

## Immunotoxic Effect of (Organophosphorous Insecticides) (Chlorpyrifos, Profenofos) and Possible Ameliorative Role of Propolis and Ginseng

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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, the present study sought 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 Immunological function parameters like IgG, IgM and by measuring TNF- $\alpha$  level. Animals were divided into nine groups; The 1<sup>st</sup> (Control group), The 2<sup>nd</sup> (Chlorpyrifos treated group) Animals for 60 days, The 3<sup>rd</sup> (Profenofos treated group), The 4<sup>th</sup> (Propolis treated group), The 5<sup>th</sup> (Ginseng treated group), The 6<sup>th</sup> (Chlorpyrifos + Propolis treated group)A, The 7<sup>th</sup> (Chlorpyrifos+Ginseng treated group), The 8<sup>th</sup> (Profenofos +Propolis treated group), The 9<sup>th</sup> (Profenofos +Ginseng treated group). Results showed that there was a correlation between CPF and PRF administration and the highly significant increase of the TNF- $\alpha$ , while administration of both propolis and ginseng highly ameliorate these dangerous toxicity markers and also increase the immune capacity by significant increasing of immunoglobulins.

**Key words:** Organophosphorous insecticides , Propolis, Ginseng, Immunoglobulin, Tumour necrosis factor.

OPI exposures have been associated with immunotoxicity (Galloway and Handy, 2003), reproductive toxicity (Joshi *et al.*, 2007) and oxidative stress characterized by increased lipid peroxidation and alterations in the status of enzymatic antioxidant defense mechanisms in humans (Shadnia *et al.*, 2005).

Mecdad *et al.*, (2011) selected IgG and IgM as markers of humoral immunity, and tumor necrosis factor alpha (TNF- $\alpha$ ) as a marker of cell-mediated immunity. The results showed statistical significant lower levels in immunoglobulin G and

immunoglobulin M levels in chlorpyrifos-exposed workers compared to control group. These findings run in parallel with those previously obtained (Liu *et al.*, 2006).

Mecdad *et al.*, (2011) conducted an experiment on 95 adult males in age 38 years from Al – Salheya Al gadeeda- sharkeya governorate Al-salheya farm–Zagazig exposed in range from 4-30 years to organophosphorous insecticides and the study revealed statistically significant reduction of the activity of (AChE), IgM, and IgG in insecticides exposed workers.

Profenofos -induced immunosuppression was associated with severe cholinergic stimulation (Pruett *et al.*, 1992).The immunosuppression may result from direct action of acetylcholine upon the immune system or it may be secondary to the toxic chemical stress associated with cholinergic

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poisoning (Zahran *et al.*, 2005). The increase in  $\alpha$ -globulin might be attributed to tissue destruction and inflammatory reaction as mentioned by Peace and Kaplan, (1987).

Recent studies have shown that propolis increases the natural killer activity against tumor cells (Sforcin *et al.*, 2002), modulates both *in vitro* and *in vivo* nitric oxide and hydrogen peroxide production by peritoneal macrophages (Orsi *et al.*, 2000), and increases the fungicidal activity of these cells (Murad *et al.*, 2002).

For centuries, Asian cultures have used ginseng as a low-dose tonic. Consumed over a long period of time it is thought to strengthen the immune system (Lok 2001).

## MATERIALS AND METHODS

### Test insecticide

Chlorpyrifos 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<sub>50</sub> (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<sub>50</sub> of CPF induced biochemical alterations in rats without morbidity (Mansour and Mossa 2009). 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 (Mahmut *et al.*, 2005).

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<sub>50</sub>, where the LD<sub>50</sub> value of Profenofos is (200 mg/Kg) according to (Weil, 1952) and this selected dose of the insecticide was based on Weil studies in which 1/10 LD<sub>50</sub> 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 (Andreson *et al.*, 1977).

### Propolis extract preparation

In this study, Propolis powder extract (70% ethanolic extract) was obtained from (Dasic 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 (Yousef and salama (2009).

### Ginseng extract preparation:

Red Ginseng powder (Supplied by Tsumura Pharmaceutical Co., Tokyo, Japan) was administered orally at dose (200 mg/Kg) (Zhang *et al.*, 2005) 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.

### 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.

## EXPERIMENTAL

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

### The 1<sup>st</sup> (Control group)

Animals received 1ml of distilled water orally daily for 8 weeks.

