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Nutritional and Biochemical Parameters of Honey Contaminated with Insecticide Residues in Male Albino Rats

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Abstract: The effect of insecticides contaminating honey on nutritional and biochemical parameters were investigated in male albino rats. Administration of honey containing malathione and dimethoate at levels of 0.003±0.24 and 0.006±0.02 ppm, respectively, significantly decreased body weight of the animals. These levels also significantly decreased the weight of testis, epididymis and suprarenal gland, while it significantly increased the weight of the parenchymatous organs (heart, liver, kidney and spleen). Serum levels of triglycerides, albumin, total protein, alanine transaminase (ALT), creatinine and bilirubin were significantly increased, while there were non-significant differences in levels of cholesterol, aspartate transaminase (AST), alkaline phosphatase (AP), urea and glucose. These results suggest that further studies should be conducted on various nutritional, biochemical, physiological, hormonal and immunological parameters to confirm the adverse impact of insecticide contaminated honey on animals and humans. Moreover, intensive studies are required on the types and levels of honey flavonoids and their impact against harmful action of the insecticides. Additional studies should also be conducted on different vegetables and fruits that could be contaminated with different insecticides and other contaminants.

Key words: Honey, malathione, dimethoate and albino rats

INTRODUCTION

It is often difficult to separate the toxicities of insecticides on honey bees from toxicities on mammals. One misconception is that substances that are poisonous to livestock are also poisonous to bees. It has been fifty years since the natural poisoning of honey bees was first seriously investigated in the United States (Maurizio, 1945; 1968). Throughout history, humans have battled against other forms of life that compete for food sources or interfere with our health. This has resulted in the use of substances destined to eliminate or control these plagues. Initially, poisons with general characteristics were used, but these were gradually replaced with more specific drugs in order to reduce the harmful effects on humans and the environment (Fernandez-Muino *et al*., 2010a).

It is important to note the discovery of the insecticide properties of DDT in 1938, which began a new phase in the control of plagues through a large number of studies and the consequent appearance of synthetic chemical compounds effective against insects. This resulted in widespread use of DDT with notable positive effects in agriculture, animal husbandry and public health. Besides direct poisoning, the phenomenon of bioaccumulation was observed, which is caused by the high degree of affinity of these pesticides for adipose tissue (Tsvetkova *et al*., 2011). Due to the lipophilic nature of some pesticides, they can readily enter the food chain by accumulating in fats, such as vegetables oils and animal fat. They can also appearing water and vegetables even in the absence of direct contact. For instance, pesticides can appear in honey though the treatment of some plants. Pesticides might be introduced into honey bees through the nectar or pollen of contaminated blossoms. Pesticides and their metabolites can accumulate within the wax of the comb (Fernandez-Muino *et al*., 2010b). It is known that bees are very sensitive to toxic materials, such as malathion (Organophosphorus compounds) and dimethoate (dimethoxy compounds; category IV). These pesticides are widely used in Egyptian culture for controlling mosquitoes, flies, household insects, animal ectoparasites, human head and body lice and insects infesting vegetables and fruits. Honeybee hives are usually located near such orchards and the insecticides can be brought into the hive with nectar (Illarionova, 2007).

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This study investigated the effect of honey collected from hives located near plant orchards sprayed with insecticides on the nutritional and biochemical parameters of male albino rats fed for 4, 9 and 14 weeks.

MATERIALS AND METHODS

Experimental animals: A total of 40 adult male albino rats, weighing approximately 179.5±7.02 g, were obtained from the Animal house laboratory of the National Research Centre, Dokki, Giza, Egypt. Selected animals were assigned to four groups (10 rats/group). The animals were housed in stainless steel wire mesh cages on a bedding of wood chips (one animal/cage). They were kept in an ambient temperature of 25±3°C on a light/dark cycle of 12/12 h. Animals received basal diet *ad libitum* formulated from natural ingredients to meet nutrient requirements (Table 1) according to the formulation diets of the National Research Council (1987).

Sample collection: The animals were anesthetized by deep ether and then sacrificed. Orchiectomy was performed using an open castration method. A pre-scrotal incision was made and the testicles were removed through the incision site and weighed on an OHAUS electric weighing balance. The testicles were exposed by incising the tunica vaginalis. The spermatic cord was exposed, ligated and incised. Semen samples were then collected from the caudal epididymis. The samples was analyzed immediately after collection.

