Hyperglycemic effect of Chlorpyrifos, Profenofos and possible ameliorative role of Propolis and ginseng

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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 using of propolis and ginseng in this study to be effective antidiabetic agents, therefore, the present study 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 glycemic parameters like Blood glucose and Insulin. 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 weeks, 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 (Chlorpyrifos+Ginseng treated group)Animals were given orally Chlorpyrifos (6.75 mg/Kg) and then co-administered with Ginseng extract (200mg/kg b.wt.) daily for 8 weeks, The 8th (Profenofos +Propolis treated group)Animals were given orally Profenofos (20 mg/Kg) and then co-administered with Propolis extract (70mg/kg b.wt.) 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 60 successive days . Results showed that there was a correlation between CPF and PRF administration and the highly significant increase of blood glucose level and decreasing Insulin level, while administration of both propolis and ginseng highly ameliorate this hyperglycemic effect.

Key words: Chlorpyrifos, Profenofos, Propolis, Ginseng, glucose, Insulin

Introduction

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 (Lasram et al., 2009).

Hyperglycemic potential is another fact of OPI toxicity that has been receiving greater focus from researchers’ all over gluconeogenesis and a state of insulin resistance as underlying mechanism of OPI-induced hyperglycemia (Rahimi and Abdollahi, 2007). Among other biochemical parameters that be altered after Chlorpyrifos administration, the increase in glucose (Kalender et al., 2005).

In addition, the increased free fatty acid flux accelerates pancreatic beta-cell apoptosis (lipotoxicity) (Pinget & Boullu-Sanchis, 2002). On the other hand, it has been suggested that chlorpyrifos induces a kind of insulin resistance that cannot overwhelm hyperglycemia. This action is mediated through disruption of islets mitochondria function (Pournourmohammadi et al., 2007).
Liver, muscle, and brain are organs involved in glycogenesis, glycogenolysis, gluconeogenesis, and glycolysis and on the other hand pancreas keeps hormonal control of glucose homeostasis by secretion of glucagon and insulin. OPs may influence pathways involved in glucose homeostasis in these organs (Rahimi and Abdallahi 2007).

Fuliang et al., (2005) have reported a decrease in blood glucose level of rats that were administered Propolis. (Ginseng) Panaxan B, a glycan, has been reported to elevate plasma insulin and increase insulin sensitivity in mice (Suzuki et al., 2006). Along these same lines, human beings given ginseng extracts showed a lowering of fasting blood glucose levels and HbA1c.

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 LD50 (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 LD50 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 LD50, where the LD50 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 LD50 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).

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

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 8 weeks using metallic stomach tube.

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

The 4th (Propolis treated group): Animals were received orally Propolis extract (70mg/kg b.wt.) daily for 8 weeks using metallic stomach tube.
The 5th (Ginseng treated group): Animals were given orally Ginseng extract (200mg/Kg b.wt.) for 8 weeks daily using metallic stomach tube.

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.

The 7th (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 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.

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.

Determination of serum glucose concentration

Serum glucose level was determined using diamond kit, according to method described by (Trinder, 1969).

Determination of insulin hormone

Insulin was assayed using insulin-\textsuperscript{125}I kit according to Woodhead et al. (1974) using Radioimmunoassay kit obtained from Radioassay System Laboratories Inc (England).

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 Cochran, (1982) to estimate the effect of different treated groups.

Results

Effect on serum Glucose level

Table 1 and Fig. (1) illustrates that the administration of Chlorpyrifos and/or Profenofos in their recommended doses for successive 60 days afforded a significant elevation in serum glucose level when compared with normal control group. Whereas, non-significant changes were observed in serum glucose level of normoglycaemic rats treated with either Propolis or Ginseng when compared with normal control group. A significant increase in blood glucose level was also reported in response to all combinations when compared with control group, yet the effect was much lesser than that produced with each insecticide alone.

