Possible Ameliorative Role of Propolis and Ginseng against Hepatotoxicity of Chlorpyrifos and Profenofos in Male Rats

Abd El-Aziz A. Diab [1], El-Sayed A. Abd El-Aziz [2], Ahmed A. Hendawy [1] and Reham Z. Hamza [1*]

[1]Zoology Department, Faculty of Science, Zagazig University, Sharkia, Egypt
[2] Pharmacology Department, Faculty of Veterinary Medicine, Zagazig University, Sharkia, Egypt
dr_reham_z@yahoo.com

Abstract: The present study was an attempt to evaluate the toxic effect of both Chlorpyrifos and Profenofos (organophosphorous insecticides) each alone and in their combinations with either propolis or ginseng and as well known that propolis and ginseng have been reported to be effective antioxidant, therefore, the present study is aimed to elucidate the possible ameliorative role of propolis and ginseng in alleviating the toxicity of both Chlorpyrifos and Profenofos when given to male rats. This was done through studying the effects of both Chlorpyrifos and profenofos on some liver function parameters like liver enzymes, total protein, and antioxidant enzymes in liver homogenates and by making protein electrophoresis as well as histopathological changes in vital organ like liver. Animals were divided into nine groups; The 1st (Control group): Animals received 1ml of distilled water orally daily for 8 weeks, The 2nd (Chlorpyrifos treated group)Animals were daily received oral doses of Chlorpyrifos (6.75 mg/Kg b.wt.) for 60 days , The 3rd (Profenofos treated group)Animals were received orally Profenofos (20 mg/Kg b.wt.) daily for 8 weeks , The 4th (Propolis treated group)Animals were received orally Propolis extract (70mg/kg b.wt.) daily for 8,The 5th (Ginseng treated group)Animal were given orally Ginseng extract (200mg/Kg b.wt.) for 8 weeks daily, The 6th (Chlorpyrifos + Propolis treated group)Animals were given orally Chlorpyrifos (6.75 mg/Kg) and then co-administered with Propolis extract (70mg/kg b.wt.) for 8 weeks daily, The 7th (Chloropyrifos+Ginseng treated group)Animals were given orally Chlorpyrifos (6.75 mg/Kg b.wt.) and then co-administered with Ginseng extract (200mg/Kg) for 8 weeks daily , The 8th (Profenofos +Propolis treated group)Animals were given orally Profenofos (20 mg/Kg) and then co-administered with Propolis extract (70mg/kg) for 8 weeks daily, The 9th (Profenofos +Ginseng treated group)Animals were given orally Profenofos (20 mg/Kg) and then co-administered with Ginseng extract (200mg/Kg) as mentioned above for 8 weeks daily . Results showed that there was a correlation between CPF and PRF administration and the highly significant increase of the liver enzymes, lactate dehydrogenase enzyme and some antioxidant enzymes with decreasing other enzymes, as well as decrease of total proteins. In contrary to these actions, co-administration of propolis and ginseng to CPF and PRF-treated rats retrieved almost most of these biochemical parameters to normal levels. On the other hand, CPF and PRF showed histopathological alterations in liver of male rats like necrosis and hydrobic degeneration and highly fatty change, while administration of both propolis and ginseng highly ameliorate these dangerous hepatotoxicity markers.
[Abd El-Aziz A. Diab , El-Sayed A. Abd El-Aziz , Ahmed A. Hendawy, Reham Z.Hamza. **Possible Ameliorative Role of Propolis and Ginseng against Hepatotoxicity of Chlorpyrifos and Profenofos in Male Rats.** *J Am Sci* 2012;8(8):645-664]. (ISSN: 1545-1003). http://www.jofamericanscience.org. 100

Keywords: Chlorpyrifos, Profenofos, Propolis, Ginseng, Hepatotoxicity, Liver enzymes, protein electrophoresis.
Abbreviations: CPF, Chlropyrifos; PRF, Profenofos; AST, aspartate transaminase; ALT, alanine transaminase; LDH, lactate dehydrogenase ; MDA, Malondialdhyde enzyme ; SOD , Superoxide dismutase ; CAT , Catalase ; NO ; Nitric oxide , GPX;Glutathione peroxidase ,GSH;Glutathione reduced , G-6-Ph;Glucose -6- phosphate .

1. Introduction

During the last decades the use of pesticides has increased steadily in developing countries in an effort to increase food production and control vector-borne diseases. At present, there are more than 65,000 chemicals that are classified as pesticides. Because large amounts of these chemicals are released into the environment daily and many of them affect non-target organisms unfortunately, this has resulted in some negative side effects on human health and the environment **[1,2]**.Widespread use of organophosphate pesticides by public health and agricultural programs has led to severe environmental pollution that constitutes a significant potential health hazard because of the possibility of the acute or chronic poisoning of humans **[3]**. Organophosphorus pesticides (OPs) continue to be a potential human health concern due to their continued use worldwide and their potential for human exposure **[4-6].**OP insecticides are extensively used throughout the world to control major arthropods in public health programs, animal ectoparasites, human head and body lice, household insects and to protect grain in storage **[7].**

Chlorpyrifos (CPF) (O.O-diethyl0-3,5,6-trichloro-2-pyridyl phosphorothioate) is a broad spectrum organophosphorus (OP) insecticide widely used for pest control and in agriculture and sanitation industries worldwide **[8].** Many studies have assessed the effect of Chlorpyrifos on the health and safety of mammals **[9, 10].** Chlorpyrifos, produce a wide range of toxicity in mammals by inhibiting acetyl-cholinesterase (AChE), and the consequent accumulation of the neurotransmitter acetylcholine (ACh) in synaptic junctions leads to excessive stimulation of postsynaptic cells causing cholinergic toxicity **[11]**.

It has also been shown that repeated doses of chlorpyrifos caused significant hepatic atrophy, CPF elicity number of other effects including hepatic dysfunction, genotoxicity, embryotoxicity, teratogenicity, neurobehavioral and neurochemical changes **[12].**

The organophosphorus (OP) insecticide Profenofos (o-4-bromo -2- chlorophenyl-O- ethyl S-propyl) phosphorothioate is used heavily in cotton-growing areas of wide area of the world such as; Eastern Australia, Northern Africa and various areas of America **[13]**. Besides Profenofos is a broad-spectrum organophosphate insecticide and acaricide used widely used for agricultural and household purposes **[14].**

One of the organophosphorothiolate insecticides used in agriculture for pest control is profenofos. Due to its wide use in control of insects and mites on many different crops, humans are inevitably exposed to its residues in various ways **[15]**.Profenofos has been classified as moderately hazardous (toxicity class II) pesticide by WHO and it has a moderate order of acute toxicity following oral and dermal administration **[16].**

Natural products are a promising source for the discovery of new pharmaceuticals. In the last decades, several works dealing with propolis' composition and biological properties have been published, revealing the interest of researchers on this bee product and its potential for the development of new drugs **[17].**

Propolis is a resinous material collected by bees from bud and exudates of plants, which is mixed with products of their salivary glands and wax. Its color varies from green, red to dark brown. Propolis has been used in folk medicine since ancient times. Propolis has attracted researchers' interest in the last decades because of several biological and pharmacological properties, such as immunomodulatory, antitumor, antimicrobial, anti-inflammatory, and antioxidant, among others **[18, 19].** The use of Propolis goes back to ancient times, at least to 300 BC, and it has been used as a medicine in local and popular medicine in many parts of the world, both internally and externally **[20]**.

Propolis has been used from ancient ages owing to its beneficial properties and for corps mum-mification in the ancient Egyptian **[21]*;*** in the last century it has been employed in the popular medicine for the cure of many diseases **[22].** Many studies have showed that propolis improved the effect of vaiproate on LPO level towards the normal values and this means that propolis has strong antioxidant activity. This antioxidant activity may he attributed to the caffeic acid phenethyl ester.

its pharmacological activities as antiseptic, antimycotic, bacteriostatic, astringent, choleric, spasmolytic, anti-inflammatory, anaesthetic and antioxidant **[23-25] .**The main chemical classes found in propolis are flavonoids, phenolics and various aromatic compounds. However, propolis contains many of the B-complex vitamins, important minerals and trace elements. But its bio-flavonoid content is now receiving attention. Bioflavonoids are antioxidant molecules that play very important roles in scavenging of free radicals, which are produced in degenerative heart diseases, atherosclerosis, aging and effects of toxic substances **[26].**

Ginseng is a group of plants belonging to the genus Panax, consisting of several species with fleshy roots, in the family Araliaceae. Ginseng grows in Korea, China, Japan, Siberia, Vietnam, and North America and is considered to be one of the most important plants in herbal medicine, with many health benefits arising from consumption of the root and its extractives **[27]**.

In oriental medicine, ginseng is extracted with boiling water and used for medicinal purposes. Aqueous extracts of ginseng are composed of a mixture of glycosides, ginsenosides, trace minerals and a variety of complex carbohydrates as well as proteins, peptides and amino acids **[28]**. The main pharmacologically active constituents of ginseng are believed to be ginsenosides **[28, 29]**. The roots of Panax ginseng C.A. Meyer (Araliaceae) have been used as a traditional medicinal herb worldwide. P. ginseng roots have been reported to include amino acids, fatty acids, carbohydrates, alkaloids, triterpene saponins, polysaccharides, sesquiterpenes, polyacetlyenes, peptidoglycans, minor elements, vitamins, and phenolic compounds. The major biochemical and pharmacological activities of P. ginseng have been attributed to triterpene saponins such as ginsenosides, and it was reported that the content of ginsenosides in root and root-hair increases with increasing age of P. ginseng from one to five years. P. ginseng roots exhibit a wide variety of pharmacological effects, such as cardiovascular control of blood pressure, increasing learning,

increasing cognitive performance, antiaging, antioxidative, anticancer, and immune stimulating activities **[30]**.

So, we want to pass through the light of safety and reality to make sure of these side effects of both Chlorpyrifos and profenofos and after assuring from that we decided to use two natural extracts propolis and ginseng to test whether propolis and ginseng could reduce the side effects of both insecticides on liver , the main vital organ of all biochemical processes in the body and also, this study was conducted to compare and evaluate the best ameliorative role of either propolis or ginseng in restoring these biochemical and histopathological variations to their normal values in liver in treated male rats.

2. Materials and Methods

2.1. Test insecticide

2.1.1Chlorpyrifos was produced by Misr for Agricultural Development Company, Cairo, Egypt. Under trade name Dursban and was stored at 4°C until stock solution preparation. The insecticide (CPF) was orally administered at a dose level equivalent to 1/20 LD_{50} (6.75 mg/kg b.wt.) in distilled water for 60 successive days, this selected dose of the insecticide was based on previous studies in which 1/20 LD_{50} of CPF induced biochemical alterations in rats without morbidity **[31]**. Stock solution was prepared by bringing Chlorpyrifos to room temperature then taking a certain amount by pipette from the Chlorpyrifos bottle and dilute it in distilled water (0.25 ml of Chlorpyrifos was dissolved in 250 ml dist. water) and diluting it in tween 80 to ensure rapid and complete absorption and we prepare 250 ml only to prepare the working solution freshly for each day of dosing **[32, 33].**

2.1.2 Profenofos is a pale yellow liquid; it was produced by Ciba-Geigy, Pharmacological Company, Scientific office Cairo, Egypt. under trade name: Selecron 72% EC , Profenofos was given at a dose of (20mg/Kg b.wt.) which represent 1/10 LD_{50}, where the LD_{50} value of Profenofos is (200 mg/Kg) according to **[Weil][34]** and this selected dose of the insecticide was based on Weil studies in which 1/10 LD_{50} of Profenofos induced biochemical alterations in rats without morbidity. Tap water was used for preparing emulsion of Profenofos immediately before use, Stock solution was prepared by bringing Profenofos to room temperature then taking a certain amount by pipette from the Profenofos bottle and diluting it in distilled water (1.97 ml of Profenofos was diluted in 250 ml dist. water) we prepare 250 ml only of working solution freshly for each day of dosing **[35]**.

2.2. Extracts

2.2.1 Propolis extract preparation:

In this study, Propolis powder extract (70% ethanolic extract) was obtained from (Dosic IMP &EXP. Co, Ltd) China .Propolis was dissolved in dist. water and administered orally for 60 successive days via gastric tube at dose 70 mg/ Kg b.wt. **[36,37].**

2.2.2Ginseng extracts preparation*:*

Red Ginseng powder (Supplied by Tsumura Pharmaceutical Co., Tokyo, Japan) was administered orally at dose (200 mg/Kg) **[38]** for 60 successive days via a gastric tube. The Ginseng extract was suspended in tap water just before use and the dose was calculated according to the animal's body weight on the week before using.

