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Effect of carbaryl on some biochemical changes in rats: The ameliorative effect of bee pollen

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ABSTRACT

In this study, 42 female Wistar albino rats, weighing between 200 and 250 g, were used and they were divided into six equal groups. Group 1 was allocated as the control group. Rats included in groups 2 and 3 were administered a water-solubilized extract of bee pollen at a dose of 50 mg/kg bw/day and 100 mg/kg bw/day, respectively. Group 4 received 225 mg/kg bw/day carbaryl. Groups 5 and 6 were given a water-solubilized extract of bee pollen at a dose of 50 mg/kg bw/day and 100 mg/kg bw/day, respectively, plus 225 mg/kg bw/day carbaryl. The indicated administrations were continued for 21 days for groups 1–6 by gavage. MDA levels and the activities of CAT, SOD and GSH-Px were analysed in blood and tissues (liver, kidney, brain and heart). At the same time, levels/activities of total protein, albumin, glucose, triglyceride, T-cholesterol, T-bilirubin, BUN, creatinine, uric acid, GGT, LDH, AST, ALT and ALP, magnesium, sodium, potassium and chloride were evaluated in serum samples. In conclusion, carbaryl was determined to cause negative changes in most of the oxidative stress markers and serum biochemical parameters investigated. These effects were observed to alleviate with the administration of bee pollen.

1. Introduction

Carbaryl is a carbamate pesticide used both in agricultural and house pest control and against ectoparasites of animals. Carbaryl exhibits a toxic effect characterized by the inhibition of the enzyme cholinesterase. Although mildly toxic for humans and domestic animals (class II), carbaryl is moderately toxic for fish, and non-toxic for poultry. The mean oral lethal dose of this insecticide for rats is 948 mg/kg. Carbaryl is toxic for honeybees (Jeleniewicz et al., 1992; Kaya, 2002; Wang et al., 2005; Mahajan et al., 2007).

Bee pollen, which is a floral type of pollen collected by honeybees, is known to be of particular essentiality for the reproduction and survival of these creatures. Pollens contain high levels of protein, amino acids, lipids, saccharin, vitamins and other compounds. These compounds bear great significance not only for the activities of honeybees but also for consumer health due to their presence in the composition of honey. Pollens are also rich in flavonoid and phenolic compounds. Their antioxidant effects are largely related to their free radical scavenging activity. The composition, which varies with botanical origin, is at the same time responsible for a high level of antioxidant activity (Sahinler, 2000; Campos et al., 2003; Arraez-Roman et al., 2007).

The present study was aimed at the evaluation of the negative effects of carbaryl, administered for a period of 21 days, based on tissue and blood analyses of certain biochemical parameters. Furthermore, the second and more important objective of the study was to determine the alleviating or curative effect of bee pollen, administered for the same period, on the possible changes in the parameters investigated.

2. Materials and methods

2.1. Animal material and drug administration

The study was carried out in 42 female Wistar albino rats, weighing 200–250 g. The animals were divided into six equal groups, one for control and five for experimental purposes. The control group received 1 ml of soy oil once per day for the entire trial period. Groups 2 and 3 were given a water-solubilized extract of bee pollen at doses of 50 and 100 mg/kg bw/day, respectively, in 1 ml volume. Group 4 was administered carbaryl at a dose of 225 mg/kg bw/day in 1 ml of soy oil. Groups 5 and 6 were given 225 mg/kg bw/day carbaryl in 1 ml soy oil plus 50 and 100 mg/kg bw/day bee pollen, respectively, in 1 ml volume. All treatments were performed by gavage and were continued for 21 days. Bee pollen was administered eight hours after carbaryl treatment in the experimental groups on each day of the trial. Drinking water and pellet feed were provided *ad libitum* throughout the study. The animals were housed under stable conditions, at 22–24 °C, and were maintained on a 12 h light/12 h dark cycle. The present study was approved by the Ethic Committee of the Faculty of Veterinary Medicine of Erciyes University.

