Ameliorative Role and Antioxidant Effect of Propolis and Ginseng against Reproductive Toxicity of Chlorpyrifos and Profenofos in Male Rats

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Abstract: The present study was aimed 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 seeked 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 testosterone hormone and by measuring reproductive performance parameters as well as histopathological changes in vital organ like testis. 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 W, 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 decrease of the testosterone hormone and deficiency of reproductive performance in male rats. In contrary to these actions, co-administration of propolis and ginseng to CPF and PRF-treated rats recovered almost most of these biochemical parameters to normal levels. On the other hand, CPF and PRF showed histopathological alterations in testis of male rats like spermatogenic arrest and odema and degeneration of spermatids, while administration of both propolis and ginseng highly ameliorate these dangerous reproductive toxicity markers.
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Keywords: Chlorpyrifos, Profenofos, Propolis, Ginseng, Testosterone, Reproductive toxicity, Reproductive performance.
Abbreviations: CPF, Chlropyrifos; PRF, Profenofos.

1. Introduction

The testes of humans and other mammals are highly susceptible to damage produced by genetic to chemical or other means. It has been reported that pesticides have been shown to cause overproduction of reactive oxygen species (ROS) in both intra and extra cellular spaces, resulting in a decline of sperm count and infertility in wildlife human **[1].**

Histological examination revealed that Chlorpyrifos caused testicular lesions characterized by markedly decreased testes weight with moderate to severe widening of interstitial spaces and partial arrest of spermatogenesis at the high level dose of 25 mg/kg.d .Histological epididymal changes were occurred in the high and middle dose groups characterized by severe oedema and congestion between epididymis tubes and lacking of sperm number. Lesser histological changes were noted at 5 mg/kg-d, where minimal histological evidence of damage was observed **[2]**.

The reduction in fertility index may simply represent the effects of Chlorpyrifos exposure on sperm parameters, and testis histological changes. Spermatogenesis and fertility are critically dependent upon the maintenance of adequate levels of testosterone [3]. Therefore, the effects of Chlorpyrifos on the fertility can be attributed to its ability to reduce serum testosterone levels and sperm

counts. Reduction in male body weight in dose group of 25 mg/kg-d can be attributed to the reduction in feed consumption and systemic toxicity of Chlorpyrifos in male mice.

It has been demonstrated that Propolis provides protection against infertility by improving sperm production, motility, count and quality, and increased the process of steroidogenesis and hence testosterone production [4]. Furthermore, Propolis protects sperm DNA from the oxidative damage caused by thiobarbituric acid-reactive substances (TBARS).

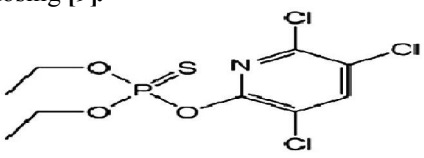
Propolis increased the levels of testosterone, relative weight of testes and epididymis and alleviated the negative effects of triphenyltin chloride (TPTC1) and this is in accordance with Shalmany and Shivazad [5] who suggested that the increase in weight of testes treated with propolis is due to high content of flavonoids.

A 2002 study by the Southern Illinois University School of Medicine (published in the annals of the New York Academy of Sciences) found that in laboratory animals, both Asian and American forms of ginseng enhance libido and copulatory performance. These effects of ginseng may not be due to changes in hormone secretion, but to direct effects of ginseng or its ginsenoside components on the central nervous system and gonadal tissues [6] in males, ginsenosides can facilitate penile erection.[7] This is consistent with traditional Chinese medicine and Korean medicine.

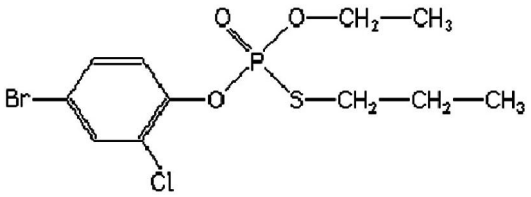
2. Materials and Methods

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 **[8]**. 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 **[9].**



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 **[10]** 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 **[11]**.



