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Therapeutic effects of olive leaves extract on rats treated with a sublethal concentration of carbendazim

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Abstract. – *Objectives:* **The present study was carried out to establish the possible protective effects of administration of olive leaves extract on carbendazim induced physiological and histopathological alterations in male rats.**

Materials and Methods: **The experimental rats were divided randomly into five groups and kept at ten rats per group. The first group was untreated and served as a control. The second group was orally administered with carbendazim (200 mg/kg) for one month. The third group was supplemented with olive leaves extract and exposed to carbendazim at the same dose given to the second group. Rats of the fourth group were supplemented with olive leaves extract at the same dose given to the third group. Rats of the fifth group were supplemented with only corn oil.**

Results: **Carbendazim induced statistically declines in the values of red blood corpuscles (RBC) count, hemoglobin (Hb) concentration, hematocrit (Hct) and the level of plasma and liver total protein, while the value of white blood cells (WBC) count, the levels of plasma glucose, triglycerides, cholesterol, creatinine, alanine aminotransferase (ALT), aspartate aminotransferase (AST), and liver glycogen and total lipid were elevated. Moreover, after one month of carbendazim exposure, there were severe changes in the structures of liver, kidney and testis. Pretreatment of carbendazim-exposed rats with olive leaves extract showed marked improvement in both physiological and histopathological alterations.**

Conclusions: **We conclude that olive leaves extract is a promising chemotherapeutic agent for reducing the toxicity of carbendazim and may be for other pesticides and toxicants.**

Key Words:

Carbendazim, Olive leaves, Physiological parameters, Histopathology, Rats.

Introduction

The pollution of the environment plays a crucial role in the occurrence of many diseases affecting plants, animals and man. One of the main factors causing pollution of the environment is the irrational use of pesticides[1]. Pesticides differ from any other chemical substances because they are deliberately spread into the environment. As a consequence, a great part of the human population may be exposed either in the general environment or in the working settings. While the environmental exposure, involving general population is mainly due to the ingestion of the contaminated foods, water and respiratory route[2]. Recently, the pesticides' problem has been in the focus of public interest. While the usage of pesticides is still the most effective and accepted means to protect plants from the pests and to increase productivity. The wide spread of pesticides is connected with serious problems of pollution and health hazards. All pesticides have the potential to be harmful to humans, animals, other living organisms, and the environment if used incorrectly. Carbamates, all derived from the basic structure of carbamic acid (NH_2COOH), represent a broad variety of compounds, which have a number of applications. They are widely applied as insecticides, herbicides, and fungicides. Carbendazim ($C_9H_9N_3O_2$) also known as methyl-2-benzimidazole carbamate (MBC) is one of the most widely used agricultural systemic fungicides. It is a primary metabolite of degradation of the broad-spectrum fungicide benomyl [methyl 1-(butylcarbamoyl)2-benzimidazol carbamate]. This compound is used as a preservative in paint, textile, paper and leather industry as well as a

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preservative of fruits[3]. Carbendazim is well absorbed (80-85%) after oral exposure and is subsequently metabolized into many compounds within the organism. The main metabolites are 5-hydroxy-2-benzimidazole carbamate (5-HBC) and 5,6-hydroxy-2-benzimidazole carbamate-*N*-oxides (5,6-HOBC-*N*-oxides). Carbendazim has low acute toxicity. The LD_{50} values range from >2000 to 15,000 mg/kg in a wide variety of animals and routes of administration[4]. However, carbendazim and its metabolites are poorly catabolized and are retained in tissues[4]. Several reproductive and developmental toxicity studies have been conducted where rats, mice and hamsters were administered carbendazim or benomyl with single or repeated doses[5-8]. Affected male fertility (reduced sperm count), embryotoxicity and malformations (exencephalia, hydrocephalia, microphthalmia, fused ribs, etc.) were observed at doses somewhat lower than those inducing paternal toxicity. Additionally, different studies showed that carbendazim administration caused several physiological and histopathological alterations in experimental animals[9-11].

Nature has been a source of medicinal treatments for thousands of years, and plant-derived products continue to play an essential role in the primary health care of about 80-85% of the world's population. Despite the trends of molecular biology and chemistry providing fast escalation of synthesized *de novo* drugs, plants still remain a traditional source of medicinal compounds; up to 40% of modern drugs may directly or indirectly be related to natural compounds[12]. Recent years have witnessed a renewed interest in plants as pharmaceuticals. This interest has been focused not only on the discovery of new biologically active molecules by the pharmaceutical industry, but also on the adoption of crude extracts of plants, such as infusions, for self-medication by the general public[13]. Within this context, considerable interest has arisen in the possibility that the impact of several diseases may be either ameliorated or prevented by improving the dietary intake of natural nutrients with antioxidant properties, such as vitamin E, vitamin C, β-carotene and plant phenolics such as tannins and flavonoids[14].

The olive tree (*Olea europaea* L.), family: *Oleaceae*, and in particular, its leaves have been used for the treatment of wounds, fever, diabetes, gout, atherosclerosis and hypertension since ancient times[15]. Olive leaf has been traditionally used for centuries to prevent and treat different diseases. It is used to enhance the immune system, in heart disease and as an antimicrobial agent. Folk medicine uses also include hypertonia, arteriosclerosis, rheumatism, gout, diabetes mellitus, and fever[16], and the most known feature of olive leaf is cardioprotection. Experimental animal studies on different total olive leaf extract or their constituents have demonstrated hypoglycemic[17-19], hypotensive[20,21], antiarrhythmic[22], anti-atherosclerotic[23], and vasodilator effects[24]. Antimicrobial[25-28], antiviral[29,30], anti-tumor[31,32] and anti-inflammatory activity[33] were also reported. Moreover, antihypertensive and cholesterol-lowering actions of olive leaf extract EFLAR®943 were confirmed in a clinical study[34].

This study is, therefore, aimed at providing information on the effect of administration of olive leaves extract on male rats exposed to a sublethal concentration of carbendazim. Physiological parameters, including the values of red blood cells (RBC) count, hemoglobin (Hb) concentration, hematocrit (Hct), white blood cells (WBC) count, blood glucose, total protein, triglycerides, cholesterol, creatinine, alanine aminotransferase (ALT), aspartate aminotransferase (AST), liver glycogen, total protein and total lipid were chosen as specific indicators of animal response to the experimental treatments. Furthermore, the histopathological alterations of liver, kidney and testis were evaluated.