Sperm count and motility assay: Immediately after dissection, the epididymal content was placed on a glass slide and viewed under the light microscope to enumerate motile and non-motile sperm cells. The motile and non-motile sperm cells were distinguished by movement (WHO, 1996). The spermatozoa was counted using a hemocytometer on the improved Neubauer (Deep 1/10 mm, LABART, Germany) chamber as described by Pant and Srivastava (2003).

Experimental design: The animals were divided into 4 groups as follows:

1: Group I (control): Fed the basal diet+2 cc of the blank honey samples daily through by oral gavage
2: Group II: Fed the basal diet+2 cc honey daily by oral gavage for 4 weeks
3: Group III: Fed the basal diet+2 cc honey daily by oral gavage for 9 weeks
4: Group IV: Fed the basal diet+2 cc honey daily by oral gavage for 12 weeks

Individual rats from each group as well as control rats were sacrificed on the next day of the experimental period to determine the tested parameters.

Table 1: Composition of the basal diet

Ingredients/nutrient composition	Percentage
Corn yellow	52.5
Soy bean meal	27.0
Poultry by-products	03.0
Blood meal	03.0
Fish meal	03.0
Animal fat	04.0
Lime stone	00.5
Bone meal	01.0
Wheat bran	03.5
Methionine	00.4
Sodium chloride	00.6
Vitamin and mineral mixture*	00.5
Crude protein	17.88
Energy (ME/kg)	3315
Crude fiber	05.50
Ether extract	06.97
Lysin	00.72
Methionine	00.89
Calcium	02.22
Phosphorus	01.43

*Vitamin and mineral mixture:

Vit. A: 5000 IU	Ca-pantothionate: 18 mg
Vit. D_3: 1500 IU	Nicotinamide: 39 mg
Vit. E: 45 mg	Folic acid: 1.00 mg
Vit. K_3: 1.5 mg	Manganese: 1700 mg
Vit. C: 75 mg	Zinc: 1400 mg
Vit B_1: 1.5 mg	Iron: 150 mg
Vit. B_2: 4.5 mg	Copper: 600 mg
Vit. B_6: 3.0 mg	Selenium: 20 mg
Vit B_{12}: 0.015 mg	Iodine: 40 mg
Biotin: 0.04 mg	

Test articles

Honey: Honey samples (100 g each) were randomly collected from different locations of El-kalubia Governorate. Sub-samples were taken from the original samples (10 g each) to determine the existence of any residues of either dimethoate or malathione using the analytical method described by El-Nabrawy and Carey (1988).

Dimethoate: Standard material 99.5% O, O-dimethyl S-(N-methyl/carbamoil methyl).

Malathion: Standard material 99.5% O, O-dimethyl phosphorothionate of diethyl.

Preparation of the standard solution: A stock solution of each insecticide was prepared by adding a specific weight of the standard material in n-hexane to obtain a stock solution of 100 µg/ml. This solution was serially diluted with the same solvent to obtain working solution of 1 ng/µl to be injected into the gas chromatography apparatus equipped with a flame photometric detector to detect the retention time.

Analytical method

Extraction of insecticide residues: Each 100 g honey sample was dissolved in 10 ml of water. The sample

was then mixed with 50 ml acidic acetone for 2 min and evaporated using a rotary evaporator in a 45°C water bath until the sample was free of any acetone traces. The samples were then transferred into a 250 ml separating funnel using 50 ml petroleum ether. The funnel was shaken and vented for two minutes. The ether layer was then removed and placed into a new 250 ml conical flask. The extract was repeated using another 50 ml of petroleum ether. The extract was then evaporated using the rotary evaporator in a 45°C water bath. The residue was finally dissolved in 10 ml n-hexane and kept in a deep freezer until clean up.

Clean-up: Clean-up was performed using a chromatographic column (30 x 1 cm). The column was backed using 0.5 cm glass wool activated florist 60-100 mesh and 2 g sodium sulphate anhydrous. This step was completed as described by the method adopted by El-Nabrawy and Carey (1988).

Residues determination: All cleaned samples were dissolved in a specific volume of n-hexane and injected in a gas chromatography apparatus (HP 5890) equipped with a flame photometric detector and capillary column (HP 101). All program equipment conditions followed those described by El-Nabrawy and Abou-Donia (2012).