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. **[4,12].**

2.2.2Ginseng extracts preparation*:*

Red Ginseng powder (Supplied by Tsumura Pharmaceutical Co., Tokyo, Japan) was administered orally at dose (200 mg/Kg) **[13]** 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 Semen analysis:

(Sperm count and motility) were performed by dissecting out the Cauda epididymus and teasing it in a known volume of normal saline at 37°C. Sperm counting was done using a haemocytometer according to the method of Gerberding *et al.***[14]**

. The right testes were kept in a deep freezer (-40°C) for biochemical estimations and microelements detection. Left testes were removed and fixed in 10 % formalin for routine histopathology.

2.6Evaluation of sperm motility and morphology:-

The testis and epididymis were excised immediately, cleaned of the adhering tissues and weighed. The right caudal epididymis was used for collecting semen for sperm counting and the left one for studying fertility –related parameters (Sperm count, motility and abnormal forms) and morphology analysis.

The total number of sperm in the ejaculate= Calculation the mean number of sperm.

Total count = the mean no. of sperm X 104= / ml.

The total count X dilution factor (100) = /ml.

2.7 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 **[15]** .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 Testosterone hormone The biochemical measurements were performed according to the details given in the kit's instructions.

2.7.1 Determination of Testosterone hormone*:*

The assay used for the determination of testosterone hormone in plasma was enzyme linked immunosorbent assay (ELISA), using available kit purchased, from Diagnostic laboratories Inc (England). **[16]**.

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 **[17]**.

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 **[18]** 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 Testosterone:

(Table1) and (Fig 1) demonstrates that treatment of normal rats with either Chlorpyrifos or Profenofos exhibited a marked decrease ($P<0.05$) in serum testosterone level after the end of the experiment when compared with normal control group. At the mean time, treatment of normal rats with Propolis or ginseng each alone induced a significant increase ($P<0.05$) in serum testosterone after the end of the experiment when compared with normal control group. Whereas, a significant decrease in testosterone level was also recorded in response to combinations of the insecticides with either Propolis or ginseng when compared with normal control group, yet this effect was much better than that produced with each insecticide alone.

3.4 Effect on male fertility and sperm count:

(Table 2) and (Fig. 2) showed that the administration of either propolis or ginseng in their recommended doses for 60 successive days to mature rats afforded a slight and marked increase ($P<0.05$) in total sperm count respectively compared to normal control group. Whereas, the administration of either chlorpyrifos or profenofos each alone and their combinations with either propolis or ginseng elicited a significant decrease ($P<0.05$) in total sperm count when compared with normal control group. But the decrease was more profound with the insecticides used.

3.5 Effect on sperm motility:

It was appearant from (Table 2) and (Fig.2) that the insecticides under investigation elicited a significant decrease ($P<0.05$) in sperm motility % compared with the control group after the end of the study .Whereas, Propolis or ginseng each alone afforded non significant increase in sperm motility % compared with control group as well as a significant increase ($P<0.05$) when compared with the groups treated with each of the insecticides used. The combinations of each of propolis or ginseng with either chlorpyrifos or profenofos elicited a non significant decrease except combination of Chlorpyrifos with propolis which showed a significant decrease when compared with normal control group.

3.6 Effect on abnormal forms of sperms:

It was apparant from (Table 2) and (Fig. 2) that the administration of either chlorpyrifos or profenofos for 60 successive days in their recommended doses to normal male rats elicited a significant increase ($P<0.05$) in the abnormal forms of spermatozoa. Whereas, non significant changes were reported in other groups when compared with normal control group.

3.7 Effect on viability % of sperms:

It was apparant from (Table 2) and (Fig. 2) that the administration of either chlorpyrifos or profenofos for 60 successive days in their recommended doses to normal male rats elicited a significant decrease ($P<0.05$) in the viability of spermatozoa. Whereas, non significant changes were reported in other groups except groups were given the combinations of propolis with either chlorpyrifos or profenofos which showed a significant decrease when compared with normal 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 Testosterone hormone in male albino rats (mean ± SE). (N = 7).

