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#### **Research Article**

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# Modulatory effect of *Jatropha gossypifolia* leaf on testicular lipidomics and plasma lipoproteins of rats exposed to cypermethrin

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# ABSTRACT

The use of pesticides such as cypermethrin (Cyp) has become hazardous resulting into public and environmental health issues in the world with several studies pointing to a decline in reproductive health coupled with increased risk of infertility. Thus, pesticide safety and effects on human exposure in relation to general and reproductive health are of paramount concern. This study was aimed at investigating the biochemical alterations in the lipidomics of plasma components and reproductive organ of male rats exposed to cypermethrin. The animals were grouped into eight comprising seven animals each and orally treated for 28 days: control, 20 mg/kg body weight of Cyp, Cyp+JG(50 mg/kg), Cyp+JG(100 mg/kg), Cyp+ALA(50 mg/kg), JG(50 mg/kg), JG(100 mg/kg) and ALA(50 mg/kg). Lipid profile analyses were carried out on the plasma, HDL, vLDL+LDL, and testis homogenate of the rats as described by the diagnostic kit manual. Results obtained from this study showed that cypermethrin administration significantly elevated triglyceride (105.22%) and not significant cholesterol level (15.28%) in the plasma; significantly increased testicular triglyceride (96,46%) and cholesterol (64,99%) concentrations; significantly decreased HDL triglyceride (46.57%) and cholesterol (38.37%); significantly increased the concentration of vLDL+LDL triglyceride (126.28 %) and cholesterol (76.09%) in comparison with the control. Co-administration with both doses of J. gossypifolia (JG) leaf extract and alpha-lipoic acid (ALA) significantly reversed these alterations. Thus, the phytoconstituents of Jatropha gossypifolia plant extract possesses modulatory potentials that can mitigate the effect of cypermethrin on the reproductive organ and plasma lipidomics especially in the treatment of heart related disorders caused by hyperlipidemia.

Keywords: Cypermethrin, Jatropha gossypifolia, Alpha-lipoic acid, Testes, Lipoproteins, Lipidomics.

# **INTRODUCTION**

The domestic needs for pest control in agricultural industries and demand for various pesticides including cypermethrin have increased. Due to its biodegradability and low toxicity, cypermethrin, a synthetic pyrethroid, has been involved in the control of pests invading cotton, fruits and vegetable crops <sup>[1]</sup>. However, studies have shown that synthetic pyrethroids including cypermethrin exhibit the ability to disrupt biochemical, hematological, and reproductive parameters in mammals <sup>[2]</sup>, interacting with the voltage-gated sodium channels in the neurons through which sodium ions enter the cell in order to transmit a nerve impulses. This interaction causes prolonged opening of the sodium channels for up to seconds compared to the normal period of a few milliseconds, after a signal has been transmitted, thus leading to the depolarization of the nerve membrane <sup>[3]</sup>. The lipophilic nature of cypermethrin may have conferred on it the ability to interact with the serum and tissue lipids especially the testes. Reports from several investigations suggested that pesticides adversely affect testicular functions and rats exposed to cypermethrin have been reported to have developed increased number of abnormal sperms <sup>[4]</sup>.

Various parts of *Jatropha gossypifolia* plant have been implicated in many preparations employed traditionally to treat different ailments. Pharmacological studies have ellucidated the efficacy of the various extracts and isolated compounds obtained from this plant species as they have been shown to exhibit antimicrobial <sup>[5]</sup>, repro-protective <sup>[6]</sup>, anti-inflammatory, antihypertensive and anticlastogenic <sup>[7]</sup> properties. According to Balee <sup>[8]</sup>, this plant has been used as a blood purifier, treatment of toothache and as an antibiotic. Also, the presence of phytoconstituents such as flavonoids, tannins, cardiac glycosides and saponins have been reported to lower blood cholesterol levels <sup>[9]</sup>.

Alpha-lipoic acid (ALA) is a unique and potent antioxidant. It is also known as thioctic acid.

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In many tissues of the body, ALA is readily converted to its reduced form, dihydrolipoic acid (DHLA). Synthesized majorly by the liver, ALA functions as a cofactor within pyruvate dehydrogenase and  $\alpha$ keto-glutarate dehydrogenase. Due to its amphipathic nature, it is readily distributed in the cellular membranes and cytosol of both plants and animals. It is naturally found in human diet as well as organs with high metabolic activity such as liver, kidney and heart, and to a lesser extent in fruits and vegetables <sup>[10]</sup>. Studies by Morikawa *et al.* <sup>[11]</sup> showed that in mammals, ALA is not adequately supplied by the diet thus de novo synthesis is required to take place in the heart, liver and testis to form ALA needed for purposes such as incorporation into enzyme complexes. Several studies have reported the protective effects of alpha-lipoic acid in cases such as vascular and neurodegenerative diseases, aging and diabetes mellitus <sup>[12]</sup>.