2.3. Animals

The present study was carried out at Zoology Department, Faculty of Science - Zagazig University, using (one hundred and ten) (110) clinically healthy mature adult male albino rats. The animals were obtained from the Animal House of Faculty of Veterinary Medicine, Zagazig University, Their weights ranged from (200-250gm) each. The animals were housed in standard conditions, where the animals were housed in metal cages and bedded with wood shavings and kept under standard laboratory conditions of aeration and room temperature at about 25°C. The animals were allowed to free access of standard diet and water *ad libitum*.The animals were accommodated to the laboratory conditions for two weeks before being experimented.

2.4. Experimental design

After the period of acclimation, animals were divided into nine groups with 10 animals in each as :

I) The 1st (Control group): Animals received 1ml of distilled water orally daily for 8 weeks.

II) The 2nd (Chlorpyrifos treated group): Animals were daily received oral doses of Chlorpyrifos (6.75 mg/Kg b.wt.) for 8 weeks using metallic stomach tube.

III) The 3rd (*Profenofos treated group*): Animals were received orally Profenofos (20 mg/Kg b.wt.) daily for 8 weeks using metallic stomach tube.

IV) The 4th (Propolis treated group): Animals were received orally *Propolis* extract (70mg/kg b.wt.) daily for 8 weeks using metallic stomach tube.

V) The 5th (*Ginseng treated group*): Animals were given orally Ginseng extract (200mg/Kg b.wt.) for 8 weeks daily using metallic stomach tube.

VI) The 6th (Chlorpyrifos + Propolis treated group): Animals were given orally Chlorpyrifos (6.75 mg/Kg b.wt.) and then co-administered with *Propolis* extract (70mg/kg b.wt.) for 8 weeks daily.

VII)* The 7th (Chloropyrifos+Ginseng treated group*): Animals were given orally Chlorpyrifos (6.75 mg/Kg b.wt.) and then co-administered with

Ginseng extract (200mg/Kg b.wt.) for 8 weeks daily.

VIII) **The 8th (Profenofos +Propolis treated group*):*** Animals were given orally Profenofos (20 mg/Kg b.wt.) and then co-administered with *Propolis* extract (70mg/kg b.wt.) for 8 weeks daily.

XI) **The 9th (Profenofos +Ginseng treated group*):*** Animals were given orally Profenofos (20 mg/Kg) and then co-administered with *Ginseng* extract (200mg/Kg b.wt.) as mentioned above for 8 weeks daily.

2.5 Biochemical Assays

Blood samples were collected after the end of the experiment from the retro-orbital vein, which is a simple, convenient and successful procedure that allows bleeding of the same animal more than one time with minimal stress **[39]** .After the last administration of the drug at the end of 8th week, individual blood samples were drawn by orbital puncture (from eye plexus) using microhematocrit capillary tubes (Lancer, Athy, County-Kildare, Republic of Ireland), Serum was harvested from blood without EDTA and then blood samples were transferred into Eppendorf tubes and subsequently used for the determination of (Albumin, Globulin, Protein electrophoresis, Total protein, Aspartate amino transferase (AST), Alanine amino transferase (ALT), Lactate dehydrogenase (LDH) , The biochemical measurements were performed according to the details given in the kit's instructions.

2.5.1 Determination of serum Aminotransferase enzymes activities*:*

Activities of AST and ALT in the serum were determined colorimetrically by using bio Merieux kit (France), using method adopted by**[Reitman et al][40].**

2.5.2 Determination of serum total protein concentration:

Serum total proteins were determined by Biuret method, using the Diamond kit **[41].** According to this method, protein forms a colored complex with cupric ions in an alkaline medium.

2.5.3 Determination of serum lactate dehydrogenase enzyme (LDH):

The reaction velocity is determined by a decrease in absorbance at 340 nm resulting from the oxidation of NADH. One unit causes the oxidation of one micromole of NADH per minute at 25°C and pH 7.3, under the specified conditions **[42]** ***by*** using Diamond kit.

2.6 Protein electrophoresis

Serum protein electrophoresis on cellulose acetate strips using barbital buffer at PH: 8.6, compared with standard protein electrophoresis to reveal our results, this method is intended to be used for *in Vitro* diagnosis and they enable the quantitative and qualitative estimation of proteins in serum and other biological materials **[43]**.

2.7 Preparation of Tissue Homogenate:

The remainder tissues of liver were used for the analyses of oxidative stress parameters. They were washed with saline and distal water for the removal of blood, and later the fatty parts were removed and blotted over a piece of filter paper. Prior to dissection, tissue was perfused with a 50 mM (sodium phosphate buffer saline (100 mM Na_2HPO_4 / NaH_2PO_4) (PH 7.4) in an Ice containing medium containing 0.16 mg / ml heparin or containing 0.1 mM ethylene di amine tetra acetic acid (EDTA)to remove any red blood cells and clots. Then tissues were homogenized in 5 – 10 ml cold buffer per gram tissue and Centrifuged at 5000 r.p.m for ½ hours. The resulting supernatant was transferred into Eppendorf tubes, and preserved at -80°Cin a deep freezer until used for various biochemical Assays **[44].**

2.7.1 Determination of Catalase activity:

Catalase (CAT) activity was determined by biodiagnostic kit method (Biodiagnositc Company, Dokki, Giza, Egypt), according to the method of **[Aebi][45].**

2.7.2 Determination of Superoxide dismutase activity:

Superoxide dismutase (SOD) activity was determined by biodiagnostic kit (Biodiagnositc Company, Dokki, Giza, Egypt), according to the method of **[Nishikimi et al].[46].**

2.7.3 Determination of Reduced Glutathione (GSH) activity:

Glutathione reduced (GSH) activity was determined by biodiagnostic kit (Biodiagnositc Company, Dokki, Giza, Egypt), according to the method of **[Beutler et al][47].**

2.7.4 Determination of Glutathione Peroxidase (GPX) activity:

Glutathione peroxidase activity was determined using biodiagnostic kit (Biodiagnositc Company, Dokki, Giza, Egypt), according to the method of **[Paglia and Valatine] [48].**

2.7.5 Determination of Lipid peroxide (Malondialdhyde) activity:

Malondialdehyde (MDA) was determined by using Biodiagnostic kit (Biodiagnostic Company, Dokki, Giza, Egypt), according to the method of **[Satoh and Ohakawa et al][49, 50].**

2.8 Preparation of tissues for histopathological examination

After 8 weeks post drug administration, animals were sacrificed and samples from heart, liver, brain, kidney and testis were fixed in 10% formalin for histopathological studies. Parts of liver were transferred into 10% buffered formalin for

histopathological examination, and the remainder tissue was used for the analysis of oxidative stress parameters. Tissue samples were taken from the liver of the necropsied animals and fixed in 10% formalin saline. The trimmed tissues were first washed with tap water followed by dehydration through a graded alcohol series and then passed through xylol and paraffin series before finally blocked in paraffin. The paraffin blocks were cut into 5-6 µm sections using a microtome stained using hematoxylin and eosin and examined under a light microscope **[51]**.

2.9 Statistical analysis

Data were collected, arranged and reported as mean ± standard error of mean (S.E.M) of nine groups (Each group was considered as one experimental unit), summarized and then analyzed using the computer program SPSS/ version 15.0) The statistical method was one way analyzes of variance ANOVA test (F-test), and if significant differences between means were found, Duncan's multiple range test (Whose significant level was defined as (P<0.05) was used according to**[Snedecor and Cochra] [52]** to estimate the effect of different treated groups.

3. Results

3.1 Morbidity and mortality:

Male rats orally administered Chlorpyrifos, profenofos in doses of (6.75 mg/kg b.wt.) and (200 mg/Kg b.wt.) respectively for 60 days have shown signs of toxicity (Diarrhea, myosis, increased urination, diaphoresis,nose and eye bleeding and salivation) and no deaths were recorded throughout the experimental groups.

3.2 Effect on serum total protein:-

The administration of Chlorpyrifos and/or Profenofos in their recommended doses for successive 60 days into normal rats elicited highly significant decrease (P<0.05) in serum total protein level compared with normal control group. Whereas, non significant changes were observed in the groups treated with either Propolis or Ginseng after 8th week when compared with normal control group (Table 1) and (Fig. 1).Whereas combinations of Chlorpyrifos and/or Profenofos with either Propolis or Ginseng afforded non significant changes when compared with control group, except combination of Chlorpyrifos and ginseng which showed a significant decrease in serum total proteins compared with normal control group. However, the combinations of the test plant extracts appear to ameliorate the hypoproteinaemia produced by each insecticide alone.

3.3 Effect on serum GOT and GPT (AST & ALT):-

Serum transferases (AST &ALT) levels were markedly elevated after 8th week post Chlorpyrifos and Profenofos administration to normal rats when compared with normal control group. Meanwhile significant elevation (P<0.05) in serum AST&ALT were recorded after 8th week in normal rats in response to administration of Propolis and/or Ginseng in their combinations with Chlorpyrifos and/or Profenofos, but this effect was less intense than that produced by each insecticide alone except group treated with propolis & ginseng alone which revealed non significant change in serum ALT when compared with normal control group (Table.1) and (Fig. 2).

3.4 Effect on serum Lactic dehydrogenase enzyme (LDH) activity:-

Treatment of normal rats with either Chlorpyrifos or Profenofos for 60 successive days in their recommended doses elicited a marked elevation in serum LDH activity after the end of the study when compared with normal control group. While the other treatments afforded a non-significant change in serum LDH activity when compared with normal control group (Table1) and (Fig. 3).

3.5 Protein Electrophoresis

3.5.1 Effect on serum Albumin:-

The administration of Chlorpyrifos and/or Profenofos each alone and their combinations with either Propolis or Ginseng to normal rats elicited a significant decrease (P<0.05) in serum albumin after two months post administration when compared with normal control group.

Meanwhile, Propolis and Ginseng treated groups showed also the same previous effect, yet their effects were better than that produced by each insecticide and their combinations (Table 2) and Figs (4, 5).

3.5.2 Effect on serum Globulin α1:-

Concerning the effect on serum Globulin α1, Chlorpyrifos and Profenofos treated groups afforded a marked decrease (P<0.05) in serum Globulin α1 when compared with normal control group after the end of the experiment. Meanwhile, non significant changes were noticed in groups treated with either Propolis or Ginseng when compared with normal control group. (Table 2) and (Figs. 4, 5) showed also that combinations of Propolis or Ginseng with either Chlorpyrifos or Profenofos elicited a significant decrease (P<0.05) in serum Globulin α1 when compared with normal control group.

3.5.3 Effect on serum Globulin α2:-

Concerning the effect on serum Globulin α2, Chlorpyrifos and Profenofos treated groups showed a marked decrease (P<0.05) in serum Globulin α2 when compared with normal control group after the end of the experiment, the same response was obtained in groups given either Propolis or ginseng but the decrease was much lesser than that produced by the insecticides used (Table 2) and (Figs 4, 5). The same previous response was recorded in groups given

the combinations of the insecticides used with either propolis or ginseng when compared with the normal control group. Yet, they showed a significant elevation when compared with the insecticide treated groups, indicating an ameliorative effect.

3.5.4 Effect on serum Globulin β and γ:-

Concerning the effect on serum Globulin β and γ, Chlorpyrifos and Profenofos treated groups afforded marked decrease (P<0.05) in serum Globulin β and γ when compared with normal control group after the end of two monthes post administration. Meanwhile, non significant changes were noticed in groups treated with either Propolis or Ginseng when compared with normal control group (Table 2) and (Figs4, 5). The same table and figure showed also that combinations of Propolis or Ginseng with either Chlorpyrifos or Profenofos elicited a significant decrease (P<0.05) in serum Globulin β and γ when compared with normal control group but this effect was much lesser than that produced by the insecticides alone.

3.6 . Effect on Antioxidant enzymes:

3.6.1 Effect on Catalase:

Regarding the effect of profenofos and Chlorpyrifos on catalase activity of normal rats, Chlorpyrifos and profenofos afforded a marked decrease (P<0.05) in liver homogenates catalase after the end of the study when compared with control group, whereas, Treatment of normal rats with either propolis or Ginseng alone exhibited non significant changes in Catalase of liver after the end of the experiment when compared with control group (Table 3) and (Fig 6)While combinations of Chlorpyrifos, Profenofos with either Propolis or ginseng exhibited a significant decrease in Catalase activity of liver after the end of the study compared with normal control group.

