



Protective Effect of *Alchornea laxiflora* Methanolic Leaf Extract on CCL4-induced Hepatotoxicity and Reproductive Toxicity in Male Wistar Rats

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Authors' contributions

This work was carried out in collaboration between all authors. Author ESU designed the study, wrote the protocol and wrote the first draft of the manuscript. Author NOR managed the literature searches, analyses of the study and performed the spectroscopy analysis. Author OO managed the experimental process. All authors read and approved the final manuscript.

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ABSTRACT

Background: *Alchornea laxiflora* is a medicinal plant with immense health benefits.

Aim: To verify the protective effects of methanol extract of *Alchornea laxiflora* (MEAL) leaf on hepatotoxicity and reproductive toxicity induced by CCl₄.

Settings and Design: Extraction and administration of Bioactive extract.

Place and Duration of Study: Department of Biochemistry, University of Medical Sciences, Ondo City, Ondo State, P.M.B 536. Between November 2015 - July 2016.

Materials and Methods: Thirty five Albino Wistar rats were randomized into seven groups of five animals each, group 1 animals which served as positive control, received normal saline, group 2 animals which served as negative control, received 1 ml/kg body weight of CCl₄ only for a day while groups 3-7 received 0.1, 0.5, 1.0, 10.0 and 50 mg/kg body weight of methanol extract *Alchornea laxiflora* (MEAL) leaf respectively for seven days after receiving a single dosage CCl₄ (1 ml/kg body weight) for a day. Activities of Gamma glutamyl transferase (GGT),

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Glutathione-S-transferase (GST), Alanine aminotransferase (ALT) and Alkaline phosphatase (ALP) were assayed to verify the effects of the plant extract on the liver while percentage sperm motility and sperm morphology was assayed to check the effects of the plant extract on reproductive system/organs.

Statistical Analysis: The data were analysed by SPSS (version 2.1).

Result and Conclusion: Graded dosage (0.1-50 mg/kg bodyweight) of methanol extract of *Alchornea laxiflora* leaf significantly ($p \leq 0.05$) reversed the toxic effects of CCl_4 on the liver and reproductive organs of wistar rats in a dose dependent manner as compared to group 2 animals. This may be due to the secondary metabolites content of *Alchornea laxiflora*, which have been shown to have profound antioxidant potentials. It could be deduced that *Alchornea laxiflora* leaf extract can reverse hepatotoxicity and reproductive toxicity and by extension cure infertility in rats.

Keywords: *Alchornea laxiflora*; hepatotoxicity; reproductive toxicity; medicinal plant; antioxidant potentials.

1. INTRODUCTION

Medicinal plants have been used in the treatment of diseases such as HIV/AIDS, malaria, diabetes, sickle-cell anaemia, mental disorders [1] and microbial infections [2]. One of such medicinal plant is *Alchornea laxiflora* (Benth) pax and Hoffman, a plant used locally for the preservation of food items in Nigeria [3,4]. *Alchornea laxiflora* is a shrub belonging to the family Euphobiaceae and is widely distributed throughout tropical Africa. The anti-toxicity activity of hexane root extract of *Alchornea laxiflora* was reported by Uhunmwangho et al. [5]. The present study is aimed at investigating the protective effects of methanol leaf extract of *Alchornea laxiflora* on hepatotoxicity and reproductive toxicity induced by CCl_4 in male Wistar rats.

2. MATERIALS AND METHODS

2.1 Plant Extraction

Fresh leaves of *Alchornea laxiflora* were obtained from local farms in Ondo and authenticated at the Department of Botany, University of Medical Sciences, Ondo City, Nigeria. (Voucher no UMB1505) The leaves were washed with distilled water, air dried and were extracted with 500L of 95% methanol (Sigma, Chemical Co. London) Utilizing a giant Soxhlet apparatus for continuous extraction at 50°C for 72 hours [6]. In each case, the crude extracts were collected and concentrated to low volumes with rotatory evaporator and the concentrated

extracts were weighed and transferred to labelled sample tubes.

2.2 Experimental Animals

Thirty five adult male albino rats of Wistar strain weighing between 120-150 g were obtained from the animal house in the Department of Biochemistry, University of Medical Sciences, Ondo City, Nigeria. The animals were kept in clean disinfected cages and were allowed to acclimatize for 2 weeks before commencement of experiment. They were fed on standard rat chows from Pfizer feeds, Nigeria and were allowed free access to water. The animals were randomized into seven groups with each group having five animals each.