2.2. Collection, extraction and analysis of bee pollen

Bee pollen (*Brassica napus* L., Brassicaceae) was collected by means of special traps, aimed at foraging bees, placed at the entrance of honeybee hives within the vicinity of Kayseri province in Central Anatolia. The pollen collected was first grounded, and then dried at 30 °C without exposure to light, and preserved at

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2–8 °C in desiccators, until extraction. The water-solubilized extract of pollen was prepared as described by Yamaguchi et al. (2007). The total protein analysis of the extract was performed spectrophotometrically, in compliance with the method first described by Lowry et al. (1951) and later modified by Miller (1959). The compounds of pollen were determined by the GC–MS analysis method used by Bankova et al. (2002).

2.3. Collection and processing of blood and tissues

At the end of the trial, blood specimens were collected into dry and heparin-coated tubes from the heart in all animals, under light ether anaesthesia. Subsequently, certain tissues (liver, kidney, heart and brain) of the rats were excised. The blood samples collected were centrifuged at 3000 rpm for 10 min to separate plasma and serum. Leucocytes and thrombocytes were removed from the pellet formed in heparinized tubes following centrifugation, and the remaining layer of erythrocytes was washed three times with phosphate buffered saline and diluted with an equal volume of this solution. Ice-cold deionised water was used for the haemolysis of erythrocytes and for this purpose water was added instantly to the tubes at a proportion of 1:5 (Winterbourn et al., 1975). The tissues excised were first removed from fat and other tissues and washed with deionised water, at a temperature of +4 °C, for the cleansing of blood. The tissues were homogenised using phosphate buffer (1:4) prepared beforehand with a pH fixed at 7.4. Following centrifugation at 20,000 rpm for 1 hour, the supernatant was collected. The tissue supernatant and hemolysate obtained were used for the analyses of the antioxidant enzymes investigated. Furthermore, as required for calculation, haemoglobin and protein levels were determined for the hemolysate and supernatant, respectively. Plasma was used for MDA measurements, whilst serum was used for the analyses of certain other blood biochemical parameters.

2.4. Determination of tissue protein and erythrocyte haemoglobin levels

Tissue protein levels were determined in compliance with the method described by Lowry et al. (1951) and modified by Miller (1959). Erythrocyte haemoglobin levels were ascertained as described by Fairbanks and Klee (1987). Measurements were performed spectrophotometrically. Calculations were expressed as mgHb/ml hemolysate and mg^{-1}-protein/ml homogenate (0.1 mg-protein/ml homogenate).

2.5. Measurement of oxidative stress markers and serum biochemical parameters

Plasma malondialdehyde (MDA) levels were determined as described by Yoshioka et al. (1979). Tissue MDA levels were measured in compliance with the method described by Ohkawa et al. (1978). The measurements were performed using a spectrophotometer. Calculations were expressed as nmol/ml and nmol/mg^{-1}-protein (nmol/0.1 mg-protein).

As regards the measurement of antioxidant enzyme activities in analysis materials, catalase (CAT) activity was determined as described by Luck (1965), superoxide dismutase (SOD) activity was ascertained in compliance with the method described by Sun et al. (1988) and glutathione peroxidase (GSH-Px) activity was measured according to Paglia and Valentine (1967). Calculations were expressed as U/mgHb and U/mg^{-1}-protein (U/0.1 mg-protein).

The measurement of serum biochemical parameters (T-protein, albumin, glucose, triglyceride, T-cholesterol, T-bilirubin, blood urea nitrogen (BUN), creatinine, uric acid, gamma glutamyl transpeptidase (GGT), lactate dehydrogenase (LDH), aspartate aminotransferase (AST), alanine aminotransferase (ALT), alkaline phosphatase (ALP), magnesium, sodium, potassium and chloride levels/activities) was performed using a Konelab 60i model auto-analyser and a kit of the same brand.

