

Suppressive Effect of Propolis In Collagen Induced Arthritis In Female Rats

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Abstract- Collagen induced arthritis in female rats was used as a study model in this study. Rheumatoid arthritis is a representative chronic inflammatory disease of the joints resulting in destruction of bone and cartilage. Propolis is a natural product derived from plant resins collected by the honey bees. In this study, we assay the effects of propolis extract on the production of key markers of liver and kidney released during chronic inflammatory event as SGOT, SGPT, Bilirubin and Creatinine in serum of collagen induced arthritic female rats. Observation reveals that this natural compound and its active principle, caffeic acid phenethyl ester (CAPE), were able to contrast the harmful effects of arthritis

Keywords- Caffeic acid phenethyl ester (CAPE); arthritis, propolis extract, SGOT, SGPT, Bilirubin, Creatinine

I. INTRODUCTION

The honeybees exhibit a combination of individual traits and social cooperation which is unparalleled in the animal kingdom. The multiple levels at which the honey bee expresses adaptation to its world provide one of the richest source for study and knowledge among all organisms. Propolis has potent biological and pharmacological properties and can be considered as drugs from the bee hive. The extract contains amino acids, phenolic acids, phenolic acid esters, flavonoids, cinnamic acid, terpenes and caffeic acid, possesses several biological activities such as anti-inflammatory, immuno-stimulatory, anti-viral and anti-bacterial. Rheumatoid Arthritis is a chronic inflammatory disease, producing joint damage mediated by cytokines, chemokines, and metalloproteases. The disease is systemic, characteristically affecting the synovial joints and peri articular synovial structures (bursae and tendon sheaths) in particular. The trigger for the disease is not known, but is thought to be autoimmune (Hansen, 1995). The protective effects of propolis against collagen induced arthritis in rats were examined.

II. MATERIALS AND METHODS

2.1. Chemicals

Propolis was collected from an apiary at village Tierra near Chandigarh. For induction of arthritis Bovine type II collagen and Incomplete Freund's Adjuvant (IFA) were sourced from SRL and Sigma respectively.

2.2. Experimental animals

Normal Sprague-Dawley female rats weighing between 220gm-250gm 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 Mani *et al.*, 2006.

2.4. Dose and mode of administration

The selected dose of propolis was 250mg/kg b.wt. was administered orally to each experimental animal by gavage with the help of cannula fixed on a syringe. In order to induce arthritis, collagen in Incomplete Freund's Adjuvant (IFA) was used at a dosage of 3mg/kg body weight of collagen was suspended in 0.9% NaCl added 0.2 ml of Freund's Incomplete Adjuvant, kept overnight at 4°C and was injected intradermally into the tail region. Intra-dermal injection of 0.3 ml was given into the root of the tail on day 0, on same region on the 7th day followed by 14th day.

2.5. Experimental design

All animals were fed on standard diet. The animals were divided into 3 groups having 5 animals in each group:-

- Group (1): Control rats
- Group (2): Collagen induced arthritic rats.

After induction of arthritis treatment was started.

- Group (3): Rats administered propolis extract 250mg/kg b.wt for 5 weeks.

After the end of the experimental period all animals were sacrificed.

III. BIOCHEMICAL PARAMETERS

I Serum Glutamate Oxaloacetate Transaminases (SGOT/AST)

The most sensitive and widely used liver enzymes are the aminotransferases. AST is normally found in a variety of tissues including liver, heart, muscle, kidney, and the brain. It is released into the serum when any one of these tissues got damaged. In the present study SGOT was assayed, showing that collagen induced arthritis caused significant elevation in its activity with respect to control. The elevated level observed in arthritis is 63.604 ± 2.95 IU/L which is significantly higher than control 54.178 ± 1.204 IU/L. This shows a 17.39% increase in AST serum level which is highly significant ($p \leq 0.001$). Our results revealed that propolis given to disease rat shows a reduction in AST level by 7.07%. Data revealed that propolis treatment 50.346 IU/L is significantly ($p \leq 0.5$) similar to control. Similar work done by Zhu *et al.* (2010) investigated that ALT and AST levels in diabetic rats increased as compared with normal rats ($p < 0.01$). Treatment of Brazilian propolis significantly suppressed AST and ALT levels.

