



Research Article

Protective effect of *Moringa oleifera* Crude Leaf Extract on Chlorpyrifos-induced Liver damage in Wistar Albino Rats

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ABSTRACT

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Chlorpyrifos is a broad spectrum insecticide that is most frequently used in food production in the European Union (EU) until 2020. This compound exhibits a wide range of biological activity. It is applied in most agricultural products and households to combat pests. Chlorpyrifos acts by inhibiting cholinesterase activity that can lead to the alteration of normal biochemical and physiological conditions of the body. This study is aimed at investigating the effect of Crude Leaf extract of *Moringa oleifera* on Chlorpyrifos induced Liver injury in Wistar Albino Rats. Sixteen (16) Wistar Albino Rats weighing between 100g-250g were divided into four (4) Groups (Grp) of four (4) Rats each. Grp I serves as Normal Control (feed and water *ad libitum*), Grp II Negative Control (10mg/kg Chlorpyrifos mixed feed), Grp III serves as Extract grp (10mg/kg *Moringa oleifera* mixed feed) and Grp IV serves as Treatment grp ((10mg/kg Chlorpyrifos + 10mg/kg *Moringa oleifera*) mixed feed). The feeds were administered in pellets. The experiment lasted for twenty eight (28) days, after which serum was obtained for biochemical parameter (Liver function). Grp II shows an increased liver marker enzymes AST ($173.75 \pm 21.84 \mu\text{L}$) when compared to Grp I with value of ($146.60 \pm 21.94 \mu\text{L}$) and Grp III with a value of ($140.75 \pm 11.12 \mu\text{L}$) and ALT ($40.00 \pm 5.83 \mu\text{L}$) compared to grp I and grp III with values of ($33.80 \pm 4.49 \mu\text{L}$) and ($33.25 \pm 7.41 \mu\text{L}$) respectively. ALP (Grp II) with a value of ($155.00 \pm 16.23 \mu\text{L}$) compared to Grp I and Grp III ($69.00 \pm 24.71 \mu\text{L}$) and ($52.00 \pm 17.06 \mu\text{L}$) respectively. ALB ($30.50 \pm 2.08 \text{g/L}$) when compared to Grp I and Grp III shows values of ($28.60 \pm 2.41 \text{g/L}$) and ($29.50 \pm 0.58 \text{g/L}$) respectively. There was no significant difference at $P > 0.05$. However, ALP shows a significantly higher value at $P < 0.001$. Values for all Liver enzymes were lower with $P > 0.05$ in Grp IV when compared with Grp II. There is also a reduced feed intake in Grp II at different days with $P > 0.05$ when compared with Grps III and IV. The Extract was found to have improved the hepatic disorder that was induced by Chlorpyrifos. The result showed some level of hepato-protective activities of *Moringa oleifera* Crude Leaf extract in Rats exposed to Chlorpyrifos.

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INTRODUCTION

Alternatively, herbal medicine is being used worldwide for the treatment of toxicity and its associated disorders (Wang *et al.*, 2018; Bedi *et al.*, 2016). Herbal medicines are used as antioxidants for all organs. They generally improve the vitality and stability of organs by inducing organ regeneration and protection (Bedi *et al.*, 2016, Cuellar-Nuñez *et al.*, 2018). The extracts of most of these herbs are used in alternative medicine in an increasingly higher rate, and the presence of many of these plants with medicinal reference became conventional in therapeutic medicine (Gupta *et al.*, 2005). The therapeutic use of *Moringa oleifera* parts in the Indian subcontinent dates back to Antiquity (Thurber and Fahey, 2009; Torimiro *et al.*, 2009). In other parts of the world, particularly the sub-Saharan Africa, the therapeutic use of *Moringa oleifera* is not known by the wide public, as it is being promoted by diverse organizations as an untapped opportunity (Thurber and Fahey, 2009; Torimiro *et al.*, 2009).