Lipids are very important macromolecule whose presence is vital to virtually all aspects of the body's biological processes. Alterations in the serum and tissue lipidomics have been associated with various abnormalities such as coronary heart disease, angina, and atherosclerosis. One of the organs involved in active lipid metabolism is the testis with two percent of its total wet weight made of lipids <sup>[13]</sup>. Research showed that eighty percent of the total lipids are produced by the testicular phospholipids while the cholesterol and triglycerides are responsible for the remaining twenty percent. However, human testis have been shown to contain lesser amount of total lipids when compared with other species due to the presence lower levels of testicular phospholipids <sup>[14]</sup>. This present study was designed to investigate the modulatory effect of the methanolic extract of *Jatropha gossypifolia* leaf and alpha-lipoic acid on the lipidomics of the testes and plasma lipoproteins of rats exposed to cypermethrin.

# MATERIALS AND METHODS

Samples were weighed using analytical weighing balance (Optima OPD-E) after excision from the animals. All samples and chemicals were preserved in the deep-freezer (HTF-319H) and refrigerator. The chemical reactions were monitored spectrophotometrically using UV-visible spectrophotometer (Jenway 6300/05/20D). Separations were performed using cold ultra-centrifuge (H-103NR). Heparinised and plain tubes were used for blood collection and organ sampling respectively. Water bath (HHS-4), was also used to apply certain amount of temperature on the samples during analysis. All chemicals used in this study were of analytical grade and were used without further purification. Alpha-lipoic acid was imported from Hangzhou Zhenghan Biological Technology Co, Limited, China. Cypermethrin (10% EC) (trade name: Cyperforce) was obtained from agricultural store in Abeokuta, Nigeria.

# **Plant Material**

*Jatropha gossypifolia* was collected at Opa street, Ile-ife, Osun State, Nigeria. The plant was authenticated by Mr. F. O.Omotayo, a botanist at the Department of Plant Science, Ekiti State University, Nigeria. It was allocated an electronic voucher number UHAE163.

# **Extraction Procedure**

Fresh leaves of *J. gossypifolia* were harvested, rinsed and air-dried under the shade on a clean dry surface for about two weeks. It was then grinded into fine powder using a blender. Exactly 388 grams of the powdered leaves was measured into a conical flask and soaked with 3.5 litres of methanol. The mixture was allowed to stand for 72 hours. The mixture was decanted and filtered using whatman No 1 filter paper. The filtrate was subjected to evaporation in a water-bath at 45°C to obtain the viscous extract which was stored at -4°C until use. The percentage yield of the extract was 10.57 % w/w.

# **Experimental Animals**

Fifty-six male albino rats weighing between 150-250 g were purchased from Olu research animals, Ibadan, Nigeria. They were allowed to

acclimatize at the animal house of the Biological Science Department of Wesley University Ondo, for two weeks under standard environmental conditions, with approximately twelve hours light/dark cycle. They were fed a standard laboratory diet and water for a period of thirty days. All experiments were carried out in accordance with guidelines of the Experimental Animal Ethic's committee.

#### **Experimental Design**

Based on the weight of the animals, they were randomly divided into eight groups of seven animals each administration was done orally with the aid of orogastric canula for thirty (30) days. Cypermethrin (Cyp), methanolic leaf extract of *Jatropha gossypifolia* (JGLE) and alphalipoic acid (ALA) were all dissolved in corn oil and administered to the rats once daily all through the duration of the experiment. Based on the dose and weight of animals, 0.5 ml of the stock solution was administered to the animals. The rats were weighed daily from the period of acclimatization to the last day of the experiment when they were sacrificed. The rats were euthanized on the last day of the experiment following anaesthesia with diethylether and the relative weights of the testis was taken (as a percentage of their body weight).

# **Blood Collection**

Blood samples were obtained from the rats through ocular puncture into well-labelled anticoagulant bottles using heparinized capillary tubes. They were then centrifuged at 4000 rpm for 10 minutes to separate the whole blood into plasma and erythrocytes. The plasma was carefully removed into labelled sample bottles using a syringe and stored in the freezer until further analysis.