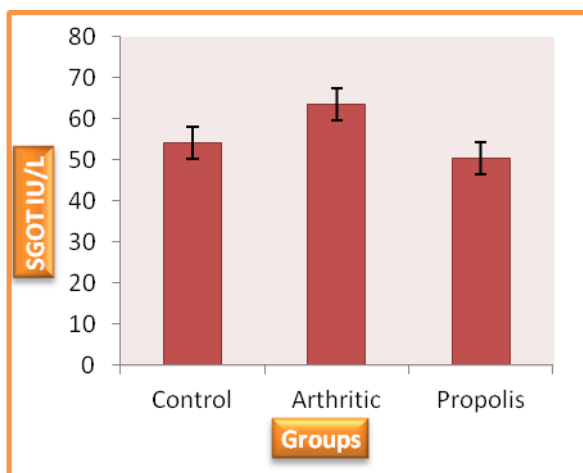


Fig. 1. Activity of alanine transaminase (serum) in rats

II Serum glutamic pyruvic transaminase (SGPT) / ALT

Serum glutamic pyruvic transaminase is also known as Alanine aminotransferase (ALT). It is found in serum and in various body tissues, but is most commonly associated with the liver. In the present study when SGPT was assayed it was observed that collagen induced arthritis caused significant elevation in its activity with respect to control. The elevated level of arthritis is 63.106 ± 5.04 IU/L when compared with control 53.606 ± 0.35 IU/L. This shows a 17.72% increase in ALT serum level which is highly significant ($p \leq 0.001$) with respect to control. Our results revealed that propolis given to disease rat shows a reduction in ALT level by 4.8%. Data revealed that propolis treatment 51.032 ± 2.41 IU/L is significantly ($p \leq 0.5$) similar to control.

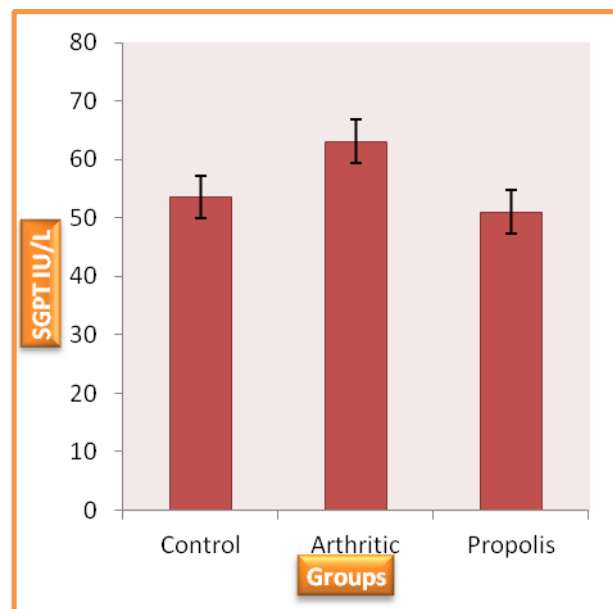


Fig 2. Activity of alanine aminotransferase (serum) in rats

III Creatinine

The main function of the kidney is excretion of water soluble waste products from our body. The kidney performs functions like filtration, excretion and secretion. Derangement in any of the function of kidney leads elevated level of function tests. Measuring serum creatinine is the most commonly used indicator of renal function. Creatinine is a waste product that comes from the normal wear and tear on muscles of the body. While studying the renal parameter during the present study, the creatinine concentration was 0.206 ± 0.02 mg/dl in normal rat (control), the elevated level of creatinine is observed in disease rat 0.306 ± 0.03 mg/dl. The elevation in level of creatinine of arthritic rats is increased by 48.54% as compared to healthy control rats showing a highly significant change ($p \leq 0.001$) with respect to control. Our results revealed that propolis given to disease rat shows increase in creatinine level with respect to control by 3.88%.

