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Ameliorative Role and Antioxidant Effect of Propolis against Hepatotoxicity of Fenvalerate in Albino Rats

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Abstract

The present work studied the effect of Pyrethroid Insecticidefenvalerate on albino rats' liver and possible ameliorative roles played by Propolis. In this study the Fenvalerate treatment has confirmed to induce many histological changes in the liver of albino rats including congestion of blood vessels, cytoplasmic vacuolization of the hepatocytes, necrosis and fatty degeneration. Biochemical results showed that fenvalerate caused marked elevation in serum ALT and AST. It also caused an increase in malondialdehyde and depletion in activity of the antioxidant enzymes, catalase and superoxide dismutase in the liver organ. Treating animals with fenvalerate and Propolis led to an improvement in both histological and biochemical alterations induced by fenvalerate. Moreover, Propolis was found to reduced the level of malondialdehyde and increased the activity level of antioxidant enzymes, SOD and CAT. These results indicated that Propolis ameliorated liver damage induced by fenvalerate and this could attribute to Propolis antioxidant activity and free radicals scavenging properties.

Keywords: Fenvalerate; Propolis; Hepatotoxicity; Transaminases; Antioxidants

Introduction

The wide spread utilization of insecticides in insect control has performed the need for evaluation of the hazards caused by such substances. Pyrethroids have been known as insecticides for many years and being used as highly active insecticides. The source of Pyrethroids is the flowers of the pyretherum plant Chrysanthemum cinerariafolium [1]. Due to the persistence of these insecticides in the environment, structures similar to Pyrethroids have been synthesized and proved to be effective against different insects [2]. On the other hand, exposure to Pyrethroids was found to produce serious side effects. It has been showed that animals exposed to these insecticides revealed disturbance in their physiological activities beside other histopathological alterations [3,4]. Fenvalerate is a cyanophenoxy-benzyl group of the synthetic pyrethroid pesticides used extensively in agriculture to protect a wide variety of crops and as indoor pest control because of its high toxicity to insects [5]. Exposing animals to Fenvalerate caused different toxic effects. High doses of fenvalerate was reported to cause reduction of body mass, increase in liver mass, and proliferation of the smooth endoplasmic reticulum in hepatic cells, and induction of the activity of microsomal enzymes [6]. It was reported by Waheed and Mohamed [7] that fenvalerate could induce hepatotoxicity in experimental animals. Prasanthi et al. [8] has demonstrated that fenvalerate caused oxidative stress in rats. El-Demerdash et al. [9] reported that fenvalerate induced hemato-biochemical changes in male rats.

Propolis is the substance that honeybees produce by mixing their own waxes with resins collected from plants. It has been used widely as a folk medicine from ancient times. Recently, it has gained popularity as a healthy food in various parts of the world because it promotes health and prevents diseases [10]. It has different biological activities such as antibacterial [11], anti-inflammatory [12-16] and hepatoprotective effects [10]. Propolis contains more than 300 components including phenolic aldehydes, polyphenol, sequiterpene quinines coumarins, steroids, amino acids and inorganic compounds [17]. The present investigation was designed to study the effect of Propolis against fenvalerate insecticide hepatotoxicity in albino rats.

Materials and Methods

Male Wistar rats aged three months weighing (120 ± 5 g) were obtained from animal house of King Abdel Aziz University, Jeddah, Saudi Arabia. They were housed in good aerated chambers and food and water were allowed ad libitum. Animals were divided into 4 groups:

Group 1 (control group): Animals were orally administrated with saline solution.

Group 2: These animals were orally given Propolis at a dose of 100 mg/kg b.w. 3 times /week for 4 weeks

Group 3: Animals were orally administrated with 5 mg/kg Fenvalerate (1/10 LD50) 3 times/week for 4 weeks.

Group 4: Animals were given orally administrated with Fenvalerate and after one hour they were given propolis, 3 times/week for 4 weeks.

At the end of this study all rats were decapitated and liver pieces were collected to be subjected for assessment of histopathological and biochemical examinations.

Histological studies

For light microscopic examination, the treated animals and their controls were sacrificed by decapitation. The livers were removed and fixed in 10% neutral formalin for 24 h. Tissues were rinsed three times in 70% ethanol, dehydrated using a graded ethanol series and then

embedded in paraffin wax. Paraffin sections were cut into 5 micrometers thick slices and stained with haematoxylin and eosin and examined under light microscope.

