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Studies on the Hepatoprotective Effect of Propolis on Arsenic Induced Toxicity in Male Sprague-Dawley Rats

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Abstract—*Honey bee products have been used by man since ancient times in traditional and alternative medicine. One of these is propolis or bee glue.* Propolis offers antiseptic, antibiotic, anti-fungal and even antiviral properties. *The present investigations were directed towards evaluating this product for countering the effect of the highly prevalent problem of heavy metal toxicity. Arsenic was chosen because it exhibits a complex metabolism, is the most abundant and potential carcinogen and has also been reported as an environmental pollutant that causes environmental tragedy. The present study was therefore designed to investigate the potential of propolis in decreasing changes induced by the arsenic trioxide in rat model. Biochemical parameters were used to assess toxicity caused by arsenic trioxide in liver of male rats. Co-administration of propolis with arsenic was tested for ameliorative effects.*

1. INTRODUCTION

The hive of the honeybee is a treasure house of useful products for which man has found many applications. Honey is just one of them. Others are beeswax, pollen, propolis, royal jelly and bee venom. *Propolis originates as the sticky resinous sap which seeps from the buds of certain trees and oozes from the bark of others. With its antiseptic properties it provides a hospital clean environment for the rearing of brood. Propolis is a very complex mixture that varies according to the source it comes from. Research shows that propolis offers antiseptic, antibiotic, anti-fungal and even antiviral properties [1].Arsenic is a highly poisonous metallic element in the nitrogen family of group VA in the periodic table.* Its compounds are used as pesticides, fungicides and rodenticides. It is used in glass, electroplating, dyestuff, paint and cosmetic industries. It is potential environmental pollutant and is responsible for induced toxicity in some areas of the world where a large population is drinking Arsenic contaminated ground water [2].The protective effects of propolis against arsenic induced toxicity in rats were examined.

2. MATERIALS AND METHODS

2.1. Chemicals

Arsenic trioxide was purchased through the Department of Zoology Panjab University Chandigarh from Sigma-Aldrich. Propolis was collected from an apiary at village Tierra near Chandigarh.

2.2. Experimental animals

Normal Sprague-Dawley male rats weighing between 200gm-290gm were selected for experiment. Rats were procured from the Central Animal House of Panjab University, Chandigarh. Animals were maintained in an environmentally controlled animal house (temperature 24 ± 3°C) in a 12 h light/dark schedule. The rats were reared in polypropylene cages and fed chow diet *ad libitum.*

2.3 Preparation of propolis extract solution

The propolis should be clean and free of wax, paint, wood etc. It should be in small pieces. Aqueous extract solution was prepared by following protocol of [3].

2.4. Dose and mode of administration

The selected dose was administered orally to each experimental animal by gavage with the help of cannula fixed on a syringe. Saline was used as carrier. Arsenic trioxide was given at concentration of 3mg/kg b.wt. The dose of propolis was 250mg/kg b.wt.

2.5. Experimental design

All animals were fed on standard diet. The animals were divided into 3 groups having 6 animals in each group:-

- Group (1): Control rats (without any treatment): 1ml saline for 14 days.
- Group (2): Rats administered with arsenic trioxide alone 3mg/kg b.wt for 14 days.
- Group (3): Rats administered arsenic trioxide 3mg/kg b.wt plus propolis extract 250mg/kg b.wt for 14 days.
- After the end of the experimental period (14days), all animals were sacrificed on 15th day under light ether anesthesia.

2.6 Biochemical parameters

2.6.1. Serum Glutamate Pyruvate Transaminase (SGPT/ALT)

Serum Glutamate Pyruvate Transaminase (SGPT) is also called as Alanine Transaminase (ALT). It is found in serum and in various body tissues, but is most commonly associated with the liver. Levels of enzyme are elevated in blood under certain diseased conditions of liver. ALT was estimated by following standardized protocol of diagnostic kit (Reckon).

2.6.2. Serum Glutamate Oxaloacetate Transaminase (SGOT/AST)

Serum Glutamate Oxaloacetate Transaminase (SGOT) also called as Aspartate Transaminase (AST) belongs to the transferase class of enzymes. This enzyme shows the high levels of activity in kidneys, liver and heart.AST was estimated by following standardized protocol of diagnostic kit (Reckon).

2.6.3. Bilirubin

Bilirubin reacts with diazotized sulfanilic acid to form an azo dye which is red in neutral and blue in alkaline solutions. Water-soluble bilirubin glucuronides react “directly”, whereas, the free “indirect” bilirubin reacts only in the presence of an accelerator. The bilirubin in serum was determined using the Reckon kit.

3. RESULTS

3.1 Serum glutamate Pyruvate Transaminase (SGPT / ALT)

Arsenic is known to produce disturbances in liver function. Activity of ALT significantly increased ($p<0.01$) in arsenic treated rats, indicating liver dysfunction. In arsenic and propolis treated rat, values of ALT were lower as compared to group II but were significantly more ($p<0.001$) than control.

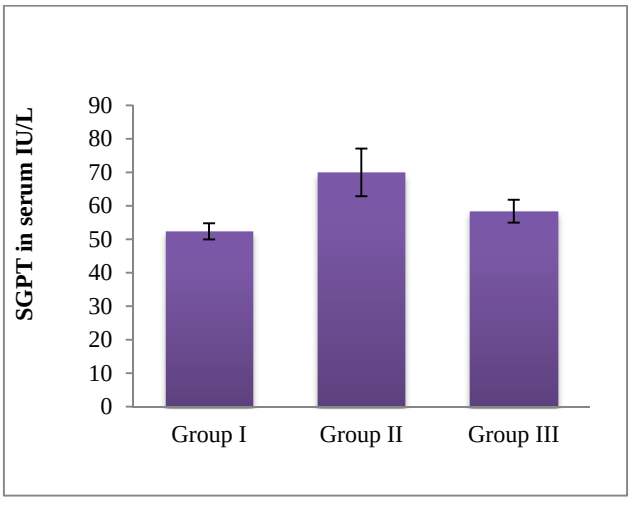

Fig. 1: Effect of different treatments given orally for 14 successive days on the level of SGPT in serum.