Groups	Testosterone (µIU/ml)
Control group	4.24 ± 0.08^{c}
Chlorpyrifos	1.68 ± 0.07^{g}
Profenofos	1.18 ± 0.05^{h}
Propolis	5.46 ± 0.10^{a}
Ginseng	4.62 ± 0.09^{b}
Chlorpyrifos + Propolis	3.16 ± 0.05^{d}
Chlorpyrifos + Ginseng (mg/Kg)	2.64 ± 0.05^{e}
Profenofos + Propolis	2.20 ± 0.07^{f}
Profenofos + Ginseng mg/Kg)	2.20 ± 0.15^{f}

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

Groups	Total countX106	Motility%	Abnormal forms%	Viability% after 1/2 h
Control group	79.8 ± 3.05^{b}	60 ± 3.25^{ab}	30 ± 6.35^{cd}	75 ± 6.35^{ab}
Chlorpyrifos	40.42 ± 2.05^{e}	40 ± 2.10^{d}	60 ± 2.36^{a}	35 ± 3.32^{d}
Profenofos	35.21± 2.03^{e}	30 ± 2.03^{e}	55 ± 5.36^{ab}	30 ± 1.52^{d}
Propolis	85.92 ± 5.96^{ab}	61.21± 3.33^{ab}	35 ± 5.32^{cd}	73 ± 1.35^{ab}
Ginseng	108.51± 6.78^{a}	70 ± 4.31a	25 ± 3.32 ^{cd}	80 ± 6.35^{a}
Chlorpyrifos + Propolis	50 ± 2.68d	45.91± 2.52^{cd}	40 ± 8.25^{bc}	45± 8.75^{cd}
Chlorpyrifos + Ginseng	65 ± 3.01^{bc}	50 ± 2.95^{bc}	39 ± 4.23 ^{bc}	55 ±1.20b^{c}
Profenofos + Propolis	60 ± 2.02^{c}	51.52 ± 3.05^{bc}	45 ± 5.36 ^{bc}	40 ± 2.51^{cd}
Profenofos + Ginseng	49 ± 2.65^{d}	58.43 ± 4.62^{b}	40 ± 8.62 ^{bc}	49 ± 6.32^{bc}

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.

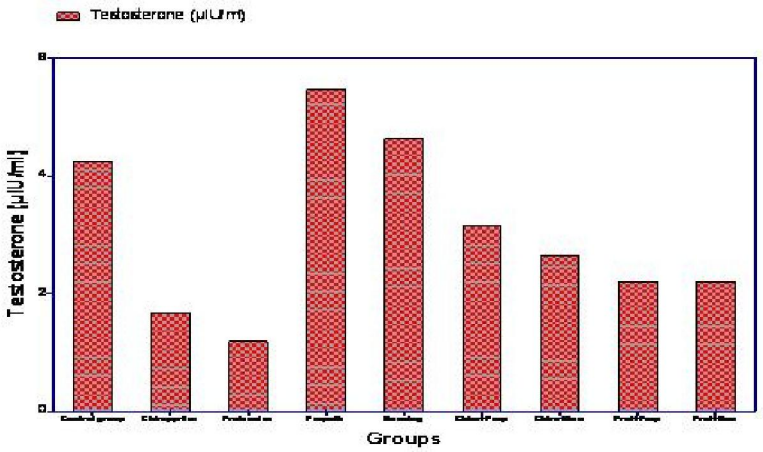


Fig (1): Effect of Chlorpyrifos (6.75 mg/kg), Profenofos (20mg/Kg) , Propolis (70 mg/ Kg), Ginseng (200 mg/Kg) and their combinations on Testosterone hormone in male albino rats.