The drumstick tree (*Moringa oleifera* Lam.) member of the *Moringaceae* family is widely spread from India to Africa and numerous other tropical and arid countries, mainly utilized as food and medicine (A husnan and Akahtani, 2016). *Moringa oleifera* is an edible plant. A wide variety of nutritional and medicinal virtues have been attributed to its roots, bark, leaves, flowers, fruits, and seeds (Kumari, 2010; Anwar *et al.*, 2007; Ramachandran and Peter, 1980). *Moringa oleifera* is viewed as a most valuable plant because all parts can be utilized for food, medication, and other industrial and household purposes (Padayachee and Baijnath, 2020; Falowo *et al.*, 2018). The leaves, in particular, may be consumed as a salad, roasted, or stored as dried powder for a long period without losing nutritious content (Amaglo *et al.*, 2010, 9). Phytochemical analyses carried out have shown that its leaves are particularly rich in potassium, calcium, phosphorous, iron, vitamins A and D, essential amino acids, also

rich in some known antioxidants such as β -carotene, vitamin C, and flavonoids (Amaglo *et al.*, 2010; Gowrishankar, 2010; Manguro and Lemmen, 2007; Aslam *et al.*, 2005; Bennett *et al.*, 2003).

Chlorpyrifos is a broad-spectrum organophosphate insecticide that belongs to a group of organophosphorus pesticides, which exhibit a wide spectrum of biological activity (Perry *et al.*, 2020). Chlorpyrifos is applied in various agricultural and horticultural crops and in households to combat the effect of pests. They act on pests by contact, ingestion, and inhalation, they also act on plant surface and inside (George *et al.*, 2014). Upon entering organisms, Chlorpyrifos acts by inhibiting acetylcholinesterase (AChE) activity, one very important enzyme influencing the peripheral and central nervous system in pests. It can also affect the human nervous system in a similar way, leading to the alteration of normal biochemical and physiological conditions (Perry *et al.*, 2020).

The liver is an organ that is capable of activating and detoxifying organophosphorus compounds with the metabolite being eliminated through the kidney (Eddleton, 2000). Repeated exposure of Chlorpyrifos may lead to the accumulation of toxic substances in the liver, which may lead to pathological alterations in the cells of the liver (Muller and Lloyd, 1994). These alterations may cause increased permeability of cellular necrosis, leading to increased release of tissue specific enzymes into the blood (Miller and Zachary, 2017).

Therefore, the current study examined the effect of *Moringa oleifera* crude leaf extract on Chlorpyrifos-induced hepatotoxicity on Wistar albino rats.

MATERIALS AND METHODS

Materials

Glass wares

Test-tubes, Beaker, Mortar and Pestle, Measuring cylinder

Chemicals and reagents

The chemical and reagents used in this study was of analytical grade. Assay kits for AST, ALT, ALP and Albumin were bought from RANDOX laboratories, India. Chlorpyrifos was obtained from reliable form at Nantong Jinling Agricultural Chemical Co. Ltd Jiansu China.

Methodology

Preparation of the Plant Extract

Approximately 1kg of fresh tender leaves of *Moringa oleifera* was collected during January – February, 2012 at Maiduguri Metropolis. The plant was authenticated by a taxonomist, in the Department of Biological Sciences, University of Maiduguri, Nigeria. The plant material was shed-dried at room temperature. The dried leaves were grounded to a fine powder and stored in an air-tight container. About two hundred and fifty grams (250g) of the dry powder was obtained.

Animals

The experiment was carried out using a total of 16 wistar albino rats of either sex, weighing between 100g – 250g. They were obtained from the Animal House, Department of Biochemistry, University of Maiduguri, Borno State, Nigeria. They were kept in metallic cages bedded with dry cleaned wood shavings. The animals were acclimatized for one week under laboratory conditions. They were fed with standard (Growers mesh) diet, and water was provided *ad libitum*.

Acute Toxicity Study

Acute oral toxicity test of crude leaves of *Moringa oleifera* was carried out in accordance with Lorke's method (Lorke, 1983). The study was carried out in two (2) phases using sixteen (16) Wistar Albino Rats. In Phase one (1), a total of nine (9) rats were divided into three (3) groups of three (3) rats each. Groups 1, 2 and 3 were given a single dose of 10mg/kg, 100mg/kg and 1000mg/kg body weight of the extract respectively. They were observed for 24 hours. This is to determine or establish the range of dose producing any toxic effect. Additionally,

another 4th group of three (3) rats was also set as control group. This 4th group was not given the extract.

In the second phase, three (3) rats were divided into three (3) groups, one (1) rat per group. They were administered the extract at higher doses of 1600mg/kg, 2900mg/kg and 5000mg/kg body weights respectively and were observed for 24 hours. This is to further determine the correct LD₅₀ value.

The LD₅₀ is calculated as below;

$$LD_{50} = \sqrt{D_0 \times D_{100}}$$

Where D₀ = highest dose that gave no mortality,

D₁₀₀ = lowest dose that produced mortality

The Crude Leaves of *Moringa oleifera* was found to be safe even at higher doses as no sign of toxicity or mortality was recorded.

