Anti-Pesticidal Effects of Ginger Oil Extract on the Body Mechanism of Male Albino Rats (Wistar Strain)

Ashade Olufemi Olukayode and Joseph Alphonsus Mamza

1Environmental Biology Unit, Department of Biological Science, Yaba College of Technology, Yaba, Lagos - Nigeria
2Microbiology Unit, Department of Biological Science, Yaba College of Technology, Yaba, Lagos - Nigeria

Abstract: The unrelenting drive to providing organic solution to inorganically created problem prompted this research. Chlopyrifos, classic broad based spectrum pesticide and suppressant, ginger oil were used for the study with Wistar albino rats as experimental animals. Research was carried out to analyze the efficacy of ginger oil to reduce the effects of pesticides in the system of an organism. One hundred and eight (108) Wistar albino rats weighing (140g -165g) were used. Rats were acclimatized for two weeks and grouped into 4 rats in triplicates for each concentration. Group A served as the control, B in subgroups B , B  and B containing rats administered with 1.0ml/75mg/kg, 1.0ml/110mg/kg and 1.0/147mg/kg respectively. While C , C and C represents 1.0ml/75mg/kg with 0.5/ginger oil, 1.0ml/110mg/kg with 0.5ml ginger oil and 1.0ml/147mg/kg with 0.5ml/ginger oil respectively. Administration of Chlorpyrifos lasted 14 days and treatment with ginger oil lasted for 14 days post Chlorpyrifos administration at 3 days interval. Average weights were recorded. Rats were sacrificed and blood samples collected for liver function, lipid profile and renal function tests. Histopathology studies of the liver and kidney was conducted as well. Results revealed some decrease in ALT, ALP and ASP in B , B  and B  groups. Mild improvement in C , C  and C groups were however recorded. There was graded reduction in albumin level despite increased creatinine and urea levels in treatment groups C , C  and C compared with the control group (A). Decreased total cholesterol level with corresponding HDL was observed. There was a significant difference (p<0.05) between the observed parameters in pesticide and ginger oil treated groups compared to control. Moderate oedema within the glomeruli and tubule was observed in C , while relatively massive glomeruli oedema seen in C . Hepatocytes appeared normal in liver with ginger oil treatment. In all, ginger oil was found to have a moderately suppressive effects on pesticides compromised tissues.

Key words: Ginger Oil - Chlopyrifos - Liver - Glomeruli

INTRODUCTION

The clarion call for the use of medicinal plants to suppress to a large extent or perhaps knock off toxicity stimulated this study. Most of these plants have been used indiscriminately by many local populations for managing various diseased states without actually knowing how relief is brought about or its safety risks. Pesticides hold a unique position among environmental contaminants, hence constitutes major public and scientific concern. Chlopyrifos is a colorless to white crystalline solid with mercaptan odor. It is a broad-spectrum insecticide belonging to organo-phosphate family with great effects on the nervous system of target organisms and non target organisms alike.

Ginger (Zingiber officinale) has attracted a wide usage in recent years. Ginger can be consumed as a fresh or dried root and it's often prepared in tea, soft drinks and

Corresponding Author: Ashade Olufemi Olukayode, Environmental Biology Unit, Department of Biological Science, Yaba College of Technology, Yaba, Lagos -Nigeria.
bread. Ginger might be useful as a potential anti-tumor agent [1]. Oil from the rhizome (Zingiber officinale) could be useful in preventing chemical hazards to health and economy their toxicology must be adequately and continuously studied.

**Aim:** To investigate the effects of ginger oil extract in pesticide laden tissues of albino rats.

**MATERIALS AND METHODS**

Fresh ginger rhizomes were purchased from Oyingbo market, Lagos, Nigeria. The rhizomes were transported to Environmental Biology Laboratory, Yaba College of Technology. The rhizomes were washed with distilled water, cut into pieces and sun dried for 5 days.

