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Clinicopathological and Cytogenetic Studies on the Ameliorative Effect of Propolis Against Profenofos Toxicity in Rats

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Abstract: Propolis, a resinous wax-like beehive product has been used as a traditional remedy for various diseases due to a variety of biological activities of this folk medicine. The concept of this study was to evaluate the ameliorative effect of propolis against profenofos induced oxidative damage in male rat. Administration of profenofos at a dose of 23 mg/kg b.wt. induced a significant increase in hepatic, renal and testicular malondialdehyde (MDA) accompanied with a significant decrease in serum total antioxidant capacity (TAC). Significant hematological changes such as anemia and leucopenia were observed. Profenofos altered various biochemical parameters. It increased serum transferase enzymes (AST and ALT) activity, glucose and cholesterol levels, while it induced a significant reduction in the total protein and serum testosterone levels. A significant increase in the percentage of sperm morphological abnormalities was observed. A significant increase in the percentage of structural and numerical chromosomal aberrations was recorded. Profenofos also induced histopathological alterations in parenchymatous organs. Concomitant administration of propolis with profenofos alleviated the induced alterations. The obtained results indicate that propolis is a valuable exogenous cytoprotective and antigenotoxic agent.

Key words: Propolis · Profenofos · Cytogenetics · Antioxidants · Sperm Morphology

INTRODUCTION

Organophosphorus pesticides are large group of pesticides which are widely used for a variety of agricultural and public health applications [1]. They are used for preventing, destroying, repelling or mitigating pests. Although there are benefits to the use of pesticides, they have a great influence on environmental pollution and their residues in feed stuffs cause health hazard in man and animals.

Exposure to organophosphorus pesticides is known to produce variety of biochemical changes, some of which may be responsible for the adverse biological effects reported in man and animals. It could induce adverse effects on the immune system, liver, pancreas, muscles, kidney, urinary and reproductive systems [2]. Moreover, it increases the incidence of chromosomal aberrations and micronuclei in bone marrow cells [3].

It has been reported that organophosphorus pesticides beside their inhibitory effect on acetylcholinestrase (AChE) [4], also induce oxidative stress by generating reactive oxygen species which may cause considerable cell damage [5]. Oxidative stress is a consequence of imbalance between the body antioxidant system and pro-oxidant state generated by pesticide toxicity. Endogenous enzymatic and non-enzymatic antioxidants are essential for the conversion of reactive oxygen species to harmless metabolites as well as to protect and restore normal cellular metabolism and functions [6].

Profenofos is a broad spectrum organophosphate pesticide. It is one of the most extensively used pesticides for protecting agricultural crops in Egypt [7]. Treatment of agricultural crops by profenofos leaves residues on crops and also contaminates surface water. The presence of these residues in water and food presents a potential hazard owing to its high mammalian toxicity. Profenofos can induce oxidative stress which may be earlier diagnostic index in case of poisoning [8].

In the recent years, extensive research work has been focused on the use of natural materials as antioxidants against the toxic oxidative materials to ameliorate their toxic and cell damaging effects.

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Propolis (bee glue) is a resinous hive product collected by honeybees from various plant sources. It is a popular folk medicine possessing a broad spectrum of biological activities [9]. It has recently gained popularity as a healthy food in various parts of the world because it promotes health and prevents diseases [10]. Propolis contains more than 300 components including phenolic aldehydes, polyphenol, sequiterpene quinines coumarins, steroids, amino acids and inorganic compounds [11]. Propolis was suggested to have potent antioxidant activity. It saves vitamin C, maintains cellular glutathione (GSH) and conserves the integrity of biomembranes [12].

The present work aimed to evaluate the ameliorative effect of propolis against oxidative damage induced by organophosphorus pesticide profenofos. The work attempts to assess the protective effect of propolis on hematological and biochemical parameters, reproductive efficiency, histopathological changes as well as its antimutagenic effect.

MATERIALS AND METHODS

Chemicals

Pesticide: Celtal: Common name:Profenofos, Polycron, Prothiofos. CAS No. 41198-08-7.

Propolis (BEE-GLUE): Propolis samples were obtained from E Katatba, Alex., Egypt. The resinous material was kept in a dark bag in the refrigerator till being extracted.

Animals: The present investigation was carried out on sixty apparently healthy male albino rats weighing 120-150g. Rats were obtained from the National Institute of Ophthamology, housed in metal cages, fed on basal diet and watered *ad-libitum*. They were left for two weeks for acclimatization before starting the experiment.

Experimental Groups: Rats were divided into (6) equal groups as follow:-Group (1) was orally administered dimethyl sulfoxide (DMSO). Group (2) was orally administered propolis at a dose of (12mg/kg b.wt.) [13]. Group (3) was orally administered propolis at a dose of (100mg/kg b.wt.) [14]. Group (4) was orally administered profenofos at a dose of (23mg/kg b.wt.) [15]. Group (5) was orally administered profenofos at a dose of (23mg/kg b.wt.) plus propolis at a dose of (12mg/kg b.wt.). Group (6) was orally administered profenofos at a dose of (23mg/kg b.wt.) plus propolis at a dose of (100mg/kg b.wt.). All treatments were daily given (5 days/week) for 2 successive months.

Extraction of Propolis: One hundred grams of crude propolis was cut into small pieces and extracted at room temperature with 50 ml of (70%) ethanol (twice with 24 hours interval). The alcoholic extract was evaporated under vacuum at 5°C until dryness. The ethanolic extract from 100 gram propolis was 66 gram and was suspended in Dimethyl sulfoxide (DMSO) to obtain 10% stock solution [16].

Collection and Preparation of Samples

Blood Samples: At the end of experimental period two blood samples were taken from retro-orbital venous plexus of each rat. The first sample was collected into vials containing (EDTA) for hematological examination. The second one was collected into plain centrifuge tubes which further subdivided into two parts. The first part was centrifuged at (2000) rpm for 10 min for separation of serum which was used for serum biochemical studies and testosterone assay. The second part was centrifuged at (4000 rpm) for 15min at (4°C) using cooling centrifuge for separation of serum to be used for determination of total antioxidant capacity.