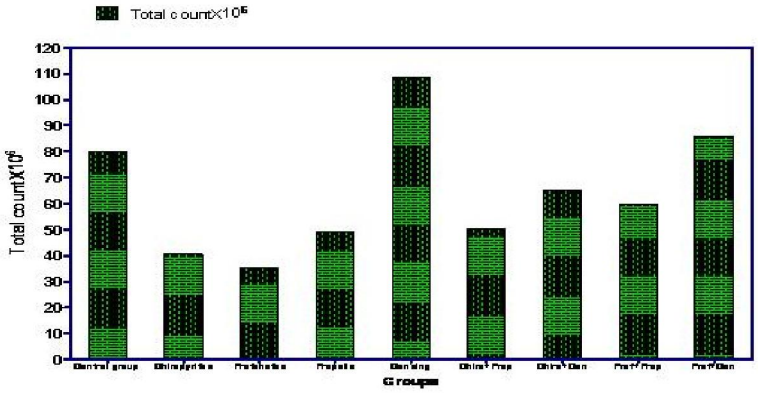


Fig (2): Effect of Chlorpyrifos (6.75 mg/kg), Profenofos (20mg/Kg) , Propolis (70 mg/ Kg), Ginseng (200 mg/Kg) and their combinations on (total count of sperms X 106) in male albino rats.

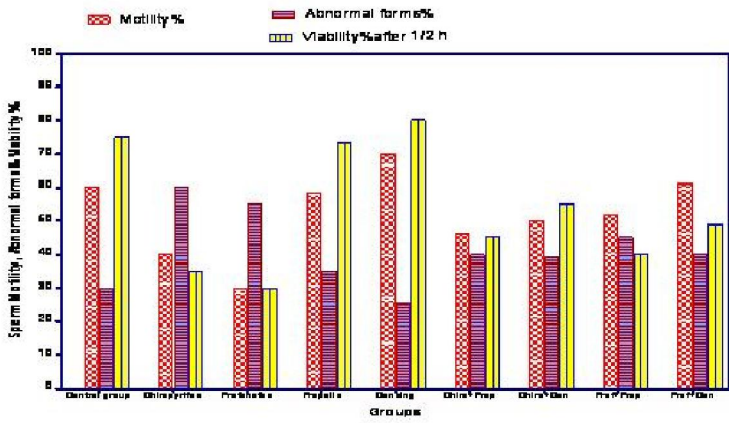


Fig (3): Effect of Chlorpyrifos (6.75 mg/kg), Profenofos (20mg/Kg) , Propolis (70 mg/ Kg), Ginseng (200 mg/Kg) and their combinations on (Sperm motility , abnormal forms & Viability) in male albino rats.

3.7: Histopathology:

(Group 1): Control group

The Testis: Microscopically: testis of the male control treated rats appears oval in size with normal testicular tissue showing normal seminiferous tubules containing small groups of leydig cells (Fig. 4).

(Group 2): **Chlorpyrifos treated group**

The testis: Microscopically, seminifeous tubules were lined by few layers of spermatogenic cells and few sperms (hypospermatogenesis) (Fig. 5), seminferous tubules lined by few layers of spermatogenic cells with no sperm formation (Testicular atrophy) were seen in (Fig. 6).

(Group 3): Profenofos treated group

The testis:Seminferous tubules were filled by primary spermatogonia only with no sperm formation (spermatogenic arrest) (Fig. 7) and a seminiferous tubule filled by spermatogenic cells up to spermatid only with no sperm formation (spermatogenic arrest) was shown in (Fig. 8).

(Group 4): **Propolis treated group**

The testis: The seminiferous tubules of this group were lined by layers of spermatogenic cells up to sperm formation and surrounded by thin basement membrane, (Fig.9).

(Group 5): Ginseng treated group

The Testis :Seminiferous tubule lined by layers of spermatogenic cells up to formation and surrounded by thin basement membrane (Fig10).

(Group 6): Chlorpyrifos + Propolis treated group

The Testis: Microscopically, seminerferous tubules lined by few layers of spermatogenic cells and few sperms (Hypopermatogenesis) (Fig. 11).

(Group 7): Chlorpyrifos + Ginseng treated group

The Testis: Microscopically, seminiferous tubules lined by several layers of spermatogenic cells up to sperm formation but surrounded by mild edematous stroma (Fig. 12).

(Group 8): Profenofos+Propolis treated group

The Testis: Microscopically, testicular tissue showing normal seminiferous tubules surrounded by edematous stroma containing small groups of leydig cells (fig. 13).