**The 2<sup>nd</sup> (Chlorpyrifos treated group)**

Animals were daily received oral doses of Chlorpyrifos (6.75 mg/Kg b.wt.) for 8 weeks using metallic stomach tube.

**The 3<sup>rd</sup> (Profenofos treated group)**

Animals were received orally Profenofos (20 mg/Kg b.wt.) daily for 8 weeks using metallic stomach tube.

**The 4<sup>th</sup> (Propolis treated group)**

Animals were received orally *Propolis* extract (70mg/kg b.wt.) daily for 8 weeks using metallic stomach tube.

**The 5<sup>th</sup> (Ginseng treated group)**

Animals were given orally Ginseng extract (200mg/Kg b.wt.) for 8 weeks daily using metallic stomach tube.

**The 6<sup>th</sup> (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.

**The 7<sup>th</sup> (Chlorpyrifos+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.

**The 8<sup>th</sup> (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.

**The 9<sup>th</sup> (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.

**Immunoglobulin G (IgG)**

The IgG is a quantitative turbidometric test for the measurement of IgG in serum or plasma by using IgG kit (GenWay Biotech, Inc).

Anti-human IgG antibodies when mixed with samples containing IgG form insoluble complexes. These complexes cause an absorbance change, dependent upon the IgG concentration of the patient sample that can be quantified by comparison from a calibrator of known IgG concentration (Peace and Kaplan, 1987).

**Immunoglobulin M (IgM)**

The IMMUNO-TEK Mouse IgM ELISA Kit (EIAab (Wuhan EIAab Science Co.,Ltd) is a rapid, easy to use enzyme-linked immunosorbent assay (ELISA) designed for the measurement of mouse IgM in plasma, serum; the kit is especially useful in monitoring the production and purification of mouse monoclonal antibodies (Pinherio and Cobber 1997).

**Tumor necrosis factor- $\alpha$  (TNF-  $\alpha$ )**

Tumor necrosis factor (TNF, formerly known as TNF- $\alpha$ ) is a potent mediator of immune and inflammatory responses. TNF is produced by many activated cell types including monocytes, macrophages, astrocytes, granulocytes, T and B lymphocytes, NK cells, keratinocytes, fibroblasts, and certain tumor cells. However, TNF has also been implicated as a central mediator in a number of pathologic responses and autoimmune diseases (Ware *et al.*, 1998).

**Statistical analysis and it is measured by using the TNF-alfa Elisa Kit**

Data were collected, arranged and reported as mean  $\pm$  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 Cochran, (1982) to estimate the effect of different treated groups.

**RESULTS****Effect on serum IGg and IGM**

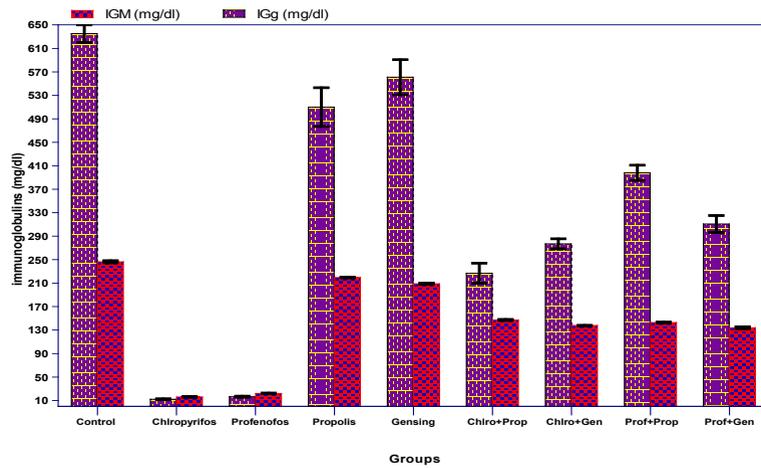
Serum IGg and IGM were markedly decreased ( $P < 0.05$ ) after 60 successive days of Chlorpyrifos and Profenofos administrations when compared with normal control group. While a significant decrease ( $P < 0.05$ ) was noticed also in groups treated with either *Propolis* or Ginseng when compared with normal control group but this effect was much lesser than that produced with the insecticides alone.

