Effect of methanolic extract of *Citrullus lanatus* Seed on lipid profile and oxidative stress in Acetaminophen intoxicated Rats

Damilola Alex Omoboyowa\(^1\) and Adekunle Adebo Ajayi\(^1\)

\(^1\)Biochemistry Research Unit, Department of Science Laboratory Technology, Akanu Ibiam Federal Polytechnic, Ebonyi State, Nigeria

*Corresponding Author: Damilola Alex Omoboyowa
Biochemistry Research Unit, Department of Science Laboratory Technology, Akanu Ibiam Federal Polytechnic, Ebonyi State, Nigeria

E-mail address: damlexb@yahoo.com; Ph: +2347032665874

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**Abstract**

The effect of methanolic extract of *Citrullus lanatus* seed on lipid profile and anti-oxidants in acetaminophen-induced hepatotoxic rats was investigated in the present study. A total of twenty (20) male Wistar rats were divided into five groups (n = 4). Group I served as normal control and were not exposed to paracetamol. Group II was exposed to 750 mg/kg body weight of paracetamol (intoxicated group). Group III was administered with 0.5 ml distilled water, 5 mg/kg b. w of silymarin and group IV and V were administered with 200 mg/kg and 400 mg/kg body weight of *C. lanatus* methanolic seed extract respectively. We found that *C. lanatus* extract is safe at a dosage of up to 5000 mg/kg body weight. The biochemical analysis of current study showed significant (\(p<0.05\)) decrease in total cholesterol of the rats exposed to Paracetamol and treated with the plant extract compared with the rats exposed to Paracetamol only. The rats exposed to 750 mg/kg b. w. of Paracetamol and treated with varying doses of the extract showed non-significant (\(p>0.05\)) increase in VLDL, HDL and TAG concentration compared with the rats exposed to Paracetamol and 0.5 ml of distilled water (Vehicle). Moreover the animals exposed to 750 mg/kg b. w. of paracetamol and treated with 200 and 400 mg/kg b. w. of the extract showed significant (\(p<0.05\)) increase in the antioxidant parameters compared to the animals exposed to 750 mg/kg b. w. of paracetamol and 0.5 ml of distilled water. The increase in the lipid profile of the treated animals reveal the ameliorating effect of dyslipidemia as observed in paracetamol intoxicated animals. Taken together methanolic seed extract of *C. lanatus* can substantiate its use as a therapeutic agent against lipid imbalance and free radical generation in traditional system of medicine.

**Keywords:** *Citrullus lanatus* Seed, Hepatotoxic, dyslipidemia, Phytochemicals, Toxicity

**Introduction**

The liver is the largest glandular organ in the body (approximately 1,500 g) among the normal adult and is located in the right upper quadrant of the abdomen. It is glossy in appearance and dark red in color due to the rich supply of blood flowing through it. Approximately 25% of the cardiac output flows to the liver. The liver has the primary metabolic function of regulating the blood concentration of most metabolites, particularly glucose and amino acids, the characteristic structure and organization of the liver enable it to perform vital roles in regulating, synthesizing, storing, secreting, transforming and breaking down different substances in the body. In addition, the liver has an ability to regulate lost tissue and helps in maintaining these functions, even in the face of moderate damage [1].

Oxidative stress has been defined as a disturbance in the balance between antioxidants and pro-oxidants (free radicals and other reactive species), with increased levels of pro-oxidants leading to potential damage [2]. This imbalance can be an effect of depletion of endogenous...
antioxidants, low dietary intake of antioxidants and/or increased formation of free radicals and other reactive species.

The hepatotoxicity of paracetamol is mediated by hepatic cytochrome P450 to a highly reactive metabolic N-acetyl-P-benzoquinone imine (NAPQI) [3]. NAPQI is initially detoxified by conjugation with reduced glutathione (GSH) to form mercapturic acid [4]. When the rate of NAPQI formation exceeds the rate of detoxification by GSH, it oxidizes tissue macromolecules such as lipid or sulphur – SH group of protein and alters the homeostasis of calcium after depleting GSH [4].

Fruits have high vitamins, minerals, fibers, phytochemicals and antioxidant in their pulps, seeds and rind but they have not been given much importance in the diet of many Nigerians especially the seeds and rind which most of the times are discarded, due to ignorance of the nutritive value and curative advantages, lack of proper storage facilities, poor distributions, rising cost of fruits, poor accessibility and affordability [5]. Citrullus lanatus contains significant amount of citrulline. Its single brown seed is high in certain fats and oils (oleic and arachinonic acid) valuable to industry, used in cooking and manufacturing of soap. Citrulline, can be metabolized to arginine. This amino acid is a substrate for the synthesis of nitric oxide and it plays a role in cardiovascular and immune function [6]. The present study is an attempt at investigating the antioxidant properties and lipid profile of methanolic extract of Citrullus lanatus seed in experimentally acetaminophen intoxicated hepatotoxic rats.

Materials and Methods

Chemicals and Reagents

The chemicals used for this study were of analytical grade in addition to the Randox, UK commercial assay kits which were used for the determination of Cholesterol, HDL, LDL, VLDL and TAG.

Plant material

The water melon (Citrullus Lanatus) used for this study was purchased from Eke market, Afikpo North Local Government Area, Ebonyi State, Nigeria. It was authenticated at Botany unit, Department of Science Laboratory Technology, Akanu Ibiam Federal Polytechnic, Unwana, Ebonyi State, where a voucher specimen with voucher number V/No. 1999011 has been deposited. The samples were bought when available in their fresh State and in sufficient quantity for the analysis. Citrullus lanatus seeds were removed from the pulp with the aid of a sterilized knife, the seeds were cleaned, washed, air dried and carefully ground into a coarse form by the use of a mechanical blender.