Semen Samples: Semen samples were collected by cutting the tail of the epididymis and squeezing it into a clean watch glass for evaluation of abnormal sperm morphology.

Bone Marrow Samples: Bone marrow samples were collected from each rat of each group (5 rats/ group) by exposing the femur and evacuating the bone marrow with syringe containing saline; pressing it into test tube containing 5ml saline for cytogenetic analysis.

Tissue Specimens: Liver, kidneys and testes were taken from the sacrificed rats of all experimental groups. Specimens were divided into two portions, the first one was immediately washed with phosphate buffered saline (PBS) containing 16mg/ml heparin, dried on filter paper and were kept in deep freezer-80 C for subsequent determination of lipid peroxidation. The second portion (Liver, kidneys, spleen and testes) was fixed in 10 % neutral buffered formalin and was used for the histopathological study.

Hematological Studies: Hemogram was done according to Feldman *et al.* [17].

Oxidant-Antioxidant Studies

Determination of Lipid Peroixdation (Malondialdehyde MDA) Level: Colorimetric determination of MDA level was done according to Satoh [18]. In this reaction, thiobarbituric acid (TBA) reacts with malondialdehyde (MDA) in acidic medium at a temperature of 95°C for 30 min with frequent shaking to form thiobarbituric acid reactive substance (TBARs). The absorbance of the resultant pink product is measured at 534 nm.

Determination of Total Antioxidant Capacity (TAC): The determination of the antioxidant capacity was performed according to Koracevic and Koracevic [19]. In this reaction, the antioxidant in the sample reacts with a defined amount of exogenously provided hydrogen peroxide (H_2O_2). The antioxidant in the sample eliminates a certain amounts of the provided H_2O_2. The residual H_2O_2 is determined calorimetrically by an enzymatic reaction which involves the conversion of 3, 5, dichloro-2-hydroxy benzensulphonate to colored product

Serum Biochemical Studies: Serum samples were used for determination of the following parameters; glucose was performed according to Trinder [20] and cholesterol according to *Watson* [21]. The activities of alanine (ALT) and aspartate (AST) amino transferases were performed according to Reitman and Frankel [22]. Total proteins were determined according to Weicheselbaun [23], albumin after Dumas and Biggs [24]. Serum globulin was calculated by subtracting the obtained values of albumin from values of total proteins. Blood urea nitrogen was estimated after Patton and Grauch [25] and creatinine according to Fabiny and Eringhausen [26]. Serum biochemical parameters were assayed using commercial diagnostic kits supplied by Stanbio-Laboratory, USA.

Total Testosterone Concentration: Total testosterone concentration was determined in the serum using radioimmunoassay (RIA) technique described by Johnson [27].

Sperm Morphological Abnormalities: Percentages and forms of sperm abnormalities were recorded as described by Barth and Oko [28] through examination of stained smear with modified eosin negrosine stain.

Cytogenetic Studies: Technique used for chromosome preparation from bone marrow cells was that of Brusick [29] with certain modification recommended by Mac Gregor and Varley [30] and Adler [31].

Histopathological Studies: Tissue specimens were obtained from liver, kidney, testes and spleen. The specimens were fixed in 10% neutral formalin solution and embedded in paraffin. Sections of 4-6 micron thickness were stained by hematoxylin and eosin for histopathological studies [32].

Statistical Analysis: The statistical analysis was computed using analysis of variance procedure described by Snedecor and Cochran [33]. The significant mean differences between treatments means were separated by Duncan's Multiple Range Test [34].

RESULTS AND DISSCUSSION

The organophosphorus, profenofos is a broad-spectrum organophosphate insecticide used widely for agricultural and household purposes. It is reported to be highly toxic to human, animals and aquatic organisms [35]. The present study was conducted to investigate the possible effect of propolis in alleviating the Profenofos toxicity in rats.

Mean values of the erythrogram; packed cell volume (PCV%), hemoglobin concentration (Hb), erythrocytes' count (RBCs), mean corpuscular volume (MCV) and mean corpuscular hemoglobin concentration (MCHC) of different experimental groups are illustrated in table (1). The obtained data revealed significant decrease in erythrocytic count, PCV% and Hb concentration with developing of macrocytic hypochromic anemia in the group of rats administered profenofos for two successive months. The observed anemia may be attributed to the interference of profenofos with Hb biosynthesis [36] and decreasing the life span of circulating erythrocytes [37]. This effect stimulates the production of reticulocytes by bone marrow, which is probably the cause of the occurrence of macrocytic hypochromic red cells. On the other hand, both groups of rats administered profenofos in combination with propolis showed a significant improvement of RBCs count, PCV% and Hb concentration in comparable to profenofos administered group. Propolis induced extensive proliferation of hematopoietic cells in the spleen and bone marrow [38]. Moreover, it improves the digestive utilization of iron and increases the regeneration efficiency of hemoglobin especially during recovery from an anemic syndrome [39]. In addition, the high content of flavonoids in propolis improves the expression level of erythropoietin hormone (EPO) and accelerates the generation of erythrocyte and hemoglobin [40].

Table 1: Effect of oral administration of profenofos, propolis and their combinations for two successive months on erythrogram of different experimental groups of rats

Groups	RBCs(X10^6 /µl)	PCV(%)	Hb(g/dL)	MCV(fl)	MCHC (%)
G1	7.16±0.63^{ab}	40.00±1.22^{a}	13.32±0.41^{bc}	56.10 ± 3.38^{bc}	33.30±0.05^{b}
G2	7.54±0.38^{a}	39.80±0.45^{a}	14.06±0.19^{a}	52.88± 2.43^{d}	35.33±0.33^{a}
G3	7.40±0.44^{a}	40.00±1.22^{a}	13.58±0.43^{ab}	54.14± 1.89^{cd}	33.95±0.57^{ab}
G4	6.06±0.19^{d}	37.80±0.45 ^{b}	11.62±0.41^{d}	62.42 ± 2.04^{a}	30.75±1.33^{c}
G5	6.62 ± 0.22^{c}	39.20±0.45 ^{a}	12.89±0.51 ^{c}	59.25 ± 1.24^{be}	32.87 ± 1.28^{b}
G6	6.68±0.29^{bc}	39.60±0.55^{a}	12.97±0.69^{bc}	59. 38± 2.88^{ae}	32.75 ± 1.87^{b}

Values represent means ± SD

$^{a-e}$ Values with different letters at the same column are significantly different at p< 0.05.