The powdered material (ginger) was extracted with double-distilled water by the hot continuous percolation method in a Soxhlet apparatus. The extract was evaporated to dryness under vacuum and over dried in a vacuum desiccator to obtain a residue (Adopted by Yogesh et al 2013)[2]

**Experimental Animals:** One hundred and eight males of Wistar strain albino rats weighing average weight of 140g – 165g were used for this experiment. They were procured from the laboratory animal house of Lagos University Teaching Hospital (LUTH), Idu abara, Lagos, Nigeria.

The experiment was largely carried out in the animal house, Biological Science Department, Yaba College of Technology. All the animals were acclimatized for two weeks. Weekly weights were taken covering two weeks of acclimatization and four weeks of experimentation.

**Experimental Process:** After two weeks of acclimatization, the rats were divided into three groups A, B and C representing the control, pesticide administered and pesticide + ginger oil administer groups respectively. Groups B and C were divided into three subgroups depending on concentrations with each in triplicates of 4 rats.

**Grouping**

Group A -- Control group with 12 rats fed with normal rat feed and water for 28 days.

Group B -- Contains subgroups B₁, B₂, and B₃

-- B₁ was administered with 1.0ml of 75mg/kg chlorpyrifos orally for 4 weeks
-- B₂ was administered with 1.0ml of 110mg/kg chlorpyrifos orally for 4 weeks
-- B₃ was given 1.0ml of 147mg/kg chlorpyrifos orally for 4 weeks.

Group C -- Contains subgroups C₁, C₂ and C₃

-- C₁ was administered with 1.0ml of 75mg/kg chlorpyrifos for 14 days after which 0.5ml of ginger oil was administered every 3 days for subsequent other 14 days.
-- C₂ was given 1.0ml of 110mg/kg chlorpyrifos for 14 days after which 0.5ml of ginger oil was administered every 3 days for subsequent other 14 days.
-- C₃ was given 1.0ml of 147mg/kg chlorpyrifos for 14 days after which 0.5ml of ginger oil administered every 3 days for subsequent other 14 days.

**Sample Collection:** Anesthetic and surgical considerations were considered. After 4 weeks, blood was collected from rats in each sub-group for liver function using method as adopted by Imafidon and Okunrobo (2012) [3]. Renal function test using method adopted by Taofeeq et al., (2010) [4] while lipid profile using method adopted by Maruthappan and Shree (2010) [5]. Liver and kidney were collected also in each subgroup for histopathology examination.

**Histopathological Protocol:**
Preparation of tissue for microtomy involved (1) Cutting up (2) Tissue processing (3) Embedding was done by Haematoxylin ad Eosin technique as adopted by (Momoh et al., 2012)[6]

**RESULTS**

**Chemistry Analysis**

**Urea and Glutamate:** There was significant (P<0.05) decrease in both the urea and creatinine levels when compared with the control group.

Histopathology Results

Kidney
Plate 1: Kidney control: Normal appearing glomeruli and tubules

Plate 2: Kidney of albino rat infected with 1ml of 147mg/kg chlorpyrifos pesticide
There is acute tubular necrosis with sloughing of tubules epithelial lining. Glomeruli still intact
Moderate edema within the glomeruli. The tubules are intact

Plate 3: Kidney of albino rat infected with 1.0ml of 75mg/kg and 0.5ml of ginger oil
There are some congestion, tubules and glomeruli is intact

Plate 4: Kidney of albino rat infected with 1.0ml of 110mg/kg and 0.5ml of ginger oil
Tubules and glomeruli still intact. Some edema
Plate 5: Kidney of albino rat infected with 1ml of 147mg/kg and 0.5ml of ginger oil

LIVER

Plate 6: Liver control
Moderate lymphocytes in the portal tract. Hepatocytes appear normal.

Plate 7: Liver of albino rat infected with 1.0ml of 75mg/kg and 0.5ml ginger oil
Moderate lymphocytes with minimal congestion in the portal tract. Hepatocytes appear normal.