3.6.2 Effect on Superoxide dismutase (SOD):

The results of the study revealed that treatment of normal rats with either of Chlorpyrifos and/or profenofos elicited a highly significant decrease (P<0.05) in liver SOD level after the end of the study when compared with control group. Treatment of normal rats with either propolis or ginseng for 8 weeks elicited a significant increase in SOD activity of the liver after the end of the study. Whereas, the combinations of the plant extracts with the test insecticides afforded non significant changes in the SOD activity of the liver compared with normal control group .Table (3) and Fig (7).

3.6.3 Effect on Malondialdhyde (MDA):

The MDA content of the liver was significantly elevated (P<0.05) in response to treatment of normal male rats with either Chlorpyrifos and/or profenofos for 8 weeks compared with normal control group. The same previous response was reported with propolis, ginseng and their combinations with either Chlorpyrifos or profenofos compared with control group (Table 3) and (Fig. 8).

3.6.4 Effect on Glutathione reduced:

It was apparent from (Table 3) and (Fig. 9) that treatment of rats with Chlorpyrifos, Profenofos each alone afforded a significant decrease (P<0.05) in liver reduced glutathione after the end of the study when compared with normal control group. On the other hand, the results revealed that Ginseng and/or Propolis induced a non significant change in reduced Glutathione content of the liver compared with control group.

Table (1): Effect of Chlorpyrifos (6.75 mg/kg), Profenofos (20mg/Kg) , Propolis (70 mg/ Kg), Ginseng (200 mg/Kg) and their combinations on Liver function in male albino rats (mean ± SE). (N = 7)

Groups	Total Protein(g/dl)	GOT(U/ml)	GPT(U/ml)	LDH(µIU/ml)
Control group	8.48±0.27^{a}	13.20±0.58^{d}	12.00±0.70^{d}	371.66±47.21^{c}
Chlorpyrifos	4.88±0.40 ^{c}	177.80±7.39^{a}	71.00±1.18^{b}	1728.40±35.37^{b}
Profenofos	4.98±0.44 ^{c}	181.80±6.02^{a}	78.80±1.82^{a}	1930.30±33.34^{a}
Propolis	8.40±0.63^{a}	13.80±1.01 ^{d}	14.20±2.10^{cd}	330.32±14.06^{c}
Ginseng	8.76±0.65^{a}	14.00±1.67 ^{d}	12.20±0.86^{d}	420.00±30.37^{c}
Chlorpyrifos + Propolis	7.74±0.51^{ab}	56.40±3.12^{b}	25.40±0.50^{c}	510.66±50.63^{bc}
Chlorpyrifos + Ginseng	7.26±0.16 ^{b}	44.20±4.61^{b}	24.60±2.73^{c}	290.31±51.26^{c}
Profenofos + Propolis	8.50±0.43^{a}	56.00±9.78^{b}	22.00±0.89^{c}	295.40±13.43^{c}
Profenofos + Ginseng	7.86±0.55^{a}	52.20±6.63^{b}	23.00±1.92^{c}	565.00±70.80^{bc}

Means within the same column in each category carrying different litters are significant at (P ≤ 0.05) using Duncan's multiple range test, where the highest mean value has symbol (a) and decreasing in value were assigned alphabetically.

Table (2): Effect of Chlorpyrifos (6.75 mg/kg), Profenofos (20mg/Kg) , Propolis (70 mg/ Kg), Ginseng (200 mg/Kg) and their combinations on Protein electrophoresis in male albino rats (mean ± SE). (N = 7).

Groups	Albumin(g/d	Globulin α1(g/dl)	Globulin α2(g/dl)	Globulin	Globulin
Control group	4.78±0.04^{a}	0.73±0.07^{a}	0.72±0.01^{a}	2.25±0.08^{a}	1.34±0.07^{a}
Chlorpyrifos	3.06±0.03^{e}	0.33±0.08^{e}	0.33±0.01^{e}	1.53±0.01^{f}	0.66±0.03^{g}
Profenofos	2.85±0.06^{ef}	0.23±0.01^{ef}	0.23±0.01^{f}	1.44±0.01^{g}	0.61±0.05^{h}
Propolis	4.40±0.07^{b}	0.62±0.05^{ab}	0.65±0.01^{b}	2.12±0.03^{ab}	1.16±0.01^{ab}
Ginseng	4.17±0.04^{b}	0.64±0.09^{ab}	0.65±0.01^{b}	2.11±0.02^{ab}	1.16±0.08^{ab}
Chlorpyrifos+	3.24±0.01^{d}	0.46±0.01^{d}	0.44±0.01^{d}	1.73±0.01^{d}	1.01±0.05^{d}
Chlorpyrifos +	3.60±0.01^{c}	0.53±0.08^{cd}	0.53±0.01^{c}	1.82±0.01^{cd}	0.87±0.05^{f}
Profenofos + Propolis	3.52±0.09^{c}	0.55±0.09^{bcd}	0.47±0.09^{cd}	1.70±0.06^{d}	1.06±0.05^{c}
Profenofos + Ginseng	3.71±0.02^{c}	0.57±0.08^{bc}	0.55±0.08^{cd}	1.64±0.01^{de}	0.94±0.01^{e}

Means within the same column in each category carrying different litters are significant at ($P \leq 0.05$) using Duncan's multiple range test, where the highest mean value has symbol (a) and decreasing in value were assigned alphabetically.

Table (3): Effect of Chlorpyrifos (6.75 mg/kg), Profenofos (20mg/Kg) , Propolis (70 mg/ Kg), Ginseng (200 mg/Kg) and their combinations on antioxidant activity in liver homogenates in male albino rats (mean±SE). (N = 7).

Groups	Antioxidant defense system		Oxidative stress marker		
	Liver Catalase	Liver SOD	Liver MDA	Liver Glutathione	Liver Glutathione
Control group	4.19±0.01^{a}	83.32±0.87^{c}	1.74±0.10^{d}	26.11±0.75^{a}	33.04±0.74^{a}
Chlorpyrifos	0.50±0.09^{c}	20.61±1.52^{de}	8.35±0.26^{a}	6.12±0.48^{c}	4.99±0.51^{c}
Profenofos	0.41±0.13^{c}	24.14±2.98^{e}	8.50±0.66^{a}	2.99±0.58^{d}	2.64±0.44^{d}
Propolis	4.33±0.03^{a}	98.82±0.91^{a}	2.81±0.12^{c}	28.30±0.94^{a}	34.50±1.34^{a}
Ginseng	4.43±0.04^{a}	92.94±1.66^{b}	3.30±0.25^{c}	27.01±0.82^{a}	35.89±0.82^{a}
Chlorpyrifos +Propolis	3.72±0.03^{b}	80.92±1.59^{c}	5.38±0.38^{b}	26.03±1.56^{a}	22.83±1.00^{b}
Chlorpyrifos +Ginseng	3.57±0.04^{b}	79.91±0.88^{c}	5.04±0.26^{b}	22.77±1.19^{b}	35.70±1.13^{a}
Profenofos + Propolis	3.72±0.05^{b}	81.18±1.68^{c}	3.66±0.26^{c}	24.47±1.70^{ab}	29.33±1.68^{ab}
Profenofos + Gensing	3.69±0.02^{b}	82.99±1.10^{c}	5.05±0.06^{b}	23.48±2.45^{ab}	32.34±2.46^{a}

Means within the same column in each category carrying different litters are significant at ($P \leq 0.05$) using Duncan's multiple range test, where the highest mean value has symbol (a) and decreasing in value were assigned alphabetically.

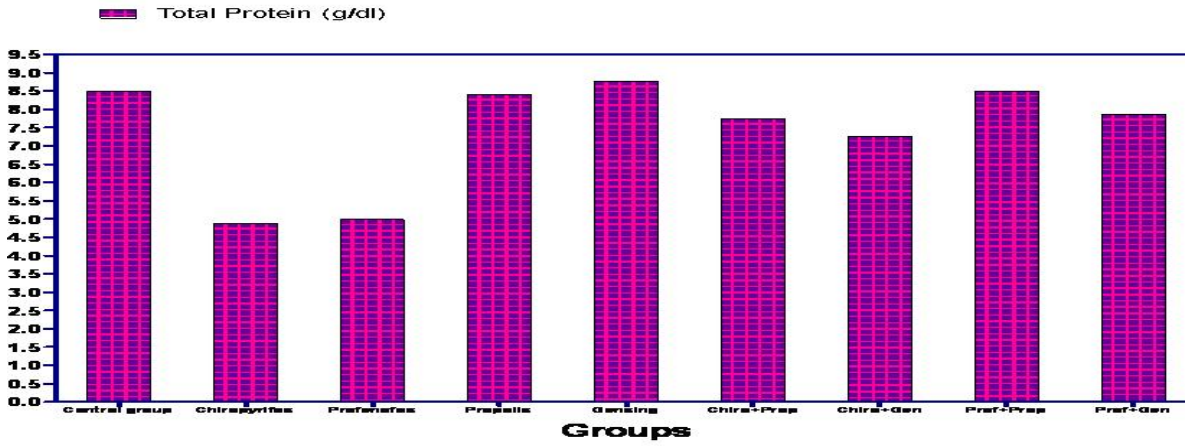

Fig (1): Effect of Chlorpyrifos (6.75 mg/kg), Profenofos (20mg/Kg) , Propolis (70 mg/ Kg), Ginseng (200 mg/Kg) and their combinations on Total protein (g/dl) in male albino rats.

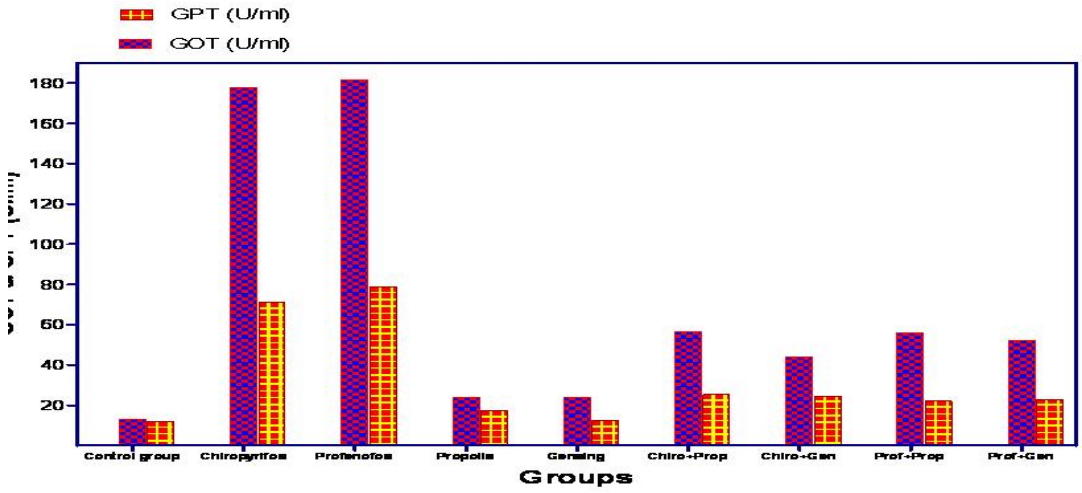

Fig (2): Effect of Chlorpyrifos (6.75 mg/kg), Profenofos (20mg/Kg) , Propolis (70 mg/ Kg), Ginseng (200 mg/Kg) and their combinations on GPT & GOT (U/ml) in male albino rats.

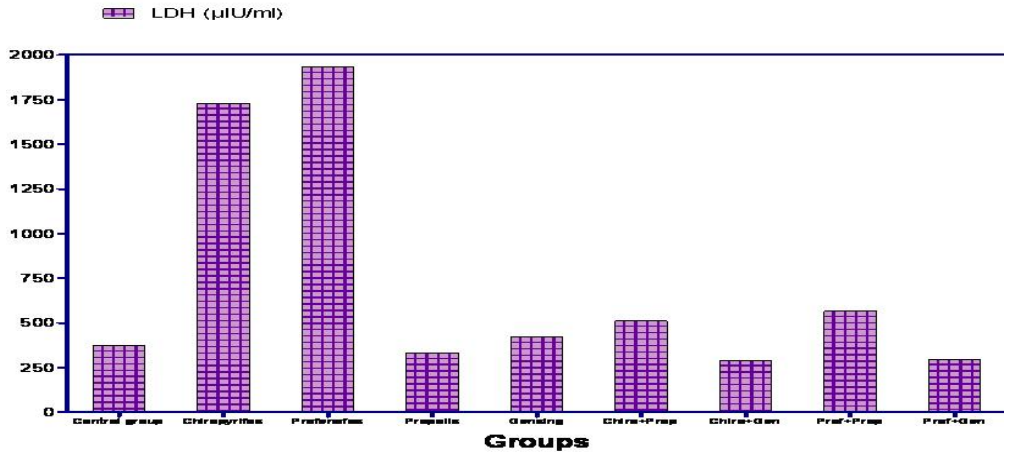

Fig (3): Effect of Chlorpyrifos (6.75 mg/kg), Profenofos (20mg/Kg) , Propolis (70 mg/ Kg), Ginseng (200 mg/Kg) and their combinations on LDH enzyme (µIU/ml) in male albino rats.