#### Lipid profile

#### Isolation of High Density Lipoprotein (HDL) from Plasma

The HDL fraction was isolated according to the method of Gidez *et al.* <sup>[15]</sup> after precipitating very low density lipoprotein (vLDL) and low density lipoprotein (LDL) with heparin-manganese chloride. An aliquot of heparin-manganese chloride solution (1.06 M, 0.025 ml) was added to 0.25 ml of plasma in an eppendorf tube. The resultant mixture was vortexed and left to stand at room temperature for 10 minutes. The clear supernatant (HDL fraction) was carefully removed into another eppendorf tube while the precipitate (vLDL+LDL) was returned to the earlier plasma volume taken with the addition of 0.1 M phosphate buffer (pH 7.4). These were then stored at -4°C until analyzed.

#### **Extraction of Lipid from Tissue Homogenates**

The method described by Rose and Oklander <sup>[16]</sup> as modified from folch extraction method <sup>[17]</sup> was employed.

#### **Triglycerides Determination**

This test was carried out spectrophotometrically on the plasma, HDL, vLDL+LDL, and testis homogenate of the rats used for this experiment according to the method of Buccolo *et al.* <sup>[18]</sup> as described by the randox diagnostic kit manual.

#### **Cholesterol Determination**

This test was carried out spectrophotometrically on the plasma, HDL, VLDL+LDL and testis homogenate of the rats used for this experiment according to the method of Nalto *et al.* <sup>[19]</sup> as described by the randox diagnostic kit manual.

#### **Statistical Analysis**

Statistical package for social sciences (SPSS -17) was used in analyzing the data collated from this study. Analysis of variance (ANOVA) was then used to compare the values in all the groups and the level of statistical significance was fixed at p<0.05.

#### RESULTS

In the plasma, cypermethrin administration significantly (p<0.05) elevated triglyceride (TAG) level (105.22 %) (Figure 1) and insignificantly (p>0.05) elevated cholesterol (CHOL) level (15.28 %) (Figure 3) when compared to control. Supplementation with the methanolic leaf extract of *Jatropha gossypifolia* (50 mg/kg and 100 mg/kg) and ALA significantly (p<0.05) decreased the level of TAG (33.45 %, 42.55 % and 44.36 % respectively) and CHOL (33.95 %, 46.88 % and 40.78 % respectively) when compared to the group administered with cypermethrin alone. Compared to control, group administered with JG50 mg alone showed a 17.92 % increase in TAG, 35.17 % decrease in CHOL; group administered with JG100 mg expressed a 20.90 % increase in TAG and 6.13 % decrease in CHOL; while the group administered with ALA exhibited a 16.42 % increase in TAG and 1.06 % increase in CHOL.

Figures 2 and 4 revealed that HDL lipid profile was significantly (p<0.05) decreased by the administration of cypermethrin when compared with the control (TAG, 46.57 % and CHOL, 38.37 %). Co-administration with the methanolic leaf extract of *Jatropha gossypifolia* (50 mg and 100 mg) and ALA significantly (p<0.05) elevated the concentration of HDL TAG (79.78 %, 81.69 % and 81.69 % respectively) and CHOL (61.11 %, 61.62 % and 62.25 % respectively) compared to the group that took cypermethrin alone. Administration of JG50 mg, JG100 mg and ALA alone caused an insignificant (p>0.05) decrease in the concentration of HDL TAG (14.45 %, 18.25 % and 8.03 % respectively) compared to control. In HDL CHOL, JG50 mg caused a 5.37 % decrease; JG100 mg caused a 4.28 % decrease; while ALA caused an increase of 8.43 % when compared to control.

There was a significant (p<0.05) increase in the concentrations of vLDL+LDL TAG and CHOL (126.28 % and 76.09 % respectively) in the group that was given cypermethrin alone when compared to control (figures 2 and 4). Amelioration with the doses of the methanolic leaf extract of *Jatropha gossypifolia* (50 mg and 100 mg) and ALA significantly (p<0.05) depressed the concentration of the vLDL+LDL TAG (55.67 %, 62.40 % and 58.44 % respectively) and CHOL (41.98 %, 38.89 % and 48.77 % respectively) when compared to the group given cypermethrin alone. Although not significant when compared to control, the group administered with only JG50 mg increased TAG concentration (2.09 %) and decreased CHOL concentration (7.83 %); the group given only JG100 mg decreased TAG and CHOL concentrations by 8.21 % and 1.63 % respectively; while the group that took only ALA showed decrease in TAG (7.46 %) and CHOL (7.97 %).