(Group 9): Profenofos + Ginseng treated group

The Testis: Microscopically, testicular tissues showing normal and some elongated seminiferous tubules surrounded by small edematous stroma (Fig. 14).

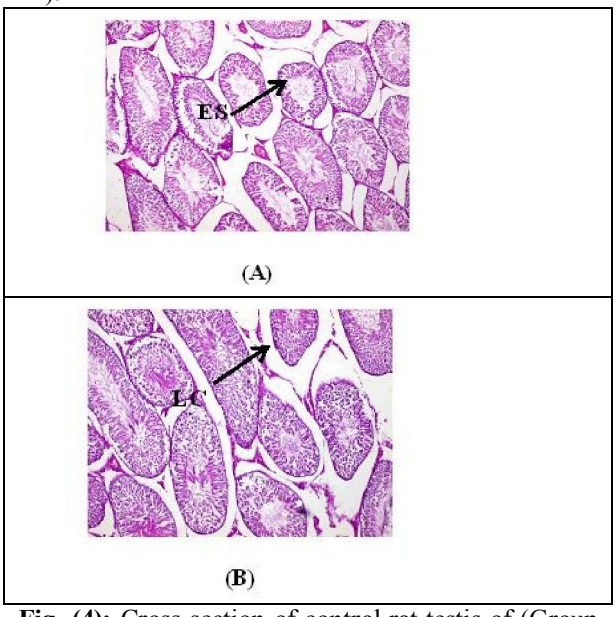
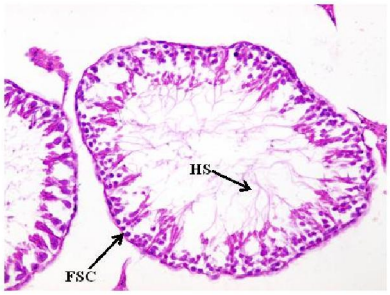
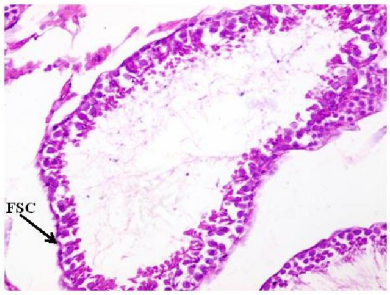


Fig. (4): Cross section of control rat testis of (Group 1) (testicular tissue) showing normal seminiferous tubules surrounded by edematous stroma containing small groups of leydig cells (H and E x 100) (SE: seminiferous tubles, ES: edematous strom, LC: Leydig cells).



(A)

Fig. (5) Cross section of rat testis of group (2) treated with chlorpyrifos (6.75 mg/kg) showing seminifeous tubules lined by few layers of spermetrogenic cells and few sperms (hypospermatogenesis) (H and E x 400) **(FSC: Few spermatogenic cells, HS: Hypospermatogenesis).**



(B)

Fig. (6) : Cross section of rat testis of group (2) treated with chlorpyrifos (6.75 mg/kg) showing semiferous tubules lined by few layers of spermatogenic cells with no sperm formation (Testicular atrophy) (H&EX400) **(FSC: Few spermatogenic cells)**.

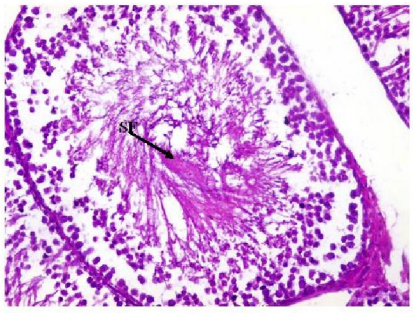


Fig.(7): Cross section of rat testis of group (3) treated with profenofos (20 mg/ Kg) showing seminiferous tubules filled by spermatogenic cells up to spermatid only with no sperm formation (spermatogenic arrest) (H and E x 400)**(SA: spermatogenic arrest)**.