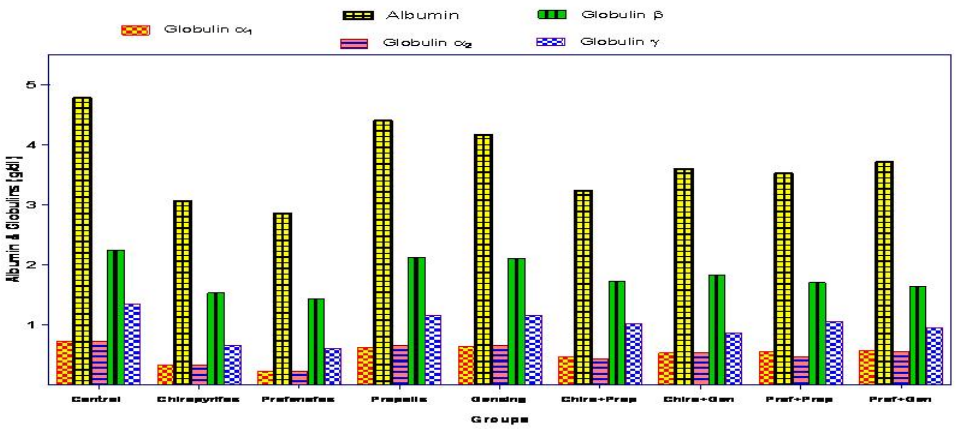

Fig (4): Effect of Chlorpyrifos (6.75 mg/kg), Profenofos (20mg/Kg) , Propolis (70 mg/ Kg), Ginseng (200 mg/Kg) and their combinations on Protein electrophoresis (g/dl) in male albino rats.

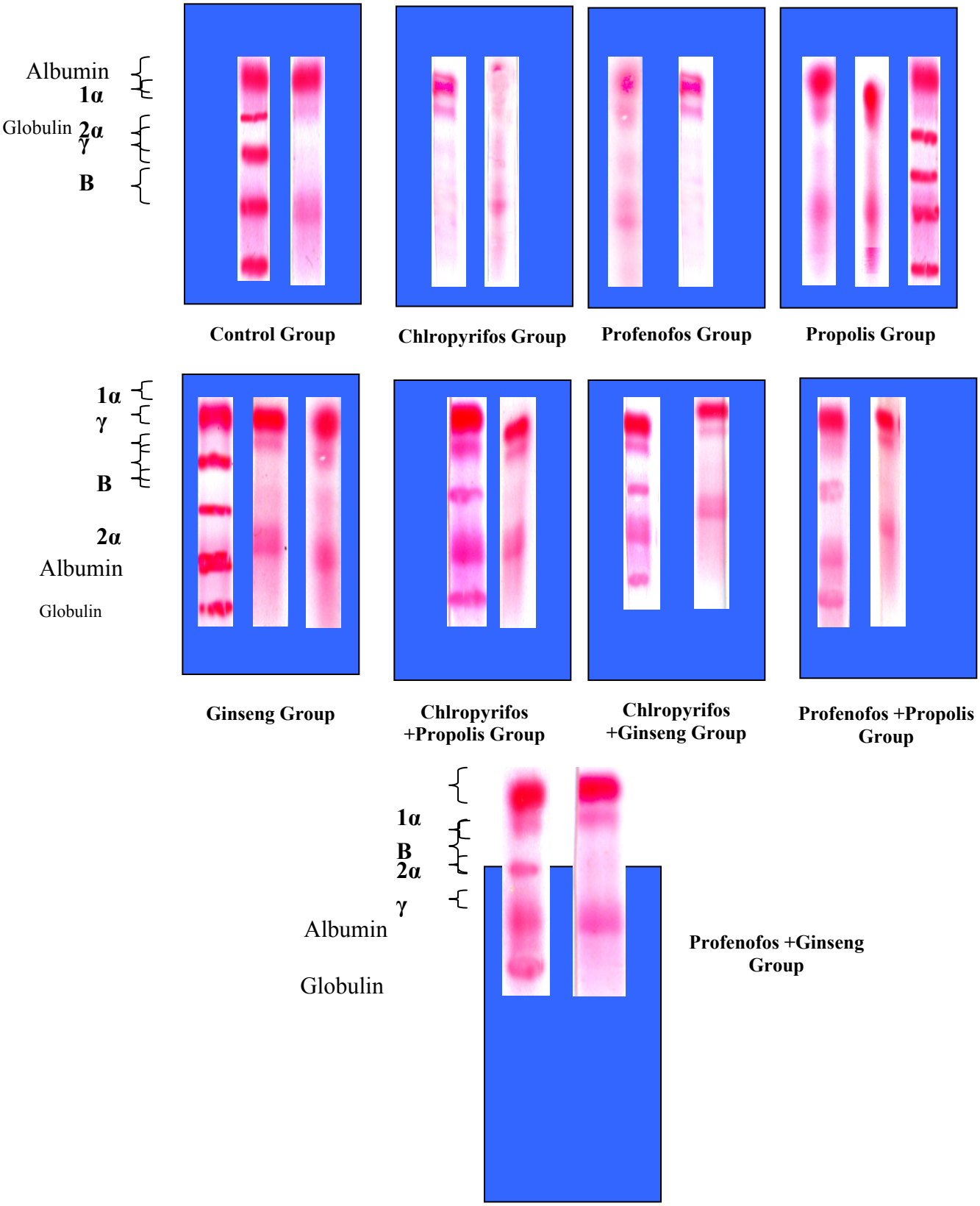

Figure (5): Strips of protein fractionations of different groups.

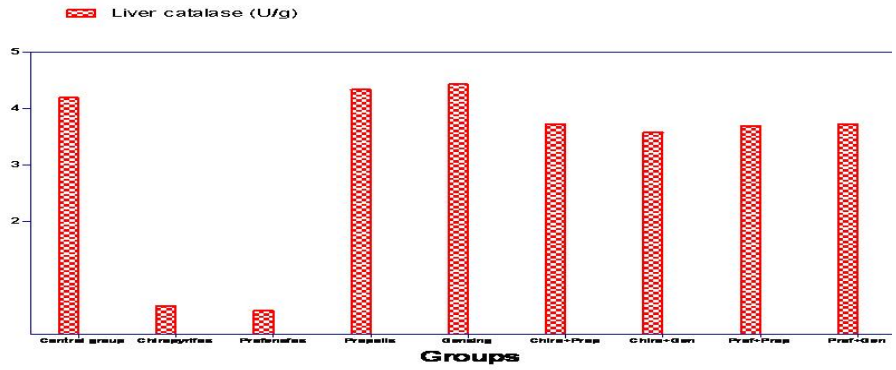

Fig (6): Effect of Chlorpyrifos (6.75 mg/kg), Profenofos (20mg/Kg) , Propolis (70 mg/ Kg), Ginseng (200 mg/Kg) and their combinations on Catalase activity (in Liver homogenates) in male albino rats .

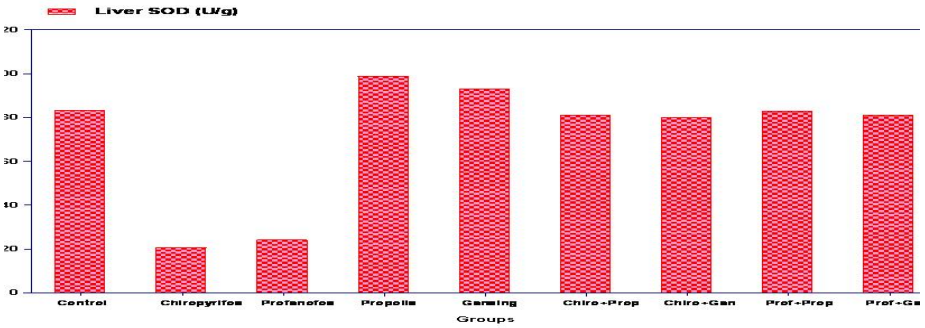

Fig (7): Effect of Chlorpyrifos (6.75 mg/kg), Profenofos (20mg/Kg) , Propolis (70 mg/ Kg), Ginseng (200 mg/Kg) and their combinations on SOD activity (in Liver homogenates)in male albino rats .

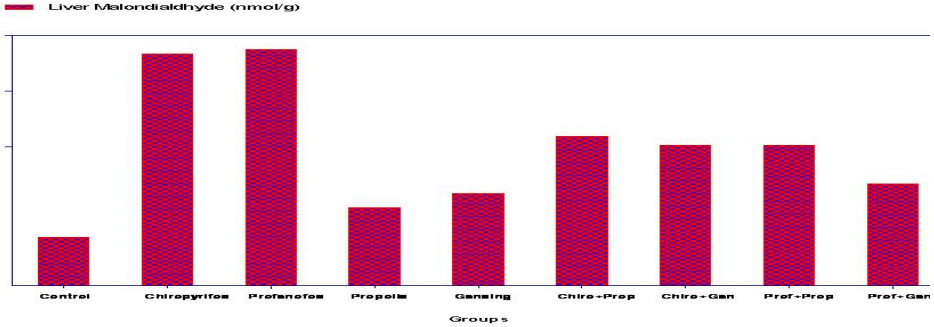

Fig (8): Effect of Chlorpyrifos (6.75 mg/kg), Profenofos (20mg/Kg) , Propolis (70 mg/ Kg), Ginseng (200 mg/Kg) and their combinations on Malondialdhyde (MDA) (in Liver homogenates) in male albino rats.

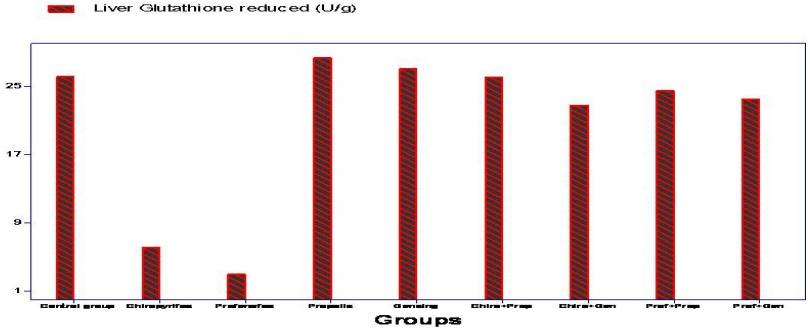

Fig (9): Effect of Chlorpyrifos (6.75 mg/kg), Profenofos (20mg/Kg) , Propolis (70 mg/ Kg), Ginseng (200 mg/Kg) and their combinations on Glutathione reduced (in Liver homogenates) in male albino rats.

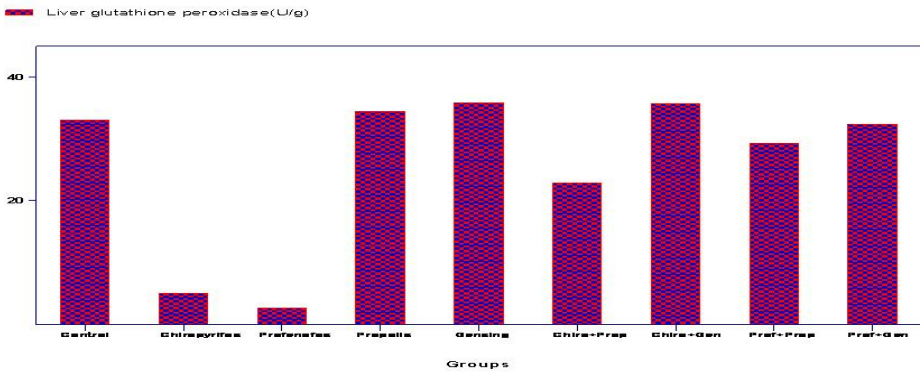

Fig (10): Effect of Chlorpyrifos (6.75 mg/kg), Profenofos (20mg/Kg) , Propolis (70 mg/ Kg), Ginseng (200 mg/Kg) and their combinations on Glutathione peroxidase (in Liver homogenates) in male albino rats.