Biochemical studies

For biochemical study, blood was also collected and sera were obtained by centrifugation the blood samples and stored at -20o for biochemical analysis. The activity of AST and ALT enzymes were determined in the sera according to Reitman and Frankel [18]. Fresh tissue samples of rat liver were homogenized in cold distilled water until a uniform suspension was obtained. The homogenate was centrifuged and the clear supernatant was separated. Catalase (CAT) activity determined from the rate of decomposition of H_2O_2 [19]. Superoxide dismutase (SOD) activity was determined according to the method described by Minami and Yoshikawa [20]. The principal of this method depends on the ability of SOD to inhibit the power of phenazine methosulphate mediated to reduce the nitroblue tetrazolium. Lipid peroxidation was estimated as the concentration of thiobarbituric acid reactive product (malondialdhyde, MDA) according to method used by Ohkawa et al. [21].

Statistical analysis

The results were expressed as mean ± SD of different groups. The differences between the mean values were evaluated by ANOVA. Data were analyzed using the computer program SPSS/ version 15.

Results

Histological observations

Histological examination of liver obtained from control rat and rat treated with propolis showed that the normal hepatic cords of the hepatocytes radiated form the central vein and no histopathological alterations were observed (Figure 1A). However, liver tissue of rats treated with Fenvalerate for 2 weeks showed apparent signs of pathological changes compared to the control group. The normal structural organization of the hepatic lobules was impaired and blood vessels were congested (Figure 1B). Infiltration of inflammatory leucocytes was also noticed (Figure 1C). In addition, the hepatocytes showed cytoplasmic vacuolation and the nuclei were pyknotic (Figure 2A). Moreover, Impairment in the hepatocytes and necrosis signs with dense inflammatory cells and other debris was detected after 4 weeks of treatment with Fenvalerate. In these tissues fatty degeneration composed of scattered fat droplets was clearly abundant (Figure 2B). On the other hand, in animals treated with Fenvalerate and Propolis, these histopathological changes became less. Kupffer cells were activated and large number of binucleated cells was noticed in these sections (Figure 2C).

Biochemical results

Figure 3 showed the effect of different treatments on serum ALT activity. Non-significant difference in serum ALT activity was recorded in rats treated with Propolis in comparison with control group. Treating rats with Fenvalerate caused a significant increase in serum ALT activity after 2 and 4 weeks of treatment. On the other hand, animals treated with Fenvalerate and Propolis revealed a significant decrease in ALT activity when compared with Fenvalerate group. In a similar manner, animals treated with Fenvalerate showed significant increase in serum AST activity while animals treated with Fenvalerate and Propolis showed a significant decrease in AST activity when compared with Fenvalerate treated group (Figure 4).

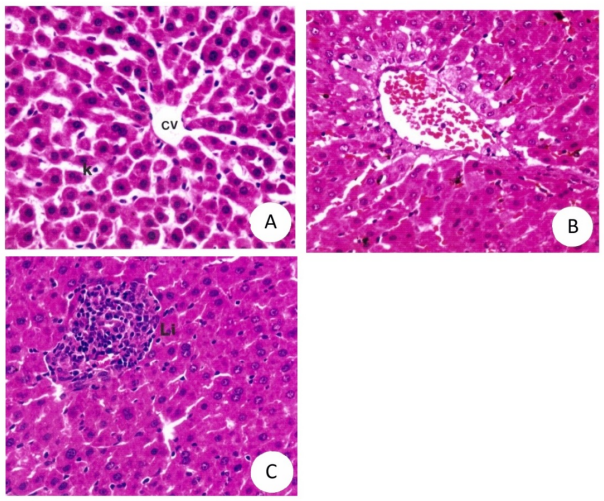

Figure 1: (A) Liver section of a control rat showing hepatic strands, kupffer cell (K) and central vein (CV). (B) Liver of a rat treated with fenvalerate showing congestion of central vein (CV). (C) Liver of a rat treated with fenvalerate showing leucocytic infilteration, (X 400).