Table 2: Effect of oral administration of profenofos, propolis and their combinations for two successive months on leukogram of different experimental groups of rats

Groups	TLC(×10^3/µl)	Neutr.(×10^3/µl)	Lymph (×10^3/µl)	Monocyte(×10^3/µl)	Esino.(×10^3/µl)
G1	13.32 ± 0.78^{ab}	2.94 ± 0.19^{a}	9.75 ± 0.50^{a}	0.29 ± 0.02^{bc}	0.34 ± 0.02^{ab}
G2	13.74 ± 0.89^{a}	2.40 ± 0.07^{b}	10.54 ± 0.60^{a}	0.45 ± 0.01^{a}	0.35 ± 0.01^{a}
G3	14.08 ± 1.38^{a}	2.86 ± 0.16^{a}	10.66 ± 1.00^{a}	0.29 ± 0.02^{b}	0.36 ±0.02^{a}
G4	10.00 ± 0.70^{c}	2.08 ± 0.14^{c}	7.10 ± 0.50^{b}	0.27 ± 0.02^{c}	0.31 ± 0.02^{b}
G5	13.85 ± 1.23^{a}	2.32 ± 0.16^{b}	10.57 ± 0.86^{a}	0.30 ± 0.03^{b}	0.37 ± 0.03^{a}
G6	12.43 ± 1.07^{b}	2.29 ± 0.15^{b}	9.60 ± 0.86^{a}	0.28 ± 0.03^{bc}	0.34 ± 0.02^{a}

Values represent means ± SD

$^{a-c}$ Values with different letters at the same column are significantly different at p< 0.05.

Table 3: Effect of oral administration of profenofos, propolis and their combinations for two successive months on hepatic, renal and testicular malondialdehyde (MDA) and serum total antioxidant capacity (TAC) in different experimental groups of rats

Groups	Hepatic MDA (µmol/g tissue)	Renal MDA (µmol/g tissue)	Testicular MDA (µmol/g tissue)	SERUM TAC (mM/L)
G1	41.53 ± 2.15 ^{b}	92.29 ± 5.98 ^{bc}	38.63 ± 3.81 ^{bc}	1.20 ± 0.03 ^{bc}
G2	31.37±2.93^{d}	78.60 ± 6.39 ^{c}	36.20 ± 3.11 ^{c}	1.32 ± 0.05 ^{a}
G3	42.61±2.44^{b}	83.67 ± 4.27 ^{bc}	34.53 ± 2.95 ^{c}	1.24 ± 0.02 ^{b}
G4	55.59 ± 3.89 ^{a}	118.60 ± 6.35 ^{a}	49.56 ± 3.98 ^{a}	1.00 ± 0.05 ^{e}
G5	37.00 ± 3.02 ^{c}	91.00 ± 2.24 ^{b}	35.20 ± 2.39 ^{c}	1.16 ± 0.07 ^{c}
G6	40.60 ± 3.64 ^{bc}	93.66 ± 5.83 ^{b}	42.58 ± 4.16 ^{b}	1.09 ± 0.07 ^{d}

Means with different superscripts (a,b,c, d and e) within the same column are significantly different at P <0.05.

Leukogram revealed significant leukopenia with neutropenia and lymphopenia in profenofos intoxicated rats compared to control group (Table 2). Profenofos exerts its toxic effect through generation of free radicals [5]. Activated neutrophils have been demonstrated to play an essential role in free radical mediated injury by inducing extracellular release of superoxide and other free radicals [41] which are toxic to host cells including neutrophils itself, thereby resulting in their decrease [42]. Moreover; the observed leukopenia could be attributed to adverse effect of profenofos on normal function of bone marrow [43]. Lymphopenia is clarified by our histopathological finding of the spleen which revealed lymphocytic necrosis and depletion (Fig.23). On the other hand rats' concurrently administered profenofos with propolis showed significant improvement in the leukocytic count in comparable to profenofos administered group Table (2). Propolis increases the proliferation of leukocyte precursors from pluripotent stem cells. Furthermore, prolonged administration of propolis elevated the myeloid and megakaryocytic type of colony forming units (CFUs) [38].

Regarding oxidant and antioxidant analysis in our study, the obtained data revealed significant increase in hepatic, renal and testicular MDA levels (indicators of lipid peroxidation) of profenofos administered rats (Table 3). This was accompanied with a significant decrease in serum total antioxidant capacity. Organophosphates exhibit their toxic effect through the generation of free radicals [44]. Free radicals are atoms or molecules which contain unpaired electrons. Since electrons have a very strong tendency to exist in a paired rather than an unpaired state, free radicals indiscriminately pick up electrons from other atoms, which in turn converts those other atoms into secondary free radicals, thus setting up a chain reaction which can cause substantial

biological damage. [45]. All the major biomolecules like lipids, protein and nucleic acids may be attacked by free radicals [46]. Due to the high concentration of polyunsaturated fatty acids in the cells, lipid peroxidation is a major outcome of the free radical-mediated injury. So the increase of MDA levels could be earlier diagnostic index in profenofos toxicity. Total antioxidant capacity (TAC) considers the cumulative effect of all antioxidants present in blood and body fluids [47].

The decreased level of serum TAC reflects a lower total antioxidant capacity. This is probably due to the depletion of the antioxidant molecules as they are consumed in the process of protecting cells against ROS generated by profenofos [48]. On the other hand, a significant decrease in hepatic, renal and testicular MDA levels associated with an increase of serum total antioxidant capacity level were recorded in rats concurrently administered propolis with profenofos compared to profenofos intoxicated rats (Table 3). Propolis is a potent antioxidant contains high content of phenolic compounds such as flavonoids which are able to scavenge free radicals [9]. Furthermore, it reserves the consumption of glutathione which has radical scavenging activity [49].

