Journal of Global Biosciences
ISSN 2320-1355
Volume 5, Number 4, 2016, pp. 3939-3947
Website: www.mutagens.co.in
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researchsubmission@hotmail.com

Research Paper

ANTIOXIDANT ROLE OF PROPOLIS AGAINST ARSENIC INDUCED NEPHROTOXICITY IN MALE RATS

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Abstract

The present study was designed to investigate the antioxidant role of propolis extract against arsenic induced nephrotoxicity in male Sprague Dawley rats. All the experimental animals were divided into three equal groups (n=5). Arsenic was administered daily at dosage 3 mg/kg b.wt orally to rats for 14 consecutive days in group II and propolis extract was given concurrently at the dosage 200 mg/kg b.wt orally in group III. On day 15th all the animals were sacrificed and kidney samples were collected for biochemical estimations. Arsenic trioxide ($As_2O_{3)}$ administration caused oxidative stress in rats manifested as significantly decreased activity of glutathione-S-transferase (GST) with decreased glutathione (GSH) content and increased level of malondialdehyde (MDA). On the other hand simultaneous administration of propolis extract help in reducing the arsenic induced nephrotoxicity by reversing the above mentioned deleterious changes near to control conditions. Therefore, we concluded propolis treatment significantly attenuated arsenic induced nephrotoxicity.

Key words: Antioxidant; Arsenic trioxide ($As_2O_{3)}$; Sprague Dawley rat; Propolis extract; Nephrotoxicity; Biochemical estimations.

INTRODUCTION

Toxic metals are a concern of the highest order with regard to exposure of living beings and pose a great threat to the present environment and human health. The kidney is the most sensitive organ and responds to metal toxication by general transport defects of the proximal tubules characterized by proteinuria, glycosuria, acidoaminuria, phosphaturia, calciuria, and reduction in glomerulus filtration, mimicking the Fanconi syndrome Buchet *et al*. (1990). Arsenic trioxide is the inorganic compound with the formula As_2O_3. Arsenic and its compounds are dangerous for the environment and human health. Arsenic exposure is known to be associated with adverse health effects on the systems of the body, but is most known for causing specific types of skin lesions and increased risks of cellular and nephrotoxicity (Ricardo *et al*., 2004; Sohini and Rana, 2007; Saxena *et al.,* 2009).

Propolis means a gum that is gathered by bees from various plants. Propolis is a multifunctional material used by bees in the construction and maintenance of their hives. It has been used as a traditional remedy for various diseases in folk medicine, as a constituent of bio-cosmetics and as health food (Kujumgiev *et al*., 1999). It is believed that it can cure heart disease, diabetes and even cancer (Hirota *et al*., 2000; Na *et al*., 2000). Several biological properties of propolis and other bee products including tumor cell arrest, antibiotic, anti-inflammatory and antioxidant

effects have also been reported (Banskota *et al*., 2000; Moreno *et al*., 2000; Cai *et al*., 2004; Singla and Kumar, 2016). It contains esters of phenolic acids and flavonoids, which have been identified as antibacterial, antiviral and antifungal agents (Marcucci, 1995; Vennat *et al*., 1995). According to Calder and Mirzoeva (1996), some of the anti-inflammatory substances present in propolis are caffeic acid, quercetin, naringenin, and caffeic acid phenethyl ester. These compounds contribute to the suppression of prostaglandins and leukotrienes synthesis by macrophages and have inhibitory effects on myeloperoxidase activity, NADPH-oxidase, ornithine decarboxylase and tyrosine-protein-kinase. The present study is designed to study the toxicity of As_2O_3 in the kidney tissue of male Sprague dawley rats by estimating the antioxidant enzymes and to test the efficacy of propolis in reducing the toxicity.

2. MATERIALS AND METHODS

2.1. Experimental Animals

Male Sprague-Dawley male rats weighing 200g-240g were obtained from the Central Animal House of Panjab University, Chandigarh. Animals were housed in polypropylene cages on rice husk and maintained at ambient temperature (22±3°C) and humidity. They were kept under hygienic conditions and fed on chow diet *ad libitum* and water throughout the treatment period. All the procedures reported here strictly followed the principals as laid down by the Institutional Animal Ethical Committee (IAEC), Panjab University Chandigarh, India on the use of the experimental animals in the Panjab University.