Plate 8: Liver of albino rat infected with 1.0ml of 110mg/kg and 0.5ml ginger oil
Normal appearing hepatocyte with minimally expanded portal tract. There are few lymphocytes in the portal tract.
Plate 9: Liver of albino rat infected with 1ml of 147mg/kg and 0.5ml of ginger oil
Reduced lymphocytes

Table 1: Liver function tests of albino rat exposed to only chlorpyrifos pesticide

<table>
<thead>
<tr>
<th></th>
<th>Control</th>
<th>B1</th>
<th>B2</th>
<th>B3</th>
</tr>
</thead>
<tbody>
<tr>
<td>AST (u/L)</td>
<td>208.5±5.61</td>
<td>108.2±4.99*</td>
<td>97.9±4.12*#</td>
<td>86.4±4.09*#</td>
</tr>
<tr>
<td>ALT (u/L)</td>
<td>65.8±2.10</td>
<td>14.6±3.06*</td>
<td>12.8±1.95*</td>
<td>10.3±2.06*</td>
</tr>
<tr>
<td>ALP (u/L)</td>
<td>111.6±4.97</td>
<td>103.3±5.13*</td>
<td>101.5±3.88*</td>
<td>92.8±3.32*#</td>
</tr>
</tbody>
</table>

*=p<0.05 when compared with control group
#=p<0.05 when compared with B1 group
β=p<0.05 when compared with B3 group

Table 2: Renal function tests of albino rat exposed to only chlorpyrifos pesticide

<table>
<thead>
<tr>
<th></th>
<th>Control</th>
<th>B1</th>
<th>B2</th>
<th>B3</th>
</tr>
</thead>
<tbody>
<tr>
<td>Creatinine (mmol/L)</td>
<td>49.86±1.56</td>
<td>25.14±2.36*</td>
<td>26.28±1.19*</td>
<td>28.92±1.79*</td>
</tr>
<tr>
<td>Urea ((mmol/L)</td>
<td>8.7±1.01</td>
<td>5.9±1.55*</td>
<td>5.3±1.00*</td>
<td>3.9±1.07*#</td>
</tr>
<tr>
<td>Glut (mmol/L)</td>
<td>4.6±0.73</td>
<td>4.3±0.23</td>
<td>4.2±0.21</td>
<td>3.9±0.16*#</td>
</tr>
<tr>
<td>ALB (g/L)</td>
<td>44.1±2.89</td>
<td>38.2±1.77*</td>
<td>37.6±1.03*</td>
<td>36.9±2.67*</td>
</tr>
</tbody>
</table>

*=p<0.05 when compared with control group
#=p<0.05 when compared with B1 group
β=p<0.05 when compared with B3 group

Table 3: Lipid profile tests of albino rat exposed to only chlorpyrifos pesticide

<table>
<thead>
<tr>
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<th>Control</th>
<th>B1</th>
<th>B2</th>
<th>B3</th>
</tr>
</thead>
<tbody>
<tr>
<td>HDL (mmol/L)</td>
<td>1.0±0.05</td>
<td>1.1±0.03*</td>
<td>1.2±0.03*#</td>
<td>1.1±0.02*β</td>
</tr>
<tr>
<td>LDL (mmol/L)</td>
<td>0.6±0.03</td>
<td>0.38±0.02*</td>
<td>0.1±0.01*#</td>
<td>0.14±0.01*#</td>
</tr>
<tr>
<td>CHOL (mmol/L)</td>
<td>1.86±0.07</td>
<td>1.63±0.04*</td>
<td>1.24±0.04*#</td>
<td>1.32±0.03*#</td>
</tr>
<tr>
<td>TG (mmol/L)</td>
<td>0.58±0.01</td>
<td>0.34±0.01*</td>
<td>0.21±0.01*#</td>
<td>0.18±0.02*#</td>
</tr>
</tbody>
</table>