Materials and Methods

Animals

Male rats (*Rattus norvegicus*) weighing 218-234 g were selected for experimentation. The rats were obtained from the Experimental Animal Unit of King Fahd Medical Research Center, King Abdul Aziz University, Jeddah, Saudi Arabia. Animals were housed at an ambient temperature of 20±1°C with a 12 h light/12 h dark cycle. Standard diet, commercial feed pellets, and tap water were freely available. The procedures used in the present study are approved by Animal Ethics Committee of King Abdul Aziz University.

Olive Leaves Extraction

Fine quality of olive leaves, 10% oleuropein, (General Nutrition Corporation, Pittsburgh, PA, USA) were used for preparation of an aqueous extract. Ten grams of olive leaves were added to

one liter cold water and mixed in an electric mixer for 15 min. The mixture was centrifuged and the clear supernatant was carefully removed and kept in a refrigerator as final extract for experimentation.

Experimental Design

Fifty rats were used in this experiment and divided randomly into five groups, ten animals for each group. Rats of group 1 were untreated and served as control. Rats of group 2 were orally administrated with 200 mg/kg of carbendazim (Sigma-Aldrich Corp., St. Louis, MO, USA) in 0.5 mL of corn oil, daily for one month. Animals of group 3 were orally given 1 mL of olive leaves extract and after 3 h treated with carbendazim at the same dose given to group 2, daily for one month. Experimental rats of group 4 were exposed to olive leaves extract at the same doses given to group 3, daily for one month. Rats of group 5 were orally supplemented with 0.5 mL of corn oil, daily for one month. At the end of the experimental period, rats were fasted for 6 h, anaesthetized using diethyl ether and blood samples were collected from orbital venous plexus in vacuum tubes containing EDTA (K_3) as anticoagulants. RBC count, Hb concentration, Hct and WBC count were estimated using ADVIA Hematology Automatic System, (USA). Furthermore, blood samples were centrifuged at 2000 rpm for 10 min for plasma separation. Plasma glucose, total protein, triglycerides, cholesterol, creatinine, alanine aminotransferase (ALT) and aspartate aminotransferase (AST) were assessed using an automatic analyzer (Architect *c*8000 Clinical Chemistry System, USA). After blood draining, the liver samples from each group were taken and homogenized. The homogenates were centrifuged at 2000 rpm for 15 min. The clear supernatants were carefully removed and kept frozen till the time of biochemical estimations of glycogen, total protein and total lipid. Liver glycogen was measured using the method of Barnes et al[35]. The method of Gornall et al[36] was carried out to measure the level of total protein. Liver total lipid was estimated according to the method of Entenman[37]. For histopathological examinations, liver, kidney and testis sections were taken from all groups. The tissues were fixed in 10% neutral formalin, dehydrated with different ethanol solutions and embedded in paraffin, then cut into 4 μ thick sections, stained with hematoxylin-eosin and observed under a photomicroscope.

Statistical Analysis

Data are reported as the means ± standard error (SE). Differences between means were evaluated by one-way analysis of variance (ANOVA). Statistical significance of the differences between the means was assessed by Student's *t*-test. The level of significance was set at $P<0.05$.

Results

As it is shown in Table I, RBC count, Hb concentration, Hct value and the level of plasma total protein were significantly decreased in rats treated with only carbendazim (group 2). Furthermore, the WBC count and the levels of plasma glucose, triglycerides, cholesterol, creatinine, ALT and AST were statistically increased in comparison with control and other treated groups. In rats exposed to olive leaves extract and carbendazim (group 3), the WBC count and the levels of plasma glucose, cholesterol, creatinine, ALT and AST were elevated significantly compared to control, olive leaves extract (group 4) and corn oil (group 5) treated rats. The value of plasma triglycerides was increased in olive leaves extract plus carbendazim treated rats compared with groups 4 and 5. Moreover, RBC count, Hb concentration, Hct value and the level of plasma total protein were statistically unchanged in rats supplemented with olive leaves extract and exposed to carbendazim. Insignificant changes of hematological and plasma biochemical parameters were observed in rats treated with olive leaves extract (group 4) or corn oil (group 5) compared to control group. The levels of liver glycogen and total lipid were statistically increased, while the level of liver total protein was significantly declined in rats exposed to only carbendazim compared to control and other treated groups. There was no significant difference in mean values of liver glycogen, total protein and total lipid in rats exposed to olive leaves extract plus carbendazim (group 3), olive leaves extract (group 4) or corn oil (group 5) compared to control group (Table II).

Normal liver sections from control (untreated) rats showed a radial arrangement of hepatocytes around the central vein along with Kupffer cells (Figure 1A). Liver sections from carbendazim treated rats showed tissues damage characterized by disarrangement of hepatic strands, an enlargement of the sinusoids, vacuoles formation, dila-

Table I. The values of RBC count, Hb concentration, Hct, WBC count, plasma glucose, total protein, triglycerides, cholesterol, creatinine, ALT and AST of control, carbendazim, olive leaves extract plus carbendazim, olive leaves extract and corn oil treated rats after one month.

Parameters	Treatments				
	Control	Carbendazim	Leaves extract+ carbendazim	Leaves extract	Corn oil
RBC (10^6/mm^3)	8.08 ± 0.08	6.97 ± 0.17[ab]	7.61 ± 0.20	8.05 ± 0.14	8.18 ± 0.16
Hb (g/dL)	15.30 ± 0.20	12.84 ± 0.34[ab]	15.44 ± 0.23	15.62 ± 0.14	15.84 ± 0.19
Hct (%)	39.94 ± 0.65	36.72 ± 1.02[ab]	39.34 ± 0.65	39.42 ± 0.66	39.62 ± 0.46
WBC (10^3/mm^3)	11.78 ± 0.32	14.28 ± 0.71[ab]	13.88 ± .64[ac]	11.66 ± 0.19	11.80 ± 0.18
Glucose (mg/dL)	98.61 ± 1.50	186.4 ± 7.59[ab]	115.00 ± 4.44[ac]	100.40 ± 1.69	101.2 ± 4.13
Total protein (g/dL)	6.04 ± 0.14	5.40 ± 0.13[ab]	6.02 ± 0.12	6.14 ± 0.10	6.20 ± 0.20
Triglycerides (mg/dL)	64.86 ± 1.45	104.48 ± 3.16[ab]	74.76 ± 4.12[c]	63.64 ± 1.61	66.08 ± 0.49
Cholesterol (mg/dL)	84.16 ± 1.42	199.80 ± 6.88[ab]	124.86 ± 13.26[ac]	82.20 ± 2.28	87.70 ± 2.39
Creatinine (mg/dL)	0.68 ± 0.03	1.36 ± 0.07[ab]	0.79 ± 0.02[ac]	0.66 ± 0.03	0.65 ± 0.02
ALT (U/L)	45.80 ± 1.93	223.80 ± 8.41[ab]	89.80 ± 6.69[ac]	48.80 ± 1.86	46.40 ± 1.72
AST (U/L)	67.60 ± 0.93	279.80 ± 11.04[ab]	133.60 ± 11.83[ac]	66.60 ± 1.12	64.40 ± 3.19