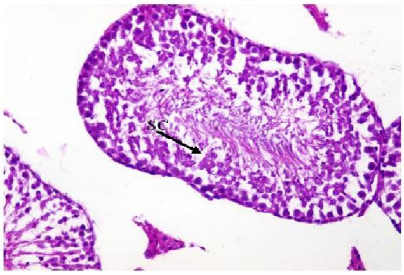
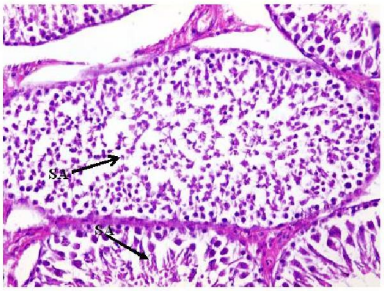


Fig. (8) : Cross section of rat testis of group(4) treated with propolis(70 mg/ Kg) showing seminiferous tubules lined by layers of spermatogenic cells up to sperm formation and surrounded by thin basement membrane (H and E x 400) **(SC: spermatogenic cells)** .



(B)

Fig. (9): Cross section of rat testis of group(5) treated with ginseng (200 mg/ Kg) showing seminiferous tubule lined by layers of spermatogenic cells up to sperm formation and surrounded by thin basement membrane (H and E x 400) **(SF: sperm formation)**.

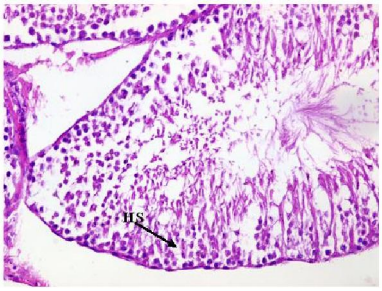


Fig. (10): Cross section of rat testis of group (6) treated with (Chlorpyrifos +Propolis) (6.75 mg/kg) & (70 mg/kg) respectively showing semineferous tubule lined by few layers of spermatogenic cells and few sperms (Hypospermatogenesis) (H&EX400) **(HS: Hypospermatogenesis)**.

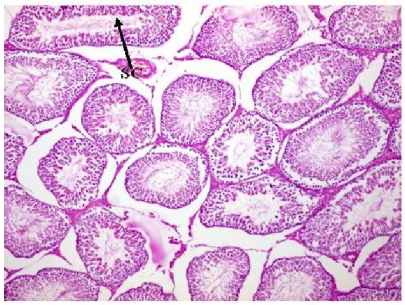
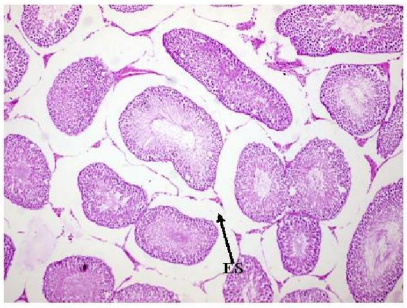


Fig. (11): Cross section of rat testis of group (7) treated with (Chlorpyrifos + ginseng) (6.75 mg/kg) & (200 mg/kg) respectively showing seminiferous tubules lined by several layers of spermatogenic cells up to sperm formation but surrounded by mild edematous stroma (H and E x 100) **(SF: Sperm formation, SC: Speromatogenic cells)** .



(B)

Fig. (12): Cross section of rat testis of group (8) treated with (profenofos + propolis) (20 mg/kg) & (70 mg/kg) respectively showing testicular tissue with normal seminiferous tubules surrounded by edematous stroma containing small groups of leydig cells (H&EX400) **(ST: Seminiferous tubules, ES: Edematous stroma).**

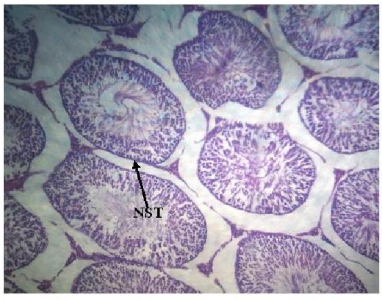


Fig. (13): Cross section of rat testis of group (9) treated with (profenofos + ginseng) (20 mg/kg) & (200 mg/kg) respectively showing testicular tissues with normal and some elongated seminiferous tubules surrounded by small edematous stroma (H and E x 200) **(NST: Normal seminiferous tubules).**