2.2. Propolis Extract

In the present study, propolis was collected from bee hives in apiary at village Tierra near Chandigarh. Ethanolic extract of propolis was prepared by following method of Mani *et al.* (2006). For this propolis samples were grounded to fine pieces. Placed the proper amount of propolis and alcohol (30gm propolis and 70% ethanol to make 100ml volume) in the container and sealed the top. Store this container in dark place at room temperature. The contents were shaken two or three times per day for two weeks. After two weeks the propolis extract was filtered, dried and stored in sealed bottles.

2.3. Experimental Design

The dose of As_2O_3 and propolis was chosen on the basis of previous reports suggested by different workers. As_2O_3 was dissolved in normal saline at a dose of 3 mg/kg b.wt given orally for 14 days by intragastric gavage. Propolis extract was given at the dose of 200 mg/kg b.wt once daily for a period of 14 days. All the animals were divided into 3 groups having 5 animals each. Control group (I) animals were administered with 0.9% normal saline. In group II animals were administered with As_2O_3 at the dosage of 3 mg/kg b.wt orally for period of 14 days. In group III animals were treated with As_2O_3 at the above mentioned dose plus propolis extract at dosage 200 mg/kg b.wt orally daily for period of 14 days. After the end of the experimental period all animals were sacrificed on day 15^{th} under light ether anesthesia. Kidney samples were collected from each animal for the biochemical analysis.

2.4. Biochemical assays

Total protein was estimated by the method of Lowry *et al*. (1951) using bovine serum albumin as standard. Lipid peroxidation (LPO) was measured by the estimation of malondialdehyde (MDA) content as described by the method of Begue and Aust (1978). Glutathione (GSH) level was measured by the method of Sedlak and Lindsay (1968) and activity of glutathione-S-transferase (GST) was determined using spectrophotometry by the method of Habig *et al.* (1974).

2.5. Statistical analysis

Statistical analysis was performed by student t- test to estimate whether the differences between the mean values of two groups are statistically significant or not.

3. RESULTS

3.1. Assessment of antioxidant enzymes

Lipid peroxidation in kidney homogenate was estimated as formation of MDA (nmol/mg protein). In As_2O_3 treated animals (Group II), the MDA level was increased significantly ($p \leq 0.001$) as compared to the control animals (Group I). However level of MDA in As_2O_3+propolis treated animals (Group III) decreased significantly when compared to the group II with respect to control as seen in Table 1 and Fig. 1. In case of estimation of GSH in kidney homogenate results revealed that GSH level reduced significantly ($p \leq 0.01$) in As_2O_3 treated animals (Group II) as compared to the control group. However level of GSH in As_2O_3+propolis treated animals (Group III) increased as compared to the group II, but were still less than the control group (Table 1 and Fig. 2). Similarly, when the activity of GST was measured in kidney post mitochondrial supernatant results showed that in As_2O_3 treated animals (Group II), the activity was reduced significantly as compared to the control animals (Group I). Propolis co-treatment restored enzyme activity partially but still remained lower than the control value as seen in Table 1 and Fig. 3.

3.2. Histopathology

Group I: Light microscopic examination of kidney of control group revealed typical general organization consisting of inner medulla and outer cortex. The medulla consisted of renal pyramids. Cortex consisted normal glomerulus surrounded by the Bowman's capsule, proximal and distal convoluted tubules without any inflammatory changes as shown in Fig 4(A-B).

Group II: Rats treated with a dose of 3 mg/kg b.wt of As_2O_3 for 14 days showed variable pathological changes in glomeruli and some parts of the urinary tubules. The tubules got significantly distorted and dilated with proteinaceous casts. Congestion and constriction of glomerulus was another prominent change. Increase in number of nuclei in glomerular tufts was observed. Mesangial space was also increased. Microscopic analysis of kidney further showed structural alterations-mainly reduced diameter of glomeruli and renal corpuscles, damaged tubules with affected quality, loss of brush border of proximal convoluted tubules and thickening of the basement membrane as shown in Fig 5(C-D).

