



HEPATO-AND NEPHRO-PROTECTIVE EFFECTS OF METHANOL EXTRACT OF *CITRULLUS LANATUS* RIND IN WISTAR RATS FED WITH USED MOTOR ENGINE OIL CONTAMINATED FEED.

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ABSTRACT

The present study investigated the hepato- and nephroprotective effects of methanol extract of *Citrullus lanatus* rind in Wistar rats fed with used motor engine oil contaminated feed (UMEO). Twenty-five (25) adult male Wistar rats were randomly assigned to five groups of five rats each. One group served as normal control, the remaining groups were fed 4 ml UMEO/kg feed, 100 mg/kg body weight (bwt) of vitamin C, 250 mg/kg bwt of extract, and 500 mg/kg bwt of extract respectively. After 28 days of treatment, the rats were euthanized, and blood and kidney samples collected for biochemical analysis. Results showed that the oral LD₅₀ of methanol extract of *C. lanatus* rind was greater than 5000 mg/kg bwt. Treatment with methanol extract of *C. lanatus* significantly ($p < 0.05$) reduced the activities of alanine aminotransferase (ALT), aspartate aminotransferase (AST), alkaline phosphatase (ALP), and concentration of total bilirubin. There were no significant differences in the concentrations of total protein (TP) and creatinine among the groups ($p > 0.05$). The concentration of urea was significantly ($p < 0.05$) increased in groups treated with the extract and vitamin C compared to the untreated animals. The activities of superoxide dismutase (SOD) and catalase were significantly reduced in negative control group compared to the control and treatment groups. Treatment with the extract significantly reduced UMEO-induced increase in malondialdehyde (MDA) concentration. The results of this study indicate that methanol extract of *Citrullus lanatus* rind may protect liver and kidney against injuries imposed by foreign compounds.

KEYWORDS: Antioxidants, *Citrullus lanatus*, Kidney, Liver, Oxidative Stress.

INTRODUCTION

Used motor engine oil (UMEO), also known as used mineral-base crankcase oil, is a brown-to-black liquid produced when motor engine oil is subjected to high temperature and high mechanical strain (ATSDR, 1997). It is a mixture of several chemicals such as low and high molecular weight aliphatic hydrocarbons (C₁₅-C₂₀), aromatic hydrocarbons, polychlorinated biphenyls, chlorodibenzofurans, lubrication additives, decomposition products, and heavy metal contaminants (aluminum, chromium, tin, lead, manganese, silicon, and nickel) (ATSDR, 1997; Wang *et al.*, 2000). Used motor engine oil (UMEO) is a common environmental pollutant, and contains toxic and carcinogenic substances that can be harmful to the ecosystem (Dominguez-Rosado and Pichtel, 2004). It is usually released to the environment during disposal into gutters, drains, empty plots and farmland; a common practice by motor and generator mechanics (Odjegba and Sadiq, 2002). This practice leads to soil and water pollution via spillage and surface run-off. This affects the growth of plants and aquatic organisms, as well as humans who consume food from the environment. The poor

disposal of UMEO by mechanics in many parts of Nigeria has led to intensified efforts by environmentalists in creating awareness on its possible harmful effects on humans and the environment.

The use of medicinal plants in the management of diseases is as old as man (Akuodor *et al.*, 2010; Grabley *et al.*, 2010). These plants which abound in our environment enjoy wide acceptability by locals and serve as cheap alternative to orthodox medicine (Sofowora, 1993; Akah and Nwabie, 1994). One of such plants is watermelon. Watermelon (*Citrullus lanatus*, family *Cucurbitaceae*) fruit has a deep green or yellow-colored smooth thick exterior rind, with gray or light green vertical stripes. The endosperm (flesh) of the fruit may be pink, red or yellow with small black seeds embedded in the middle third of the flesh. Generally, watermelon flesh is the main consumable portion; however, the outer rind is also consumed in some parts of the world (Touhami *et al.*, 2007). Watermelon flesh (endosperm) has been reported to contain antioxidant molecules, such as carotenoids (lycopene and β -carotene), amino acids like citrulline (Rimando and Perkins, 2005),

minerals such as potassium (Perkins and Collins, 2006), and antioxidant enzymes (Bueno and Gimeenez, 1995). The rind has been shown to contain alkaloids, saponins, cardiac glycosides, flavonoids, phenols, lipid, protein, fiber and carbohydrates (Ercal *et al.*, 2001). This study investigated the hepato- and nephroprotective effects of methanol extract of *Citrullus lanatus* rind in Wistar rats given feeds with used motor engine oil contaminated feed.

MATERIALS AND METHODS

Plant Material and Authentication

Fresh fruits of *Citrullus lanatus* (water melon) were obtained from a local market in Umuahia South Local Government Area of Abia State. It was identified and authenticated by Dr. Garuba Omosun of the Department of Plant Science and Biotechnology, Michael Okpara University of Agriculture, Umudike, Abia State, Nigeria with a voucher number, MOUAU/COLNAS/PSB/18/045.