Table (1) and Fig. (1) revealed also that the combinations of *Propolis* and Ginseng with either Chlorpyrifos or Profenofos elicited significant

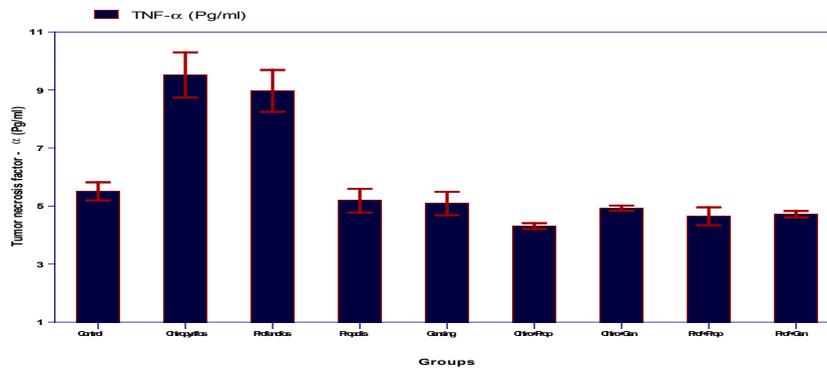
**Table 1.** Effect of Chlorpyrifos (6.75 mg/kg), Profenofos (20mg/Kg) , Propolis (70 mg/ Kg), Ginseng (200 mg/Kg) and their combinations on Immunoglobulin in male albino rats (mean ± SE). (N = 7)

Groups	IGg (mg/dL)	IGM (mg/dL)	TNF- $\alpha$ (Pg/ml)
Control group	635.00±14.83 <sup>a</sup>	246.40±1.80 <sup>a</sup>	5.51±0.31 <sup>b</sup>
Chlorpyrifos	12.20±0.66 <sup>e</sup>	16.20±0.58 <sup>i</sup>	9.52±0.78 <sup>a</sup>
Profenofos	16.60±0.92 <sup>c</sup>	22.00±0.70 <sup>h</sup>	8.97±0.72 <sup>a</sup>
Propolis	510.00±33.01 <sup>b</sup>	219.60±0.50 <sup>b</sup>	5.19±0.41 <sup>b</sup>
Ginseng	561.00±29.82 <sup>b</sup>	209.00±1.30 <sup>c</sup>	5.09±0.40 <sup>b</sup>
Chlorpyrifos + Propolis	227.00±17.14 <sup>d</sup>	147.20±0.86 <sup>d</sup>	4.31±0.10 <sup>bc</sup>
Chlorpyrifos + Ginseng	277.00±8.45 <sup>d</sup>	137.80±0.37 <sup>f</sup>	4.93±0.09 <sup>bc</sup>
Profenofos + Propolis	398.00±12.80 <sup>c</sup>	143.00±0.70 <sup>c</sup>	4.65±0.31 <sup>bc</sup>
Profenofos + Ginseng	310.80±14.46 <sup>d</sup>	134.00±1.87 <sup>g</sup>	4.72±0.11 <sup>bc</sup>

Means within the same column in each category carrying different letters 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



**Fig. 1.** Effect of Chlorpyrifos (6.75 mg/kg), Profenofos (20mg/Kg) , Propolis (70 mg/ Kg), Ginseng (200 mg/Kg) and their combinations on Immunoglobulins 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 tumor necrosis factor - $\alpha$  in male albino rats.

decrease ( $P < 0.05$ ) when compared with control group and groups treated with Chlorpyrifos, Profenofos but their effect was much more better than that produced by insecticides alone.

#### Effect on serum TNF- $\alpha$

Table (1) and Fig. (2) showed that the administration of either Chlorpyrifos or Profenofos elicited a significant increase in serum Tumor necrosis factor  $\alpha$  (TNF-  $\alpha$ ) whereas, their combinations with propolis or ginseng elicited a non significant decrease in serum Tumor necrosis factor  $\alpha$  (TNF-  $\alpha$ ) Whereas, Propolis and/or ginseng afforded a non significant decrease compared with either control group or groups given the insecticides alone.

## DISCUSSION

#### Effect on immunological activity

Serum IgG and IgM were markedly decreased after the 8<sup>th</sup> weeks of Chlorpyrifos and Profenofos administrations when compared with normal control group. While a significant decrease was noticed also in groups treated with either Propolis or Ginseng when compared with normal control group but this effect was much lesser than that produced with the insecticides alone.

The obtained results revealed also that the combinations of Propolis and Ginseng with either Chlorpyrifos or Profenofos elicited significant decrease when compared with control group and groups treated with Chlorpyrifos, Profenofos but their effect was much better than that produced by insecticides alone.