Data represent the means ± SE of 5 animals per group. [a]Indicates a significant difference between control and treated groups. [b]Indicates a significant difference between rats exposed to carbendazim and olive leaves extract plus carbendazim, olive leaves extract or corn oil. [C]Indicates a significant difference between rats treated with olive leaves extract plus carbendazim and olive leaves extract or corn oil.

tion and congestion of blood vessels with hemorrhage (Figures 1B-F). Administration of olive leaves extract before carbendazim exposure declines the histopathological alterations which evidencing by mild disarrangement of hepatic strands, sinusoids enlargement and an absence of congestion of blood vessels with hemorrhage (Figures 1G-I). Liver sections of olive leaves extract treated rats (group 4) showed a normal structure (Figure 1J). Normal histology of kidney in control rats was shown in Figure 2A and 2B. Figure 2A showed a typical structure of renal cortex and medulla. Figure 2B showed the normal structure of renal (Malpighian) corpuscle. After one month of carbendazim exposure, there were several alterations in the structure of kidney including disarrangement of renal cortex and medulla tissues with severe congestion of blood vessels with hemorrhage (Figures 2C and 2D) and abnormal structure of renal corpuscles, which appearing a highly degeneration of glomeruli and Bowman's capsules (Figures 2E and 2F). Pretreatment of experimental animals with olive leaves extract showed an obviously protection in carbendazim induced kidney damage. Histopathological findings in this group showed that as compare to control animals, the renal cortex and medulla showed a normal struc-

Table II. The values of liver glycogen, total protein and total lipid of control, carbendazim, olive leaves extract plus carbendazim, olive leaves extract and corn oil treated rats after one month.

Parameters	Treatments				
	Control	Carbendazim	Leaves extract+ carbendazim	Leaves extract	Corn oil
Glycogen (mg/g)	10.58 ± 0.34	13.08 ± 0.52[ab]	10.54 ± 0.38	10.28 ± 0.35	10.90 ± 0.34
Total protein (mg/g)	217.06 ± 2.54	190.42 ± 7.21[ab]	219.68 ± 2.16	215.10 ± 2.36	212.04 ± 4.38
Total lipid (mg/g)	118.26 ± 3.52	167.24 ± 7.08[ab]	120.90± 3.18	122.16 ± 2.28	121.32 ± 6.19

Data represent the means ± SE of 5 animals per group. [a]Indicates a significant difference between control and treated groups. [b]Indicates a significant difference between rats exposed to carbendazim and olive leaves extract plus carbendazim, olive leaves extract or corn oil.

ture with mild hemorrhage (Figure 2G) and restoring normal renal corpuscle structure (Figure 2H). Olive leaves extract treated animals showed normal kidney, renal cortex and medulla (Figure 2I) and renal corpuscle (Figure 2J) histology. Normal histology of the testis in control rats was shown in Figures 3A and 3B. Seminiferous tubules appear as rounded or oval structures, each surrounded by a thin basal membrane and contains in its wall several layers of cell representing spermatogonia, primary spermatocytes, secondary spermatocytes, spermatids and spermatozoa, which are connected to cells of Sertoli. The intertubular tissue, which is formed of connective tissue holding the seminiferous tubules with each other and contains blood vessels. It also contains Leydig (interstitial) cells, cells of endocrine secretion. In comparison with the control