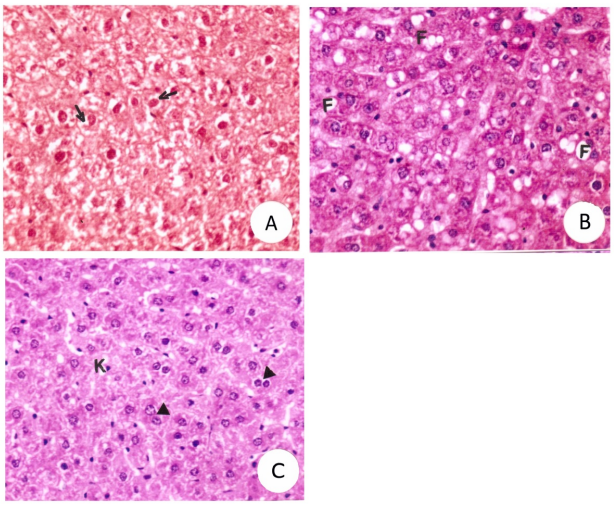

Figure 2: (A) Liver section of a treated rat showing cytoplasmic vacuolizations of the hepatocytes (arrows). (B) Liver section of a treated rat showing fat droplets (F). (C) Liver section of a rat treated with fenvalerate and propolis showing advanced degree of improvement with increase of binucleated cells (arrow head) and activate kupffer cells (K), (X400).

Table 1 showed the effect of different treatments on Malondialdehyde (MDA) (index of tissue lipid peroxidation), superoxide dismutase (SOD) and catalase (CAT) in liver of animals examined after 4 weeks. In rats treated with Fenvalerate, MDA level

was increased significantly, whereas the activity of SOD and CAT was found to be decreased. Treating rats with Fenvalerate and Propolis decreased MDA level and increased SOD and CAT activity to nearly that of the control.

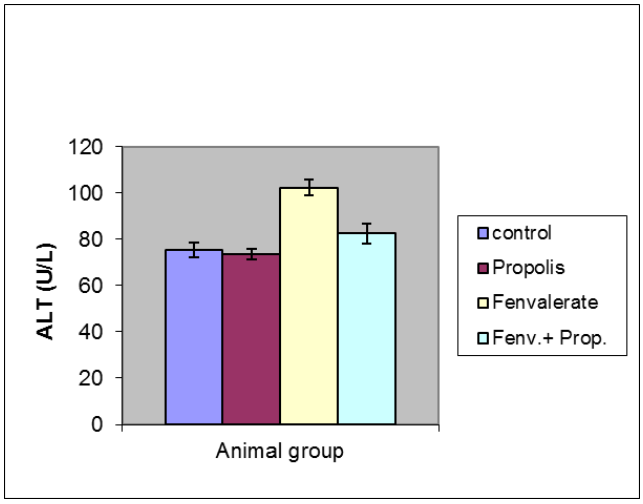

Figure 3: Change in activity of ALT in different animals group.

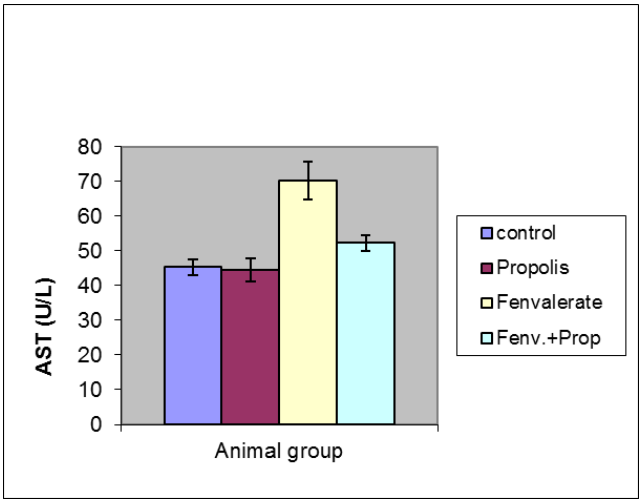

Figure 4: Change in activity of AST in different animals group.

		Treatment			
		Control	Propolis	Fenvalerate	Fenvalerate + Propolis
Parameters	MDA (u mole/g tissue)	133.5 ± 5.2	135 ± 6.3	192 ± 5.5	140.5 ± 2.3*
	SOD (Units/g tissue)	45.6 ± 2.8	44.7 ± 1.5	28.4 ± 2.5	36.5 ± 3.4*
	CAT (mole/min/g tissue)	0.33 ± 0.01	0.30 ± 0.02	0.12 ± 0.01	0.23 ± 0.01*

- Values are expressed as mean ± SD
- (*) Significant at in comparison with fenvalerate group at p<0.05

Table 1: Changes in MDA, CAT and SOD in different animals group.