4. Discussion:

Effect on Sex hormone and male fertility:

Our results demonstrated that treatment of normal rats with either Chlorpyrifos or Profenofos exhibited a marked decrease in serum testosterone level after the end of the experiment when compared with normal control group. At the mean time, treatment of normal rats with Propolis or ginseng each alone induced a significant increase in serum testosterone after the end of the experiment when compared with normal control group. Whereas, a significant decrease in testosterone level was also recorded in response to combinations of the insecticides with either Propolis or ginseng when compared with normal control group, yet this effect was much better than that produced with each insecticide alone.

At the meantime, our results showed that the administration of either propolis or ginseng in their recommended doses for 60 successive days to mature rats afforded a slight and marked increase in total sperm count respectively compared to normal control group. Whereas, the administration of either Chlorpyrifos or profenofos each alone and their combinations with either propolis or ginseng elicited a significant decrease in total sperm count when compared with normal control group. But the decrease was more profound with the insecticides used.

It was appearant from our results that the insecticides under investigation elicited a significant decrease in sperm motility % compared with the control group after the end of the study .Whereas, Propolis or ginseng each alone afforded non significant increase in sperm motility % compared with control group as well as a significant increase when compared with the groups treated with each of the insecticides used. While the combinations of each of propolis or ginseng with either Chlorpyrifos or profenofos elicited a non significant decrease except combination of Chlorpyrifos with propolis which showed a significant decrease when compared with normal control group.

It was obvious from our results that the administration of either Chlorpyrifos or profenofos for 60 successive days in their recommended doses to normal male rats elicited a significant increase in the abnormal forms of spermatozoa. Whereas, non significant changes were reported in other groups when compared with normal control group.

It was appearant from our results that the administration of either Chlorpyrifos or profenofos for 60 successive days in their recommended doses to normal male rats elicited a significant decrease in the viability of spermatozoa. Whereas, non significant changes were reported in other groups except groups

given the combinations of propolis with either Chlorpyrifos or profenofos which showed a significant decrease when compared with normal control group.

The testes of humans and other mammals are highly susceptible to damage produced by genetic, to chemical or other means. It has been reported that pesticides have been shown to cause over production of reactive oxygen species (ROS) in both intra and extra cellular spaces, resulting in a decline of sperm count and infertility in wildlife human **[1]**.

Our results are greatly supported by **[19]**. They concluded that combined exposure of toxic doses of Chlorpyrifos and Malathion induced significant reproductive dysfunction in the offspring of rats.

At the meantime, Chlorpyrifos can induce adverse effects on reproductive performance; it showed fetotoxic and teratogenic effects at a maternal dose of 25 mg/kg-d, a dose that also produced maternal toxicity **[20]**.

Histological examination revealed that Chlorpyrifos caused testicular lesions characterized by markedly decreased testes weight with moderate to severe widening of interstitial spaces and partial arrest of spermatogenesis at the high level dose of 25 mg/kg.d .Histological epididymal changes were occurred in the high and middle dose groups characterized by severe oedema and congestion between epididymis tubes and lacking of sperm number. Lesser histological changes were noted at 5 mg/kg-d, where minimal histological evidence of damage was observed **[2]**.

Testicular spermatide and epididymal sperm counts indicated that spermatogenesis was partially arrested at the middle and high dose groups (15 and 25mg/kg-d). These results are in consistent with the published data reported that Chlorpyrifos at 17.5 mg/kg-d caused adverse reproductive effects in male mice included severe testicular damage and resulted in reduction in sperm count and thus affect the fertility **[2]** and these results are in full agreement with our results.

Indeed, decreased sperm number and ventral prostate weight of male mice observed are all related indicators for hypothyroidism and lowered testosterone level at 17.5 mg/kg-d. Chlorpyrifos was reported to be a potential endocrine disrupter by depression of sperm T4 level **[21].**

More recently, **[22]**, reported that the Chlorpyrifos treated group showed that there were necrosis, degeneration, decreasing number of spermatogenic cells in some seminiferous tubules, separating of cells from basal region of seminiferous tubules and edema in interstitial tissue of testis.