Statistical analysis of serum biochemical parameters are illustrated in table (4). A significant increase in blood glucose level was observed in rats exposed to profenofos compared to control group. The observed hyperglycemia may be attributed to the effect of profenofos on the pathways involved in glucose homeostasis in liver, muscle, brain and pancreas [50]. The concomitant administration of propolis with profenofos induced a significant decrease in glucose level. This effect referred to the modulatory effect of propolis on glucose metabolism. It modulates antioxidant enzymes and decrease lipid peroxidation process in plasma, liver, muscle, brain, lung and pancreas [51]. Similar results were previously obtained by Fuliang *et al.* [52] who reported that propolis induced decrease in glucose levels when administered to diabetic rats.

Significant hypercholesterolemia was observed in profenofos administered group compared to control group. Hypercholesterolemia may be attributed to the generation of free radicals induced by profenofos that cause lipid peroxidation [53]. Peroxidation of membrane phospholipids alters lipid milieu and increases the supply of non-essential fatty acids which in turn increases cholesterol level. Our results are in accordance with [54]. Co-administration of propolis with profenofos induced a significant reduction in the level of cholesterol. This may be attributed to high content of flavonoids in propolis. Flavonoids act as a scavenger of polyunsaturated fatty acids and interrupt the chain reaction [55].

In the present study, rats administered profenofos showed a significant elevation of serum activities of ALT and AST. The elevated transferaes enzymes denoted the adverse effect of profenofos on hepatic function. Our results are confirmed histopathologically as liver showed congestion of central veins and hepatic sinusoids as well as necrosis of hepatocytes (Fig.15). Regarding to the effect of propolis on profenofos intoxicated rats, propolis induced frank improvement (decrease) on the elevated enzyme activities. Such improvement supports the hepatoprotective effects of propolis. This might be due to the accelerated regeneration of hepatic parenchymal cells under the influence of various bioactive compounds of propolis like flavonoids and their esters. They prevent membrane fragility and subsequently decreased the leakage of marker enzymes into the circulation [56]. Our results are in accordance with Kanbur *et al.* [57] who recorded that propolis reduced the elevated activities of serum ALT and AST in propetamphos intoxicated rats.

Table 4: Effect of oral administration of profenofos, propolis and their combinations for two successive months on some serum biochemical parameters of different experimental groups of rats

Groups	Glucose(mg/dl)	Choles.(mg/dl)	ALT(IU/L)	AST(IU/L)	T.prot.(g/dl)	Albumin(g/dl)	Globu.(g/dl)	(A/G)Ratio	BUN(mg/dl)	Creatin.(mg/dl)
G1	81.01±5.18^{c}	75.52±4.83^{c}	49.20±3.15^{c}	62.40±3.99^{c}	7.88±0.55^{b}	4.39 ±0.28^{a}	3.48±0.24^{cd}	1.27 ±0.08^{a}	20.63±1.45^{ab}	0.62 ±0.04^{a}
G2	80.74±2.20^{c}	77.80±2.12^{c}	52.60±1.43^{c}	60.00±1.63^{c}	8.65 ±0.50^{a}	4.36 ±0.12^{a}	4.30 ±0.28^{a}	1.03 ±0.03^{b}	19.24±1.17^{b}	0.64 ±0.02^{a}
G3	83.75±4.83^{c}	74.39±4.29^{c}	50.00±2.88^{c}	59.00±3.40^{c}	8.12±0.76^{ab}	3.90 ±0.22^{b}	4.22 ±0.34^{a}	0.96±0.06^{bc}	18.81 ±1.76^{b}	0.64 ±0.03^{a}
G4	104.78±6.91^{a}	115.77±7.64^{a}	67.00±4.42^{a}	73.80±4.87^{a}	6.28±0.49^{c}	2.93±0.24^{d}	3.35 ±0.22^{d}	0.87±0.08^{d}	22.75 ±1.66^{a}	0.64 ±0.04^{a}
G5	93.25 ± 6.48^{b}	101.32±7.04^{b}	61.20±4.25^{b}	68.55±4.78^{b}	7.42±0.61^{bc}	3.51 ±0.24^{c}	3.92±0.32^{ab}	0.92±0.06^{cd}	22.08±1.72^{a}	0.61 ±0.04^{a}
G6	97.02±7.04^{b}	81.63 ± 5.67^{c}	59.20±4.11^{b}	63.20±4.39^{c}	7.38±0.66^{bc}	3.59±0.25^{c}	3.80±0.30^{bc}	0.99±0.07^{bc}	21.55±1.99^{a}	0.60 ±0.03^{a}

Values represent means ± SD

Means with different superscripts (a,b,c,and d) within the same column are significantly different at P <0.05.

Table 5: Effect of oral administration of profenofos, propolis and their combinations for two successive months on serum testosterone level and percentage of total sperm abnormalities of different experimental groups of rats

		Percentage of Sperm Morphological Abnormalities					
			Tail abnormalities				
Groups	Testosterone (ng/ml)	Head abnormalities (%)	Coiled (%)	Bent (%)	Distal protoplasmic droplet (%)	Neck abnormalities (Bent neck) (%)	Total sperm abnormalities (%)
G1	1.18±0.02^{a}	0.60±0.98^{d}	2.80±0.45^{cd}	15.60±1.09^{c}	2.40±0.15^{d}	3.20±0.22^{c}	24.60±3.05^{d}
G2	1.24±0.11^{a}	0.40±0.55^{d}	1.80±0.45^{d}	13.20±0.86^{d}	2.40±0.07^{d}	2.40±0.16^{c}	20.20±0.84^{e}
G3	1.24±0.11^{a}	1.00±0.71^{cd}	2.60±1.14^{d}	12.16±0.99^{d}	1.24±0.07^{e}	3.24±0.26^{c}	20.80±1.10^{e}
G4	0.75±0.05^{d}	5.00±1.58^{a}	6.60±1.14^{a}	24.40±1.61^{a}	5.80±0.38^{a}	13.20±1.17^{a}	55.00±3.32^{a}
G5	0.99±0.08^{b}	2.00±0.71^{bc}	4.60±2.97^{bc}	17.60±1.41^{b}	4.00±0.28^{c}	11.80±0.96^{b}	40.00±1.58^{c}
G6	0.86±0.06^{c}	3.00±0.71^{b}	5.20±1.30^{ab}	18.80±1.54^{b}	4.60±0.32^{b}	12.40±0.99^{ab}	44.00±3.16^{b}

Means with different superscripts (a,b,c,d and e) within the same column are significantly different at P <0.05.