Recovery studies: Blank honey samples were fortified by adding a specific amount of the insecticide standard material to obtain concentrations ranging between 0.1-1.0 ppm. They were then processed through all of the steps of the analytical method to validate the assay procedure.

Parameters studied

Body and internal organs weight: The weights of individual animals were recorded weekly. Internal organs were placed in saline after sacrificing the animals and weighed.

Biochemical parameters: On the 5, 10 and 15th weeks of the experiment, blood samples were collected from the orbital plexus into suitable clean dry centrifuge tubes, incubated for 30 min to ensure complete clotting and then centrifuged for 10 min at 300 RPM. The separated serum was stored for subsequent analysis. The following biochemical parameters were studied; cholesterol, albumin, total protein, AST, ALT, alkaline phosphatase (AP), urea, Creatinine, Bilirubin and glucose using commercial diagnostic kits (Bio-Merieux, France).

RESULTS AND DISCUSSION

The effect of feeding honey contaminated with insecticides on male albino rats was investigated. We found that the average malathione and dimethoate recovery rates were 90.0 and 91.5, respectively (Table 2). These results validate the procedure used in this study. The concentration of malathione and dimethoate residue in honey ranged from 0.003 to 0.24 ppm and 0.003 to 0.02 ppm, respectively. The lowest amount of insecticide that could be detected by the apparatus was 0.001 ppm. These results are in agreement with those of El-Nabrawy (2012) who found that honey samples were contaminated by malathione and dimethoate at levels ranging from 0.24 to 0.49 ppm and 0.005 to 0.024 ppm, respectively. Furthermore, El-Nabrawy (2012) demonstrated that honey samples collected from El-Gharbia Governorate were contaminated with chlorpyrifos and sumothion residue. The permissible limits for these compounds in honey are currently unknown (Federal Register, 2014). Indeed, honey should be free from any contaminant or insecticides residues. It is well known that therapeutic value of honey necessitates the absence of any insecticide residues due to the harmful effect on humans, which in turn decreases its economics value. Pesticides may be brought to the hives through bees that feed on the nectar when the crops are sprayed or when crops are sprayed that, although not flowering, contain numerous flowering weeds. El-Nabrawy and Carey (1988) found that honey from a study in the US was contaminated with chlorothalonil and its related metabolites. Moreover, Bigazzi-Grasso and Capri (2013) detected DDT residue in honey from Italy (28.4 to 1018 μg/kg of honey). In addition, Dizilinski and Szymanowska (2010) detected ppDDT, ppDDE and ppDDD residues in honey from Poland with traces of Lindan in some samples. Tsvetkova *et al*. (2011) detected trichlorfon and dichlorvos in 8 samples, HCH in 7 samples and DDE in 7 samples from a total of 40 samples obtained in Bulgaria (2-6 μg/kg of honey). In 2013, Serra Bonhevi (2005) and Fernandez-Muino *et al*. (2010a,b) from Spain carried out a similar study on 25 honey samples. They detected Lindan (0.07-0.12 μg/kg), Aldrin (0.05-0.07 μg/kg), Dieldrin (0.05-0.1 μg/kg) and Endrin (0.005 μg/kg). Moreover, in a study by Rexillius (2006) of 56 honey samples from Germany, vinclozoline was detected at concentrations of 0.5-62 μg/kg in 50 samples, dialiphos in 41 samples at levels of 2.92 μg/kg, Methoxchlor in 6 samples at concentrations of 2-40 μg/kg and endosulfan in 4 samples at levels of 7 μg/kg.

We found significant differences in average and total body weight gain between treated and control groups in this study (Table 4). This result confirmed the pesticide contamination in honey and underscores the physiological impact, which could be due to stimulation of the paraventricular and supra optic nuclei in the hypothalamus thus leading to a decrease in appetite and consequently in body weight gain (Pizzi *et al*., 2007). We identified a significant increase in the weight of the

Table 2: Percentage of dimethoate and malathione recovered in honey

Added ppm	Dimethoate	Malathione
1.0	93.0	97.0
0.5	91.0	93.0
0.1	86.0	85.0
Average	90.0	91.5