2.3 Experimental Design

Animals were post-treated with methanol leaf extract of *Alchornea laxiflora* for seven days after receiving 30% carbon tetrachloride (CCl_4) (hepatotoxicant) in olive oil intra-peritoneally at a dose of 1 ml/kg body weight for a day [7]. The plant extract was administered (orally) to all groups except the positive and negative controls (groups 1&2), group 1 (positive control) received only normal saline, while the negative control (group 2) received only 30% CCl_4 in olive oil (1 ml/kg) of toxicant intra-peritoneally for 1 day all through the course of the experiment. Group 3-7 received graded doses of the plant extracts (0.1, 0.5, 1.0, 10, and 50) mg/kg body weight respectively after receiving the toxicant for a day.

That is:

Group	Treatments
Group 1	Received normal saline only (Positive control)
Group 2	Received 5 ml/kg CCl_4 only (negative control)

Group	Treatments
Group 3	Received CCl ₄ and 0.1 mg/kg body weight of methanol extract of <i>Alchornea laxiflora</i> leaf
Group 4	Received CCl ₄ and 0.5 mg/kg body weight of methanol extract of <i>Alchornea laxiflora</i> leaf
Group 5	Received CCl ₄ and 1.0 mg/kg body weight of methanol extract of <i>Alchornea laxiflora</i> leaf
Group 6	Received CCl ₄ and 10 mg/kg body weight of methanol extract of <i>Alchornea laxiflora</i> leaf
Group 7	Received CCl ₄ and 50 mg/kg body weight of methanol extract of <i>Alchornea laxiflora</i> leaf

2.4 Tissue Preparation and Sample Collection

The animals were sacrificed 24 hours after the last administration by cervical decapitation. Liver samples were isolated from sacrificed animals, washed in ice cold 1.15% KCl and homogenized in homogenizing buffer (50mM Tris.Hcl, 1.15% KCl, pH7.4). The homogenate was centrifuged at 9 kg for 20 minutes and the resulting supernatant further centrifuged at 105 kg for 1hour at 4°C to obtain the microsomal fraction. This fraction was re-suspended in 0.25 M sucrose and stored frozen. Laparotomy was carried out and the right epididymis was dissected out for sperm motility and sperm morphology tests.

2.5 Biochemical Analysis

Liver Marker Enzymes: serum and liver homogenate were analyzed for the following parameters: Gamma-glutamyl transferase (GGT) [8], Alanine Aminotransferase (ALT) [9], Alkaline phosphatase (ALP) [10] and Glutathione-S-transferase (GST) by Meyer et al. [11]. These were carried out using standard reagent and kits from Sigma Aldrich Laboratory limited, U.S.A.

2.6 Sperm Morphology Assay

This was determined according to Narayana, [12]; Bairy et al. [13]. Sperm suspension was made and stained with 0.2 ml of 1% aqueous eosin. About one drop of stained suspension was placed on a clean slide and was dried. Slides were examined for abnormalities in five hundred sperms per animal and were classified into normal and abnormal sperms. Further, the abnormal sperms were designated under head abnormalities and tail abnormalities. The abnormalities were further sub-grouped as cut, bent, loop, and detached.

2.7 Sperm Motility

This was determined according to Narayana, [12]. Sperm suspension was prepared in 1 ml of phosphate buffered saline (PBS) at pH 7.2. About 1 ml of the suspension was diluted in a ratio of 1:40 with PBS. A sample of the diluted

suspension was charged into a hemocytometer. The eight squares were examined for motile sperms and the percentage of motile sperms was recorded.

2.8 Statistical Analysis

All analyses were carried out in triplicates. The data were recorded as mean ± standard deviation and analysed by SPSS (version 2.1). One way analysis of variance (ANOVA) was carried out and Tukey's Post hoc test was performed to test any significant difference between column means. Values of p≤0.05 were considered significant.

3. RESULTS

3.1 Effects of Methanol Extract of *Alchornea laxiflora* Leaf on the Levels of GGT, GST, ALT and ALP in CCl₄ Induced Hepatotoxicity

Administration of a single dose (1 ml/kg) of CCl₄ significantly increased (P≤0.05) the levels of GGT, GST, GPT and ALP of the treatment groups compared to the positive control. However, administration of graded dosage (0.1-50 mg/kg body weight) of methanol extract of *Alchornea laxiflora* (MEAL) leaf significantly (P≤0.05) reduced the levels and brought it closer to that observed for the positive control. For both GGT and GST, the greatest reduction was observed at 50 mg/kg of the plant extract (Table 1).

3.2 Effects of Methanol Extract of *Alchornea laxiflora* leaf on Sperm Motility in CCl₄ Induced Reproductive Toxicity

Administration of a single dose (1 ml/kg) of CCl₄ significantly decreased (P≤0.05) percentage sperm motility of the treatment group compared to the positive control. However, administration of graded dosage (0.1-50 mg/kg body weight) of methanol extract of *Alchornea laxiflora* (MEAL) significantly increased (P<0.05) the percentage sperm motility of the treatment group in a dose dependent manner (Table 2).