Significant hypoproteinemia associated with hypoalbuminemia were observed in profenofos exposed rats comparable to control group. This recorded reflect the hepatocellular injury and disturbed amino acid metabolism induced by profenofos [58]. These changes could be attributed to the adverse effect of profenofos on the absorption and assimilation of protein from gastrointestinal tract. The observed hyporoteinemia may also be attributed to the reactive oxygen species (ROS) generated by profenofos and induced damage to the cellular macromolecules as protein [59]. Similar findings were reported by Attia and Nasr [54] and Mohamed *et al*. [60]. Co-administration of propolis with profenofos induced a significant increase in the total protein level compared to the rats treated with profenofos. Propolis could modulate protein metabolism [61]. This may be referred to the high antioxidant capacity of propolis [9].

Values of blood urea nitrogen and serum creatinine showed insignificant increase in profenofos intoxicated rats. However, an increased level of renal MDA was observed. The previous findings denote that renal MDA may be considered as an earlier marker in detecting renal damage induced by profenofos.

Profenofos intoxicated rats exhibited significant increase in total sperm abnormalities with decreased level of testosterone hormone (Table 5). The most pronounced sperm malformations were head abnormalities, tail abnormalities (coiled and bent tail), neck abnormalities (bent neck) and protoplasmic droplet (distal) Fig. [9-14]. The previous findings reflect the adverse effect of Profenofos on testicular function [62]. It has been shown that organophosphorus pesticides can cause various histopathological and cytopathological changes in the reproductive system of male mammals [63]. Organophosphorus pesticides have the ability to cross the blood-testis barrier inducing oxidative stress and lipid peroxidation that damage the biological membranes in the testes [63]. This in turn may cause degeneration of spermatogenic and leydig cells, which disrupt spermatogenesis. The sperms themselves may also be damaged by oxidative effects of organophosphorus pesticides. The previous results were supported histopathologically as testicular degeneration and intraluminal accumulation of necrosed germ cells as well as interstitial oedema were observed in the testes of Profenofos intoxicated rats (Fig. 27).

On the contrary, Co-administration of propolis with profenofos showed a significant reduction of morphological sperm abnormalities with a significant increase in serum testosterone level in comparison with profenofos exposed group (Table 5). Propolis has free radical scavenging activity that protects sperm membrane from the deleterious action of oxidative attack and reduces thiobarbituric acid reactive substances (TBARS) formation [64]. Moreover, propolis induces a significant increase in the level of antioxidant enzymes [65].

Cytogenetic analysis in our study revealed that profenofos at a dose of (23 mg/kg b.wt.) induced a significant increase in the percentage of bone marrow chromosomal aberrations (Table 6). The recorded chromosomal aberrations were categorized into structural and numerical aberrations. Fig.[1-8]. The structural aberrations were in the form of deletion, break, fragment, gap, centromeric attenuation and ring. Profenofos is an alkylating agent that chemically altered polynucleated chains. This essentially inhibits DNA synthesis in the alkylating region inducing one or more type of

Table 6: Effect of oral administration of profenofos, propolis and their combinations for two successive months on structural and numerical chromosomal aberrations of different experimental groups of rats

		Percentage of metaphase cells with chromosomal aberrations										
		Structural aberrations								Numerical aberrations		
Groups	M.No.	Deletion	Break	Fragment	Gap	CA	Ring	MOA	TSA	Polyploidy	Peridiploidy	TNA
G1	50	3.40±0.32^{d}	1.00 ±0.06^{d}	1.20 ±0.10^{d}	0.40 ±0.02^{e}	4.00 ±0.33^{c}	0.00 ±0.00^{b}	1.40 ±0.04^{d}	11.40±0.94^{d}	0.20±0.01^{c}	4.20±0.29^{d}	4.40±0.28^{d}
G2	50	3.20 ±0.22^{d}	0.60 ±0.04^{e}	0.40 ±0.03^{e}	0.20 ±0.05^{d}	2.20 ±0.15^{d}	0.00 ±0.00^{b}	1.20 ±0.08^{e}	7.80±0.50^{e}	0.20±0.01^{c}	4.40±0.29^{d}	4.60±0.13^{d}
G3	50	2.80 ±0.16^{d}	0.60 ±0.02^{e}	0.60 ±0.04^{e}	0.40 ±0.02^{e}	3.80 ±0.25^{c}	0.00 ±0.00^{b}	1.20 ±0.07^{e}	9.40±0.49^{de}	0.20±0.01^{c}	5.00±0.41^{d}	5.20±0.30^{d}
G4	50	12.00 ±0.84^{a}	3.80 ±0.25^{a}	3.60 ±0.32^{a}	3.20 ±0.21^{a}	10.00 ±0.89^{a}	2.00 ±0.14^{a}	2.80 ±0.18^{a}	37.40±2.42^{a}	2.00±0.13^{a}	18.00±1.60^{a}	20.00±1.32^{a}
G5	50	5.60 ±0.46^{c}	1.60 ±0.11^{c}	2.60 ±0.21^{c}	1.80 ±0.13^{c}	6.40 ±0.52^{b}	0.00 ±0.00^{b}	2.00 ±0.14^{b}	20.00±1.50^{c}	0.40±0.03^{b}	7.40±0.60^{c}	7.80±0.54^{c}
G6	50	7.80 ±0.70^{b}	2.80 ±0.19^{b}	3.00 ±0.25^{b}	2.00 ±0.14^{b}	9.60 ±0.77^{a}	0.00 ±0.00^{b}	1.60 ±0.11^{c}	26.80±2.22^{b}	0.40±0.03^{b}	12.40±0.99^{b}	12.80±0.89^{b}

CA: Centromeric attenuation MOA: More than one aberration

TSA: Total structural aberrations TNA: Total numerical aberrations

Values represent means ± SD

Means with different superscripts (a,b,c,d and e) within the same column are significantly different at P value <0.05.