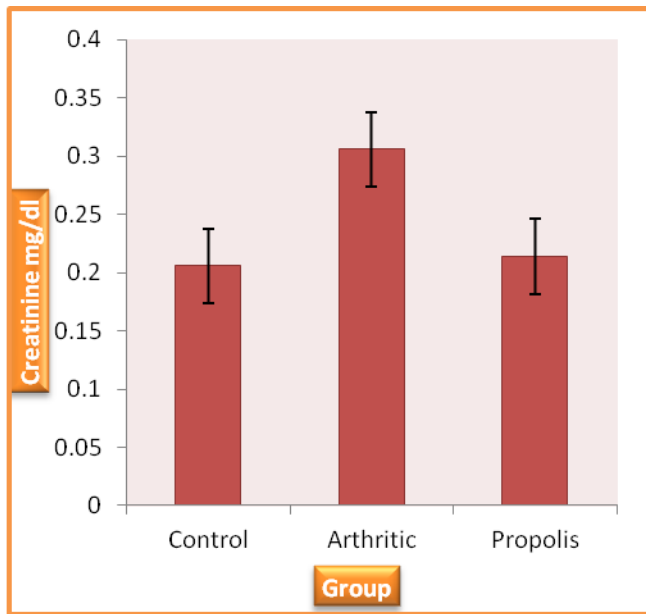


Fig 3. Activity of creatinine (serum) in rats

IV Bilirubin

Bilirubin is a breakdown product of haemoglobin. Bilirubin formed in the reticulo-endothelial system is transported to the liver bound to albumin. This bilirubin is water insoluble and is known as indirect or conjugated bilirubin. Total bilirubin concentration in healthy rat was (C) 0.254 ± 0.02 mg/dl the elevated level of total bilirubin is observed in disease rat (T) 0.332 ± 0.09 mg /dl increase by 30.70% with respect to control. Present studies showed that when propolis was administered alone in arthritic rats 0.294 ± 0.03 mg/dl showed an increase of percentage change by 15.74 % with respect to control, showing a significant change ($p \leq 0.05$) with respect to arthritis (T) and significant change ($p \leq 0.05$) with respect to control. Studies done by Mahmoud and Elsoadaa (2013) showed the effectiveness of ascorbic acid (AA), propolis and royal jelly in alleviating the toxicity of Aluminum chloride ($AlCl_3$) on liver and kidney. The level of urea, and the activities of aspartate aminotransferase (AST), alkaline phosphatase (ALP), and alanine aminotransferase (ALT) were increased, Ascorbic acid, Propolis and royal jelly had beneficial influences and could antagonize $AlCl_3$ toxicity.

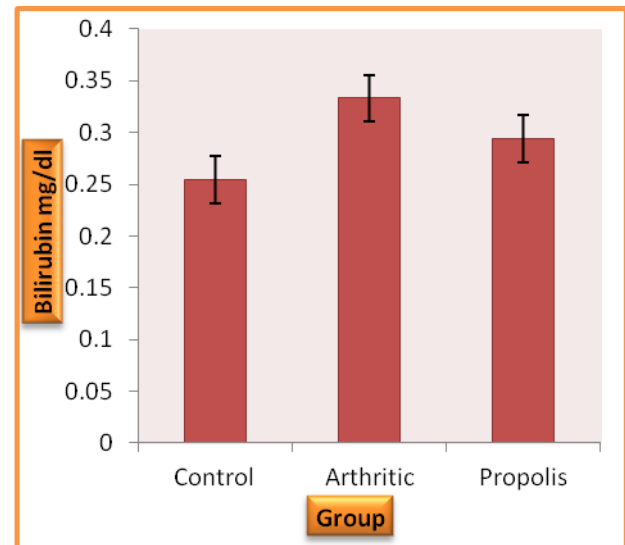


Fig 4. Activity of Bilirubin (serum) in rats

IV. 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 (Krell, 1996; Itavo *et al.* 2011). The present investigations were directed towards evaluating this product for countering the effect of the highly prevalent problem of rheumatoid arthritis. The protective effects of propolis against collagen induced arthritis in rats were examined. Reactive oxygen species (ROS) are chemically reactive molecules containing oxygen. During times of stress ROS levels can increase. This may result in significant damage to cell structures which cumulate into a situation known as oxidative stress (Bulger and Helton, 1998). The liver is the main site of metabolism and sensitive organ to per oxidative damage because it is rich in oxidizable substances. Propolis which contains flavonoids was evaluated for its antioxidant properties against collagen induced arthritis in present study. The animals that were administered collagen had a detrimental effect on the serum markers. 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 collagen 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 collagen induced arthritis administration was significantly reduced by administration of propolis (Fig. 2 and 3) 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.

V. 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 arthritic 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 propolis group rats ($p < 0.01$). Bilirubin levels in serum of propolis group indicated reduction on administration of propolis at a dosage of 250mg/kg b.wt.

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