Plant Extraction

The fruits were thoroughly washed to remove sand debris and peeled. Methanol extraction of the rind was by maceration over a 48h period. The filtrate was concentrated using a rotary evaporator and freeze-dried via lyophilization (Sukhdev *et al.*, 2008).

Determination of Oral Lethal Dose (LD₅₀)

Acute toxicity study was carried out on the methanol extract according to the method described by Lorke's method (1983).

Experimental Rats

Adult male Wistar albino rats weighing 150 to 180 g were used for this study. The rats were obtained from the animal house of the Department of Veterinary Medicine, Michael Okpara University of Agriculture, Umudike, and housed in metal cages in a well-ventilated room. They were allowed one week for acclimatization before commencement of study and had free access to feed and clean drinking water. The rats were maintained according to the recommendations of the guide for care and use of laboratory animals (NIH, 2002) in which proper consideration was given to their care and use while minimizing discomfort, distress or pain to them.

Experimental Design

A total of twenty-five rats were used for this study. They were allowed to acclimatize for one week, during which time they were maintained on grower's mash and water *ad libitum*. At the end of this period, the rats were divided into five groups of five rats each. Control received grower's mash and water only. Negative control received feed contaminated with 4ml of UMEO per kg of feed, while the treated groups received feed contaminated with 4ml of UMEO per kg of feed and 100 mg/kg bwt of Vitamin C, 250 mg/kg and 500mg/kg bwt of methanol extract of *C. lanatus* rind respectively. The methanol extract of *C. lanatus* rind was administered to the rats once daily using a gavage. The study lasted 28 days.

Biochemical Analyses

Blood Sample Collection and Preparation

Blood samples were collected from anesthetized rats through cardiac puncture into heparinized sample bottles, and centrifuged at 2000 rpm for 10 min to obtain plasma which was used for biochemical analysis. The kidneys were excised and used to prepare homogenate used for antioxidant assays.

Liver and Kidney Function Tests

The activities of AST, ALT, and ALP, concentrations of total bilirubin and total protein (TP) were determined using their respective Randox kits. Creatinine and urea concentrations were also determined using Randox kits.

Antioxidant Assays

Estimation of Catalase Activity

Catalase activity in kidney homogenate was determined using the modified method described by Atawodi (2011).

Estimation of SOD Activity

Superoxide dismutase (SOD) activity in kidney homogenate was determined using the method described by Sun *et al.* (1988).

Assessment of Lipid Peroxidation

The concentration of MDA in kidney homogenate was determined using the method described by Draper and Hadley (1960).

Statistical analysis

Data are presented as mean \pm SEM. Statistical analysis was performed using SPSS (22.0). Means were compared using Duncan's multiple test range. Values of ($p < 0.05$) were considered statistically significant.

RESULTS**Oral LD₅₀ of Methanol Extract of *C. Lanatus* Rind**

The oral LD₅₀ of methanol extract of *C. lanatus* rind was greater than 5000 mg/kg bwt (Table 1).

Table 1: Outcome of Acute Toxicity Study of Methanol Extract of *C. lanatus* Rind

Dose (mg/kg bwt)	No. of rats	No. of deaths	Survival	Mortality ratio
10	3	0	3	0/3
100	3	0	3	0/3
1000	3	0	3	0/3
1600	1	0	1	0/1
2900	1	0	1	0/1
5000	1	0	1	0/1

Data are number of death and survival of rats.

Indices of Liver Function

Treatment with methanol extract of *C. lanatus* rind significantly reduced ($p < 0.05$) the activities of ALT, AST and ALP and concentration of total bilirubin compared with the untreated group. There were no significant differences in the concentrations of TP among the groups ($p > 0.05$). These results are shown in Table 2.

Table 2: Activities and Concentrations of Liver Function Markers

Group	ALT (IU/L)	AST (IU/L)	ALP (IU/L)	BIL (mg/dL)	TP (mg/dL)
A (normal control)	19.18±0.29 ^c	18.76±1.26 ^c	51.57±0.93 ^b	0.21±0.02 ^c	5.07±0.06
B (negative control)	31.45±1.33 ^a	32.57±1.68 ^a	61.22±2.05 ^a	0.85±0.04 ^a	4.79±0.13
C(100 mg/kg bwt vitamin C)	23.51±1.89 ^b	24.85±1.75 ^b	53.43±1.70 ^b	0.55±0.02 ^b	5.29±0.27
D (250 mg/kg bwt of extract)	24.57±0.40 ^b	25.35±1.27 ^b	52.32±1.42 ^b	0.51±0.04 ^b	5.46±0.33
E (500 mg/kg bwt of extract)	25.38±0.50 ^b	26.78±1.20 ^b	53.08±1.62 ^b	0.51±0.02 ^b	5.11±0.08

Data are mean ± SEM (n =5). Within each column superscripts with different letters are significantly different at $p < 0.05$.