2.6. Statistical analysis

The SPSS 13.0 statistical software programmee for windows was used for statistical calculations. Data was given in form of arithmetical mean and ± standard deviations. Differences between groups were evaluated according to the ANOVA and Duncon test.

3. Results

The total protein content of bee pollen was determined as 74.70 mg-total protein/g pollen. Furthermore, based on the results of GC–MS analysis for pollen composition, pollen was ascertained to contain organic acid, flavonoids, esters, aldehydes, hydrocarbons, terpenes, alcohol, ketone and others (Table 1).

The comparison of the plasma and tissue (liver, kidney, heart and brain) MDA and erythrocyte and tissue SOD, CAT and GSH-Px values of the groups which were administered pollen alone at two different doses with the controls demonstrated statistically

Table 1
Chemical composition assessed by GC–MS of bee pollen.

Compounds	R.T.	TIC
Organic acids		
2-Methylheptanoic acid	15.62	5.85
Dodecanoic acid	18.83	0.71
Flavonoids		
Apigenin	20.41	0.89
Pinobanksin	34.11	0.91
Esters		
Octyl acetate	13.60	0.21
Methyl valerate	13.82	2.17
Ethyl octanoate	13.33	3.08
Ethyl nonanoate	15.13	1.99
Ethyl laurate	16.81	5.77
Aldehydes		
Heptanal	6.24	2.16
n-Decanal	13.47	0.34
Nonanal	11.41	15.13
Hydrocarbons		
n-Octane	3.45	4.38
6,8-Dioxabicyclo octane	7.37	1.62
n-Uncdecane	11.30	0.15
n-Eicosane	19.94	0.33
n-Octadecane	21.35	0.45
Pentadecane	22.69	0.09
Cyclopentasiloxane	12.53	3.62
n-Tetracosane	14.83	0.12
Cyclohexane	15.30	0.30
Benzene	18.24	0.36
Naphthalene	17.95	0.06
Terpenes		
Valencene	17.07	0.52
Farnesol	17.51	0.27
Thujopsene	17.67	0.26
Gamma-terpinene	10.36	0.45
Alpha-cubebene	16.55	0.70
Beta-caryophyllene	17.29	0.87
Alpha-pinene	7.10	1.93
Alcohol, ketone and others		
Geranylaceton-6,10-dimethylundeca-5,9-dien-2-one	17.76	0.97
Eucalyptol	9.69	7.52
Lauryl alcohol	10.85	0.36
Lauryl alcohol	16.74	0.07
1-Decanol	19.24	0.21
Dimethyl sulfide	1.49	1.47
2,4-Nonadienal	8.74	1.40
Nonanal	11.41	15.13

[a]The ion current (TIC) generated depends on the characteristics of the compound concerned and it is not a true quantitation. RT: retention time.

significant differences not to exist between the two groups (Groups 2 and 3) (Tables 2–5). The comparison of the values pertaining to the group which received carbaryl alone (Group 4) with the control group has caused significant increase in both tissue and plasma MDA levels, excluding heart tissue (Table 2). The statistically significant changes determined in antioxidant enzyme activities when compared to the controls, included decrease in the SOD activity of erythrocytes and liver, kidney and brain tissue (Table 3). While CAT activity was determined to have decreased in erythrocytes, it had increased in all tissues excluding liver tissue (Table 4). GSH-Px activity was ascertained to have decreased in liver, kidney and brain tissue, and to have increased in erythrocytes (Table 5). Values pertaining to the remaining two groups, which were administered carbaryl plus bee pollen at two different doses (Groups 5 and 6) were observed to have drawn closer to those of the control group. Statistically significant differences were determined in plasma and tissues (liver, kidney and brain) MDA levels (Table 2), erythrocyte SOD activity (Table 3) and erythrocyte, liver and kidney GSH-Px activities (Table 5) in the group which received