Table 3: Residues (ppm) of dimethoate and malathione in honey

Sample number	Dimethoate	Malathione
1	0.000	0.000
2	0.020	0.200
3	0.100	0.015
4	0.006	0.240
5	0.005	0.013
6	0.005	0.003
Blank	0.000	0.000

parenchymatous organ, liver (0.01), kidney (0.05) and heart (0.05) in group II, which consisted of animals sacrificed during the 5th week of the experimental period. The spleen weight in this group significantly increased in a time dependent manner. This result indicates that the administration of honey containing insecticide resulted in a disturbance in the metabolic state, which led to catabolism despite the apparent gain in body weight in some treated groups. It appears that the decrease in body weight gain with concomitant increase in serum lipids (triglycerides and cholesterol) and decrease in internal organ weight suggest a decrease in muscle mass and increase in body fat. Our results are in agreement with those by Lohiya *et al.* (2012) who showed that body weight was adversely affected in male albino rats that received honey contaminated with insecticides.

This study found no significant increase in blood glucose levels (Table 6) in rats fed honey containing insecticides compared to controls. The increase in serum glucose may be a result of enhanced gluconeogenesis and tissue catabolism derived from disturbances in thyroid gland status (non-significant increase in thyroid gland weight), which plays a major role in carbohydrate metabolism. We also found no significant difference in the weight of the prostate and seminal vesicles glands, while there was a significant decrease in the weight of testis, epididymis and supra-renal gland. These results were in agreement with the results of Lohiya *et al.* (2012) who reported that oral administration of honey contaminated accidentally with insecticides led to a significant decrease in cauda epididymal sperm motility, testis mass and sperm count of male albino rats. However, our results are not in agreement with those of Chinoy *et al.* (2007), who demonstrated that there was no change in body weight and reproductive organ weight of male albino rats receiving 20 mg/kg honey contaminated with insecticide for 30 days. Moreover, the same authors reported no apparent histochemical changes in the liver and kidney. Serum cholesterol, AST and ALT AST levels were also unaltered.

Our biochemical data clearly showed no significant differences in levels of serum cholesterol, AST, AP and urea. Therefore, the low levels of pesticides found in honey might not affect these parameters or the flavonoid content of honey may overcome the adverse effect of these pesticides on those biochemical parameters (Siess *et al.*, 2006). Furthermore, Plochberger (2009) reported that no clear differences could be identified in AST, ALT and AP levels in chicks fed honey experimentally contaminated with pesticides. Chinoy *et al.* (2007) also found no apparent changes in serum cholesterol, SGOT and SGPT levels in female albino rats receiving 20 mg/kg of contaminated honey with insecticides for 30 days. The same authors noted no changes in the liver or kidney of these rats by histochemical analysis. Murphy (2007) noted that a metabolite of malathione and certain impurities in technical malathione inhibit the hydrolysis of malathione in rats and this hydrolysis is relatively less important as a detoxification mechanism in rats. Moreover, dimethoate is rapidly excreted from the body, where by 50. 5 of ingested dimethoate is excreted through urine and 25% in the feces in the first 24 h after ingestion (Sanderson and Edson, 2006). Nine days after dosing, only 0.9-1.1% of the dimethoate remained in the animal body. In general, insecticides becomes more toxic when administered in association with a diet severely deficient in protein, but the diet provided in our study had a sufficient amount of protein according to established guidelines.

Our study found significant increases in serum triglycerides in rats receiving honey containing insecticides ($p<0.01$), with a significant increase in serum levels of albumin, total protein, creatinine and bilirubin ($p<0.001$). The higher level was recorded for group II followed by group III and group IV, respectively. It is easy to notice that these parameters increase in an independent manner, increase time and dosage of honey administration, improve the levels of these biochemical parameters. The explanation may be due to the influence of honey flavonoids against the adverse effect of the insecticides present (Siess *et al.*, 2006). Furthermore, a single dose of 58 mg (0.84 mg/kg) of honey containing malathione produced no clinical effect in humans and 23. 5 of it was recovered from the urine in the form of organic phosphorus (Mattson and Sdlok, 2006). The adverse effect of honey containing insecticides on albumin, total protein and bilirubin might be due to the excretion of the insecticides via liver and feces. Moreover, its adverse effect on creatinine levels may be due to the excretion of more than 50 5 of the insecticides through the kidney and urine. Based on

Table 4: Body weight of male albino rats fed contaminated honey with insecticides