3.6.5: Effect on Glutathione Peroxidase:

The plasma Glutathione peroxidase level was significantly reduced (P<0.05) in all groups treated with Chlorpyrifos, Profenofos each alone, propolis, ginseng and their combinations for successive 60 days when compared with normal control group. Whereas, a non significant change to slight decrease was reported in response to treatments with all combinations used except combination of Chlorpyrifos with propolis which showed a significant decrease compared with normal control group (Table 3) and (Fig.10).

3.7 :Histopathology:

(Group 1): Control group

The Liver: Microscopically: normal liver tissue formed of small central vein surrounded by cords of hepatocytes showing central vesicular nuclei and eosinophilic cytoplasm (Fig.11).

(Group 2): Chlorpyrifos treated group

The Liver: Microscopically, liver tissue showing markedly dilated central vein filled by large number of red blood cells and surrounded by hepatic cords (Fig.12) and showing liver cells necrosis in the form of pyknotic nuclei (↑) and more eosinophilia of the cytoplasm was also seen (Fig. 13).

(Group 3): Profenofos treated group

The liver: Microscopically liver tissue showing severe fatty change in the hepatocytes (H and E x 400) (Fig 14,15) and liver tissue showing necrosis in liver cells in the form of pyknotic nucleic (↑) and more eosinophilia of the cytoplasm (H and E x 400) was seen in (Fig. 16).

(Group 4): Propolis treated group

The Liver :Microscopically: normal liver tissue formed of central vein surrounded by cords of hepatocytes showing central vesicular nuclei and eosinophilic cytoplasm (Fig. 17).

(Group 5): Ginseng treated group

The Liver: The liver tissue of this group formed of central vein surrounded by cords of hepatocytes (Fig. 18).

(Group 6): Chlorpyrifos + Propolis treated group

The Liver: Microscopically, liver tissue cords of hepatocytes showing mild fatty change in the form of central nuclei surrounded by vacuolated cytoplasm (↑) (Fig. 19).

(Group 7): Chlorpyrifos + Ginseng treated group

The Liver: Microscopically, liver tissue cords of hepatocytes showing mild fatty change in the form of central nuclei surrounded by vacuolated cytoplasm (↑), (Fig. 20).

(Group 8): Profenofos+Propolis treated group

The Liver: Microscopically, liver tissue was showing moderate fatty change in the hepatocytes (Fig.21) and liver tissue cords of hepatocytes showing mild fatty change in the form of central nuclei surrounded by vacuolated cytoplasm (Fig.22).

(Group 9): Profenofos + Ginseng treated group

The Liver: Microscopically, liver tissue showing cords of hepatocytes with mild hemorrhage in the center, (Fig. 23).

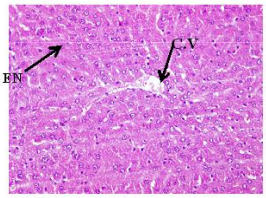

Fig. (11): Cross section of control rat liver of (Group 1) formed of small central vein surrounded by cords of hepatocytes showing central vesicular nuclei and eosinophilic cytoplasm (H and E x 400) **(CV: central vein, EN: esoinophilic nuclei)**.

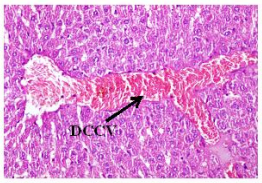

Fig. (12) : Cross section of liver tissue from group (2) treated with chlorpyrifos (6.75 mg/kg) showing markedly dilated central vein filled by large number of red blood cells and surrounded by hepatic cords (H and E x 400) **(DCCV:Dilated congested centeral vein)**.

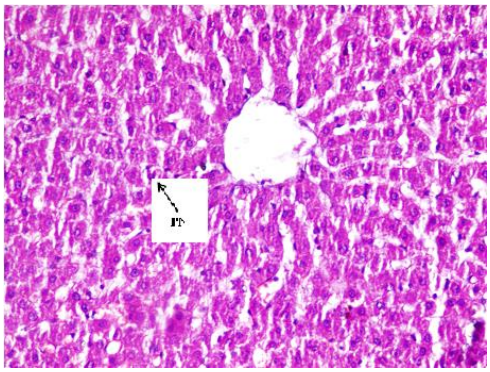

Fig. (13) : Cross section of liver tissue of group (2) treated with chlorpyrifos (6.75 mg/kg) showing liver cells necrosis in the form of pyknotic nuclei (↑) and more eosinophili or of the cytoplasm (H and E x 400). **(PN: Pyknotic nuclei)**

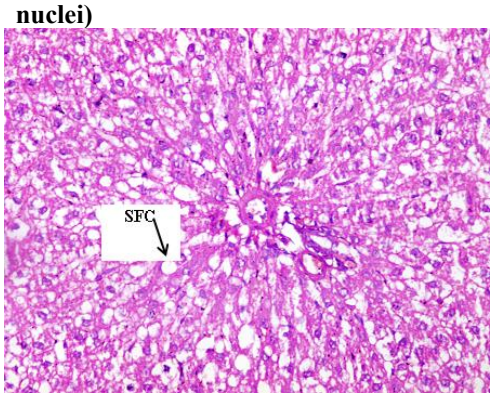

Fig. (14: Cross section of rat liver of group (3) treated with profenofos (20 mg/ Kg) showing severe fatty change in the hepatocytes (H and E x 400) **(SFC: Severe fatty change)**.

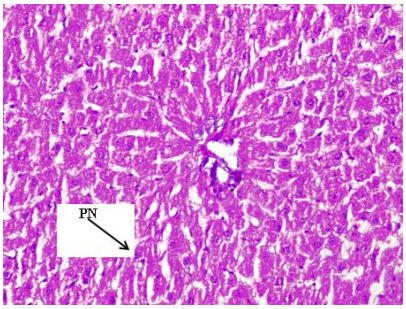

Fig. (15) : Cross section of rat liver of group (3) treated with profenofos (20 mg/ Kg) showing necrosis in liver cells in the form of pyknotic nuclei (↑) and more eosinophilia of the cytoplasm (H and E x 400) **(PN: Pyknotic nuclei)** .

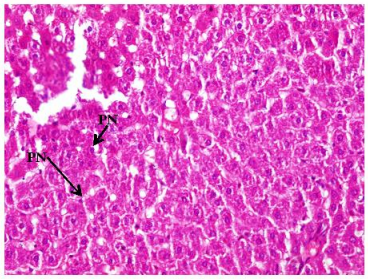

Fig. (16) : Cross section of rat liver of group (3) treated with profenofos (20 mg/ Kg) showing necrosis in liver cells in the form of pyknotic nuclei (↑) and more eosinophilia of the cytoplasm (H and E x 400) **(PN: Pyknotic nuclei)** .

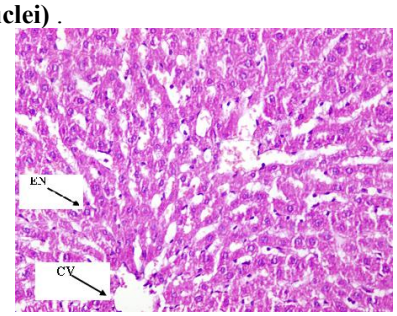

Fig. (17) Cross section of rat liver of group(4) treated with propolis(70 mg/ Kg) showing normal liver tissue formed of control vein surrounded by cords of hepatocytes with normal vesicular nuclei and eosinophilic cytoplasm (H and E x 400) **(EN: Eosinophilic nuclei, CV: Central vein).**

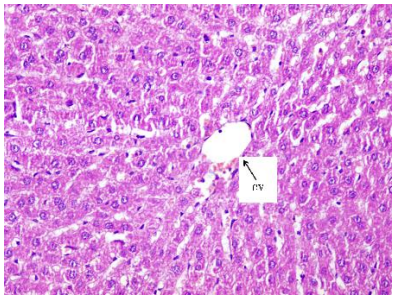

Fig. (18) : Cross section of rat liver of group(5) treated with ginseng (200 mg/ Kg) showing central vein surrounded by cords of hepatocytes (H and E x 400) **(CV: Central vein)**.

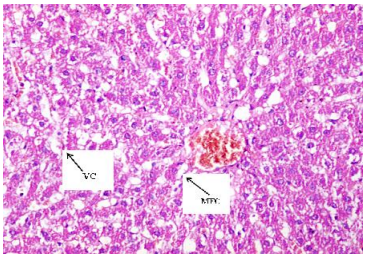

Fig. (19): Cross section of rat liver of group (6) treated with (Chlorpyrifos +Propolis) (6.75 mg/kg) & (70 mg/kg) respectively showing tissue cords of hepatocytes with mild fatty change in the form of central nuclei surrounded by vacuolated cytoplasm (↑) (H and E x 400) **(MFC: Mild fatty change, VC: vacuolated cytoplasm)**.

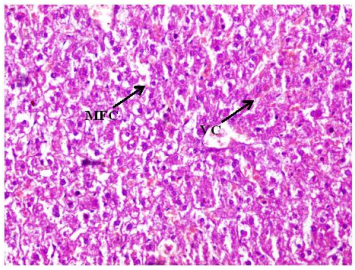

Fig. (20): Cross section of rat liver of group (7) treated with (Chlorpyrifos +ginseng) (6.75 mg/kg) & (200 mg/kg) respectively showing cords of hepatocytes with mild fatty change in the form of central nuclei surrounded by vacuolated cytoplasm (↑) (H and E x 400) **(MFC: Mild fatty change, VC: Vacuolated cytoplasm)**.

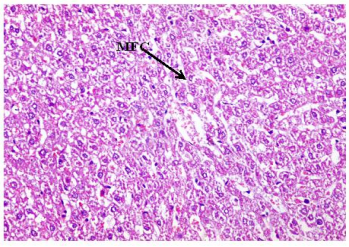

Fig. (21): Cross section of rat liver of group (8) treated with (profenofos + propolis) (20 mg/kg) & (70 mg/kg) respectively showing moderate fatty changes in the hepatocytes (H and E x 400) **(MFC: Moderate fatty change)**.

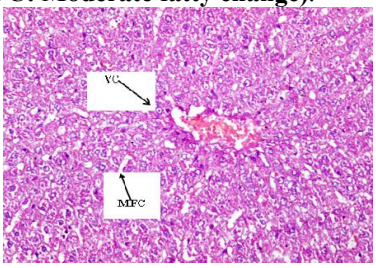

Fig. (22): Cross section of rat liver of group (8) treated with (profenofos + propolis) (20 mg/kg) & (70 mg/kg) respectively showing tissue cords of hepatocytes with mild fatty change in the form of central nuclei surrounded by vacuolated cytoplasm (↑) (H and E x 400) **(MFC: Mild fatty change, VC: Vacuolated cytoplasm).**

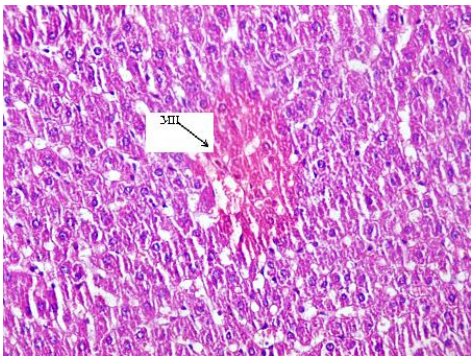

Fig (23): Cross section of rat liver of group (9) treated with (profenofos + ginseng) (20 mg/kg) & (200 mg/kg) respectively showing cords of hepatocytes with mild hemorrhage in the center (H and E x 400) **(MH: Mild Hemorrhage)**.

4. Discussion:

Chlorpyrifos (CPF) is an effective organophosphate ;(OP) pesticide used heavily throughout the world for agriculture and domestic purposes. The main target of OP pesticides is acetylcholinesterase (AChE), which hydrolyses acetylcholine (ACh) in cholinergic synapses and at neuromuscular junctions [53].This results in the accumulation of ACh in the synapses which in turn induces hyperactivity in cholinergic pathways.

Profenofos caused different symptoms of toxicity and revealed some biochemical changes especially in the enzymes activity of the liver and brain following two sublethal doses of profenofos in mice [54].

Natural products are a promising source for the discovery of new pharmaceuticals. In the last

decades, several works dealing with propolis' composition and biological properties have been published, revealing the interest of researchers on this bee product and its potential for the development of new drugs [17].

