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Effects of Methanol Leaf Extract of *Alchornea laxiflora* on Antioxidant Enzymes, Triacylglycerol and Cholesterol Levels in Male Wistar Rats

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Abstract: ***Alchornea laxiflora*** **belongs to Euphorbiaceae family and it is mainly cultivated for its ethno-medicinal uses. It is used for the treatment of malaria, inflammation and infectious diseases in Nigeria. The aim of this work was to investigate the effects of the methanol extract of the leaf (M_L), extracted with 95% methanol using soxhlet extractor at 65^oC, on antioxidant enzymes, triacylglycererol (TAG) and cholesterol levels in male Wistar rats. The animals (30) weighing between 120g-150g were divided into five groups of six rats each. Group 1 served as control and received distilled water, p.o. Groups 2, 3, 4 and 5 received 0.5, 1.0, 10.0 and 50.0mg/kg body weight dose of the M_L respectively (p.o) for fourteen consecutive days. Results showed that the rats fed with the higher concentrations of M_L had significant increase ($p<0.05$) in glutathione content (GSH), SOD and catalase activities relative to control. Also, there were no significance changes in the TAG and the cholesterol level in all the concentrations of M_L extracts examined. These results suggest that *Alchornea laxiflora* M_L extracts possess varied degree of potent antioxidant activity and may serve as important sources of antioxidants in food, cosmetics and pharmaceutical industries.**

Keywords**: *Alchornea laxiflora*, antioxidant, cholesterol, triacylglycerol.**

1. INTRODUCTION

Reactive oxygen species are not only produced naturally in cell following stress or respiration but also have been reported to be produced by radiation, bacterial and viral toxin, smoking and alcohol. Overproduction of ROS or inadequate antioxidants has been implicated in the pathogenesis and complications of some disease conditions like diabetes, Alzheimer's disease, cancer, atherosclerosis, arthritis, neurodegenerative disease, and aging process (Khalaf *et al*., 2008). Antioxidants act as a defense mechanism that protect against deleterious effects of oxidative reaction produced by reactive oxygen species (ROS) in a biological system thus, they are absolutely critical for maintaining optimal cellular and systemic health and well-being (Jayachitra and Krithig, 2010). A number of medicinal plants have shown their beneficial effect on the cardiovascular disease by virtue of their lipid lowering, antianginal, antioxidant and cardioprotective effects (Dwivedi, 2004). Recently, there has been an upsurge of interest in the therapeutic potential of plants as antioxidants in reducing oxidative tissue injuries (Patel *et al*., 2010). Plants, herbs, and spice, rich in phenolic compounds like flavonoids, have been demonstrated to have anti-inflammatory, antiallergenic, antiviral, antiaging, antihyperlipidaemic and anticarcinogenic activities which can be attributed to their antioxidant properties (Aqil *et al*., 2006).

Alchornea laxiflora belongs to Euphorbiaceae family. It is a deciduous shrub and about 6-10 m high. It grows naturally in Nigeria, in DR Congo, in Ethiopia, and throughout East Africa to Zimbabwe (Burkill, 1994). *Alchornea laxiflora* is called 'ububo' in *Igbo, 'pepe'* or 'ijan' among the Yoruba tribe in Nigeria. The leaf infusion of the plant is often used in folklore

medicine as antimalarial (Adeloye *et al*., 2005). The stem, especially the branches, is used in Nigeria as chewing sticks (local tooth brush) for cleaning teeth while the leaves are used to preserve kola nut and other perishable fruits and vegetables. Decoction of the leaves is usually administered to treat inflammatory and infectious diseases (Ogundipe *et al*., 2001). The leaves of *Alchornea laxiflora* have also been reported to possess hepatoprotective property (Oloyede, *et al*., 2011). The leaf extract has been reported to contain alkaloids, flavonoids, saponins, tannins, carbohydrates, cardioactive glycosides, steroids, phenols and reducing sugars as phytochemicals resident in the plant (Oloyede *et al*., 2010), this suggests that the leaf of *Alchornea laxiflora* possess antioxidant properties. The aim of this study was therefore to investigate the effects of methanol leaf extract of *Alchornea laxiflora* on antioxidant parameters, triacylglyceride and cholesterol levels in male Wistar rats.

2. MATERIALS AND METHODS

2.1 Experimental Animals:

Thirty Male Wistar rats weighing between 120g -150g were obtained from the animal house of University of Medical Sciences, Ondo State, Nigeria. They were allowed to acclimatize for two weeks before the commencement of the experiment, during which they were kept in ventilated cages, and were given rat chows and water *ad libitum*.

