on a wide variety of substrates in preparation for excretion from the cell. This activity is critical in the detoxification of peroxidised lipids as well as the metabolism of xenobiotics. GSTs are present in eukaryotes and in prokaryotes, and they catalyze a variety of reactions and accept endogenous and xenobiotic substrates. During the present study it was seen that arsenic trioxide inhibited glutathione-S-transferase activity significantly in testes. However propolis restored the GST activity near to control. Increase in LPO with concomitant decrease in GSH level and antioxidant enzymes was a clear indication of oxidative stress produced by acute exposure to arsenic. In fact therapy with propolis extract seemed to afford protection against this noxious stimulus. Because of high affinity of arsenic to thiol groups, it affects living organisms by damaging thiol proteins and enzymes. In the present study when blood parameters were measured it was seen that administration of arsenic caused elevation of serum AST (69.07 ± 5.50 IU/L), and ALT (66.97 ± 7.3 IU/L) activities compared with control group (52.52 ± 1.95 IU/L for AST and 49.37 ± 2.2 IU/L for ALT). Co-treatment of propolis with arsenic caused reduction in the level of AST (58.79 ± 3.40 IU/L) and ALT (55.37 ± 3.40IU/L) suggesting protective activity of propolis against arsenic. Another parameter evaluated in serum was bilirubin .Its level in the blood was elevated in the arsenic treated group (0.565 ± 0.156 mg/dl) as compared to control group (0.275 ± 0.035 mg/dl). Co–treatment of propolis decreased the bilirubin level (0.370 ± 0.04 mg/dl) when compared to arsenic group. El Mazoudy et al., (2011) studied the protective role of propolis against reproductive toxicity of chlorpyrifos (CPF) in male rats. Rats exposed to CPF showed an increase in the serum enzyme activities of ALT and AST level compared to control. Propolis treated group did not show any changes as compared to control. Combination treatment (CPF with propolis) decreased the enzymes activity as compared to rats that were treated with CPF only.

V. CONCLUSION

The present study demonstrates that arsenic trioxide administration induced oxidative stress in rats as studied by the parameters such as lipid peroxidation, reduced glutathione (GSH) and drug detoxification enzyme glutathione-s-transferase (GST). Propolis could improve the antioxidant status and level of detoxification enzyme GST. Further studies done in serum such as bilirubin and enzymes ALT and AST showed that propolis could improve physiological function by reducing the toxicity parameters to some extent.

REFERENCES

[1] Attia A.A., ElMazoudy R.H. and Nahla S. E.(2012). Antioxidant role of propolis extract against oxidative damage of testicular tissue induced by insecticide chlorpyrifos in rats. Pestic Biochem Physiol., 10: 5-15.

[2] Burdock G.A. (1998). Review of the Biological Properties and Toxicity of Bee Propolis. Food Chem Toxicol., 36: 347-363.

[3] Ellman G.L. (1959).Tissue Sulfhydryl Group. Arch Biochem., 82: 70.

[4] ElMazoudy, Reda H. Attia A.A. and Shenawy N.S.E. (2011).Protective role of propolis against reproductive toxicity of chlorpyrifos in male rats. Pesticide Biochem Physio., 101.(3): 175-181.

[5] Habig W. H., Pabst M.T. and Jakoby W.B. (1974). Glutathione – S – Transferases. The first enzymatic step in mercapturic acid formation. J Biol chem., 249:7130-7135.

[6] Hsu P.C., Hsu C.C., Liu M.Y., Chen L.Y. and Guo Y.L. (1998). Lead-induced changes in spermatozoa function and metabolism. Toxicol Environ Hlth., 55: 45–64.

[7] Isla M.I., Moreno N., Sampietro A.R. and Vattuone M.A. (2001). Antioxidant activity of Argentine propolis extracts. J. Ethnopharmacol., 76: 165– 170.

[8] Mani F., Damasceno H.C.R., Novelli E.L.B., Martins E.A.M. and Sforcin J.M. (2006). Propolis effect of different concentrations, extracts and intake period on seric biochemical variables. J.Ethnopharma., 105: 95–98.

[9] Mahran A.A., Rashidy A.H.R. and Mawla A.M.A. (2011). Role of propolis in improving male rat fertility affected with aluminum chloride cytotoxicity. Spatula DD., 1(4): 189 – 198.

[10] Pompella A., Visvikis A., Paolicchi A., Tata D. and Casini V. (2003). The changing faces of glutathione, a cellular protagonist. Biochem Pharmacol ., 66 (8): 1499–503.

[11] Wills E.D. (1971). Measurement of lipid peroxidation. Biochem