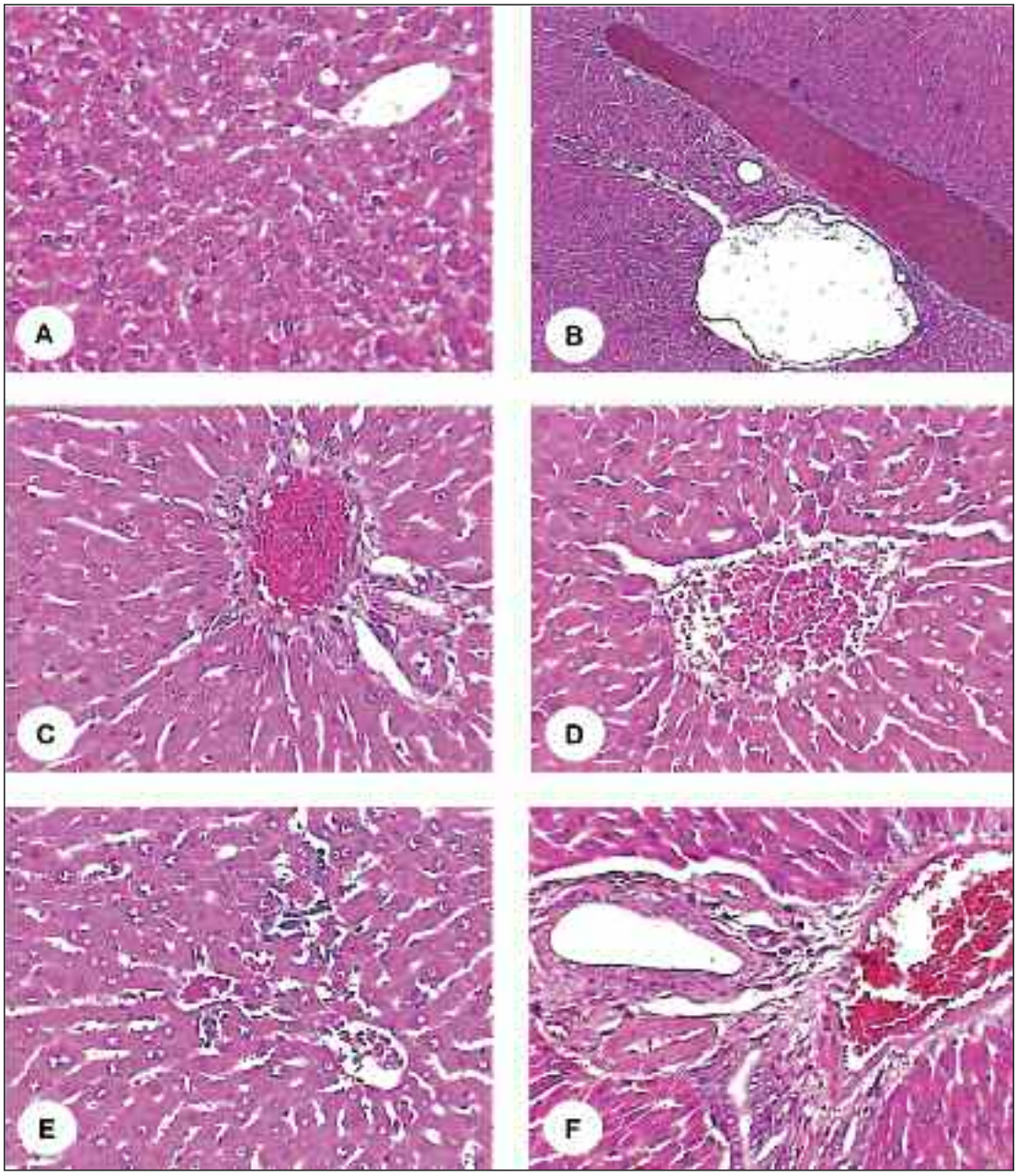

Figure 1. ***(A-F)*** Liver histopathological alterations. Representative microscopic photographs. Normal liver structure of control rats (A, ×400). Carbendazim treated rats (B, ×100; C-F, ×400). Olive leaves extract plus carbendazim treated rats (G-I, ×400). Olive leaves extract treated rats (J, ×400).

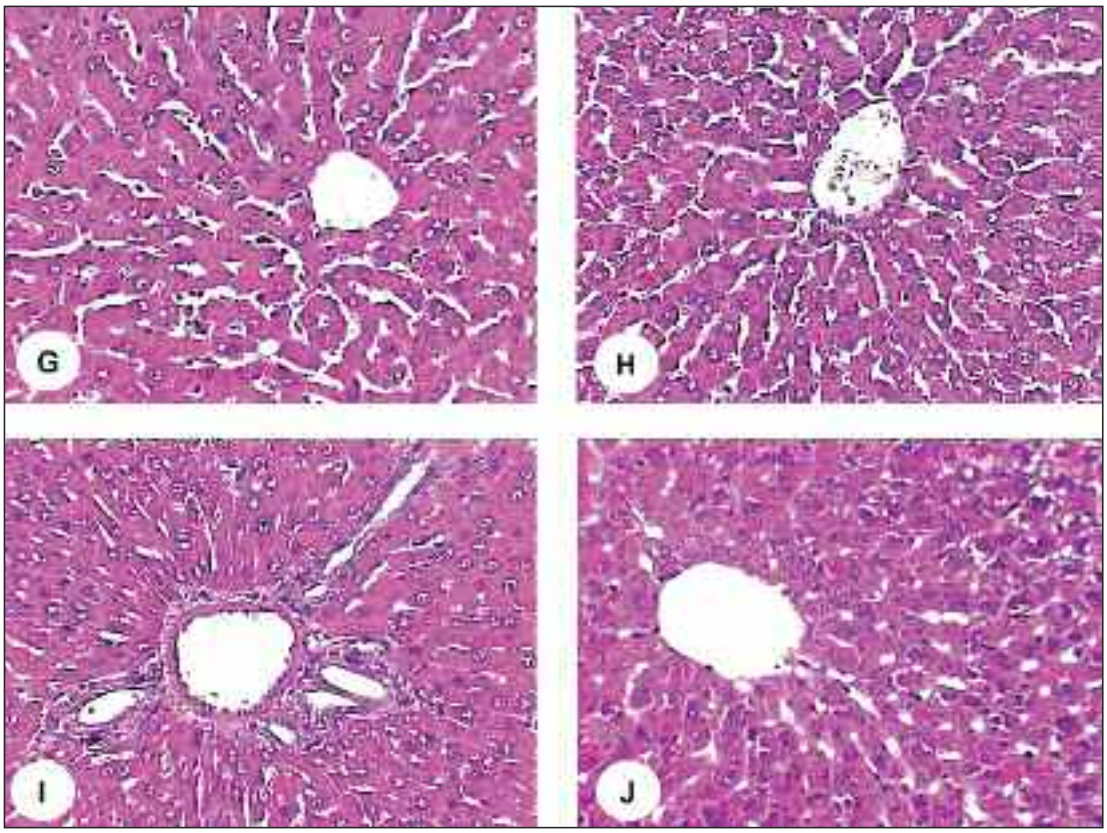

Figure 1. *(G-J)*

group, the findings of testis histopathological examination showed that treatment with carbendazim for one month resulted in severe damage and completely absences of spermatogonia, primary spermatocytes, secondary spermatocytes, spermatids and spermatozoa and losses of the spermatogenesis process (Figures 3C and 3D). Supplementation with olive leaves extract showed a marked recovery from severe damages, the absences of spermatogonia, primary spermatocytes, secondary spermatocytes, spermatids and spermatozoa; and losses of spermatogenesis process (Figures 3E and 3F). Furthermore, some of seminiferous tubules in this group (Figures 3G and 3H) showed similar histological structure which observed in rats treated with only carbendazim. Figures 3I and 3J represented the normal structure of the testis and its seminiferous tubules in rats supplemented with only olive leaves extract (group 4). It is worth mentioning that the histological structures of liver, kidney and testis (not included) in rats treated with corn oil (group 5) showed normal appearance compared to control group.