Table 1. The effects of methanol extract of *Alchornea laxiflora* on the levels of GGT, GST, ALT and ALP in CCl₄ induced hepatotoxicity

Treatment	GGT	GST	ALT	ALP
Positive control (normal saline)	0.251±0.03	0.361±0.36	0.069±0.08	0.126±0.03
Negative control (5 ml/kg CCl ₄)	0.400±0.48	0.467±0.57	0.096±0.10	0.497±0.04
0.1 mg/kg	0.225±0.15	0.386±0.47	0.072±0.11	0.250±0.02
0.5 mg/kg	0.174±0.21	0.381±0.56	0.071±0.07	0.188±0.03
1.0 mg/kg	0.129±0.15	0.370±0.45	0.068±0.08	0.160±0.02
10.0 mg/kg	0.130±0.16	0.356±0.48	0.070±0.10	0.129±0.02
50.0 mg/kg	0.125±0.11	0.354±0.44	0.072±0.12	0.136±0.06

Results are presented as mean ± SD for five animals in each group, for 5 animals in each group

3.3 Effects of Methanol Extract of *Alchornea laxiflora* Leaf on Sperm Morphology in CCl₄ Induced Reproductive Toxicity

Administration of a single dosage (1 ml/kg) of CCl₄ significantly (P≤0.05) altered the morphology of sperm in the treatment groups compared to the control as evident in the increased levels of Revoleventory tail (LT), Looped Tail (LT), Cut Midpeice (CMP) and Detached Tail (DT). However, administration of graded dosage (0.1-50 mg/kg body weight) of methanol extract of *Alchornea laxiflora* (MEAL) significantly reduced (P≤0.05) the abnormalities in sperm morphology of the treatment group and even more the positive control group (Table 3).

Table 2. The effects of methanol extract of *Alchornea laxiflora* leaf on sperm motility in CCl₄ induced reproductive toxicity

Treatment	% mortality
Positive control (normal saline)	80
Negative control (5 ml/kg CCl ₄)	38
0.1 mg/kg	64
0.5 mg/kg	57
1.0 mg/kg	75
10.0 mg/kg	80
50.0 mg/kg	80

Results are presented as mean ± SD for five animals in each group, for 5 animals in each group

4. DISCUSSION

Healthy state in living organisms is a complex interplay of many factors which must be geared towards the maintenance of well-being. The living organism is constantly faced with lots of life-threatening situations ranging from attacks from predators, insects, disease harbouring hosts, and even some of the food consumed by them may pose some dangers to them. It is also noteworthy that some of the drugs used by

human beings have adverse effects on them. The literature is replete with the toxic effects of toxicants on the liver being the major organ for xenobiotic metabolism, however, it has been discovered that some of these toxicants have toxic effects on reproduction and reproductive organs [14]. This is especially very crucial to note giving the rise in the incidence of infertility among human populations [15]. Thankfully, the plant kingdom is a repertoire of medicinal plants whose therapeutic benefits have been used in the treatment of myriads disease conditions [16]. *Alchornea laxiflora* is one of such beneficial medicinal plants whose anti-toxicity potential was reported by Uzunmwangho et al. [5]. The present study was designed to verify the ameliorative potentials of *Alchornea laxiflora* methanol leaf extract on hepatotoxicity and reproductive toxicity induced by CCl₄. From the result obtained, Table 1 shows the GGT, GST, ALT and ALP activities of the control and the treatment groups in CCl₄-induced hepatotoxicity. CCl₄ administration significantly (P≤0.05) increased the activities of these enzymes in group 2 (CCl₄ only). This is to confirm that CCl₄ induction actually damaged the livers of the animals and this is not surprising given the fact the metabolism of CCl₄ leads to the generation of free radicals through its intermediate metabolite CCl₃[•] [17,18]. Gamma glutamyl transferase (GGT) is an intracellular enzyme that helps transfer G-glutamyl moieties to acceptor molecules and more importantly in the gamma glutamyl cycle for glutathione (an important antioxidant) homeostasis. Increased activity of GGT has been reported in liver injury [19]. However, administration of graded dosage (0.1-50 mg/kg body weight) of *Alchornea laxiflora* methanol leaf extract significantly (P≤0.05) reduced the activities of GGT with the 50 mg/kg giving the greatest reduction. Even more than the positive control (0.125±0.11 and 0.251±0.03) respectively, Glutathione-S-transferase (GST) is a family of intracellular enzyme that transfer thiol group of Glutathione to other cell components in