Group III: Co-treatment with propolis led to the attenuation of degenerative processes of the kidney. Renal corpuscles having glomerulus and Bowman's capsule were clearly observed. Glomerulus revealed normal morphology. Protein cast formation was decreased in this group. Histology of this group showed the normal basement membrane as shown in Fig 4(E-F).

Table 1. Antioxidant status in kidney tissue of treated experimental rats

Treatment Groups	MDA (nmol/mg)	GSH (µmol/g)	GST (µmol/min/mg)
Control (I)	2.27 ± 0.80	0.02 ± 0.008	0.16 ± 0.014
As_2O_3 (II)	4.42 ± 1.13*** + 94%	0.01 ± 0.006** -50%	0.08 ± 0.017*** -50%
As_2O_3+Propolis (III)	2.37 ± 0.51* + 4.40%	0.017 ± 0.005** -15%	0.13 ± 0.020*** -18.75%

Values are expressed as means ± SD; n=5 for each treatment group
*Represents $p \leq 0.05$; **Represents $p \leq 0.01$; ***Represents $p \leq 0.001$
(+) Indicates % increase; (-) Indicates % decrease

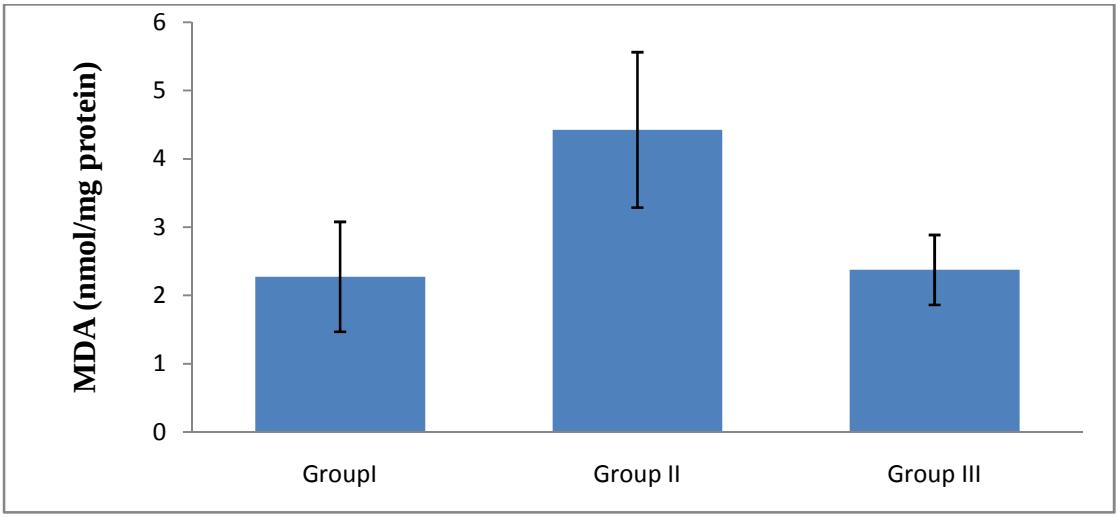


Figure 1. Effect of As_2O_3 and propolis on the level of lipid peroxide in 14 days experiment