2.2 Plant Extraction:

Fresh leaves of *Alchornea laxiflora* were exhaustively extracted with 500ml of 95% methanol utilizing a giant Soxhlet apparatus at 65^{o} C for 60 minutes. The crude extracts were collected and concentrated with rotary evaporator; the concentrated extracts were further dried in a desiccator.

2.3 Experimental Design:

The animals were divided into 5 groups of 6 animals each. Animals in group 1 served as control and received distilled water. Groups 2, 3, 4 and 5 received 0.5mg/kg, 1.0mg/kg, 10.0mg/kg and 50.0mg/kg body weight dose of the extract respectively. The administration was done for fourteen consecutive days.

2.3.1 Sample Collection:

Animals were fasted overnight and sacrificed by cervical dislocation on day fifteen of the experiment. Blood was collected from the heart (using a syringe) into plain sterile bottles, allowed to stand for 30 minutes to clot and centrifuged at 2,000 rpm for 10minutes at room temperature. The samples were stored at 8°C before use.

2.3.2 Preparation of Tissue Homogenates:

Liver was harvested after sacrificing the animals and placed in plain, sterile containers which contained 25ml of normal saline and stored in ice (4^{o}C). 1g of liver and was homogenized in 10ml of ice cold physiological saline. The resulting homogenates were centrifuged at 1,000g of 10 minutes and the supernatant obtained was used for subsequent analyses.

2.3.3 Estimation of Antioxidant Parameters:

2.3.3.1 Estimation of Reduced Glutathione (GSH):

Reduced glutathione (GSH) in the liver was estimated according to the method of Ellman (Ellman, 1959).

2.3.3.2 Estimation of Catalase Activity:

Catalase activity was determined according to the method of Sinha, (1971).

2.3.3.3 Estimation of Superoxide Dismutase Activity:

The level of SOD activity was determined by the method of Misra and Fridovich (1972).

2.3.4 Estimation of Cholesterol and Triacylglycerol:

The serum cholesterol and triacylglycerol were estimated using Randox colorimetric kits. (Crumlin Country, Antrim, United Kingdom).

2.4 Statistical Analysis:

Results were expressed as mean ± SEM. One way analysis of variance (ANOVA) followed by Turkey's test was used to determine significance of differences between the test groups and the control .Statistical significance was declared when p value was less than 0.05. The statistical analysis was performed using the graph pad instat (version 3.0).

3. RESULTS

The antioxidant properties of methanol leaf extract of *Alchornea laxiflora* was evaluated by determining the activities of catalase and superoxide dismutase (SOD) in serum as well as the concentration of reduced glutathione (GSH) in liver homogenate.

Table 1 shows the concentration of GSH in the liver homogenate for the various groups. There was dose-dependent significant ($p<0.05$) increase in GSH concentration across the test groups administered the extracts compared to the control given distil water.

Table 2 shows serum catalase activity of the rats subjected to fourteen days administration of *Alchornea laxiflora* extracts. A statistically significant increase ($p<0.05$) was observed in the catalase activity of the test groups in contrast to the control group in dose-dependent fashion.

Table 3 shows the result of serum superoxide dismutase (SOD) activity of the rats placed on fourteen days administration of *Alchornea laxiflora* extracts. There exists significant elevation ($p<0.05$) in the SOD activity in groups administered 0.5mg/ml and 50mg/ml *Alchornea laxiflora* extracts relative to the control group. Conversely, the test groups given 1mg/kg and 10mg/kg showed no significant deviation ($p<0.05$) from the control group.

Table 4 shows the effect of *Alchornea laxiflora* methanol extract on the level of serum triglyceride and cholesterol in rats for a period of fourteen days. Significant decrease was observed in the triglyceride level of the groups given 0.5mg/kg and 1mg/kg relative to the control. In contrast, there was no statistical significant difference ($p<0.05$) in the groups administered 10mg/kg and 50mg/kg of the extract in comparison to the control group. Serum cholesterol of all the test groups displays no significant difference ($p<0.05$) compared to the control.

4. DISCUSSION

Plant biomass has been known overtime for their healing properties which may be due to the presence of the phytochemicals and this may lead to groundbreaking in the area of new drug discovery and design (Madhavan *et al*., 2010). The phytochemicals are the secondary metabolites also referred to as antioxidants. Studies have shown the role of antioxidants (drug and plant-derived antioxidant) in scavenging free radicals and ultimately preventing oxidative stress resulting from the effects of pro-oxidants on the cells, thereby abrogating the disease-causing potentials of free radicals in the cells (Hamid *et al*., 2010; Nwaneri-Chidozie *et al*., 2016).