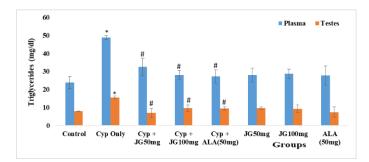
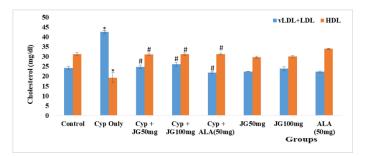
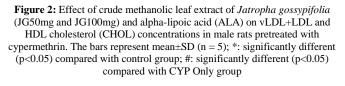
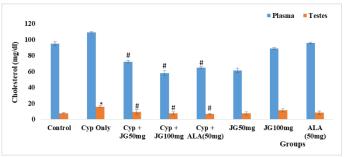


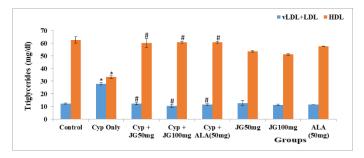
Figure 1: Effect of crude methanolic leaf extract of *Jatropha gossypifolia* (JG50mg and JG100mg) and alpha-lipoic acid (ALA) on plasma and testes triglycerides (TAG) concentrations in male rats pretreated with cypermethrin. The bars represent mean $\pm$ STD (n = 5); \*: significantly different (p<0.05) compared with control group; #: significantly different (p<0.05) compared with CYP Only group







**Figure 3:** Effect of crude methanolic leaf extract of *Jatropha gossypifolia* (JG50mg and JG100mg) and alpha-lipoic acid (ALA) on plasma and testes cholesterol (CHOL) concentrations in male rats pretreated with cypermethrin. The bars represent mean $\pm$ STD (n = 5); \*: significantly different (p<0.05) compared with control group; #: significantly different (p<0.05) compared with CYP Only group



**Figure 4:** Effect of crude methanolic leaf extract of *Jatropha gossypifolia* (JG50mg and JG100mg) and alpha-lipoic acid (ALA) on vLDL+LDL and HDL triglycerides (TAG) concentrations in male rats pretreated with cypermethrin. The bars represent mean±SD (n = 5); \*: significantly different (p<0.05) compared with control group; #: significantly different (p<0.05) compared with CYP Only group

Figures 1 and 3 showed that cypermethrin administration caused a significant (p<0.05) increase in testes lipid profile (TAG, 96.46 % and CHOL, 64.99 %) when compared with the control group. Co-administration with the methanolic leaf extract of *Jatropha gossypifolia* (50 mg and 100 mg) and ALA significantly decreased the concentrations of testes TAG and CHOL (p<0.05) by 55.86 %, 38.51 % and 38.85 % respectively; and 40.66 %, 51.52 % and 57.57 % respectively when compared with the group administered with cypermethrin only. Evaluation of the doses of the methanolic leaf extract of *Jatropha gossypifolia* (50 mg and 100 mg) and ALA alone in comparison with the control revealed that JG50 mg decreased CHOL (22.19 %) concentration and increased TAG (20.79 %) concentration;

JG100 mg increased TAG (17.45 %), CHOL (18.75 %) concentrations; and ALA decreased TAG and CHOL (7.09 % and 14.37 % respectively) concentrations. However, statistically, these increase and decrease were not significant.

# DISCUSSION

This present study showed that cypermethrin resulted in a marked increase of plasma triglycerides, vLDL+LDL cholesterol, vLDL+LDL triglycerides and a marked reduction in high density lipoprotein cholesterol (HDL-c) and triglyceride (HDL-TG) when compared to the control. Similar trend was observed by Newairy and Abdou<sup>[20]</sup> and Athesh et al. [21] using chlorpyrifos and diazinon respectively. Previous studies have shown increase in the concentration of serum or plasma triglycerides and cholesterol in experimental animals exposed to different insecticides such as organophosphate, dichlorvos and carbamate furadan <sup>[22]</sup>. This rise has been imputed to an inhibition of the lipase enzyme activity of both the hepatic triglycerides and plasma lipoproteins <sup>[23]</sup>, as well as increase in both de novo synthesis and intestinal absorption of cholesterol. The decrease observed in HDLcholesterol and HDL-triglycerides was similar to what was obtained by Newairy and Abdou<sup>[20]</sup> in which oral administration of chlorpyrifos resulted in a significant reduction in serum HDL-cholesterol. HDLcholesterol is a lipoprotein majorly produced in the liver and intestinal cells playing a crucial role in cholesterol efflux from tissues (especially the heart) and back to the liver for removal as bile acids. It has been established that high levels of triglycerides, total cholesterol, vLDL+LDL accompanied with a low level of HDL-cholesterol are associated with an increased risk for coronary heart disease <sup>[24]</sup>. It is well documented that an increase in HDL-cholesterol level could potentially contribute to anti-atherogenicity [25]. Therefore, the elevation of plasma vLDL+LDL in combination with a reduction in HDL fraction as seen in this study as a result of cypermethrin administration showed that exposure to cypermethrin could result in heart-related diseases such as atherosclerosis, stroke, angina and hypertension.