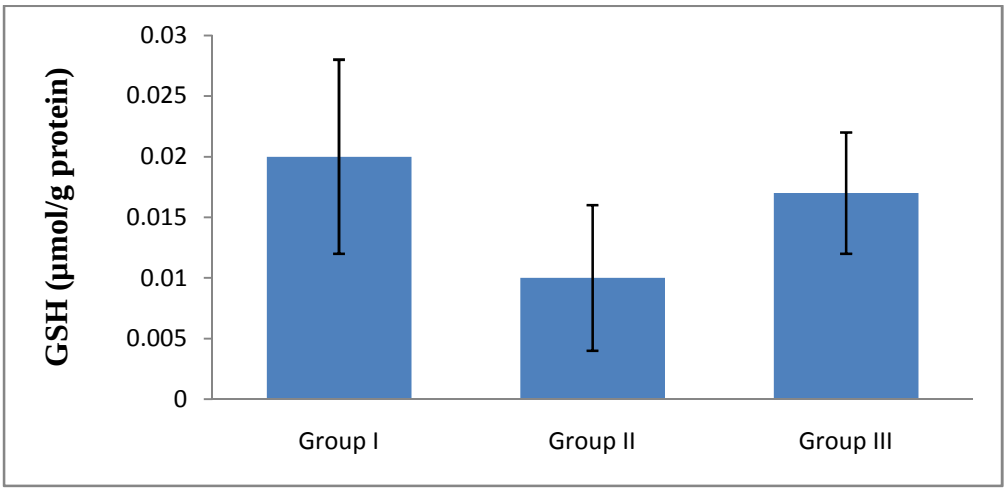


Figure 2. Effect of As_2O_3 and propolis on the level of glutathione in 14 days experiment

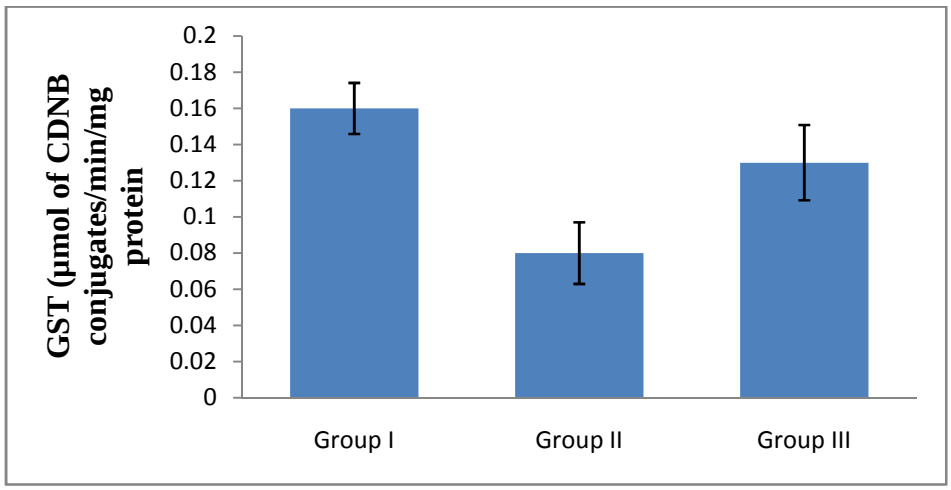
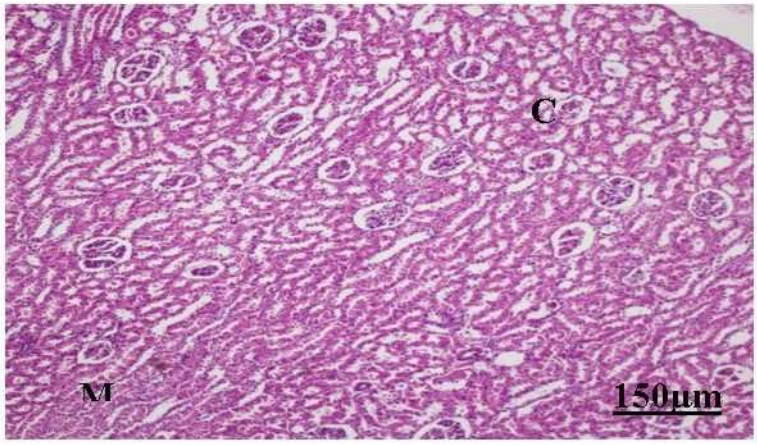
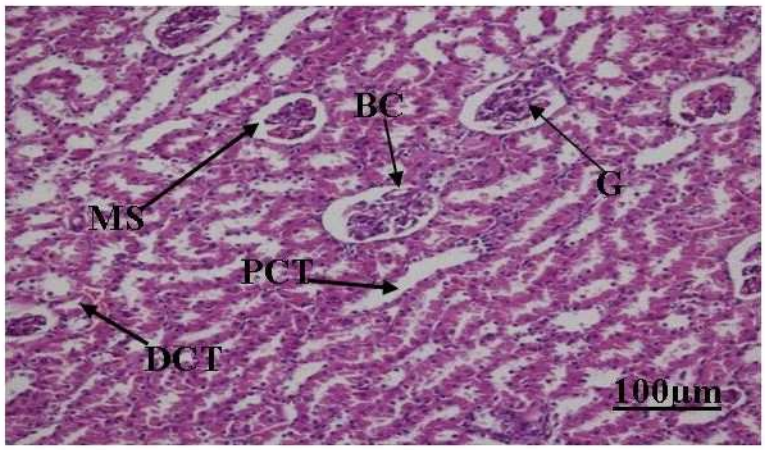