Testosterone is produced mostly in the leydig cells of the testis in response to hormonal signals from the hypothalamus and pituitary. And it was further metabolized to dihydrotestosterone (DTH) by 5a-reductase, a highly lipophilic enzyme found on intracellular membrane **[23]**. The reduction in fertility index may simply represent the effects of Chlorpyrifos exposure on sperm parameters, and testis histological changes. Spermatogenesis and fertility are critically dependent upon the maintenance of adequate levels of testosterone **[24]**. Therefore, the effects of Chlorpyrifos on the fertility can be attributed to its ability to reduce serum testosterone levels and sperm counts. Reduction in male body weight in dose group of 25mg/kg-d can be attributed to the reduction in feed consumption and systemic toxicity of Chlorpyrifos in male mice.

Our results are in full accordance with **[25]**. They demonstrated that 60 day's exposure of male rats to profenofos at the dose 23.14 mg/kg body weight (4 doses/week) resulted in decreased the testes and epididymus weights, male fertility indices (sperm count and motility).Moreover, the authors showed that the weights of testes and epididymus were significantly lower in the profenofos-treated rats than in the controls. The decrease in testicular weight in treated rats may be due to reduced tubule size, spermatogenic arrest and inhibition of steroid biosynthesis of leydig cells, a site of steroid biosynthesis **[26]**.

The decrease in testicular weight in profenofos-treated rats may indicate impairment at testicular, pituitary, or hypothalamic level **[27]**. Similar results were recorded by [2], who mentioned that Chlorpyrifos (OPIs) at dose levels of 7.5, 12.5 and 17.5 mg/kg b.wt./day, for 30 days, decreased significantly the weight of testes. The epididymus is androgen-dependant organ, relying on testosterone for its growth and function **[28]**. They proposed that profenofos probably reduced the activity of testes and epididymus by inhibition of androgen production or its direct action on these organs **[29]**, the reduction in the weights of testes and epididymus in their study may be due to lower bioavailability of androgen **[26]**. Moreover, the deleterious effects of profenofos on reproductive organ weights might be due to a decrease in the testosterone (T) and thyroid hormone levels after 60 days from the onset of the treatment **[30]**.

Furthermore, **[25]** confirmed the previous reports of [31] who mentioned that administration of rats with profenofos at 23.14 and 46.30 mg/kg body weight for 28 days and 60 days, respectively, induced significant decrease in thyroid hormone levels. There is ample evidence that thyroid hormone is essential to the normal development of testes in the neonate **[32]** and these results are a new support for our obtained results.

Sperm count is one of the most sensitive tests for spermatogenesis and it is highly correlated with fertility. Our results go in full agreement with **[25]**. They revealed that, treatment of rats with profenofos significantly reduced the sperm count and motility. The decreased sperm motility and density (count) after oral administration of profenofos may be due to androgen insufficiency **[33]** which caused impairment in testicular functions by altering the activities of the enzymes responsible for spermatogenesis **[34]**.

Moreover, histological structure of the testes confirmed the before mentioned results, where it revealed degeneration in some of seminiferous tubules associated with low luminal spermatozoal concentration. It is tempting to speculate that the decreased sperm motility in the present study may /have been related to earlier studies on profenofos.

At the same time, it had been reported that profenofos brought about marked reduction in epididymal and testicular sperm counts in exposed males **[2]** .Also, testicular atrophy and degenerative changes in the seminiferous tubules had been reported by **[35]** which are in accordance with our obtained results.

Moustafa et al., **[36]** reported also that Profenofos is considered as one of the male reproductive toxicants. ALP is primary of testicular and epididymal origin and, therefore, suitable for differentiation of oligo-and izospermia.Similar results were recorded by **[37]**, who mentioned that profenofos produced atrophy, morphological changes and impaired spermatogenesis in testes of experimental animals.

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 lower level of testosterone hormone and decreasing total sperm count and increasing abnormal forms. Moreover, the damage in tissues of Testis.

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.

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