<table>
<thead>
<tr>
<th>Groups</th>
<th>Insulin (IU/ml)</th>
<th>Glucose (mg/dl)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control group</td>
<td>42.00±0.27\textsuperscript{a}</td>
<td>95.40±1.88\textsuperscript{d}</td>
</tr>
<tr>
<td>Chlorpyrifos</td>
<td>22.60±0.50\textsuperscript{e}</td>
<td>169.80±6.42\textsuperscript{a}</td>
</tr>
<tr>
<td>Profenofos</td>
<td>19.20±0.86\textsuperscript{e}</td>
<td>173.20±7.67\textsuperscript{a}</td>
</tr>
<tr>
<td>Propolis</td>
<td>36.00±1.58\textsuperscript{b}</td>
<td>111.60±4.16\textsuperscript{d}</td>
</tr>
<tr>
<td>Ginseng</td>
<td>42.80±1.65\textsuperscript{e}</td>
<td>94.00±4.50\textsuperscript{d}</td>
</tr>
<tr>
<td>Chlorpyrifos+ Propolis</td>
<td>30.60±0.87\textsuperscript{e}</td>
<td>126.80±11.79\textsuperscript{b}</td>
</tr>
<tr>
<td>Chlorpyrifos + Ginseng</td>
<td>34.80±0.58\textsuperscript{d}</td>
<td>122.60±5.89\textsuperscript{b}</td>
</tr>
<tr>
<td>Profenofos + Propolis</td>
<td>30.00±0.70\textsuperscript{d}</td>
<td>125.00±4.90\textsuperscript{b}</td>
</tr>
<tr>
<td>Profenofos + Ginseng</td>
<td>26.20±0.86\textsuperscript{d}</td>
<td>140.80±3.78\textsuperscript{b}</td>
</tr>
</tbody>
</table>

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.

Effect on serum Insulin

The effect of Chlorpyrifos, Profenofos, Propolis and Ginseng each alone and their combinations on serum insulin level were illustrated in Table (1) and Fig. (2). The results showed that serum insulin level was significantly decreased (P<0.05) in groups treated with Chlorpyrifos and/or Profenofos when compared with normal control group. Whereas all treated groups with combinations of either Propolis or Ginseng with either Chlorpyrifos or Profenofos revealed a significant decrease (P<0.05) in serum insulin level when compared with control group after the end of the experiment. Whereas, Ginseng treated group showed non significant change when compared with normal control group. Concerning the effect of Propolis on serum insulin level, Table (1) and Fig. (2) showed that rats treated with Propolis afforded significant decrease in serum insulin when compared with control group.
Control group  Chlropyrifos  Profenofos  Propolis  Ginseng  Chlro+Prop  Chlro+Gen  Prof+Prop  Prof+Gen
0 50 100 150 200
Glucose (mg/dl)

Figure 1. Effect of Chlorpyrifos (6.75 mg/kg), Profenofos (20mg/Kg) , Propolis (70 mg/ Kg), Ginseng (200 mg/Kg) and their combinations on blood glucose level (mg/dl) in male albino rats.

Control group  Chlropyrifos  Profenofos  Propolis  Ginseng  Chlro+Prop  Chlro+Gen  Prof+Prop  Prof+Gen
0 10 20 30 40 50
Insulin (Iu/ml)

Figure 2. Effect of Chlorpyrifos (6.75 mg/kg), Profenofos (20mg/Kg) , Propolis (70 mg/ Kg), Ginseng (200 mg/Kg) and their combinations on Insulin level (Iu/ml) in male albino rats.

Discussion
Effect on immunological activity
Effect on Glucose level

Our results showed that serum insulin level was significantly decreased in groups treated with Chlorpyrifos and/or Profenofos when compared with normal control group. Whereas all treated groups with combinations of either Propolis or Ginseng with either Chlorpyrifos or Profenofos revealed a significant decrease in serum insulin level when compared with control group after the end of the experiment. Whereas, Ginseng treated group showed non significant change when compared with normal control group. Concerning the effect of Propolis on serum insulin level, our results showed that rats treated with Propolis afforded significant decrease in serum insulin when compared with control group.