	G-I	G-II	G-III	G-IV
Zero time	173.55±6.75	180.17±8.42	189.2±11.6**	175.25±7.41
1st week	184.25±7.15	190.17±8.82	197.6±12.82*	177.50±7.0*
2nd week	193.78±6.88	198.70±10.6	204.2±14.48	181.00±7.79*
3rd week	203.55±7.21	205.33±13.77	212.2±15.79	183.25±7.85**
4th week	210.64±6.78	211.83±20.93	216.2±13.33	190.25±9.88
5th week	217.57±6.65		223.2±12.68	194.75±12.95
6th week	225.25±6.71		230.8±16.21	199.00±14.00
7th week	232.35±6.59		239.2±19.01	199.50±15.07
8th week	240.51±7.14		246.4±21.30	204.50±16.5
9th week	247.75±6.84		250.4±20.07	206.75±18.93
10th week	254.54±7.42			214.25±19.41
11th week	260.25±6.93			218.75±20.76
12th week	267.38±7.23			228.30±22.48
13th week	272.54±7.90			228.75±22.44
14th week	279.97±7.73			230.00±23.24
Total body gain	106.42±2.35	31.66±3.44**	61.2±4.15**	54.75±3.55**
Average daily body gain	1.086	1.131	0.97	0.559

Table 5: Internal organs weight of male albino rats fed honey contaminated with insecticides (mean±SD)

	G-I	G-II	G-III	G-IV
Testis	1.0210±0.0207	0.9115±0.022*	0.891±0.0403*	0.893±0.0305*
Epididymis	0.3410±0.0066	0.291±0.006**	0.288±0.006**	0.292±0.006**
Vas-deference	0.0598±0.0018	0.072±0.002**	0.0655±0.0042	0.0616±0.0039
Prostate gland	0.2101±0.0069	0.2042±0.0091	0.2056±0.0073	0.2104±0.0070
Seminal vesicles	0.4164±0.0315	0.3223±0.0656	0.3785±0.0592	0.3696±0.0421
Supra-renal	0.0118±0.0017	00.070±0.0005*	0.0088±0.0007	0.0109±0.0012
Thyroid gland	0.2951±0.0085	0.3217±0.0097	0.3068±0.0078	0.2969±0.0081
Heart	0.3991±0.0325	0.6175±0.051*	0.4343±0.0388	0.4118±0.0319
Spleen	0.2028±0.0135	0.570±0.0242**	0.358±0.021**	0.2325±0.0222
Kidney	0.7225±0.0316	0.9115±0.056*	0.7400±0.0356	0.7217±0.0324
Liver	6.1225±0.2075	8.094±0.236**	6.5063±0.2596	6.4545±0.1119

*Significant, p>0.05, **Significant, p>0.01

Table 6: Biochemical parameters of male albino rats fed honey contaminated with insecticides (mean±SD)

	G-I	G-II	G-III	G-IV
Triglycerides (mg/dl)	36.5±3.36	71.72±4.58*	59.75±4.49*	52.65±3.54*
Cholesterol (mg/dl)	29.7±9.35	65.71±6.24	91.86±9.53	95.75±5.35
Albumin (g/dl)	3.35±0.21	3.99±0.28**	2.78±0.34**	2.66±0.24**
Total protein (g/dl)	7.64±0.62	7.59±0.58**	6.96±0.55**	6.84±0.57**
AST (U/L)	66.5±7.66	156.5±2.65	132.5±1.29	110.5±1.65
ALT (U/L)	25.6±1.85	51.60±1.62**	46.30±1.51**	39.6±1.71**
Alk. Phos. (U/L)	83.4±10.5	177.5±9.67	159.7±8.56	155.5±8.55
Urea (mg/dl)	30.7±3.26	32.32±1.59	32.14±2.61	32.45±1.38
Creatinine (mg/dl)	0.36±0.15	0.55±0.16**	0.490±0.13**	0.41±0.11**
Bilirubin (mg/dl)	0.32±0.03	0.45±0.03**	0.410±0.02**	0.37±0.04**
Glucose (mg/dl)	76.7±8.50	93.5±6.67	85.80±5.65	81.7±5.44

*Significant, p>0.05, **Significant, p>0.01

these results, it can be concluded that honey contaminated with insecticides has a slightly harmful effect on the carbohydrate (6.52-21.9%), cholesterol (121.25-22.39%), triglycerides (44.25-96.5%) and protein (0.66-10.47%) metabolism as well as liver and kidney function. Further research studies are needed to assess the effect of contaminated honey with insecticides on other nutritional, biochemical, hormonal and immune parameters of various animal models.

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