Discussion

Synthetic pyrethroids account for more than 30% of insecticide use worldwide in household and agricultural application. On the other hand, exposure to these chemicals was accompanied with several toxicities. Results obtained in the present work showed that administration of fenvalerate to rats resulted in many histopathological alterations in the liver and increase in liver function enzymes, ALT and AST. In agreement with this result, Amaravathi et al. [4] has reported that treating rats with Fenvalerate caused degenerative changes in the liver, haemorrhages, mild fatty changes, infiltration of mono nuclear cells and proliferation of bile duct. Inhalation of Fenvalerate resulted in liver necrosis and fatty degeneration in rats [6]. Ali [22] has observed histopathological changes in liver of rats given Fenvalerate such as degeneration and proliferation of hepatocytes forming acinar and pseudoglandular pattern. The effect of fenvalerate on ALT and AST was recorded by many investigators. Mani et al. [6] demonstrated elevated levels of SGOT and SGPT in rats treated with Fenvalerate. Prasanthi et al. [8] reported that fenvalerate administration caused significant increases in activities of hepatic transaminases, ALP and LDH. Administration of fenvalerate to rats resulted in induction of toxicity to the liver as reflected by elevation of liver damage marker enzymes like alkaline phosphatase, aspartate transaminase, alanine transaminase, Gamma Glutamyl Transferase and lactate dehydrogenase [7].

The current results showed that fenvalerate led to an increase in the lipid peroxidation marker, MDA and decrease of the antioxidant enzymes SOD and CAT. Similarly, Waheed and Mohamed (2012) reported that the fenvalerate treated rat showed decreased activity

levels of SOD, CAT, GSHPx and GSH in the liver homogenate while the amount of lipid peroxidation was high as evidenced by increase in the level of MDA. Thus, the increase in MDA and depletion in the content of SOD and CAT may be related to the oxidative stress generated in hepatocytes of rats treated with fenvalerate.

Treating animals with fenvalerate and propolis caused an amelioration of hepatotoxicity of fenvalerate as indicated by reduction of histopathological alterations and decrease of ALT and AST. Moreover, fenvalerate and propolis-treated rats showed significant increase in the activities of SOD and CAT while the MDA content in the liver was significantly reduced compared to fenvalerate-treated rats. Several studies showed the hepatoprotective effect of propolis. The aqueous propolis extract was shown to have a protective effect on hepatocytes against carbon tetrachloride (CCl)-induced injury *in vitro* [23] and *in vivo* [24]. Kolankaya et al. [25] reported that the treatment with propolis significantly prevented the release of transaminases and significantly enhanced protein towards control, suggesting its hepatoprotective potential.

Propolis induced reduction of the increased activity of AST and ALT in plasma of rats treated with $AlCl_3$ [26]. Saleh [27] reported that Administration of aqueous propolis extract combined with octylphenol ameliorated the hepatotoxicity induced by octylphenol.

Propolis is known to have antioxidant effect and free radical scavengers. It detoxifies a variety of free radicals and reactive oxygen intermediates. The antioxidant activities of propolis and its polyphenolic flavonoid components are related to their ability to chelate metal ions and scavenge singlet oxygen, superoxide anions, proxy radicals, hydroxyl radicals and peroxynitrite [28]. The present results showed that propolis decreased lipid peroxidation possibly by its antioxidant activity. Propolis was found to modulate antioxidant enzymes and decrease lipid peroxidation processes in plasma, liver, lungs, and brain of mice in a dose- and tissue-dependent manner [29]. Luan et al. [30] reported that propolis improve lipid profile, MDA and SOD activity in mice. Propolis can also reduce the levels of ROS; such as H_2O_2 and NO, that might be responsible for its antiinflammatory effects [31]. Saleh [26] demonstrates the scavenging effect of propolis on free radicals produced by liver in response to octylphenol toxicity. Benguedouar et al. [32] reported that propolis decreased superoxide anion radicals and inhibited the lipid peroxidation in rats given doxorubicin and vinblastin. The present findings constitute evidence that the antioxidative properties of the propolis contribute to the prevention of damage induced by fenvalerate in albino rats.

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