Experimental Design

Sixteen Healthy Wistar Albino Rats were randomly divided into four (4) groups of four (4) rats each (n=4) as follows;

Group I (Normal Control): received standard diet (growers mesh) which is about 94.20% dry matter, 11.85% crude protein, 7.95% crude fibre.

Group II received 10mg/kg of Chlorpyrifos

Group III received 10mg/kg crude *Moringa oleifera* leaf.

Groups IV received 10mg/kg crude *Moringa oleifera* leaf + 10mg/kg Chlorpyrifos.

All the feed were administered in pelleted form after being weighed (20g per pellet) and the animals were fed as above for a period of four weeks (28days). Feed consumed (subtracting feed remnants weight from initial feed weight) was also obtained. On the 29th day, the animals were sacrificed and blood samples collected for some liver marker enzymes assay; Aspartate aminotransferase (AST), Alanine aminotransferase (ALT), Alkaline phosphatase (ALP) and Albumin (ALB).

Biochemical analysis

Serum Aspartate aminotransferase, Serum alanine aminotransferase and Albumin were

determined by the method described by (Reitman and Frankel, 1957) while serum alkaline phosphatase was determined according to the standard method (Rec, 1972).

Statistical analysis

The data was analyzed using Statistical Package for Social Science (SPSS) and was expressed as Mean \pm SEM (standard error of the mean). comparison between the groups was done using one-way analysis of variance (ANOVA).

RESULTS

Acute Toxicity Study conducted showed no sign of toxicity and/or mortality with both the first and second phases given a dose of (10, 100 and 1000) mg/kg and (1600, 2900 and 5000) mg/kg respectively of the crude leaves extract of *Moringa oleifera* administered orally. This is shown in Table 1.

Results for mean daily feed consumption by rats was shown in table 2. The table shows increase in *Moringa oleifera* consumption from Day I to Day III. It also shows a decrease in drug consumption (Chlorpyrifos) from Day III to Day IV and V.

Table 3 shows the mean value of ASAT, ALAT, ALP and ALB, in normal and treated rats. The results shows that for ASAT, the control group ($146.60 \pm 21.94 \mu\text{L}$) when compared with the *Moringa oleifera* group ($140.75 \pm 11.12 \mu\text{L}$) showed no significant difference ($P > 0.05$). No significant difference

($P > 0.05$) was observed when control group was compared with the Chlorpyrifos group ($173.75 \pm 21.84 \mu\text{L}$). Also, the comparison between the control group and the *Moringa* – Chlorpyrifos ($155.00 \pm 46.99 \mu\text{L}$) showed no significant difference in the level of the ASAT in the serum.

For ALAT, the comparison between the normal control group ($33.80 \pm 4.49 \mu\text{L}$) with *Moringa oleifera* group ($33.25 \pm 7.41 \mu\text{L}$) with Chlorpyrifos control group ($40.00 \pm 5.83 \mu\text{L}$), with *Moringa* – chlorpyrifos group ($36.75 \pm 15.56 \mu\text{L}$) also showed no significant difference ($P > 0.05$).

Alkaline phosphatase (ALP) comparison between control groups ($69.00 \pm 24.71 \mu\text{L}$) with *Moringa oleifera* group ($52.00 \pm 12.06 \mu\text{L}$) showed no significant difference ($P > 0.05$). However, the control group when compared with the Chlorpyrifos group ($155.00 \pm 16.23 \mu\text{L}$) was considered extremely significant ($P < 0.001$). The comparison between normal control groups with *Moringa* - chlorpyrifos group ($99.67 \pm 18.01 \mu\text{L}$) showed no significant difference ($P > 0.05$).

Albumin (ALB) showed no significant difference at ($P > 0.05$) when compared with control group ($28.60 \pm 2.41 \text{g/L}$) and *Moringa oleifera* group ($29.50 \pm 0.58 \text{g/L}$) and Chlorpyrifos group ($30.50 \pm 2.08 \text{g/L}$). Also, *moringa* crude leave extract has lowering effect on the level of liver parameter

Table 1: Lethal dose (LD_{50}) of the Crude Leaves Extract of *Moringa Oleifera* in Wistar Albino Rat

Groups	Dose (mg/kg)	Mortality Ratio	% Survival
1 st Phase			
1	10	0/3	100
2	100	0/3	100
3	1000	0/3	100
2 nd Phase			
1	1600	0/1	100
2	2900	0/1	100
3	5000	0/1	100