3.2. Activity of Serum Glutamate Oxaloacetate Transaminases (SGOT/AST).

Estimations done on serum to check the extent of hepatic toxicity and tissue damage revealed that activity of AST was significantly higher ($p<0.001$) in arsenic treated group as compared to control animals. Activity of AST was found to decrease in serum of group III rats as compared to group II rats.

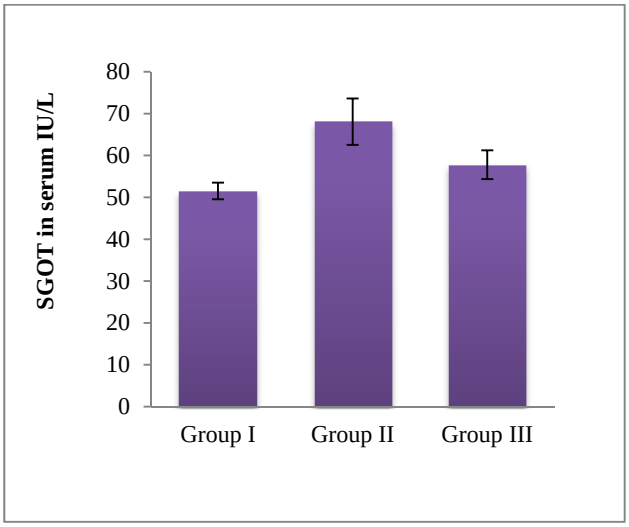

Fig. 2: Effect of different treatments given orally for 14 successive days on the level of SGOT in serum.

3.3 Bilirubin level

Determination of liver toxicity parameters such as bilirubin in all three groups of rats revealed that arsenic administration for 14 days to liver caused toxicity when compared with the control group. There is a significant increase ($P<0.001$) in bilirubin level in group II treated rats compared to control group. There was a significant decrease ($P<0.001$) in the level of the propolis + arsenic (group III) treated animals compared to the control group.

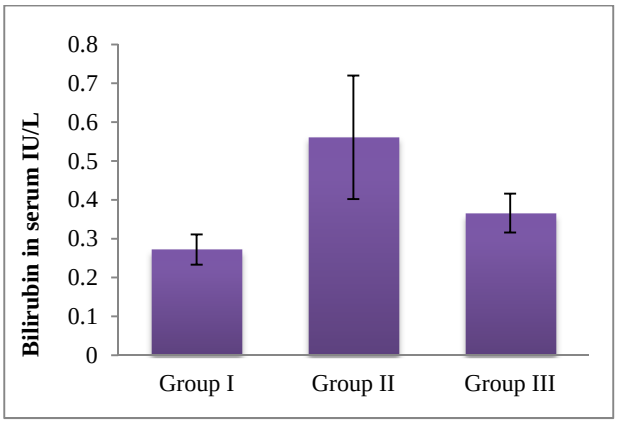

Fig. 3: Effect of different treatments given orally for 14 successive days on the level of Bilirubin in serum.

4. DISCUSSION

The hive of honey bee is a treasure house of useful products. Man has been quick to see and understand the practical applications of these. Earlier studies demonstrated and confirmed the anti-bacterial activities of propolis [4,5].The present investigations were directed towards evaluating this product for countering the effect of the highly prevalent problem of heavy metal toxicity. The protective effects of propolis against arsenic induced toxicity in rats were examined. Factors causing stress include physical conditions such as climate and environment and physiological factors such as nutrition and diseases. The liver is the main site of metabolism and sensitive organ to per oxidative damage because it is rich in oxidizable substances. The increment of oxidative stress in the cells of the liver and the consequent decrease in the antioxidant ability of the cells results in the occurrence of aggressive cellular damage to the liver cells with destruction of their membranes and the release of enzymes into the blood stream. Propolis which contains flavonoids was evaluated for its antioxidant properties against arsenic toxicity in liver during the present study. Arsenic administration had a detrimental effect on the body weights of rats. Arsenic is known to cause changes in cell permeability. Elevated levels of serum enzymes are indicative of cellular leakage and loss of functional integrity of the cell membrane in liver. In the present study, administration of arsenic caused elevation of serum AST and ALT activities compared with their respective value in control group. Further the rise in AST and ALT activities induced by arsenic administration was significantly reduced by administration of propolis when combined with arsenic suggesting the protective activity of propolis against cellular leakage and loss of functional integrity of the cell membrane in hepatocytes. Levels of bilirubin in blood were elevated in arsenic group compared with control group further indicating hepatic injury.

5. CONCLUSION

Estimations done in serum to check the extent of hepatic toxicity and tissue damage revealed that activity of ALT and AST was significantly higher in arsenic treated group as compared to control animals; bilirubin levels were found to be highly significantly increased. Activity of ALT and AST was found to be improved in serum of arsenic plus propolis group rats ($p<0.01$). Bilirubin levels in serum of arsenic + propolis group indicated reduced toxicity of arsenic on co-administration of propolis at a dosage of 250mg/kg b.wt.

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