Our results illustrated that the administration of Chlorpyrifos and/or Profenofos in their recommended doses for successive 60 days afforded a significant elevation in serum glucose level when compared with normal control group. Whereas, non-significant changes were observed in serum glucose level of normoglycaemic rats treated with either Propolis or Ginseng when compared with normal control group. A significant increase in blood glucose level was also reported in response to all combinations when compared with control group, yet the effect was much lesser than that produced with each insecticide alone.

It is a well known fact that hyperglycemic potential is another fact of OPI toxicity that has been receiving greater focus from researchers all over gluconeogenesis and a state of insulin resistance as underlying mechanism of OPI-induced hyperglycemia (Rahimi and Abdollahi, 2007).
Our results were supported with that reported by Kalender et al., (2005). They reported that among biochemical parameters that be altered after Chlorypyrifos administration, the increase in glucose.

The obtained elevation in glucose level could be possibly attributed to the increased free fatty acid flux that accelerates pancreatic beta-cell apoptosis (lipotoxicity) (Pinget & Boullu-Sanchis, 2002). On the other hand, it has been suggested that Chlorypyrifos induces a kind of insulin resistance that cannot overwhelm hyperglycemia. This action is mediated through disruption of islets mitochondria function (Pournourmohammadi et al., 2007).

The majority of studies evaluated the effects of OP on glucose homeostasis have reported that exposure to OPs induce hyperglycemia. Dimethoate has markedly increased blood glucose concentration in rats (Reena et al., 1989).

According to Gupta (1974), the hyperglycemic condition induced by OPs might be explained in part by inhibition of AChE at neuroeffector sites in the adrenal medulla, leading to hypersecretion of adrenaline. Adrenaline promotes glycogenolysis in hepatocytes and skeletal muscle cells (Gustavson et al., 2003). In addition, it has a key role in pathogenesis of insulin resistance by inhibition of glucose transport in skeletal muscle via impinging on the component of insulin signaling pathway (Hunt and Ivy 2002). Also, it promotes lipolysis which leads to enhanced free fatty acid levels. It has been demonstrated that elevated level of free fatty acid has an inhibitory effects on the insulin signaling and inhibit glycogen synthesis (Itani et al., 2002).

Further support to our results was obtained by Hsiao et al., (1996). They found that Profenofos was claimed to have a harmful effect on the endocrinal and biochemical functions of the pancreas. Chronic administration of profenofos for 2 months resulted in a significant decrease in plasma insulin level and a pronounced increase in blood glucose level. These effects persisted even after discontinuation of the insecticide. These biochemical changes were accompanied by histological and histochemical changes with destruction of the β-cells that could account for the observed elevation in blood glucose level after profenofos intoxication.

Our results were in full agreement also with Fletcher et al., (1988). They reported a pronounced increase in blood sugar level after profenofos treatment which was going parallel to the inhibition of the cholinesterase. Chronic profenofos administration for 2 months led to degenerative changes of variable degrees of the pancreatic islets as well as the exocrine acini. The degeneration of the acini might be considered as a sort of toxic inflammatory process simulating pancreatitis.

Our results go hand in hand with Fuliang et al., (2005). They have reported a decrease in blood glucose level of rats that were administered Propolis.

Furthermore, Suzuki et al., (2006) reported that ginseng is able to elevate plasma insulin and increase insulin sensitivity in mice. Along these same lines, human beings given ginseng extracts showed a lowering of fasting blood glucose levels and HbA1c.

Attele et al., (1999) showed that extract of Panax ginseng has glucose-lowering and weight-lowering effects in mice. After daily intraperitoneal injections of Panax ginseng, the obese mice showed significantly improved glucose tolerance, as shown by a 46% decrease in the overall glucose excursion compared with controls.

In addition, (Lima et al., 2009) demonstrated that a wild ginseng ethanol extract has preventive effects on diabetes and obesity.

References


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