Figure 3. Effect of As_2O_3 and propolis on the activity of glutathione-S-tranferase in 14 days experiment

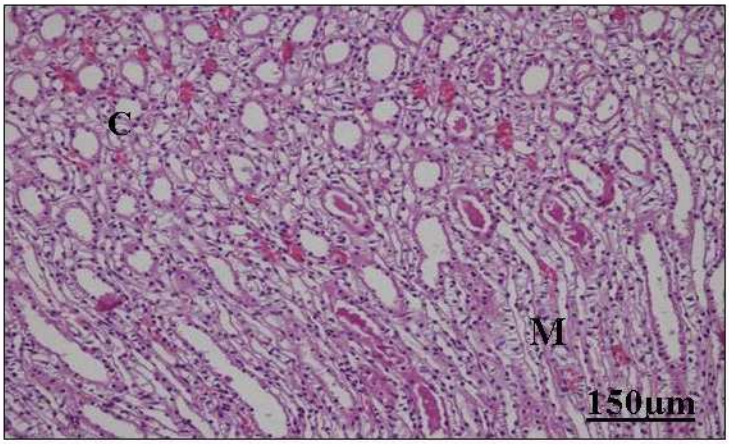


A -100X

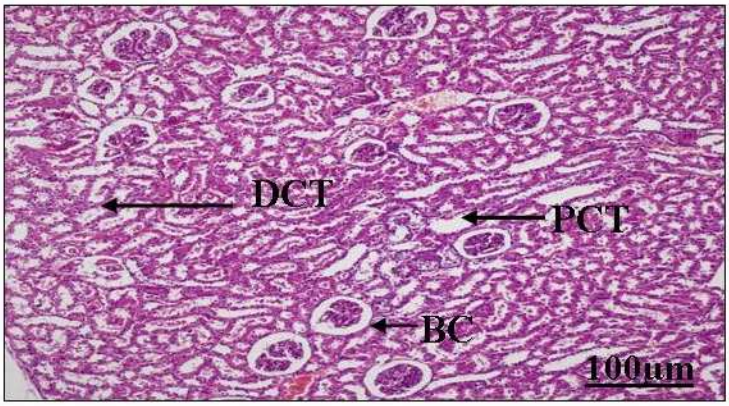


B- 200X

Figure 4. Histological features of normal rat kidney (A-B) showing following structures:-
C-Cortex, M-Medulla, BC-Bowman's capsule, G-Glomerulus, MS-Mesangial space, PCT-Proximal convoluted tubule and DCT-Distal convoluted tubule .



C– 100X



D– 200X

Figure 5. Histological features of arsenic treated kidney of rats (C,-D) showing following structures:-
C-Cortex, M-Medulla, BC-Bowman;s capsule, G-Glomerulus, MS-Mesangial space,PCT-Proximal convoluted tubule and DCT-Distal convoluted tubule.