*=p<0.05 when compared with control group
#=p<0.05 when compared with B1 group
β=p<0.05 when compared with B3 group

Table 4: Liver function tests of albino rat exposed to both chlorpyrifos pesticide & ginger oil

<table>
<thead>
<tr>
<th></th>
<th>Control</th>
<th>C1</th>
<th>C2</th>
<th>C3</th>
</tr>
</thead>
<tbody>
<tr>
<td>AST (u/L)</td>
<td>208.5±5.61</td>
<td>165.7±2.74*</td>
<td>183.7±2.09*#</td>
<td>208.2±2.58*β</td>
</tr>
<tr>
<td>ALT (u/L)</td>
<td>65.8±2.10</td>
<td>22.0±1.23*</td>
<td>83.0±2.18*#</td>
<td>143.9±2.22*#β</td>
</tr>
<tr>
<td>ALP (u/L)</td>
<td>111.6±4.97</td>
<td>250.0±3.47*</td>
<td>156.2±2.16*#</td>
<td>173.4±2.72*#β</td>
</tr>
</tbody>
</table>

*=p<0.05 when compared with control group
#=p<0.05 when compared with C1 group
β=p<0.05 when compared with C3 group
Table 5: Renal function tests of albino rat exposed to both chlorpyrifos pesticide & ginger oil

<table>
<thead>
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<th>C3</th>
</tr>
</thead>
<tbody>
<tr>
<td>Creatinine (mmol/L)</td>
<td>49.86±1.56</td>
<td>40.89±2.00*</td>
<td>40.13±2.11*</td>
<td>30.58±1.85 *#</td>
</tr>
<tr>
<td>Urea (mmol/L)</td>
<td>8.7±1.01</td>
<td>6.3±1.20</td>
<td>9.2±1.06</td>
<td>12.8±0.98 *#</td>
</tr>
<tr>
<td>Glut (mmol/L)</td>
<td>4.6±0.73</td>
<td>4.2±0.51</td>
<td>3.1±0.13 *#</td>
<td>4.2±0.26 *#</td>
</tr>
<tr>
<td>ALB (g/L)</td>
<td>44.1±2.89</td>
<td>39.7±1.09 *</td>
<td>38.9±1.02 *#</td>
<td>40.3±1.04</td>
</tr>
</tbody>
</table>

Table 6: Lipid profile tests of albino rat exposed to both chlorpyrifos pesticide & ginger oil

<table>
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<th>C1</th>
<th>C2</th>
<th>C3</th>
</tr>
</thead>
<tbody>
<tr>
<td>HDL (mmol/L)</td>
<td>1.0±0.05</td>
<td>1.2±0.03 *</td>
<td>1.5±0.02 *#</td>
<td>1.3±0.01 *#</td>
</tr>
<tr>
<td>LDL (mmol/L)</td>
<td>0.6±0.03</td>
<td>0.12±0.02 *</td>
<td>0.14±0.01 *#</td>
<td>0.27±0.01 *#</td>
</tr>
<tr>
<td>CHOL (mmol/L)</td>
<td>1.86±0.07</td>
<td>1.21±0.03 *</td>
<td>1.64±0.02 *#</td>
<td>1.75±0.02 *#</td>
</tr>
<tr>
<td>TG (mmol/L)</td>
<td>0.58±0.01</td>
<td>0.28±0.01 *</td>
<td>0.43±0.01 *#</td>
<td>0.29±0.01 *#</td>
</tr>
</tbody>
</table>

*=p<0.05 when compared with control group

#=p<0.05 when compared with C1 group

β =p<0.05 when compared with C2

DISCUSSION

Organophosphorus (OP) compounds are among the most commonly used pesticides in agriculture. Because of their wide use and easy accessibility, OP toxicity is important global health problem especially in many developing countries [7, 8]. Every year, hundreds of thousands of deaths occur worldwide due to poisoning with OP compounds [9].