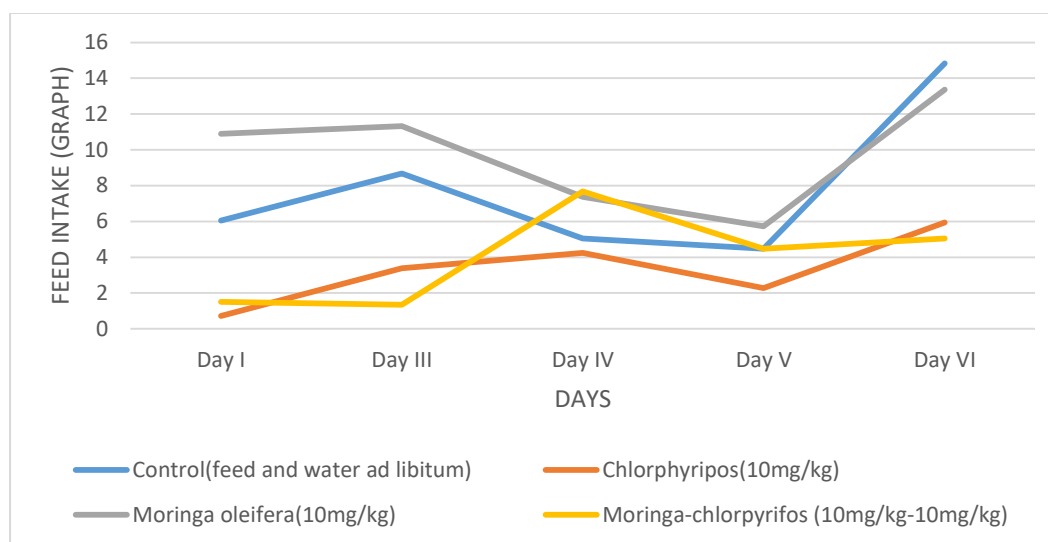
The Lethal Dose (LD_{50}) was ≥ 5000 mg/kg body weight

Table 2: Profile of mean \pm standard deviation (S. D) of feed intake in rats

Groups	Day I	Day III	Day IV	Day V	Day VI
I Control (feed and water <i>ad libitum</i>)	6.05 \pm 1.20 ^b	8.68 \pm 1.87 ^b	5.04 \pm 4.47 ^c	4.47 \pm 2.02 ^b	14.84 \pm 3.31 ^a
II Chlorpyrifos (10mg/kg)	0.72 \pm 0.68 ^d	3.39 \pm 2.70 ^d	4.25 \pm 0.07 ^d	2.27 \pm 1.51 ^c	5.95 \pm 0.64 ^c
III <i>Moringa oleifera</i> (10mg/kg)	10.90 \pm 6.36 ^a	11.32 \pm 8.29 ^a	7.38 \pm 5.83 ^b	5.72 \pm 3.30 ^a	13.37 \pm 2.07 ^b
IV <i>Moringa</i>-chlorpyrifos (10mg/kg-10mg/kg)	1.50 \pm 1.27 ^c	1.35 \pm 0.33 ^c	7.68 \pm 3.42 ^a	4.48 \pm 2.02 ^b	5.05 \pm 1.06 ^d

All values are presented as Mean \pm Standard error of mean (SEM)

Values with different superscripts along the column are significantly different (P<0.05)

**Figure 1:** Line graph showing daily feed intake in Rats**Table 3:** Profile of mean \pm S.D of ASAT, ALAT, ALK and ALB in Chlorpyrifos treats in the

Groups	ASAT (μ /L)	ALAT (μ /L)	ALK (μ /L)	ALB (g/L)
I Control (Feed and water <i>ad- libitum</i>)	146.60 \pm 21.94 ^c	33.80 \pm 4.49 ^a	69.00 \pm 24.71 ^c	28.60 \pm 2.41 ^c
II Chlorpyrifos (10mg/kg)	173.75 \pm 21.84 ^a	40.00 \pm 5.83 ^a	155.00 \pm 16.23 ^a	30.50 \pm 2.08 ^a
III <i>Moringa oleifera</i> (10mg/kg)	140.75 \pm 11.12 ^d	33.25 \pm 7.41 ^a	52.00 \pm 17.06 ^d	29.50 \pm 0.58 ^b
IV <i>Moringa</i>-chlorpyrifos (10mg/kg-10mg/kg)	155.00 \pm 46.99 ^b	36.75 \pm 15.56 ^a	99.67 \pm 18.01 ^b	30.25 \pm 2.36 ^a

presence and absence of *Moringa oleifera*

All values are presented as Mean \pm Standard error of mean (SEM)

Values with different superscripts along the column are significantly different (P<0.05)

DISCUSSION

Generally, acute toxicity studies are the first test conducted in any findings regarding toxicity study. It provides data on the relative toxicity that may likely arise from a single or brief exposure to any substance, be it a plant extract or a chemical.