Discussion

The widespread environmental pollution caused by the chemical substances such as pesticides is a serious problem for creatures, including humans38-40. Various chemical substances entering animal bodies are carried to the organs responsible for detoxification, such as liver and kidney, and excreted. On the other hand, some hydrophobic substances among these chemicals are accumulated in our body[41]. As seen in the present study, the values of RBC count, Hb concentration and Hct were statistically decreased in rats treated with only carbendazim. This may be due to the influence of carbendazim on blood-forming organs suggesting the anemic condition of the treated animals. These findings are consistent with previous studies which indicated that treatment with carbendazim caused a significant decrease in RBC count, Hb concentration and Hct value[3,10,11]. The anemia may be due to the inhibition of erythropoiesis and hemosynthesis and to an increase in the rate of RBC destruction in hemopoietic organs. In general anemia, reduction in the number

of RBC or of Hb in the blood can reflect an impaired synthesis of Hb, e.g. in iron deficiency, or impaired production of RBC, e.g. in folic acid or vitamin B_{12} deficiency[42]. Anemia, defined clinically as a decrease in Hct or Hb concentration, may be caused by blood less, excessive hemolysis, or deficient erythropoiesis[43]. Jee et al[44] studied the decline in RBC count, Hb concentration and Hct presumably reflects erythrocyte hemolysis and due to either an increase in the rate at which Hb concentration may be destroyed or a decrease in the Hb synthesis. Decrease in Hct is attributable to the reduction in RBC count caused either destruction or reduction in size.

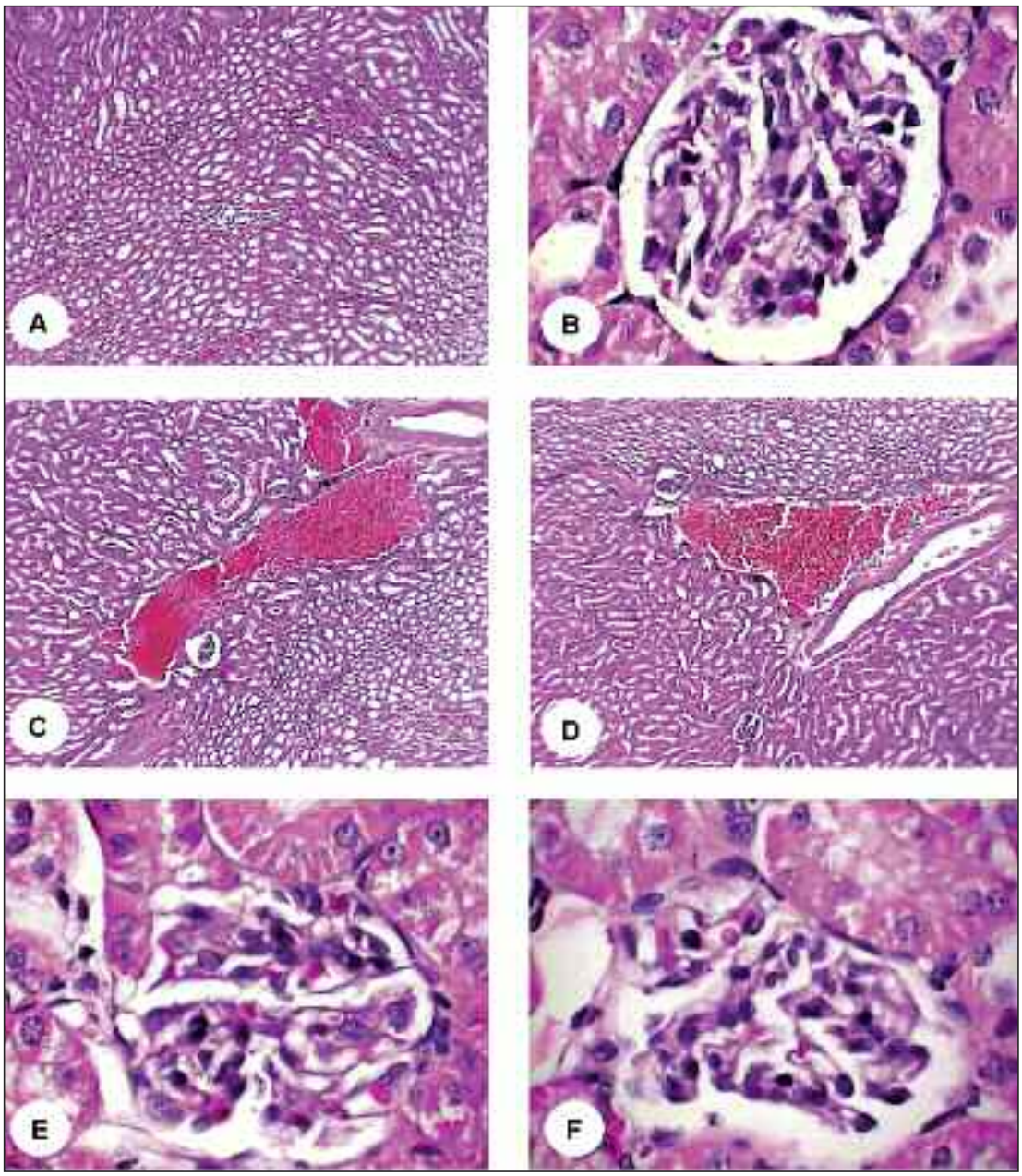

Figure 2. ***(A-F)*** Kidney histopathological alterations. Representative microscopic photographs. Normal renal cortex and medulla (A ×100) and renal corpuscle (B ×1000) structures of control rats. Renal cortex and medulla (C and D, ×100) and renal corpuscles structures (E and F, ×1000) of carbendazim treated rats. Renal cortex and medulla (G, ×100) and renal corpuscle (H, ×1000) structures in rats treated with olive leaves extract plus carbendazim. Renal cortex and medulla (I, ×100) and renal corpuscle (J, ×1000) in rats supplemented with only olive leaves extract.