**Serum Liver Enzymes:** AST is an enzyme that help metabolize alanine, an amino acid. AST is normally present in blood at low levels. An increase in AST levels may indicate liver damage or disease. This result indicates that there is an increase in the alanine metabolism in the liver indicating a hepato-protective effect. ALT is an enzyme found in the liver that helps the body metabolize protein. When the liver is damaged, ALT is released into the bloodstream and levels increase. The graded reduction in the blood levels is an indication that the hepatic protein metabolism/synthesis is greatly enhanced by the treatment.

ALP is an enzyme in the liver, bile ducts and bone. Higher than normal activity, ALP may indicate liver damage or disease, such as a blocked bile duct, or certain bone diseases. The graded reduction in the blood level activities is an indication that there was no impairment in the biliary system or bone damages which could result in a reduction in red blood cell synthesis. The present study shows that exposure to organophosphates causes a decrease in values of AST, ALT and ALP. This is similar to a report in another study by Pourgholam et al. [10]. They studied the effect of different sub-lethal concentrations of diazinon on grass carp after 45 days and found that levels of AST, ALT and ALP were lower than control.

Similar changes were also observed in *R. frisii* male brood stocks by Luskova *et al.* [11]. They examined the effect of diazinon on carp and showed that Na and K levels were higher and AST, ALT and ALP levels were lower in common carp after being exposed to insecticides.

Treatment with ginger oil resulted in a mild improvement in the liver enzymes status [12], has previously shown that there was a significant improvement in the liver enzyme status on the administration of ginger oil, but this study didn’t result in a significant improvement.

This could be due to the fact that the metabolic function of the liver had been compromised due to oral exposure to the organophosphate, the treatment with the ginger oil seems not to produce a protective effect on the animal and this might be due to the acute nature of the study.

**Serum Metabolites:** Albumin is one of several proteins made in the liver. The body needs this protein to fight infections and to perform other functions such as transportation in the blood. Lower than normal levels of albumin may indicate liver damage or disease. The graded reduction in the blood levels of albumin in this study is an indication that the treatment inhibited hepatic albumin synthesis and lowered the immune response of the animals, despite the treatment with the ginger oil; this could have been so due to the length of study period or inadequacy of the administered dose of the ginger oil. This finding is similar to previous report by Ma’chova *et al.* [13] who showed that pesticide resulted in mortality and high incidence of malformations, a decrease in growth rate and ontogenetic development [14] also demonstrated the effect of organophosphate on some blood biochemical parameters in rainbow trout (*Oncorhynchus mykiss*) after 7, 14 and 28 days. Their study showed that
acetylcholinesterase activity and the levels of total protein, albumin as well as globulin in plasma was significantly reduced showing that exposure organophosphates changes some haematological and biochemical parameters of *R. frisii* male brood stocks [15].

There was also a combined decrease in the levels of creatinine and urea in the treated group when compared with the control group; this could either be as a result of an enhanced efficiency in the clearance function of the kidney or a reduced output of these metabolites by the liver due to a suppressed metabolic rate.

Treatment with ginger oil resulted in a mild improvement in the liver enzymes status as previously demonstrated by Medhat et al. [16].