Indices of Kidney Function

The concentration of urea was significantly lower in the negative control group compared to the normal control and treated groups. There were no significant differences in the concentrations of creatinine among the groups ($p > 0.05$).

Table 3: Concentrations of Creatinine and Urea in the Rats

Group	Creatinine (mg/dL)	Urea (mg/dL)
A(normal control)	0.87±0.05 ^b	31.95±1.39 ^b
B(negative control)	1.10±0.05 ^b	27.03±0.89 ^a
C(vitamin C control)	0.91±0.08 ^b	36.58±4.22 ^b
D(250 mg/kg bwt extract)	0.91±0.05 ^b	31.09±1.70 ^b
E(500 mg/kg bwt extract)	0.92±0.07 ^b	30.61±1.82 ^b

Data are mean ± SEM (n =5). Within each column superscripts with different letters are significantly different at $p < 0.05$.

Oxidative Status in the Rats

The activities of SOD and catalase were significantly reduced in the negative control group compared to other groups, but were significantly increased ($p < 0.05$) after treatment with methanol extract of *C. lanatus* rind. Treatment with the extract also significantly reduced ($p < 0.05$) UMEO-induced increase in MDA concentration (Table 4).

Table 4: Activities of Antioxidant Enzymes and Concentration of MDA

Group	SOD (unit/mg protein)	Catalase (unit/mg protein)	MDA(μ mole/mg protein)
A(normal control)	12.76 \pm 0.61 ^b	14.35 \pm 0.35 ^b	1.96 \pm 0.05 ^b
B(negative control)	11.43 \pm 0.61 ^a	10.96 \pm 0.17 ^a	3.59 \pm 0.47 ^a
C(100 mg/kg bwt vitamin C)	12.39 \pm 0.46 ^b	13.05 \pm 0.04 ^b	2.24 \pm 0.35 ^b
D(250 mg/kg bwt extract)	12.93 \pm 0.71 ^b	14.25 \pm 0.36 ^b	1.99 \pm 0.03 ^b
E(500 mg/kg bwt extract)	12.30 \pm 0.31 ^b	14.15 \pm 0.42 ^b	2.09 \pm 0.09 ^b

Data are mean \pm SEM (n =5). Within each column superscripts with different letters are significantly different at $p < 0.05$.

DISCUSSION

Humans and animals are increasingly exposed to UMEO due to poor disposal methods by automobile mechanics. The UMEO ends up in sewage, drains, streams and rivers resulting in contamination of underground water. This may present health problems in human population in the long-run. The target organs are mostly the liver and kidney resulting in liver and kidney diseases (Hodgson, 2004). Plants are not left out as they also depend on the soil to grow, thus, UMEO finds its way into the food chain.

Antioxidant status in this study, showed an increase in lipid peroxidation in the negative control and decrease in SOD activity in the negative control compared to other groups. This indicates that the engine oil present in the feed promoted the generation of reactive species that can damage biomolecules and cell membranes. It has been reported that high lipid peroxidation products in plasma, brain and liver homogenates may be due to damage to cell membranes induced by free-radical attack (Arulselvan and Subramanian, 2007). The reduction in lipid peroxidation biomarker in the plant treated group, can be labeled to the rich phytochemicals that may be present in the extract. Plants have been reported to be a good source of polyphenols which are antioxidant molecules. Superoxide dismutase (SOD) constitutes the first line of defense against reactive oxygen species (ROS) and its activity increases under conditions of oxidative stress. Catalase catalyzes the breakdown of hydrogen peroxide (H_2O_2), a potent precursor for generation of free radicals, thereby protecting biological systems from oxidative damage (Kaushik and Aryadeep, 2014). Its activity also increases under conditions of oxidative stress.

When the liver is damaged, the activities of liver enzymes in the serum are usually increased due to increased membrane permeability and necrosis. These enzymes leak from the damaged cells and find their way into the blood. The increase in liver enzymes present in serum in the negative control is an indication that the liver membrane is damaged. Studies by

Ndukaku *et al.*, 2015 reported an increase in liver enzymes in serum once liver is compromised. The damage done on the liver can be linked to the heavy metals and poly aromatic hydrocarbons present in the used motor oil. However, the plant material effectively ameliorative the harmful effect of the engine oil as shown by the liver enzyme concentration of the treated groups.

Creatinine is synthesized in the liver and it is a breakdown product of creatine phosphate in muscle. It serves as biomarker for kidney injury. Creatinine is removed mainly through the kidney, and its concentration in biological system is altered by various muscle toxicants or decreased muscular activity (Ndukaku *et al.*, 2015). An increase in creatinine concentration is indicative of nephrotoxicity. This leads to an increase in protein loss in the urine (proteinuria). The results of this study suggest that methanol extract of *C. lanatus* rind may improve kidney function altered by UMEO.

CONCLUSION

The results of this study indicate that methanol extract of *Citrullus lanatus* rind protects liver and kidney against injury induced by UMEO.

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