The low cost of traditional medicinal plants also raise significant interest to prevent morbidity and mortality from chronic diseases in countries where low or middle income populations are important [55].

Increased utilization of medicinal plants became a World Health Organization (WHO) policy in 1970. Plants and herbs are chemical factories that directly provide about 25% of currently used drugs and another 25% of drugs comprise chemically altered natural products [56].

Propolis is a resinous hive product collected by honeybees from plants, showing a very complex chemical composition [18]. It has been used in folk medicine since ancient times, due to its many biological properties, such as antibacterial [57], antitumor [25, 58], and immunomodulatory [59], among others.

Ginseng is a well-known medicinal herb in traditional Asian medicine and is considered an adaptogen. *Panax ginseng* C.A. Meyer (Araliaceae), which grows in China and Korea, has a variety of beneficial biological actions that include anti-carcinogenic, anti-diabetic-inflammatory effects, as well as cardiovascular protection and neuroprotection [30, 27].

Concerning the effect of the test compounds on liver function parameters; it is a well known fact that Liver plays a central role in the detoxification process and the threat of maximum exposure to xenobiotics and their metabolic by-products [60].

Our results showed that the administration of Chlorpyrifos and/or Profenofos in their recommended doses for successive 60 days into normal rats elicited highly significant decrease in serum total protein level while Serum transferases (AST &ALT) levels were markedly elevated compared with normal control group.

Our results showed also that Treatment of normal rats with either Chlorpyrifos or Profenofos for 60 successive days in their recommended doses elicited a marked elevation in serum LDH activity after the end of the study when compared with normal control group.

The administration of Chlorpyrifos and/or Profenofos each alone and their combinations with either Propolis or Ginseng to normal rats elicited a significant decrease in serum albumin after two months post administration when compared with normal control group.

Concerning the effect on serum Globulin α1, Chlorpyrifos and Profenofos treated groups afforded a marked decrease in serum Globulin α1 when compared with normal control group after the end of the experiment. Concerning the effect on serum Globulin α2, Chlorpyrifos and Profenofos treated groups showed a marked decrease in serum Globulin α2 when compared with normal control group after the end of the experiment. Concerning the effect on serum Globulin β and γ, Chlorpyrifos and Profenofos treated groups afforded marked decrease in serum Globulin β and γ when compared with normal control group after the end of two monthes post administration.

While, non significant changes were observed in total proteins in the groups treated with either Propolis or Ginseng after 8th week when compared with normal control group .Whereas combinations of Chlorpyrifos and/or Profenofos with either Propolis or Ginseng afforded non significant changes when compared with control group, except combination of Chlorpyrifos and ginseng which showed a significant decrease in serum total proteins compared with normal control group. However, the combinations of the test plant extracts appear to ameliorate the hypoproteinaemia produced by each insecticide alone.

Meanwhile significant elevation in serum AST&ALT were recorded after 8th week in normal rats in response to administration of Propolis and/or Ginseng in their combinations with Chlorpyrifos and/or Profenofos, but this effect was less intense than that produced by each insecticide alone except group treated with propolis & ginseng alone which revealed non significant change in serum ALT when compared with normal control group.

While the combinations of both Chlorpyrifos and profenofos with either propolis and/or ginseng afforded a non-significant change in serum LDH activity when compared with normal control group.

Meanwhile, Propolis and Ginseng treated groups elicited a significant decrease in serum albumin after two months post administration, yet their effects were better than that produced by each insecticide and their combinations.

Non significant changes were noticed in groups treated with either Propolis or Ginseng on serum Globulin α1when compared with normal control group. While the combinations of Propolis or Ginseng with either Chlorpyrifos or Profenofos elicited a significant decrease in serum Globulin α1 when compared with normal control group.

A marked decrease in serum Globulin α2 was obtained in groups given either Propolis or ginseng when compared with normal control group after the end of the experiment, but the decrease was much lesser than that produced by the insecticides used.

The combinations of Propolis or Ginseng with either Chlorpyrifos or Profenofos elicited a significant decrease in serum Globulin β and γ when compared with normal control group but this effect was much lesser than that produced by the insecticides alone.

Our results were in full agreement with [60-63] They reported that Changes in the level of total protein reflect disorders in the synthesis and metabolism of proteins.

Further support to our results was obtained by *Bouaziz et al. [64]* as they reported that serum AST activity was determined to be statistically increased in the animals that were administered Chlorpyrifos alone. The same change was determined by in rats. Furthermore, [65] have reported that administration of Chlorpyrifos to mice cause similar differences in serum enzyme activity. On the other hand, compared to the control group, the significant decrease determined in the serum ALT activity of the group that was ingested Chlorpyrifos suggested hepatic dysfunction [66].

More recently, [67] revealed that CPF-treatment caused an increase in the activities of AST, ALT, LDH and GGT in serum of male and female rats. The increase in these enzymes may be due to liver dysfunction and disturbance in the biosynthesis of these enzymes with alteration in the permeability of liver membrane takes place.

Our results seem to be conceivable with that obtained by [Gokcimen et al][68] .They observed increase in the level of serum LDH after organophosphorous insecticide exposure. It has been suggested that when liver cells containing LDH are damaged or destroyed due to oxidative effect of organophosphorous insecticide, the integrity of cell membrane gets disturbed and it might become more porous and permeable or may rupture resulting in the leakage of this enzyme.

Kalender et al. [61] reported that among other biochemical parameters that be altered after Chlorpyrifos administration, the increase in AST and ALT activities were found to be related to damage in the liver and the change in hepatic functions. ALP activity increases in case of the damage of hepatic cells and the obstruction of bile ducts arising from cellular damage.

At the meantime, it was obvious that the activity of serum GGT, LDH; biochemical Signs of hepatocellular injury and disturbed amino acid metabolism may be of value as markers of exposure to Profenofos, [69]. Moreover, high doses of the profenofos induced tissue vacuolization, haemorrhage and hyperplasia of Kupffer cells in the liver was reported by [Fawzy et al.] [70].

Irfan et al., [71] reported that prolonged exposure of rats to profenofos was also shown to cause a significant increase in γ-glutamyl transferase (GGT) and lactate dehydrogenase (LDH) Activities, compared with the control group. The significant elevations in enzymes activities of GGT and LDH indicate damage to any or all organs producing these enzymes such as liver or kidneys injuries [72, 73].

Our results were compatible with [Mantle et al] [74]. They reported that Exposure to organophosphorous insecticides has been shown to inhibit all the cytoplasmic proteases and some of the lysosomal proteases in the liver tissue, the major site for insecticide metabolism.

The electrophoretic pattern of serum protein pointed out that profenofos provoked a significant lower serum protein concentration with higher gamma-globulins and lower albumins and therefore A/G decreased [75]. Such changes in total protein and albumin reflect hepatocellular injury and disturbed amino acid metabolism induced by profenofos [69] *Mogda et al*., [75] showed that rats treated with profenofos showed a lower concentration of serum proteins and albumin accompanied by decreased globulin alpha 1 and beta along with an increased gamma 2 globulin. After exposure to profenofos α2, β1, γ1 contents were decreased while α1, β2, γ2 globulins were increased these findings may be related to impact of profenofos towered the hepatic cells and immune system [*Yousef* et al][37].

[Nagat et al][90] Mentioned that the Chlorpyrifos-treated animals also exhibited significantly lower total protein and albumin levels than the control animals and these results are in full agreement with our findings. Albumin, which is the most abundant blood plasma protein, is produced by the liver and several studies have shown that its production can be decreased by OPs such as Chlorpyrifos Since reductions in albumin levels are generally suggestive of liver disease, it is possible that OPs like Chlorpyrifos alter protein and free amino acid metabolism and their synthesis in the liver.

[Mansour et al][69] showed that total protein and A/G ratio is done as a routine test to evaluate the toxicological nature of various chemicals. Increases of total protein and decrease of A/G ratio were observed following chlorpyrifos treatment.

On the same basis, reported that the albumin level was also slightly decreased in males from (5.65%) and increased in females from (5.30%), suggesting high serum globulin levels reflecting high protein in both male and female rats. The increase of total protein and A/G ratio in Chlorpyrifos-treated groups may be due to the liver and kidneys

dysfunctions, partially as a result of high elevation of the serum enzymes.

The hepatoprotective effect of propolis was supported by the results of [Sugimoto et al] [76]. They showed that the AST activity determined to be high in the group that was administered Chlorpyrifos and AST was demonstrated to be decreased in the groups that were administered Propolis in association with Chlorpyrifos. This decrease supports the hepatoprotective effect of Propolis and these researchers have reported Propolis to cause decrease in AST activity when administered to rats exposed to D-galactosamine.

Similarly, *Chopra et al.,* [77] reported that Propolis caused decrease in AST activity that has increased due to exposure to doxorubicin in rats. On the other hand, the difference between the groups that was administered Chlorpyrifos and the groups that were administered Propolis in association with Chlorpyrifos, with respect to ALT activity, was determined to be decrement compared to control group. The same condition was observed for ALP activity in the group that was administered long and short-term Propolis in association with Chlorpyrifos.

Ramadan et al., [78] reported that oral administration of Propolis for 70 days; decreased the activities of AST and ALT in plasma. Also, *Sforcin et al.,* [79] reported that treatment of rats with Propolis does not induce any alteration in AST level. Moreover, *Mani et al.,* [80] found no alteration in AST value in the serum of Propolis treated rats for (30 or 90 or 150 days) at doses of (1, 3 and 6 mg/kg/day).

Our results are in agreement with [ElDenshary et al] [81]. They reported that the histological examination of liver sections in rats treated with honey, KGE and honey plus KGE showed the normal hepatocytes architecture and the central vein, we want to mention that our results considered one of the first studies deals with the effect of ginseng on liver activity and parameters.

Pesticides may also affect the biochemical and physiological functions in living organisms, there by affecting the membrane integrity [82] and may induce in vivo and in vitro generation of reactive oxygen species (ROS)leading to oxidative stress [83].

Living organisms have a complex antioxidant (enzymatic and non-enzymatic) system to protect against the deleterious effects of free radicals. Activity of the antioxidant defense system can be increased or inhibited under chemical stress, and antioxidant parameters therefore represent biomarkers of interest [84]. The enzymes that provide the first line of defense include superoxide dismutase (SOD), catalase(CAT) and glutathione reductase (GR). Reduced glutathione (GSH) is the primary cellular antioxidant (non-enzymatic) and plays an important role in the antioxidation of ROS and free radicals and, as a thiol- containing co-enzyme, in the detoxification of xenobiotic compounds .Glutathione-S transferase (GST) is a group of multifunctional enzymes that catalyze the conjugation of GSH with a variety of electrophilic metabolites that are involved in the detoxification of both reactivintermediates and oxygen radicals [85].

Our results are reinforced by *Verma & Srivastava* [86]. They reported that Chlorpyrifos is known to produce oxidative stress resulting in the accumulation of lipid peroxidation products in different organs of rats; also authors [87, 88] reported that CPF and other OP pesticides have been shown to damage DNA also.

Naval et al., [89] have demonstrated the protective effect of a normalized aqueous Panax ginseng root extract on hydrogen peroxide-induced oxidative damage in astrocytic primary cultures. Their results showed that the root of Korean ginseng is endowed with significant antioxidant properties and this is the base for its glioprotection against acute oxidant stress.

In contrary to our result, CAT and SOD activities in liver were increased in chlorpyrifos treated mice probably to dismutate superoxide anions and to decompose H_20_2 according to *Nagat Aly, et al., [90]*. These data are parallel with *Yu et al.*, [91] .In contrast. *Banudevi et al.,* [92] found that bisphenol A and PCB's decreased the activity of both CAT and SOD.

Levels of MDA, a major oxidation product of poly-unsaturated fatly acids, have been considered to be the most significant indicator of membrane lipid peroxidation arising from the interaction of reactive oxygen types with cellular membranes [93].

Kumar and Ramakrishna [94] have reported an increase in MDA levels in chronic Chlorpyrifos intoxication and these findings in complete accordance with our results.

Increasing level of MDA in our study as a result of treatments with both Chlorpyrifos and profenofos go hand in hand with the results of *Mecdad et al.,* [95]. They revealed statistically significant reduction of antioxidant defense enzymes, total antioxidant capacity, while MDA levels showed significant elevations in MDA in insecticides exposed workers.