The Crude leaf extract of *Moringa oleifera* shows that it does not have any toxic effect as doses up to 5000mg/kg did not cause mortality or alterations in the behavior of the normal animals. According to Lorke's (1983), "any substance that is not toxic at 5000mg/kg is considered relatively safe. The Crude leaf extract of *Moringa oleifera* was therefore found to be relatively safe.

Food components such as natural ingredients as well as contaminants such as pesticides, can affect gut health by interfering with gut microbiota and gut barrier (Jin *et al.*, 2019). Appetite-regulating hormones interact with the gut microbiota in shaping appetite and metabolic status, which is sensitive to nutritional status and dietary factors (Jin *et al.*, 2019). Chlorpyrifos has been found to induce gut dysbiosis even at a low dose. This study could be attributed to the finding by (Jin *et al.*, 2019) that the low feed intake by the Rats at group II which were given Chlorpyrifos could be due to their appetite being regulated as a result of disruption in the gut health. However, there is no significant difference when compared with the feed intake in the subsequent groups.

Primarily, the liver is the target organ for systematic toxicity caused by chemicals, which leads to deterioration of its functions (Andres *et al.*, 2021). The prevalent use of pesticide on agricultural products in Nigeria has led to many diseases and dysfunctions which include the liver function, this is in line with the present studies. Elevated levels of liver enzymes (ASAT, ALAT, ALP and ALB) in the plasma are indicative of the condition of hepatic function (hepatotoxicity) (Oluwatoyin *et al.*, 2017). Slight cell damage can lead to

increase in the enzymes activity in the serum which can be detected (Rasha *et al.*, 2023).

From these findings, it showed that there was a rise in the levels of ASAT, ALAT, ALP, and ALB in group II, which were administered Chlorpyrifos. These could be attributed to the liver damage due to toxicity of the Chlorpyrifos.

Aspartate amino transferase (ASAT) activity is widely distributed in human tissues. Very high concentrations of the enzyme in the blood results in liver and heart, and moderately large amounts are in skeletal levels of this enzyme in the serum reflects hepatic injury. In this study, it is seen that levels of ASAT is higher in Group II in which only Chlorpyrifos was administered. Alanine amino transferase (ALAT) or Alanine transaminase is found in much higher concentrations in the liver, but the liver has about three times as much ASAT than ALAT. Like ASAT, increased serum ALAT activities are found in liver diseases due to destruction of the liver cells by either toxins or drugs. Exposure to Chlorpyrifos has led to an increased ALAT concentration in the serum as shown in Group II of this study.

Alkaline phosphatase (ALP) is widely distributed in human tissues, notably in the biliary canaliculi of the liver, proximal convoluted tubules of the kidney etc. increased serum or plasma alkaline phosphatase activity levels is also attributed to certain disorders including liver diseases or disorders that results in obstructive biliary disease causing cholestasis. Albumin (ALB) is also a marker of liver function since it is synthesized by the hepatocytes.

The administration of crude leaves extracts of *Moringa oleifera* showed to have lowering effect on the level of the liver constituents into the circulation. This could be attributed to the claims made in the interactive that leaves of *Moringa oleifera* is described to remove all kinds of excessive pains, used as disease cure and antihelmentics as correlated by Ngaha *et al.*, 1988. *Moringa oleifera* has been known to

be highly beneficial for improving health as most of its parts such as the leaves and seed is used as food sources, traditional treatment of illnesses. The leaves however are rich in protein, minerals and antioxidant compounds (Fenty *et al.*, 2022). From this, it could be said that *Moringa oleifera* leaves has some degree of hepato-protective effects on liver damage due to toxicity caused by Chlorpyrifos.

CONCLUSION

Conclusively, from this study, exposure to Chlorpyrifos has some level of hepato - toxic effects in rats as shown in elevated levels of transaminase enzymes and albumin compared to control group.

Moringa oleifera alone and with Chlorpyrifos did show lower elevation in the transaminase enzymes, albumin, compared to Chlorpyrifos group. This indicate some level of hepato - protective effect of *Moringa oleifera*.

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