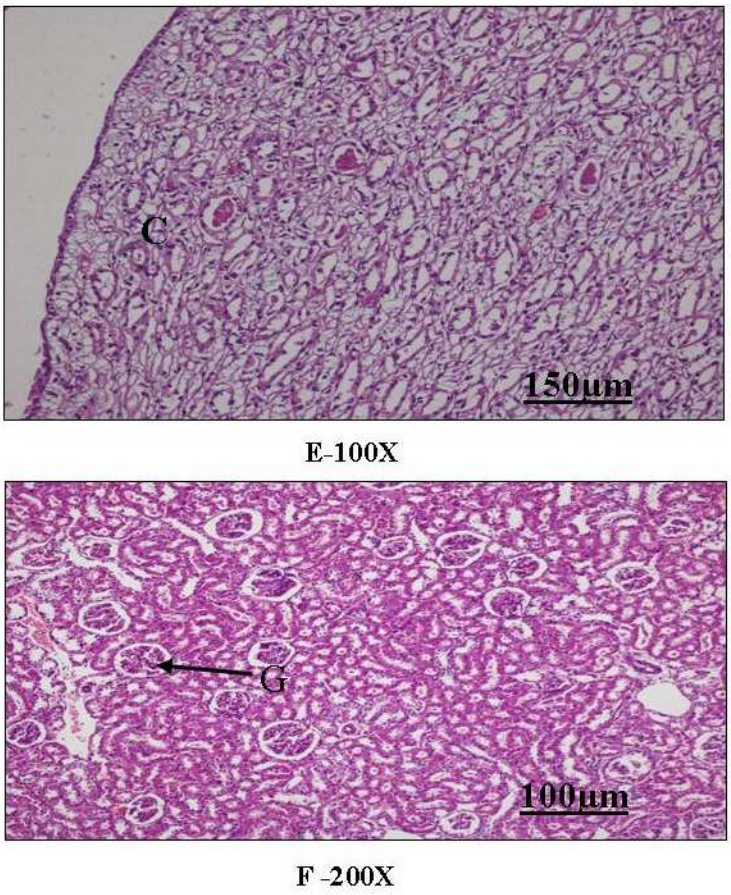


Figure 6. Histological features of arsenic + propolis treated kidney of rats (E-F) showing following structures:-
C-Cortex, M-Medulla, BC-Bowman;s capsule, P-Podocyte,G-Glomerulus, MS-Mesangial space,PCT-Proximal convoluted tubule and DCT-Distal convoluted tubule.

4. DISCUSSION

Oxidative stress represents an imbalance between the production of free radicals and the biological system's ability to readily detoxify the reactive intermediates or to repair the resulting damage (Flora, 2011). It has been reported as a major mechanism of As_2O_3 induced toxicity observed in the present study. Naturally occurring antioxidants and their role in quenching free radicals generated in the body under various pathologic conditions have been an active area of research. Studies have revealed that antioxidants possess the ability of both preventing and curing the damage caused by the generation of free radicals in the body. Propolis, a mixture of phenolic acids, flavonoids and other bioactive compounds, is known to have antioxidant effects in rats (Banskota *et al*., 2001; Fadillioglu *et al*., 2004). The present study was carried out to understand the type of adverse biochemical and histopathological changes occurred in kidney tissue of male Sprague dawley rats as a result of As_2O_3 administration and the efficacy of propolis in protecting the animals from this same. During the present investigation extensive renal toxicity caused by As_2O_3 was observed, as indicated by highly significant increase in the level of MDA with decreased level of tissue GSH as well as activity of GST. Also degenerative changes observed in the kidney histology supported the biochemical results. These findings indicated that arsenic as exposure resulted in changed levels/activity of antioxidant enzymes which was suggestive due to the arsenic induces free radical formation either through direct promotion of free radical generation (Liu *et al*., 1999) or through

inhibition of antioxidant enzymes (Ramos *et a*l., 1995). Present data further indicated that co-administration of propolis extract reduced arsenic induced nephrotoxicity as shown in Table 1 and Fig. 1-3 supported by the findings (Lahouel *et al*., 2010; Mahmoud and Elsoadaa, 2013; Singla *et al.,* 2014; Singla *et al.,* 2015).

CONCLUSION: It was observed that antioxidant dietary supplementation of propolis greatly helped in boosting the immune system and thus aided in preventing the onset of degenerative diseases due to its antioxidative and immunomodulatory properties.

ACKNOWLEDGEMENT: The authors acknowledge the financial support provided by UGC-BSR, Government of India, New Delhi, India, for this work.

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