Furthermore, Exposure of rats to Chlorpyrifos through drinking water resulted in a significant increase in lipid peroxidation and protein oxidation as indicated by the significant increase in MDA content, protein carbonyls and appotosis levels suggesting that Chlorpyrifos activated the formation of free radicals in cerebral cortex tissue. This is corroborated with the findings which demonstrated that Chlorpyrifos

exposure stimulated the generation of reactive oxygen species (ROS) in the brain [96].

Our results showed marked decrease in glutathione enzyme and these results were in full agreement with *Rana et al.,* [97] They reported that glutathione deficiency contributes oxidative stress, which plays a key role in aging and the pathogenesis of many diseases including seizures, Alzheimer's disease, Parkinson's disease, liver disease, cystic fibrosis, sickle cell anemia, AIDS, cancer, heart attack, stroke and diabetes. GSH is also a substrate of enzymes, glutathione peroxidase and glutathione-S-transferase.

The authors added that treatment of rats with both insecticides has also been reported that the long-term treatment with OP causes a gradual depletion of GPx, and GST [98].

Our results were compatible also with *Fang et al.,*[99]. They reported that a considerable decline in GSH content in the tissue may be due to its utilization to challenge the prevailing oxidative stress under the influence of ROS generated from MP and CPF oxidative stress. However, oxidative stress can induce GSH rising by protective role in the organisms exposed to chemicals. Reduced GSH and its metabolizing enzymes provide the foremost defense against ROS-induced cellular damage.

Meanwhile, the administration of profenofos caused a significant decrease in the levels of glutathione peroxidase (GPx) and reduced glutathione (GSH), and an increase in the lipid peroxidation (LPO) level [100] and this in contrary to our results.

5. Conclusions

From the obtained results, we report that both organophosphorous insecticides either Chlorpyrifos or profenofos have very dangerous and toxic effects, since they showed many side effects represented by high level of liver enzymes and decreasing total protein concentrations. Moreover, the damage in tissues of liver.

6. Recommendations

So we recommend the use of the combination of propolis and ginseng which is known as antioxidants compounds in order to ameliorate the possible side effects caused by insecticides that we exposed to them to avoid the proven hazardous effect of insecticides on biochemical parameters and to overcome the side effects of both Chlorpyrifos and profenofos on liver.

Conflict of interest

The authors declare that there are no conflicts of interest.

References

[1] Konradsen,F., van der Hoek, W., Cole ,D.C., Reducing acute poisonings in developing countries-options for restricting the availability of pesticides. Toxicology.; 192 (2003) 249–61.

[2] Hamilton, D., Ambrus, A., Dieterle, R., Pesticide residues in food—acute dietary exposure. Pest Manag Sci.; 60 (2004) 311–39.

[3] Lasram, M.M., Annabi, A.B., Elj, N., Selmi, S., Kamoun, A., El-Fazaa, S., Gharbi, N., Metabolic disorders of acute exposure to Malathion in adult Wistar rats. J. Hazard. Mater.; 163 (2009)1052–1055.

[4] Alexander, B.H., Burns, C.J., Bartels, M.J., Acquavella, J.F., Mandel, J.S., Gustin, C., Baker, B.A., Chlorpyrifos exposure in farm families: results from the farm family exposure study. J. Exp. Sci. Environ. Epidemiol.; 16(2006) 447–456.

[5] Farahat Richard, A., Fayssal, M., Fenske, N., James, R., Olson, S., Kit Galvin., Matthew, R., Bonner Diane, S., Rohlman, N., Taghreed, M., Farahat, A., Pamela, J., Lein, W., Kent Anger, A., Chlorpyrifos exposures in Egyptian cotton field workers, NeuroToxicology.; 31(2010) 297-304.

[6] Garabrant, D.H., Aylward, L.L., Berent, S., Chen, Q., Timchalk, C., Burns, C.J., Hays, S.M., Albers, J.W., Cholinesterase inhibition in chlorpyrifos workers: Characterization of biomarkers of exposure and response in relation to urinary TCPy. J. Exp. Sci. Environ. Epidemiol.;19(2009) 634–642.

[7] Mahmut Selvi., Rabia Sarıkaya., Figen Erkoç., Oner Koçak., Investigation of acute toxicity of chlorpyrifos-methyl on guppy Poecilia reticulate. Chemosphere.; 60(2005)93–96.

[8] Lemus, R., Abdelghani, A., Chlorpyrifos: an unwelcome pesticide in our homes. Rev. Environ. Health.;15(2000) 421-433.

[9] Dishburgere, H.J., McKellar, R.L., Pennington, J.Y., Rice, J.R., Determination of residues of chlorpyrifos (insecticide), its oxygen analogue, and 3, 4, 5, 6-trichloro-2-pyridinol in tissues of cattle fed. J. Agric. Food. Chem.; 25(1997)1325-1329.

[10] Johnson, D.E., Seidler, F.J., Slotkin, T.A.,Early biochemical detection of delayed neurotoxictiy resulting from development exposure to chlorpyrifos. Brain res. Bull.; 45(1998)143-147.

[11] Kalender Yusuf., Sinan Kaya., Dilek Durak., Fatma Gokce Uzun., Filiz Demir., Protective effects of catechin and quercetin on antioxidant status, lipid peroxidation and testis-histoarchitecture induced by chlorpyrifos in male rats. Environmental Toxicology and Pharmacology.; 33(2012)141–148.

[12] Cuany, J., Handani, J., Berge, D., Fournier, M., Raymond, C.P., Ccorghiou, N., Paste, A., Action of esterase Bl on chlorpyrifos in organophosphate-resistant Culex mosquitos. Pestic. Biochem. Physiol.;45(1993)1-6.

[13] Kumar, A., and Chapman, J.C., Profenofos toxicity to the eastern rainbow fish (Melanotaenia duboulayai). Environ Toxicol Chem.; 17(2002)1799–1806.

[14] Prabhavathy, E.L., Meistrich, M.L., and Bairy, K.L., Cytotoxicity and genotoxicity induced by pesticides profenofos on cultured human peripheral blood lymphocytes. Drug.Chem.Toxicol. 3(2006)313-322.
[15] Abass Khaled., Petri Reponen A.B., Jorma Jalonen B., Olavi Pelkonen A., vitro metabolism and interaction of profenofos by human, mouse and rat liver preparations. Pesticide Biochemistry and Physiology.; 87(2007)238–247.
[16] WHO, The WHO recommended classiffication of pesticides by hazard and guidelines to classiWcation , WHO/PCS/90.1, World Health Organization, Geneva, Switzerland, (1990-1991).
[17] Sforcin José MaurÍcio ., Vassya Bankova ., Review, Propolis: Is there a potential for the development of new drugs? Journal of Ethnopharmacology.; 133(2011) 253–260.
[18] Bankova, V.S., Castro, S.L., Marcucci, M.C., Propolis: recent advances in chemistry and plant origin. Apidologie.; 31(2000) 3-15.
[19] Kanbur, M., Eraslan, G., Silici, S., Antioxidant effect of propolis against exposure to propetamphos in rats. Ecotox. Environ. Saf.; 72 (3)(2009)909-915.
[20] Ghisalberti, E.L., Propolis: a review. Bee World.; 609(1979) 59-84.
[21] Grange, J.M., Davey, R.W., Antibacterial properties of propolis (bee glue), journal of the Royal Society of Medicine.; 83(1990)159-160.
[22] Castaldo, S., Capasso, F., Propolis, an old remedy used in modern medicine.Fitoterapia.;73(2002) 1-6.
[23] Marcucci, M.C., Propolis: chemical composition, biological properties and therapeutic activity. Apidologie.; 26(1995)83-99.
[24] Burdock, G.A., Review of the biological properties and toxicity of bee propolis. Food Chemical and Toxicology.; 36 (4)(1998) 347-363.
[25] Banskota, A.H., Tczuka, Y., Kadota, S., Recent progress in pharmacological research of propolis. Phytotherapy Research.; 15(2001) 561-571.
[26] Kolankaya, D., Selmanoglu, G., Sorkun, K., Salih, B., Protective effects of Turkish propolis on alcohol-induced serum lipid changes and liver injury in male rats. Food Chem.; 78(2002) 213-217.
[27] Yun, T.K., Lee, Y.S., Lee, Y.H., Kim, S.I., Yun, H.Y., Anticarcinogenic d bffect of Panax ginseng C.A. Meyer and identification of active compounds. Journal of Korean Medical Science.; 16(2001)S6-S18.
[28] Park, J.D., Recent studies on the chemical constituents of Korean ginseng (Panax ginseng C. A. Meyer). Kor. J. Ginseng Sci.; 20(1996) 389–415.
[29] Gillis, C.N., Panax ginseng pharmacology: a nitric oxide link? Biochem. Pharmacol. ;54 (1997) 1–8.
[30] Seung Phill Choid., Kyong-Hwan Bangb., Dongho Leec.,Hyung-Kyoon Choia., NMR-based metabolic profiling and differentiation of ginseng roots according to cultivation ages, Journal of Pharmaceutical and Biomedical Analysis.;58 (2012) 19–26.
[31] Mansour, S.A., and Mossa, A.M., Lipid peroxidation and oxidative stress in rat erythrocytes induced by chlorpyrifos and the protective effect of zinc. Pesticide Biochem. Physiol.; 93 (2009) 34-39.
[32] Mahmut Selvi., Rabia Sarıkaya., Figen Erkoç., Oner Koçak., Investigation of acute toxicity of chlorpyrifos-methyl on guppy Poecilia reticulate. Chemosphere.; 60(2005)93–96.
[33] Whitney, K.D., Seidler, F.J., Slotkin, T.A., Development neurotoxcity of chlopyrifos: cellular mechanisms toxicol. Appl. Pharmacol.; 134 (1995)(53-62).
[34] Weil, C.S., Tables for convenient calculation of medium effective dose (LD50) or ED50) and instruction in their use Biometrics, 8(1952)263 -294.
[35] Andreson, R.A., Aaraas, I., Gaare, G., Fonnum, F., Inhibition of acetylcholinesterase from different species by organophosphorus compounds, carbamates and methylsulphonylflouride. Genetic Pharmac.; 8 (1977)331-334.
[36] Newairy, A.S., Salama, A.F., Hussien, H.M., Yousef, M.l., Propolis alleviates aluminium-induced lipid peroxidation and biochemical parameters in male rats. Food Chem Toxicol.; 47(2009)1093-8.
[37] Yousef, M.I., Salama, A.J., Propolis protection from reproductive toxicity caused by aluminium chloride in male rats. Food Chem. Toxic.; 47(2009) 1168-1175.
[38] Zhang, Z., Lian, X.Y., Stringer, J.L., Protective effects of ginseng components in a rodent model of neurodegeneration. Annals of Neurology.; 57(2005) 642-648.
[39] Scherners, S., The blood morphology of laboratory animals . Blackwell Scientific Publication (3rd Ed); (1967)20:22.
[40] Reitman, S., and Frankel, S., A colorimetric method for the determination of serum glutamic oxaloacetic and glutamic transaminase . Amer.J.Clin . Pathology. ;(28) (1957) 56.
[41] Henry, R.G., Clinical Chemistry . Chemicals and Technique. (2nd Ed), Harper, New York .(1974) P.257 .
[42] Esakova, T., and Ivanov, M., Interaction of lactate dehydrogenase and Membranes of the sarcoplasmic reticulum .Russian, Biokhimiia.; 57(1992) 253.
[43] Jeppsson,J.O., Laurell,C.B., Franzén,B., Agarose gel electrophoresis Clin Chem 25 (1979) 629-638.
[44] Habig, W., and Pabast, M., Jakoby, W.j., The first step in mercapturic acid formation.Biol.Chem.; 249(1974) 7130-7139.
[45] Aebi, H., Catalase in vitro. Method Enzymol.; 105(1984)121–126.
[46] Nishikimi, M., Roa, N.A., and Yogi, K The occurrence of superoxide anion in the reaction of reduced phenazine methosulfate and molecular oxygen. Biochem. Bioph. Res. Common.; 46(2)(1972)849 — 854.
[47] Beutler, F., Duron, 0., Kelly, M.B., Improved method of estimation of blood glutathione. J. Lab Clin. Med.; 61(5)(1963) 882.
[48] Paglia, D.E., and Valentine, W. N., Studies on the quantitative and qualitative characterization of erythrocyte glutathione peroxidase. J. Lab. Clin. Med.; 70(1967) 158—169.
[49] Satoh.K., Serum lipid peroxide in cerebro vascular disorders: determined by a new colorimetric method Clinica chemical Acta .; 90(1978)37-43.