Our results showed that the administration of either Chlorpyrifos or Profenofos elicited a non significant increase in serum Tumor necrosis factor  $\alpha$  (TNF-  $\alpha$ ) whereas, Propolis and/or ginseng afforded a non significant decrease compared with either control group or groups given the insecticides alone.

It is a well known fact that OPI exposures have been associated with immunotoxicity (Galloway and Handy, 2003).

More recently, Meccad *et al.*, (2011) selected IgG and IgM as markers of humoral immunity, and tumor necrosis factor alpha (TNF- $\alpha$ ) as a marker of cell-mediated immunity. Their results showed statistical significant lower levels in immunoglobulin G and immunoglobulin M

levels in Chlorpyrifos-exposed workers compared to control group. These findings run in parallel with those previously obtained by Liu *et al.*, (2006) and these results were in complete agreement with the present results.

Our results seem to be conceivable with that obtained by Meccad *et al.*, (2011). They conducted an experiment on 95 adult males in age 38 years from Al – Salheya Al gadeeda- sharkeya governorate Al- salheya farm – Zagazig exposed in range from 4-30 years to organophosphorous insecticides and the study revealed statistically significant reduction of the activity of IgM, and IgG in insecticides exposed workers.

Evaluating the cell mediated immunity; further support to the present results was obtained by Meccad *et al.*, (2011). They revealed significant increase of TNF $\alpha$  secretion among Chlorpyrifos -exposed workers. Enhancement of TNF $\alpha$  release was explained by the fact that Insecticides modulate immune response via different mechanisms: Th1-like immune response was enhanced with the release of cytokines (IL-2 and TNF $\alpha$ ) affecting B-cell maturation and immunoglobulin production. The IL-2 and TNF $\alpha$  increase may result from a mechanism to compensate for the decrease in TNF-gamma after Insecticide exposure (Banerjee *et al.*, 2001) TNF $\alpha$  is also associated with the activation of repair mechanisms following xenobiotic damage.

Moreover, Meccad *et al.*, (2011) documented that there was a positive correlation between the total Chlorpyrifos residues and the decrease of immunoglobulin G and immunoglobulin M levels, respectively. The higher the Insecticide residue the more frequent infection and immunological abnormalities in the form of decreased immunoglobulin and cytokines production.

Meccad *et al.*, (2011) clarified some correlations in insecticides-exposed workers. There is a significant positive correlation between (AChE) and both IgM and total antioxidant capacity. A negative correlation between MDA and both IgG and IgM as MDA is the biomarker of the oxidative stress damage produced by Insecticides exposure which in turn had deleterious effect on humoral immune response. They stated that their results clearly stressed that Insecticides exposure in Insecticides applicators for prolonged duration leads to accumulation of Insecticides residues

in their blood, significant oxidative damage, compromised antioxidant status and modulation of the immune system involving impairment of humoral and cellular immune functions. These changes are more prominent and marked in insecticides exposed workers.

Our results disagree with Sforcin *et al.*, (2002). They showed that propolis increases the natural killer activity against tumor cells, modulates both in vitro and in vivo nitric oxide and hydrogen peroxide production by peritoneal macrophages (Orsi *et al.*, 2000), and increases the fungicidal activity of these cells (Murad *et al.*, 2002).

On the other hand, our results were in full agreement with Orsi *et al.*, (2000); Murad *et al.*, (2002). They stated that Propolis administration to rats increased antibody production. Propolis ability to modulate antibody synthesis is a part of its adjuvant activity, since it has been shown recently that propolis has a potent effect on different cells of innate immune response.

Our obtained results seem to be in consistent with Sforcin *et al.*, (2002). They revealed that Propolis increases the natural killer activity against tumor cells, modulates both in vitro and in vivo nitric oxide and hydrogen peroxide production by peritoneal macrophages (Orsi *et al.*, 2000), and increases the fungicidal activity of these cells (Murad *et al.*, 2002).

Propolis 10% treatment for 3 days increased the cytotoxic activity of NK cells against murine lymphoma (Sforcin *et al.*, 2002). This finding confirmed a previous observation that Propolis administration over a short-term leads to better results concerning the immune system, increasing the immunological response and these findings were compatible with our obtained results.

Our results seem to be compatible also with Lok (2001). They revealed that for centuries, Asian cultures have used ginseng as a low-dose tonic. Consumed over a long period of time it is thought to strengthen the immune system.

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