Reduced glutathione (GSH) is a tripeptide, non enzymatic biological antioxidant present in the liver which helps to donate reducing equivalent to $NADP^+$ and oxidant molecules. Decreased GSH level is associated with reduced antioxidant status in the living system. This reduction is as well linked to various diseases related to oxidative stress such are atherosclerosis, diabetes mellitus, carcinogenesis and others. The observed elevated GSH level (Table 1) upon administration of the plant extract may depict its ability in boosting the antioxidant defence of biological system thereby preventing diseases associated with oxidative stress (Arivazhagan *et al*., 2000).

Catalase activity is one of the indices of antioxidant status of the body. Reduction in activity of this enzyme may lead to deleterious effects as a result of superoxide and hydrogen peroxide assimilation (Oyedemi *et al*., 2010). Increased catalase activity observed in the present study (Table 2) could be an indication of chemopreventive potential of the plant in the pathogenesis of oxidative stress-related diseases which may be associated to the presence of various bioactive principles in the plant such as alkaloids, flavonoids, saponins, tannins, carbohydrates, cardioactive glycosides, steroids, phenols and reducing sugars (Oloyede *et al*., 2010). This is in consonant with the report given by Nwaneri-Chidozie *et al*., 2016, in a study on the effect of *Annona muricata* in rats.

Also, elevated serum superoxide dismutase (SOD) in groups administered 0.5mg/ml and 50mg/ml (Table 3) could depict that the extract at these doses may be effective in combating rapid generation of free radicals in the body system. Whereas, groups showing similar effect to the control group may be indicative of the safety intake of this plant extract at such doses.

Elevated serum total cholesterol, triglycerides and LDL-C is associated with narrowing of the vasculature which can lead to plaque and atherosclerosis, subsequently coronary artery disease. The observed statistical similarity in serum triglyceride and cholesterol level of the rats administered the extracts to the control group may be a pointer to its ability in maintaining the lipid profile status in the biological system (Rich-Edwards *et al*., 1999). This plant could be useful in preventing lipid peroxidation process because of the presence of some secondary metabolites and its ability to maintain lipid profile level in living system (Salemcity *et al*., 2014).

5. CONCLUSION

From the above data, it could be deduced that the methanol leaf extract of *Alchornea laxiflora* possess significant antioxidant capacity which could be good scavenger of free radicals and invariably useful in the prevention of oxidative stress related diseases. Also, the plant may serve as excellent antihyperlidemic agent as it is capable of maintaining lipid profile status.

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APPENDIX – A

List of Tables:

Table 1: GSH Content in Liver Homogenate of Rats on *A. laxiflora* Extracts for Fourteen Days

Dose (mg/kg of *Alchornea laxiflora*)	GSH content (µg/ml)
- (1 ml distilled water)	23.33 ± 0.73
0.5	27.78 ± 0.43
1.0	37.50 ± 0.29*
10.0	38.82 ± 0.09*
50.0	43.50 ± 0.29*

n= 3

Values are expressed as mean ± SEM. * denotes significant difference from the control.

Table 2: Catalase Activity in Serum for the Groups

Dose (mg/kg of *Alchornea laxiflora*)	Catalase Activity (U/ml)
- (1ml distilled water)	0.007 ± 0.002
0.5	0.011 ± 0.003*
1.0	0.011± 0.002*
10.0	0.016 ± 0.008*
50.0	0.020 ± 0.005*

Values are expressed as mean ± SEM. * denotes significant difference from the control.

Table 3: Superoxide Dismutase (SOD) Activity in Serum

Dose (mg/kg of *Alchornea laxiflora*)	SOD ACTIVITY (U/ml)
- (1 ml distilled water)	0.02 ± 0.002
0.5	0.12 ± 0.006*
1.0	0.04 ± 0.009
10.0	0.05 ± 0.004
50.0	0.08 ± 0.002*

n= 3

Values are expressed as mean ± SEM. The test groups values are significantly different ($p<0.05$) from the control

Table 4: Effects of Alchornea laxiflora Methanol Leaf Extract on the Level of Triacylglycerol (TAG) and Cholesterol In Rats Serum

Dose (mg/kg of *Alchornea laxiflora*)	TAG Level (mmol/l)	Cholesterol Level (mmol/l)
- (1 ml distilled water)	3.64 ± 0.03	14.54 ± 0.14
0.5	2.02± 0.01	13.92 ± 0.07
1.0	1.42 ± 0.07	13.46 ± 0.16
10.0	3.18 ± 0.04	13.88 ± 0.13
50.0	3.42 ± 0.02	14.00 ± 0.16

n= 3

Values are expressed as mean ± SEM

There was no significant difference ($p<0.05$) between the test groups and the control.