[50] Ohkawa, H., Ohishi, W., Yagi, K., Assay for lipid peroxides in animal tissues by thiobarbituric acid reaction. Anal Biochem.; 95(2)(1979)351-358.
[51] Carleton, H.M., Carleton's Histological Technique . (4th Ed), Pub .London, New York, Toronto, Oxford university press (1967).
[52] Snedecor, G.W., and Cochran, W.G., Statistical Methods (8thEd), Ames Iowa State University (1982).
[53] Ecobichon, D.J., Toxic effects of pesticides, in: C.D. Klaassen (Ed.), Casarett and Doull's Toxicology. The Basic Sciences of Poisons, fifth ed., McGraw-Hill, New York, pp. (1996) 643–689.
[54] Saeed, R. M., Al-Koly, M. A., and Ali, M. A., Hepatic, renal and pulmonary responses in pregnant mice and their fetuses induced by profenofos. Journal of Union of Arab Biologists: Zoology.; 3(A)(1995)359-386.
[55] Gazioano, T.A., Galea,G., and Reddy,K.S., Scaling up interventions for chronic disease prevention . Lancet ;370(2007) 1939-1946.
[56] Desmet, P.A. , The role of plant –derived drugs and herbal medicines in health care .Drugs; 54(1997)801-840.
[57] Sforcin, J.M., Fernandes J.r., Lopes, C.A.M., Bankova, V., Funari, S.R.C., Seasonal effect on Brazilian propolis antibacterial activity. Journal of Ethnopharmacology; 73(2000) 243–249.
[58] Bazo, A.P., Rodrigues, M.A.M., Sforcin, J.M., Camargo, J.L.V., Ribeiro, L.R., Salvadori, D.M.F., Protective action of propolis on the rat colon carcinogenesis. Teratogenesis, Carcinogenesis and Mutagenesis.; 22(2002)183–194.
[59] Murad, J.M., Calvi, S.A., Scares, A.M.V.C., Bankova, Y., Sforcin, J.M., Effects of propolis from Brazil and Bulgaria on fungicidal activity of macrophages against Paracoccidioides brasiliensis.Journal of Ethnopharmacology.; 79(2002)331-334.
[60] Van Lieshout, E.M., Ekkel, M.P., Bedaf, M.M., Nijhoff, W.A., Peters, W.H., Effects of dietary anlicarcinogens on ral gastroiniestinal gluta-thione peroxidase activity, Oncol. Rep.; 5(1998)959 963.
[61] Kalender, S., Ogutcu, A., Uzunhisarcikli, M., Acikgoz, F., Durak, D., Ulusoy, Y., Kalender, Y., Diazinon induced hepatotoxicity and protective effect of vitamin E on some biochemical indices and ultrastructural changes, Toxicology.; 211(2005) 197–206.
[62] Filazi, M., Sircli, F., Kalkan, M., The influence of amitraz on biochemical parameters in mice, Hum. Exp. Toxicot.; 22(2003) 99-101.
[63] Kapoor, V., Prasad, T., Paliwal, V.K., Blood biochemical constituents in calves following subclinical levels of fluoride toxicosis, Fluoride.; 34(2001)126-131.
[64] Bouaziz, H., Ketata, S., Jammoussi, K., Boudawara, T., Ayedi, F., Ellouze, F., Zeghal, N., Effects of sodium fluoride on hepatic loxicity in adult mice and their suckling pups, Pestic. Biochem. Physiol.; 86(2006)124-130.
[65] Chinuy, N.J., Memon, M.R., Beneficial effects of some vitamins and calcium on fluoride and aluminium toxicity on gastrocnemius muscle and liver of male mice. Fluoride.; 34(2001)21 -33.
[66] Birkner, E., Grucka-Mamczar, E., Machoy, Z., Tarnawski, R., Pola-nkk, R., Disturbance of protein metabolism in rats after acute poisoning with sodium fluoride, Fluoride.; 33(2000)182 186.
[67] Mansour ,Sameeh. A., and Mossa, Abdel-Tawab .H., Oxidative damage, biochemical and histopathological alterations in rats exposed to chlorpyrifos and the antioxidant role of zinc. Pesticide Biochemistry and Physiology.; 96(2010) 14–23
[68] Gokcimen, K., Gulle, H., Demirin, D., Bayram, A., Kocak, I., Altuntas, A., Effects of diazinon at different doses on rat liver and pancreas tissues, Pestic. Biochem. Physiol.; 87(2007) 103–108.
[69] Gomes, J., Dawodu, A.H., Lloyd, O., Revitt, D.M., Anilal, S.V., Hepatic injury and disturbed amino acid metabolism in mice following prolonged exposure to organophosphorus insecticides. Hum Exp Toxicol.; 18(1)(1999)33-7.
[70] Fawzy, I., Iman. Zakaria, Hamza, A., Ihab, A., The effect of an organophosphorous insecticide on the Hepatic, Renal And Pulmonary tissues of mice fetuses. Egypt J. Med. Lab. Sci.; 16(2)(2007)99 -113.
[71] Irfan, A., Namik, D., Mustafa, D., Ibrahim, K., Numan, T., the effects of methidathion on lipid peroxidation and some liver enzymes: role of vitamins e and c. Archives of Toxicology.; 76, (8), (2002): 470-473.
[72] Amacher, D.E., A toxicologist's guide to biomarkers of hepatic response Human & Experimental Toxicology.; 21(2002) 253 -262.
[73] Ncibi, S., Ben Othman, M., Akacha, A., Krifi, M.N., Zourgui, L., Opuntia ficus indica extract protects against chlorpyrifos-induced damage on mice liver. Food Chem Toxicol.; 46(2)(2008)797-802.
[74] Mantle, D., Effects of pirimiphos methyl on proteolytic enzyme activities in rat heart, kidney, brain and liver tissues in vivo. Clin Chim Acta .;262(1997) 89-97.
[75] Mogda, K. Mansour., Afaf, A.I. El-Kashoury., Rashed, M.A. Koretem, K.M., Oxidative and biochemical alterations induced by profenofos insecticide in rats, Nature and Science.; 7(2)(2009)1-15.
[76] Sugimoto, Y., Tarumi, T., Kaneko, Y., Isayama, S., Kawai, N., Sugim-oto, H., Yamada, H., Kamei, C., Effect of propolis extract on n-galactos-amine-induced hepatic injury in rats, Biol. Pharm. Bull;2 (1999) 1237-1239.
[77] Chopra, S., Pillai, K.K., Husain, S.Z., Giri, D.K., Propolis protects against doxorubicin-induced myocardiopathy in rats. Experimental and Molecular Pathology.; 62(1995)190-198.
[78] Ramadan, G., Soliman, B., Sawsan, S., Mahmoud, c., Salwa, M., Nofal, c., Rehab, F., Abdel-Rahman, c., Evaluation of the safety and antioxidant activities of Crocus sativus and Propolis ethanolic extracts.A Journal of Saudi Chemical Society.; 16(2012)13–21
[79] Sforcin, J.M., Kaneno, R., Funari, S.R.C., Absence of seasonal effect on the immunomodulatory action of Brazilian propolis on natural killer activity. The Journal of Venomous Animals and Toxins.; 8(2002)19-29.
[80]Mani,F.,Damasceno,H.C.R.,Novelli,E.L.B.,Martins,E. A.M.,Sforcin,J.M., Propolis: Effect of different

concentrations, extracts and intake period on seric biochemical variables, Journal of Ethnopharmacology.; 105(2006)95-98.

[81]ElDenshary.Ezzeldeen.S.,Mohammad,A.Al-Gahazalia., Fathia,A.Mannaa.,Hesham,A.Salem.,Nabila,S.Hassan., Mosaad,A.Abdel-Wahhab., Dietary honey and ginseng protect against carbon tetrachloride-induced hepatonephrotoxicity in rats. Experimental and Toxicologic Pathology.; (6)(2011)10-18.

[82] Aggarwal, M., Naraharisetti, S.B., Sarkar, S.N., Rao, G.S., Degen, G.H., Malik, J.K., Effects of subchronic coexposure to arsenic and endosulfan on the erythrocytes of broiler chickens: A biochemical study, Arch. Environ. Contam. Toxicol.; 56(2009)39–148.

[83] Bagchi, D., Bagchi, M., Hassoum, E.A., Stohs, S.J., In vitro and in vivo generation of reactive oxygen species, DNA damage and lactate dehydrogenase leakage of selected insecticides. Toxicol.; (1995)104-129.

[84]Doyotte,A.,Cossu,C.,Jacquin,M.C.,Babut,M.,Vasseur,P., Antioxidant enzymes, glutathione and lipid peroxidation as relevant biomarkers of experimental or field exposure in the gills and the digestive gland of the fresh water bivalve Unio tumidus. Aquat.Toxicol.;39(1997)93–110.

[85] Dickinson, D.A., Forman, H.J., Cellular glutathione and thiols metabolism. Biochem. Pharmacol.; 64(2002) 1019–1026.

[86] Verma, R.S., Mehta, A., Srivastava, N., In vivo chiorpyrifos induced oxidative stress: attenuation by antioxidant vitamins. Pestic. Biochem. Physiol.; 88(2007) 191—196.

[87] Rahman, M.F., Mahboob, M., Danadevi, K., Saleha, B., Grover, P., Assessment of genotoxicity effects of chlorpyrifos and acetaphate by the comet assay in mice leucocytes, Mutat. Res.; 516(2002)139-147.

[88] Shadnia, S., Foul addel, S., pajaoum, A., jalali, N., Abdollahi, M., Evaluation of oxidative stress and genotoxicity in organophosphorous insecticide formulators, Hum .Exp.Toxicol.; 24(2005) 439-445.

[89] Naval, M.V., GÓmez–serranillos, M.P., carretero, M.E., Villar, A.M. Neuroprotective effect of a ginseng (Panax ginseng) root extract on astrocytes primary culture, Journal of Ethnopharmacology.; 112(2007) 262-270.

[90] Nagat Aly., Kawther EL-Gendy., Fatma Mahmoud., Abdel Khalek El-Sebae., Protective effect of vitamin C against chlorpyrifos oxidative stress in male mice. Pesticide Biochemistry and Physiology.; 97(2010)7–12.

[91] Yu, Y., Yang, A., Zhang, H., Hu, S., Mjtcnul, S., Exposure in the mixture of organophosphate pesticides inducing reproductive dysfunction in the offspring. Environ. Toxicol.; 98(2011)25-35.

[92] Banudevi, S., Krishnamoorthy, G., Venkataraman, P., Vignesh, C., Aruldhas, M.M., Arunakaran, J., Role of α-tocopherol on antioxidant status in liver, lung and kidney of PCB exposed male albino rats, Food Chem. Toxicol.; 44(2006) 2040– 2046.

[93] Halliwell, S., Chirico, L., Lipid peroxidation: its mechanism, measurement, and significance. Am J. Clin. Nutr.; 57(1993) 715-724

[94] Kumar, A., and Chapman, J.C., Profenofos toxicity to the eastern rainbow fish (Melanotaenia duboulayai). Environ Toxicol Chem.; 17(2002)1799–1806.

[95] Mecdad,Alaa.A., Manal, H., Ahmed, B., Manal, E.A., ElHalwagy, C., Mostafa, M., Afify, M., A study on oxidative stress biomarkers and immunomodulatory effects of pesticides in pesticide-sprayers. Egyptian Journal of Forensic Sciences.; (2011)1:93–98.

[96] Shah, Z.A., Gilani, R.A., Sharma, P., Vohora, S.B., Cerebroprotective effect of Korean ginseng tea against global and focal models of ischemia in rats. Journal of Ethnopharmacology.; 101(2005) 299–307.

[97] Rana, S.V.S., Allen, T., Singh, R., Inevitable glutathione, then and now. Indian J. Exp. Biol.; 40(2002) 706—716.

[98] Song, S.B., Xu, Y., Zhou, B.S., Effects of hexachlorobenzene on antioxidant status of liver and brain of common carp (Cyprinus carpio), Chemosphere.; 65(2006)699–706.

[99] Fang, Y.Z., Yang, S., Wu, G., Free radicals, antioxidants, and nutrition, Nutrition.; 18(2002)872–879.

[100] Gamal, H. Abdel Rahman., Abdel Razik, H. Farrag. ,Sonya, L. El Sharkawy., Wafaa E. Abdel Aal., Effects of Profenofos on Antioxidant Enzymes Activities and Gastric Mucosa in Rats. JASMR.; 1(2)(2006)125-134l.

6/22/2012