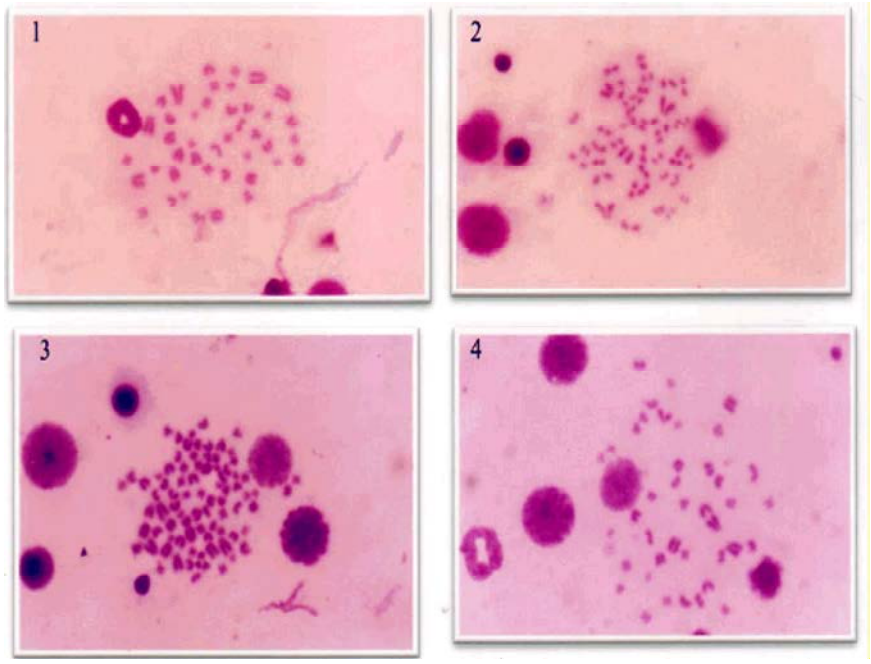

Fig. 1: Normal spread metaphase of rat bone marrow cells 2n-42.
Fig. 2: Metaphase spread of profenofos administered rat showing centromeric attenuation.
Fig. 3: Metaphase spread of profenofos administered rat showing Polyploidy.
Fig. 4: Metaphase spread of profenofos administered rat showing Peridiploidy.

chromosomal aberrations in the cells [66]. Furthermore, organophosphate toxicity may be mediated in part by the generation of reactive oxygen species and reactive nitrogen species [67]. Free radicals can damage DNA through oxidation of DNA bases or through covalent binding to DNA resulting in strand breaks and cross-linking [68]. The recorded numerical aberrations (polyploidy and peridiploidy) may be ascribed to cellular disturbances in the spindle organization induced by profenofos [69]. Our results are in accordance with El-Nahas *et al*. [70] who found that curacron induced a significant increase in structural chromosomal aberrations after acute and subacute treatment in rat bone marrow cells. On the other hand, rats administered propolis with profenofos showed a significant decrease in structural and numerical aberrations (Table 6). This finding denotes that propolis possesses an antimutagenic effect which is principally based on its radical scavenging activity [71]. It was suggested that polyphenolic components, caffeic acid (CA) derivatives and flavonoids in particular,

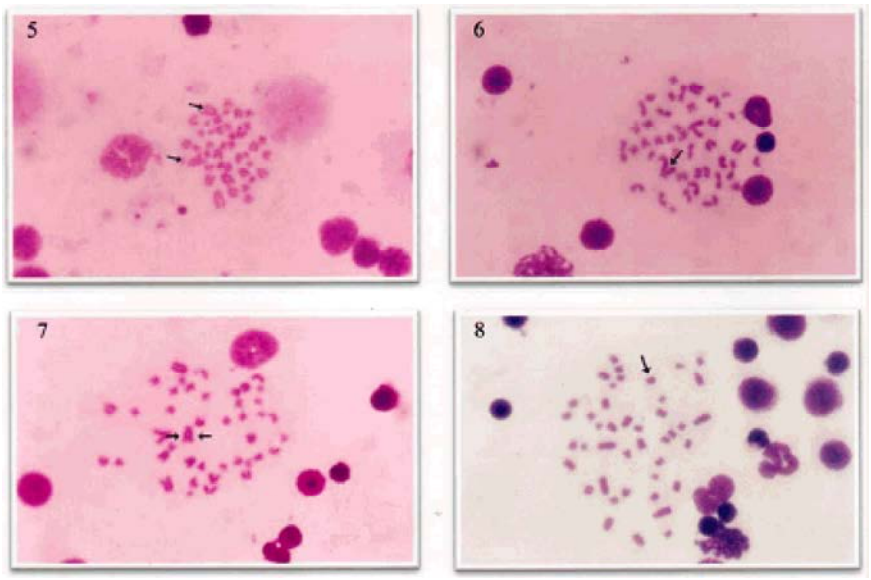

Fig. 5: Metaphase spread of profenofos administered rat showing chromatid break and fragment.
Fig. 6: Metaphase spread of profenofos administered rat showing deletions.
Fig. 7: Metaphase spread of profenofos administered rat showing chromosomal gap
Fig. 8: Metaphase spread of profenofos administered rat showing ring.