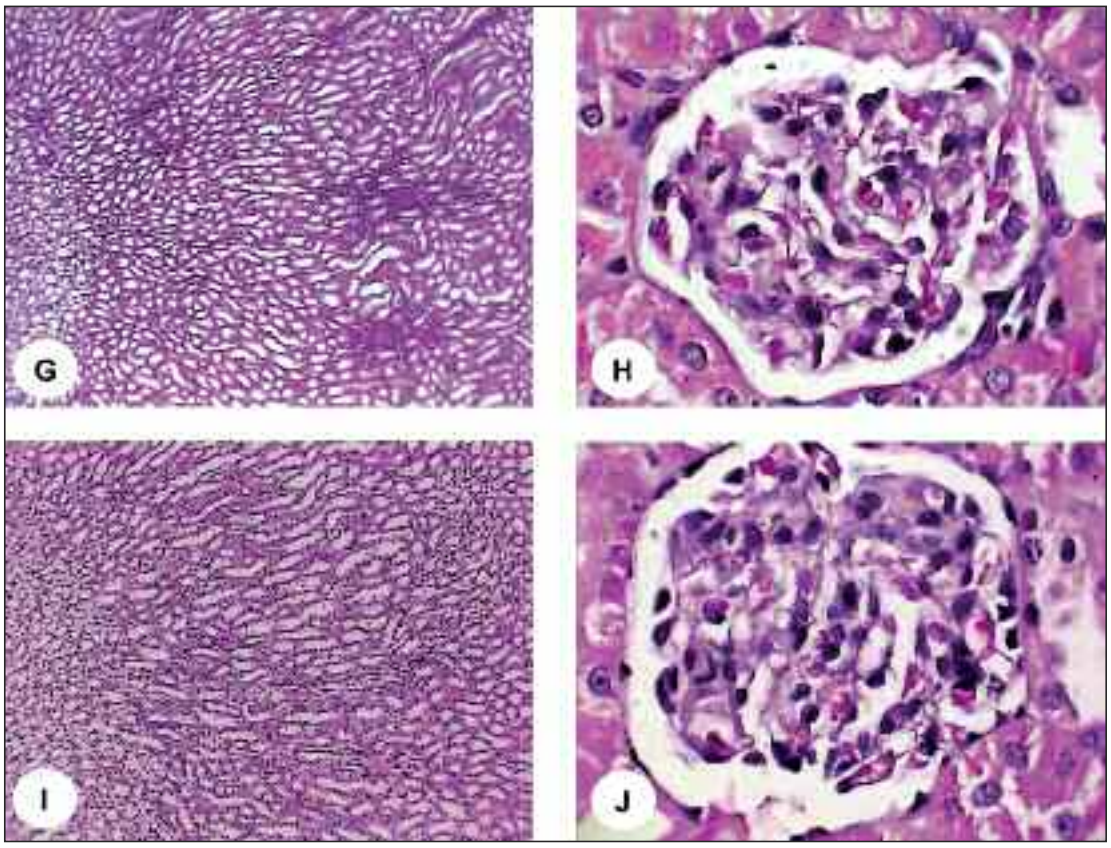

Figure 2. *(G-J)*

The present enhancement of WBC count following carbendazim intoxication could be possible due to leucocytosis as leucocytosis is an outcome of proliferation of hemopoietic cells leading to progressive infiltration in peripheral blood[45]. Additionally, Muthuviveganandavel et al[46] reported that the mean Hb, WBC, eosinophil, and platelet counts increased and total RBC, neutrophil and lymphocyte counts decreased in rats exposed to carbendazim.

Administering carbendazim to rats resulted in a statistically significant increase of plasma glucose and liver glycogen levels. The progressive accumulation of plasma glucose revealed that rats exposed to a sublethal concentration of carbendazim became hyperglycemic. The hyperglycemia observed in the present results might have been due to the lesser availability of insulin. However, this result indicates that the administration of carbendazim caused a disturbance in metabolism of glucose in the liver by inhibiting glycogenolysis and stimulating gluconeogenesis. Furthermore, with regards to the fact that carbendazim has increased both the plasma glucose level and liver glycogen content, it could be suggested that carbendazim prevented the influx of glucose into the cells of peripheral tissues. Disturbed glycogen metabolism and glucose transport have been suggested as a cause of insulin resistance. Thus any chemical factor with potential to alter hepatic glycogenolysis or gluconeogenesis might have a significant influence on glucose hemostasis. Increased blood glucose concentration results from an imbalance between the hepatic output of glucose and the peripheral uptake of the sugar[47]. Moreover, several studies showed that the levels of blood glucose were elevated in experimental animals treated with carbendazim and other pesticides[3,46,48-50].

Carbendazim intoxication caused increases in the levels of plasma triglycerides and cholesterol and liver total lipid. Similarly, several investigations showed that the values of blood triglycerides and cholesterol were evoked in carbendazim and other pesticides-treated experimental animals[3,46,51-53]. A number of hepatotoxic agents also cause accumulation of fatty deposits, predomi-

nantly triglycerides in the parenchyma cells in the liver. This accumulation of triglycerides may be as a result of an imbalance between the rate of synthesis and the rate of release of triglycerides by the parenchyma cells into the systemic circulation[54]. Additionally, Muthuviveganandavel et al[46] stated that cholesterol level increased in the serum due to liver and kidney damage by carbendazim exposure in rats. The present statistically decrease of plasma and liver total protein levels in rats exposed to carbendazim reflected a disturbance of protein metabolism.