Furthermore, increased plasma total cholesterol observed in this study due to cypermethrin administration is consistent with the findings of Newairy and Abdou<sup>[20]</sup>. This rise in plasma cholesterol could be due to an increase in the synthesis of liver cholesterol. This induced elevation in plasma cholesterol may be attributed to the effect of the pesticide on the permeability of liver cell membrane and/or to the blockage of liver bile ducts resulting in the reduction or cessation of its secretion to the duodenum <sup>[26]</sup>.

From this present study, co-administration with the methanolic leaf extract of Jatropha gossypifolia and alpha-lipoic acid normalized the derangement that was seen in the lipid profile of the plasma and its lipoprotein fractions caused by the exposure to cypermethrin. HDL lipid profile concentrations were markedly elevated while vLDL+LDL lipid profile concentrations were significantly lowered in comparison with the group administered with cypermethrin alone. These data were in agreement with the results obtained by Fuliang et al. (2005) in which there was a significant decrease in total cholesterol, triglycerides, LDLcholesterol and very low-density lipoprotein cholesterol (vLDL-c); and an increase in the serum level of HDL-cholesterol in the serum of rats orally administered with propolis. Also, a number of studies have given credence to the fact that ALA supplementation acts as a potent antilipidemic agent which lowers plasma triglycerides, total cholesterol and LDL-cholesterol, and elevates HDL-cholesterol <sup>[27]</sup>. In addition, the ability of the methanolic extract of Jatropha gossypifolia as well as the alpha-lipoic acid in attenuating the deleterious effect of cypermethrin is an indication that they could be employed in the treatment of cardiovascular diseases. The administration of quercetin against lindane-induced alterations in plasma, HDL and LDL lipid profile as reported by Viswanadha et al. <sup>[28]</sup> supported this present study.

The methanolic leaf extract of *Jatropha gossypifolia* and alpha-lipoic acid seems to lower plasma LDL-bound cholesterol by: firstly inhibiting the uptake of cholesterol in the gastrointestinal tract,

secondly by eliminating LDL-cholesterol from the blood via LDL receptor and/or thirdly by increasing the activity of cholesterol-degrading enzymes such as cholesterol-7-hydroxylase. At the same time, their ability to significantly raise the level of HDL-cholesterol is due to their elimination of cholesterol. Both the *Jatropha gossypifolia* leaf extract and ALA reduced total cholesterol and increased HDL-cholesterol indicating that they may be mobilizing cholesterol from extrahepatic tissues to the liver for catabolism <sup>[29]</sup>. The plant extract and ALA were also able to markedly lower the concentration of triglyceride in the plasma and vLDL+LDL. This decrease may be related to increase in endothelium-bound lipoprotein lipase activity that hydrolyses the triglycerides into fatty acids <sup>[21]</sup>.

Levels of lipid profile parameters were significantly elevated in the testes after cypermethrin exposure when compared to the control. This increase may be attributed to non-utilization of cholesterol esters for androgen biosynthesis. Sheriff<sup>[13]</sup> had reported that increase in neutral lipids (cholesterol and triglycerides) suggests that testicular lipids get altered whenever spermatogenesis and steroidogenesis are impaired. Thus testicular lipids may be considered as one of the biomakers or indices of testicular function. Helena et al. [30] also showed that abnormalities in HDL structure, function and concentration compromised fertility. However, these changes were normalized by the co-administration of the methanolic leaf extract of Jatropha gossypifolia and ALA. This shows that the methanolic leaf extract of Jatropha gossypifolia and ALA efficiently and effectively regulated cholesterol and triglyceride metabolism possibly through the decreased expression of genes for fatty acid synthesis and enhanced activation of expression of genes for  $\beta$ -oxidation <sup>[21]</sup>.

# CONCLUSION

In conclusion, findings from this present study further elucidates the deleterious effect of cypermethrin on the testes of rats after exposure which were ameliorated by both the *Jatropha gosypifolia* leaf extract and alpha-lipoic acid. The ability of the *Jatropha gossypifolia* extract to mitigate these side effects may be attributed to the presence of secondary metabolites such as tannins, cardiac glycosides and flavonoids embedded in it. These vital phytoconstituents could be explored and employed in the treatment of both reproductive challenges and cardiovascular diseases.

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