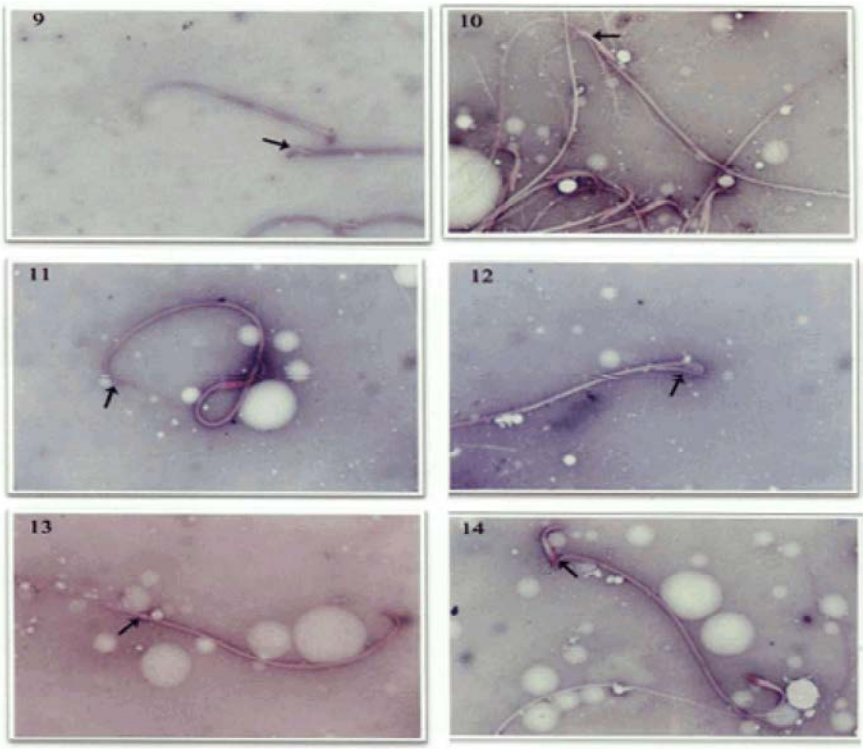

Fig. 9: Epididymal sperm of profenofos administered rat showing hockless head.
Fig. 10: Epididymal sperm of profenofos administered rat showing flattened head
Fig. 11: Epididymal sperm of profenofos administered rat showing bent tail
Fig. 12: Epididymal sperm of profenofos administered rat showing coiled tail
Fig. 13: Epididymal sperm of profenofos administered rat showing distal protoplasmic droplets
Fig. 14: Epididymal sperm of profenofos administered rat showing bent neck and detached head

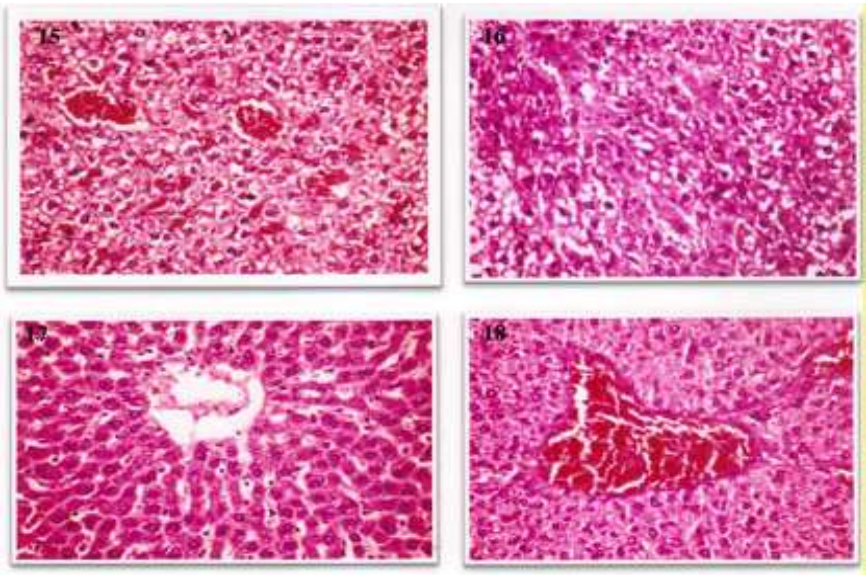

Fig. 15: Liver of profenofos administered rat showing congestion of central veins and hepatic sinusoids as well as vacuolation of hepatocytes (H and E x200).

Fig. 16: Liver of profenofos administered rat showing necrosis of hepatocytes and pyknosis of their nuclei (H and E x200).

Fig. 17: Liver of rat treated with profenofos plus propolis (12mg / kg b.wt.) showing no histopathological changes (H and E x200).

Fig. 18: Liver of rat concurrently administered profenofos and propolis (100 mg / kg b.wt.) showing dialation and congestion of central vein (H and E x200).

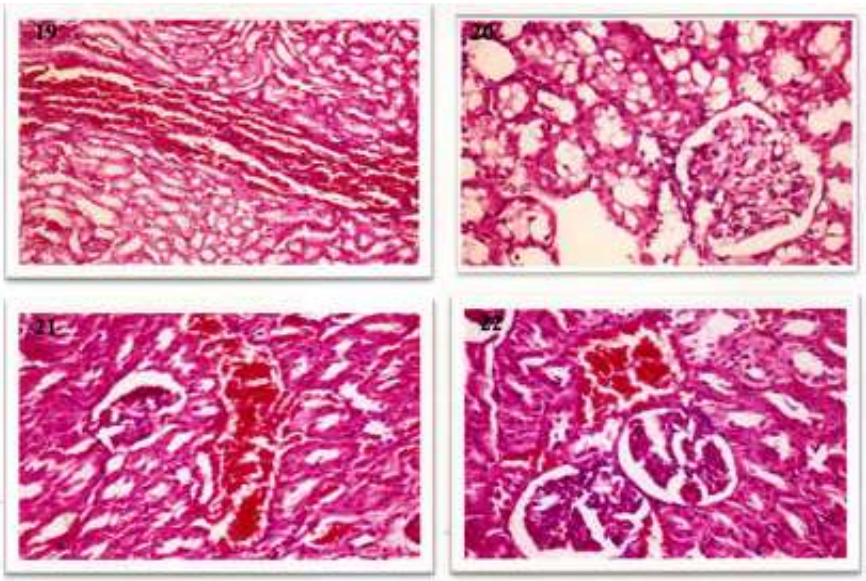

Fig. 19: Kidney of profenofos administered rat showing congestion of renal blood vessels (H and E x200).