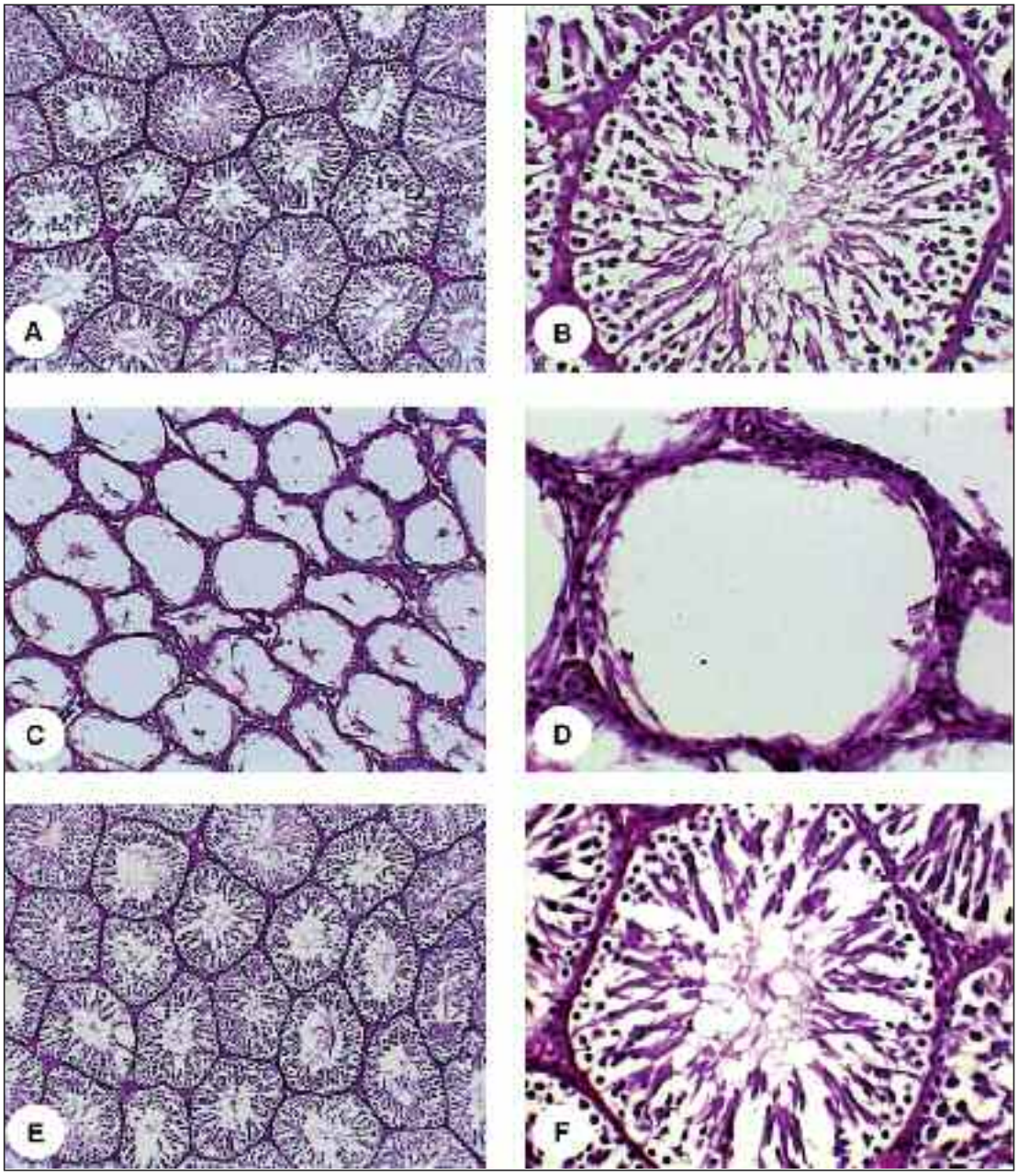

Figure 3. ***(A-F)*** Testis histopathological alterations. Representative microscopic photographs. Normal seminiferous tubules structure with normal spermatogenesis process (A, ×100 and B, ×400) in control rats. Seminiferous tubules structure with an absence of spermatogenesis process in carbendazim treated rats (C, X100 and D, ×400). Seminiferous tubules structure with normal spermatogenesis process in olive leaves extract plus carbendazim treated rats (E, ×100 and F, X400). Seminiferous tubules structure with some absences of spermatogenesis process in olive leaves extract plus carbendazim treated rats (G, ×100 and H, ×400). Normal seminiferous tubules structure with normal spermatogenesis process (I, ×100 and J, ×400) in rats supplemented with only olive leaves extract.

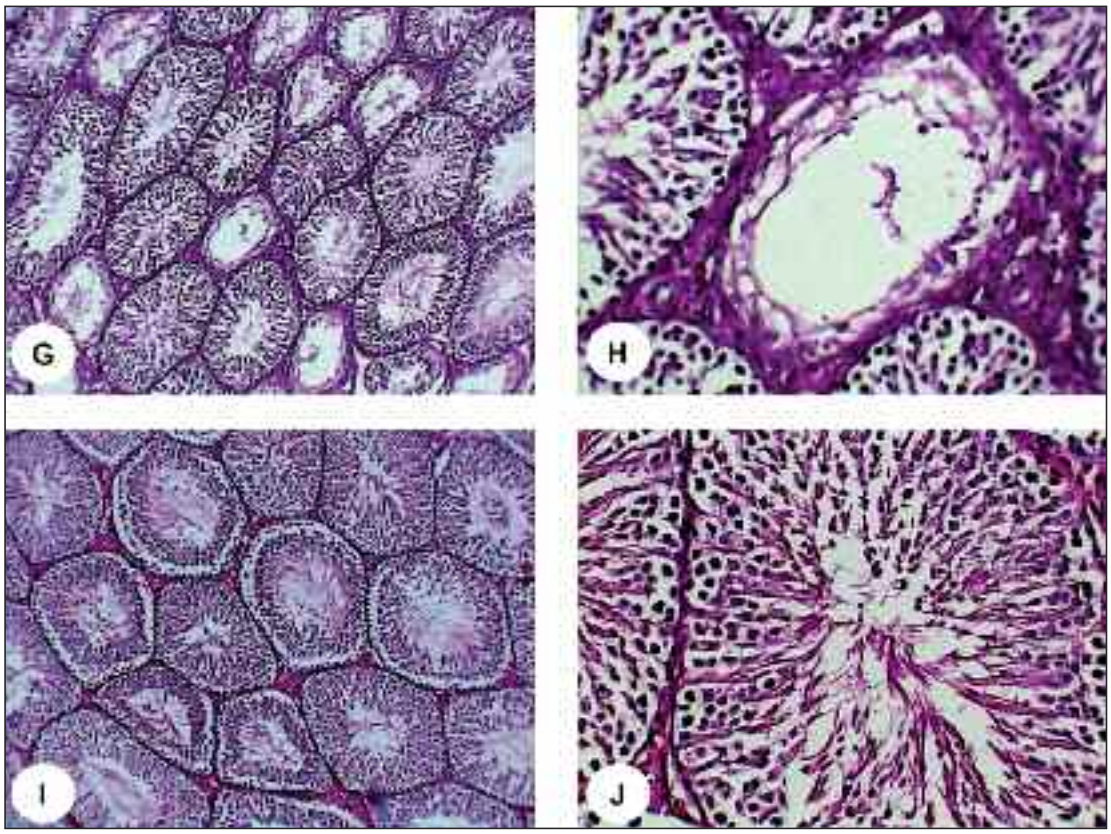

Figure 3. *(G-J)*

In the study of Muthuviveganandavel et al[46], they showed that the levels of serum total protein and albumin were decreased in carbendazim-treated rats, and they suggested that the decrease in protein content could be due to a decrease in the rate of protein synthesis.

The levels of plasma creatinine, ALT and AST increased in rats treated with carbendazim, the same trend was seen with carbendazim and other pesticides[3,11,46,50,53,55-57]. A disorder of kidney function reduces excretion of creatinine, resulting in increased blood creatinine levels. Thus, creatinine levels give an approximation of the glomerular filtration rate. However, it is known that increase of creatinine occurred with renal failure[58].