a bid to counter oxidative stress occasioned by free radical generation. Increased activities of GST have been observed in liver and kidney disease [20]. Administration of CCl_4 significantly increased ($P \leq 0.05$) GST activities of the treatment group compared to the positive control. However, post treatment with *Alchornea laxiflora* methanol leaf extract significantly ($P \leq 0.05$) decreased the activities of GST and brought it closer to the positive control group. *Alchornea laxiflora* leaf extract was able to combat the free radicals generated by CCl_4 because of its contents of secondary metabolites, which may be responsible for the decreased activities of GST in the *Alchornea laxiflora* treated group in a dose dependent manner. Assaying for activities of alanine aminotransferase (ALT), an intracellular enzyme, is one of the indices of liver function test [21,22]. Increased activities of ALT results primarily due to oxidative damage to the membrane of the liver [23], administration of graded dosage (0.1-50 mg/kg body weight) of methanol leaf extract of *Alchornea laxiflora* significantly reduced ($p \leq 0.05$) ALT activities and brought it closer to that observed for the positive control group. The therapeutic potentials of medicinal plants are tied to their contents of secondary metabolites [24]. The Phytochemistry of *Alchornea laxiflora* leaf revealed that it was rich in flavonoids, saponins etc. [25]. These secondary metabolites have profound antioxidant potentials which can combat free radicals and as a result help in resealing and healing the liver membrane damaged by CCl_4 administration [26] which may be the reason for the reduced activities of ALT in the *Alchornea laxiflora* treated groups. Alkaline phosphatase (ALP) activity is also used as an indicator of liver damage [27]. CCl_4 administration significantly increases ALP activities however; post treatment with graded

dosage (0.1-50 mg/kg body weight) of methanol extract of *Alchornea laxiflora* significantly ($P \leq 0.05$) reduced the activities of ALP in a dose dependent manner. This is to attest to the anti-toxicity potentials of *Alchornea laxiflora* as reported by Uahunmwangho et al. [5]. Reproductive toxicity is one of the dangers of drug toxicity [28], given the recent rise in the incidence of infertility [15]. Public attention has been drawn to the consumption of drugs that have adverse effects on the reproductive system and organs. Man in his quest for a solution to this problem has found the effectiveness of medicinal plants in the reversal of reproductive toxicity [29] due to their contents of secondary metabolites [24]. CCl_4 administration significantly ($p < 0.05$) affected the percentage sperm motility and morphology as evident in Tables 2 and 3. Table 2 shows that CCl_4 administration significantly reduced sperm motility which is one of the criteria that determines sperm viability. However, *Alchornea laxiflora* methanol leaf extract significantly ($p \leq 0.05$) increased percentage motility of the treatment group. This means that the plant extract contains active substances that aid sperm motility. Sperm motility is essential for the fertilization of ovum. Therefore, it may be safe to say that *Alchornea laxiflora* contains active substances that can reverse reproductive toxicity and aid fertility. Table 3 shows the sperm morphology parameters of the various groups, CCl_4 administration significantly affected the sperm morphology parameters (Revolventory Tail (R.T), Bent Tail (B.T) Looped Tail (L.T), Cut Tail (C.T), Cut Midpiece (C.M.P) and Detached Tail (D.T) which on the long run may lead to infertility. However, administration of graded dosage (0.1-50 mg/kg body weight) of methanol leaf extract of *Alchornea laxiflora* significantly reduced the effects of CCl_4 .

Table 3. The effects of methanol extract of *Alchornea laxiflora* leaf on sperm morphology in CCl_4 induced reproductive toxicity

Treatment	% R.T	% B.T	% L.T	% C.T	% C.M.P	% DT
Positive control (normal saline)	2.3	0.0	0.0	0.0	2.6	6.9
Negative control (5 ml/kg CCl_4)	7.8	6.8	5.3	4.6	4.8	11.4
0.1 mg/kg	4.6	3.2	5.3	1.5	2.2	4.8
0.5 mg/kg	2.2	2.3	2.2	0.0	1.6	2.2
1.0 mg/kg	2.3	2.6	0.0	0.0	1.6	3.2
10.0 mg/kg	1.5	1.2	0.0	0.0	0.0	3.0
50.0 mg/kg	2.1	1.0	0.0	0.0	0.0	3.0

Results are presented as mean \pm SD for five animals in each group, for 5 animals in each group Where R.T is Revolventory Tail, B.T is Bent tail, L.T is Looped Tail, C.T is Cut Tail, C. M. P is curve Midpiece, and D.T is Detached Tail

5. CONCLUSION: PROTECTIVE EFFECTS OF MEAL ON HEPATO AND REPRODUCTIVE TOXICITY

In conclusion, methanol extract of *Alchornea laxiflora* leaf extract can be used to reverse both hepatotoxicity and reproductive toxicity induced by CCl₄ and by extension aid infertility in male wistar rats. This is not surprising given the secondary metabolites contents of *Alchornea laxiflora* leaf. However, further studies are needed to verify if the same is obtainable in man.

CONSENT

It is not applicable.

ETHICAL APPROVAL

All authors hereby declare that all experiments have been examined and approved by the appropriate ethics committee and have therefore been performed in accordance with the ethical standards laid down in the 1964 Declaration of Helsinki.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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