Fig. 20: Kidney of profenofos administered rat showing hypertrophy and vacuolation of glomerular tuft as well as renal tubular epithelium (H and E x200).

Fig. 21: Kidney of rat concurrently administered profenofos and propolis (12mg/kg b.wt) showing congestion of intertubular renal blood vessels (H and E x200).

Fig. 22: Kidney of rat concurrently administered profenofos and propolis (100mg/kg b.wt.) showing congestion of renal blood vessels(H and E x200).

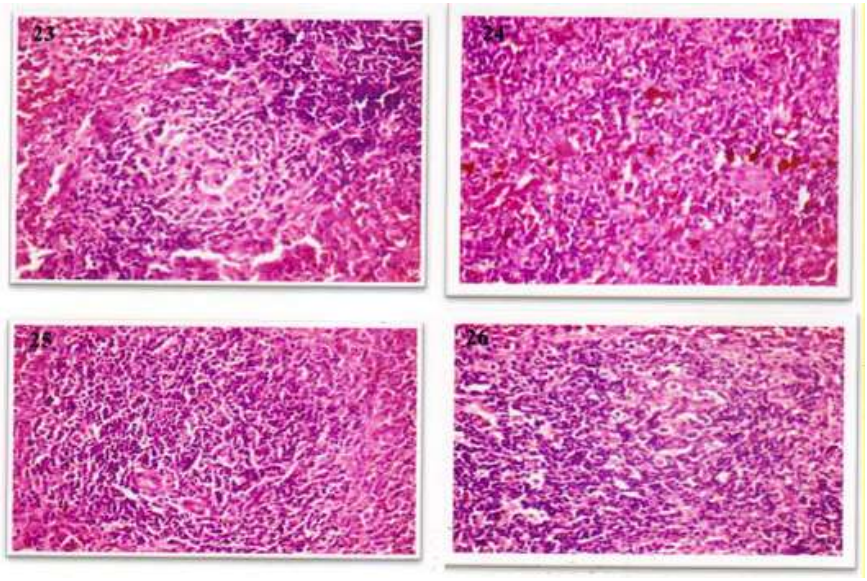

Fig. 23: Spleen of profenofos administered rat showing lymphocytic necrosis and depletion (H and E x200).
Fig. 24: Spleen of profenofos administered rat showing deposition of golden brown hemosidren pigments (H and E x200).
Fig. 25: Spleen of rat concurrently administered profenofos and propolis (12mg/kg b.wt) showing no histopathological alterations (H and E x200).
Fig. 26: Spleen of rat concurrently administered profenofos and propolis (100 mg/kg b.wt) showing slight lymphocytic necrosis and depletion (H and E x200).

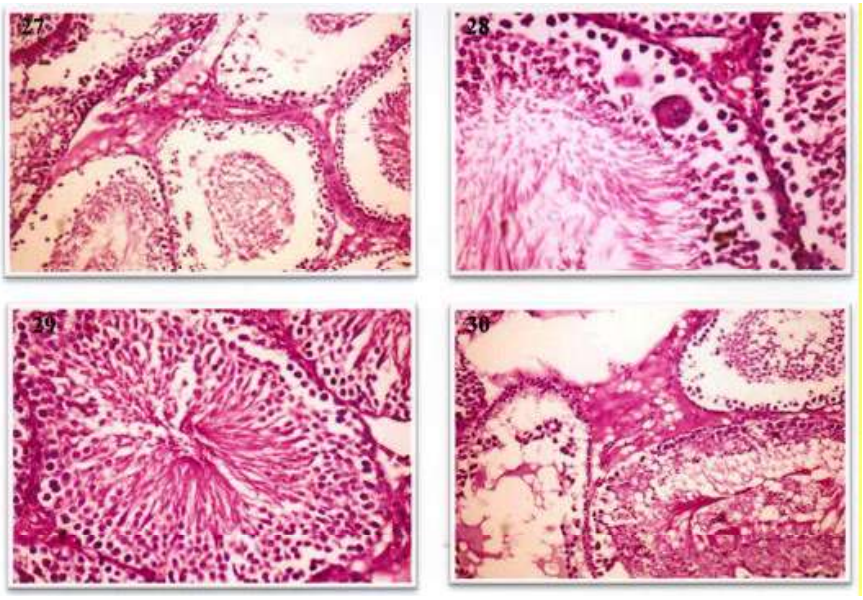

Fig. 27: Testis of profenofos administered rat showing degeneration and intraluminal accumulation of necrosed germ cells as well as interstitial oedema (H and E x200).
Fig. 28: Testis of profenofos administered rat showing multinucleated spermatid gaint cells in the lumen of seminiferous tubules (H and E x200).
Fig. 29: Testis of rat concurrently administered profenofos and propolis (12mg/kg b.wt) showing no histopathological changes (H and E x200).
Fig. 30: Testis of rat concurrently administered profenofos and propolis (100 mg/kg b.wt) showing testicular degeneration and interstitial oedema in some semineferous tubules (H and E x200).

were matter of interest for the antioxidant property of propolis [71]. Flavonoids have the efficacy in reducing genotoxic effects through scavenging ROS and enhancing the process of DNA repair [72]. Moreover, propolis increases the main antioxidant enzyme activities such as superoxide dismutase, catalase and glutathione peroxidase [74, 75].

The present investigation concluded that profenofos can induce oxidative stress by generating free radicals and altering antioxidant levels of the free radical scavenging enzymes. Propolis with its high concentration of flavonoids (polyphenolic compounds) possesses potent antioxidant and free radical scavenging activities. Therefore, it is capable of modulating the antioxidant enzymes" activities and suppressing the oxidative stress induced by profenofos.

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