The noticed increase in the levels of ALT and AST as well as the decrease in the levels of total protein in the serum, are the major diagnostic symptoms of liver diseases[59]. Furthermore, serum or plasma enzyme levels have been used as markers for monitoring chemically induced tissue damages[60-62]. The enzymes ALT and AST are important enzymes that are often employed in assessing liver injury[61-63]. Moreover, the present findings were confirmed by the histopathological alterations of kidney and liver in rats treated with carbendazim for one month.

Carbendazim induced many histopathological changes in the liver, kidney and testis. The liver of carbendazim-treated rats showed disarrangement of hepatic strands, an enlargement of the sinusoids, vacuoles formation, dilation and congestion of blood vessels with hemorrhage. There were several alterations in the structure of kidney, including disarrangement of renal cortex and medulla tissues with severe congestion of blood vessels with hemorrhage and abnormal structure of renal corpuscles, which appearing a highly degeneration of glomeruli and Bowman's capsules. Furthermore, the treatment with carbendazim for one month resulted in severe damage of testis and completely absences of spermatogonia, primary spermatocytes, secondary spermatocytes, spermatids and spermatozoa and losses of spermatogenesis process.

These results were in line with previous investigations, which studied the toxic effects of carbendazim and other pesticides[3,9-11,56,64-68]. Gray et

al[64] stated that carbendazim treatment markedly altered sperm morphology, testicular and epididymal weights, and sperm numbers and testicular histology. Fertility, sperm motility, and hormonal levels were altered, primarily in the males with very low sperm counts. Yu et al[68] showed that the fertility index was decreased, and the testis weight, the sperm counts and motility were also decreased. The levels of luteinizing hormone (LH) showed a decreasing tendency and there was a statistical difference between the carbendazim-treated rats' group and the control group. There were no obvious effects on the levels of follicle stimulating hormone (FSH) and testosterone (T). Histopathological evaluation showed atrophic seminiferous tubules, decreased germ cells, and increased sloughing of germ cells. Flow cytometric analysis of the testicular tissue revealed that carbendazim inhibited meiotic transformation and interfered with the spermatogenic process. Barlas et al[9] reported that the administration of carbendazim resulted in a decrease in serum testosterone and dihydrotestosterone hormones levels, and histopathological damage in the testis. The mechanism of action for carbendazim and other benzimidazoles is well defined and involves the binding to tubulin and subsequent inhibition of microtubular function[69-71]. Moreover, Lu et al[72] suggested that carbendazim is an endocrine disruptor, which elicits its reproductive and development toxicity through pathways involving androgen- and androgen receptor-dependent mechanisms. The spermatogenesis in mammals depends on testosterone production by Leydig's cells in response to stimulation by FSH and LH. FSH increases Sertoli cell synthesis of an androgen binding protein needed to maintain high concentrations of testosterone. LH stimulates testosterone production by the interstitial cells of the testis[73]. The effect on germ cells might be due to the decrease in testosterone level resulting due to injury of the cells of Leydig[74]. However, carbendazim is also known as an inhibitor of DNA synthesis[75].

Furthermore, carbendazim and related benzimidazoles show marked reproductive toxicity and endocrine-disrupting activity in rats[76]. Cytochrome P450 (CYP) is the primary enzyme system in metabolism and is responsible for the metabolism of drugs, carcinogens, steroid hormones, and environmental pollutants. CYP genes are markedly responsive to the stimulatory and inhibitory effects of xenobiotics and provide a powerful tool to investigate gene-environment interaction. Fu[76] investigated the ability of carbendazim to modulate CYP-dependent monooxygenases and antioxidant enzymes in rat liver, kidney, lung, and testis. Treatment of male rats with carbendazim decreased testis spermatid density dose-dependently.

In liver microsomes, the carbendazim treatment increased P450 content, NADPH-cytochrome c reductase, 7-ethoxyresorufin O-deethylase (EROD), methoxyresorufin O-demethylase (MROD), pentoxyrsorufin O-dealkylase, and 7-ethoxycoumarin O-deethylase (ECOD) activities. In kidney microsomes, carbendazim increased P450 content and EROD and ECOD activities. In lung microsomes, the treated increased EROD activity.

In testis microsomes, the treatment increased NADPH-cytochrome c reductase activity.

Furthermore, carbendazim increased glutathione S-transferase (GST) and catalase activities in liver cytosol and GST and superoxide dismutase (SOD) activities in testis cytosol. The fungicide decreased glutathione content in the kidneys and lipid peroxidation in the testes. Additionally, he reported that the results of immunoblot and RT-PCR analyses showed that carbendazim induced CYP1A1/2 and CYP2B proteins and mRNA in the liver and CYP1A1 protein and mRNA in kidneys and lungs.

The obtained results showed that the pretreatment of rats with olive leaves extract improved the hematological, biochemical and histopathological alterations induced by carbendazim intoxication. This indicated the effectiveness of olive leaves extract in prevention of carbendazim toxicity. The main constituent of the olive leaves is oleuropein, one of iridoide monoterpenes, which is thought to be responsible for pharmacological effects. Furthermore, the olive leaves contain triterpenes (oleanolic and maslinic acid), flavonoides (e.g., luteolin, apigenine, rutin), and chalcones (olivin, olivin-diglucoside)[16,34,77]. It is its chemical content that makes olive leaf one of the most potent natural antioxidants. Oleuropein has high antioxidant activity *in vitro*, comparable to a hydrosoluble analog of tocopherol[78], as do other constituents of olive leaf[79]. It was shown that total olive leaves extract had antioxidant activity higher than vitamin C and vitamin E, due to the synergy between flavonoids, oleuropeosides and substituted phenols[80]. Concerning the mechanism action of carbendazim toxicity and the antioxidant activity of olive leaf constituents and their general pharmacological properties, especially oleuropein.

We suggest that olive leaves extract exert its ameliorative effect against carbendazim-induced the hematological, biochemical and histopathological alterations by preventing the decline of antioxidant defense system and direct free radical scavenging activity.

In conclusion, this is the first study on the therapeutic effects of olive leaves extract supplementation in rats exposed to carbendazim. The present results suggest that it is worthwhile carrying out further investigations to find out whether olive leaves extract and its isolated active constituents could be a supplement, as a therapeutic agent and may be beneficial for preventing the physiological and histopathological alterations induced by carbendazim and other toxicants.

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