**Serum Lipid Profile:** The combined decrease in the levels of total cholesterol and low density lipoprotein is an indication of a cardio-protective properties of the treatment employed in this study, these lipids have been shown by various studies to promote/induce the pathogenesis of cardiovascular diseases such as arteriosclerosis, hypertension and heart failure, while there was a corresponding increase in the levels of high density lipoproteins, which is a strong indication of a beneficial consequence. This coupled effect points to the act that the treatment has no negative effect on cardiovascular functions. Increased concentration of low density lipoprotein (LDL) cholesterol or decreased level of high density lipoprotein (HDL) cholesterol are important risk factors for coronary atherosclerosis. However, an independent association of triglycerides (TG) with atherosclerosis is uncertain [17], on the other hand the treatment could have induced systemic hypotension/bradycardia in the animals, which is another index of lowered lipid profile[18]. The following treatment with ginger oil resulted in a moderate improvement in the lipid profile, this findings is similar to previous report by Hamid et al.[19] that the underlying mechanism by which cholesterol is lowered may be due to a decrease in cholesterol absorption from the intestine, through binding with bile acids within the intestine and increasing bile acids excretion[20] or via decreasing the cholesterol biosynthesis especially by decreasing the 3-hydroxy-3-methyl-glutaryl coenzyme A reductase (HMGCoA reductase) activity, a key enzyme of cholesterol biosynthesis and/or by reducing the NADPH required for fatty acids and cholesterol synthesis.

The decrease of serum TG level is another important finding; recent studies have also shown that TG are independently related to coronary heart disease[19]. The observed hypo-triglyceridemic effect may be due to a decrease of fatty acids synthesis [21], enhanced catabolism of LDL, activation of Lecithin–cholesterolacyltransferase LCAT and tissue lipases and/or inhibition of acetyl-CoA carboxylase [22] and production of triglycerides precursors such acetyl-CoA and glycerol phosphate.

The reduced levels of the liver enzymes and metabolites could be due to the following factors:

- Short duration of the study.
- Dose administrated to the animals.
- Neural compromise as a result of the exposure to the pesticide (organophosphate).

**Liver and Kidney Histopathological Discussion:** There was moderate lymphocytes in the portal tract despite the Hepatocytes appearing normal in the C1 group when compared with the control group, the C2 group showed moderate lymphocytes with minimal congestion in the portal tract. The hepatocytes also appear normal when compared with the control group, but there were expanded portal tract with lymphocytes and congestion within C3 group hepatocytes when compared with the control group. While in the kidney there was Moderate edema within the glomeruli in group C1 despite the fact that the tubules are intact when compared with the control group, in the C2 group there was some congestion though the tubules and glomeruli were still intact, while in group C3 there were some edema even with tubules and glomeruli still been intact. This is supported with previous study reported [23] that there was degeneration in the epithelial cells of renal tubules and dilation of glomerular capillaries with hypertrophied cells.

Hamid et al. [19] showed that organophosphate intoxication can lead to a damaging effect on the intracellular structure of the liver. Another finding of this study was that exposure to organophosphate in rats led to an acute renal failure. The kidney, the major detoxification organ for many xenobiotics, is frequently susceptible to their nephrotoxic effects. Acute renal failure was reported following exposure to organophosphate. The transient renal injury was attributed in these studies to both a direct action of the organophosphate, causing tubular cell necrosis and to a secondary mechanism that followed the cholinergic crisis, causing hypovolemic shock and rhabdomyolysis [24-26]. Many steps along the metabolic/detoxification pathways of organophosphate can be different in the rat. The pesticides in this mixture are metabolized via liver hydrolysis to active (and more potent) metabolites [27]. The cytochrome P450 and
flavin-containing monooxygenase (FMO) enzymes are the major oxidative enzymes in phase I metabolism. Many OP and carbamate thioether compounds are excellent substrates for these enzymes. P450 enzymes are involved in both oxidative desulfuration of the phosphorothioate to form the oxon and oxidation of the thioether group to the corresponding sulfoxide and sulfone of various OPs [28]. The FMOs are generally limited to the formation of sulfoxides [29]. A recent comprehensive study on oxidative OP metabolism by P450 and FMO isoforms found sulfoxidation of several thioether pesticides in human liver microsomes to be mainly P450-driven (85–90%), with the remainder accounted for by FMO [30].

**CONCLUSION**

Ginger oil extract can moderately help in reducing the effect of chloropyrifos insecticide (organophosphate) when use in controlling pest on farm produce which might accidentally be consumed as food in both man and animal.

**REFERENCES**