Extraction Procedure

The ground water melon seed (568.5g) was macerated in 1400 ml of methanol for 48 hours. The macerate was passed through whatman No 4 filter paper. The filtrate was concentrated in a rotatory evaporator and dried in a boiling water bath. The extract yield for the methanolic extract was calculated as:

\[
\text{Extract yield} = \frac{\text{Mass of crude plant} - \text{Mass of crude extract}}{\text{Mass of crude plant}} \times 100
\]

Experimental Design

Twenty (20) adult white male albino rats weighing 180 to 200g were used for this study. They were fed ad libitum with 18% crude protein (Guinea feed) commercial feed and were allowed to acclimatize for two weeks under standard photo periodic condition in a clean rat cage with four rats per cage in the Biochemistry animal house of Akanu Ibiam Federal Polytechnic, Unwana, Ebonyi State. All animal were maintained under the standard laboratory condition for temperature (26 ± 2°C) and light (12 hours day length) and were allowed free access to food and water ad libitum. The rats were divided into five different groups with four animals per group (n=4):

- **Group 1:** Normal/Negative control (un-induced)
- **Group 2:** Positive control (Acetaminophen challenged + Vehicle)
- **Group 3:** Acetaminophen challenged + 5 mg/kg b. w. of silymarin
- **Group 4:** Acetaminophen challenged + 200 mg/kg b. w. of methanolic seed extract of Citrullus lanatus.
- **Group 5:** Acetaminophen challenged + 400 mg/kg b. w. of methanolic seed extract of Citrullus lanatus.

Induction of Paracetamol Hepatotoxicity in Rats

The minimum dose of paracetamol that causes death in rats is 1060 mg/kg and the median lethal dose (LD₅₀) is 675 mg/kg [7]. Paracetamol hepatotoxicity was induced by single administration of solution of paracetamol at 750 mg/kg intraperitoneally. After 4 days only rats with ALT levels above 18µg/l were considered hepatotoxic and used for the study. The normal ALT standard reference range for experimental studies is 2 – 18 µg/l [8].

Acute toxicity and lethality (LD₅₀) test

The acute toxicity and lethality of methanolic extract of the C. lanatus was determined using the modified method of Lorke, (1983) [9]. The test was divided into two stages. In first stage, nine (9) randomly selected adult mice were divided into three groups (n=3) and received 10, 100 and
1000 mg/kg body weight of the methanolic extract and the signs of toxicity and number of death for a period of 24 hours were recorded. After 24 hours observation, the doses for the second phase were determined based on the outcome of the first phase. Since there was no death, a fresh batch of animals were used following the same procedure in phase I but with higher dose ranges of 1900, 2600 and 5000 mg/kg body weight of the extract. The animals were also observed for 24-hours for signs of toxicity and possible number of death. The LD_{50} was calculated as the geometric mean of the high non-lethal dose and lowest lethal dose [9].

**Phytochemical Test**
Basic quantitative phytochemical screening of the methanolic extract of the *C. lanatus* seeds was carried out by testing for the concentration of the following plant constituents: flavonoids, tannins, saponins, steroids, alkaloids, reducing sugar, cyanogenic glycosides and soluble carbohydrate. The phytochemical analysis of the sample was carried out using the procedures outlined by Harborne, (1984) [10] and Pearson, (1976) [11].

**Biochemical Assay**
The concentration of glutathione was determined by the method of Habig *et al.* (1974) [12], catalase activity was assayed spectrophotometrically according to the method described by Aebi, (1983) [13]. The concentration of malondialdehyde (MDA) was determined by the method of Wallin *et al.* (1993) [14], superoxide dismutase activity was assayed by the method of Artur and Boyne, (1985). The plasma vitamin C concentration was determined using the method of Tietz, (1983) [15]. The concentration of total cholesterol was determined by the use of Randox assay kit as prescribed by Trinder, (1969) [16]. Determination of high density lipoprotein (HDL) concentration was carried out by the method described by Assmann, (1979) [17] in Randox assay kit. Low density lipoprotein (LDL) was determined by the method described by Assaman, (1984) [18]. Determination of triacylglycerol concentration was carried out based on the methods of Fossati and Prencipe, (1982) [19] as described in Randox assay kit.

**Statistical Analysis**
The data obtained were analyzed using One Way Analysis of Variance. The data was further subjected to LSD post hoc test for multiple comparisons and differences between the mean is regarded significant at $p<0.05$. The results were expressed as Mean ± SEM.

**Results**

**Yield of the methanolic extract of *Citrus lanatus* seed**
The extract yield for the extract was 25.7 g (5.5%).

**Acute toxicity and lethality (LD50) test**
Intraperitoneal administration of up to 5000 mg/kg b.w of methanolic extract of *Citrus lanatus* seeds caused no death in the two stages of the test. Thus, the intraperitoneal LD_{50} of methanolic extract in mice was estimated to be greater than or equal to 5000 mg/kg body weight.

**Results of Phytochemical Screening**
The results of quantitative phytochemical analysis of *Citrus lanatus* seeds (table 1) indicated the presence of the phytochemical constituents of the extract in the order: Alkaloids > Steroids > Terpenoids > Flavonoids > Phenol > Saponins > Tannins > Cyanogenic glycosides.

**Table 1:** Result of the Quantitative phytochemical composition of methanolic extract of *Citrus lanatus* seeds.

<table>
<thead>
<tr>
<th>Phytochemical Compounds</th>
<th>Quantity (mg/100g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Saponins</td>
<td>1.333 ± 0.0071</td>
</tr>
<tr>
<td>Tannins</td>
<td>0.536 ± 0.057</td>
</tr>
<tr>
<td>Alkaloids</td>
<td>33.795 ± 0.0071</td>
</tr>
<tr>
<td>Steroids</td>
<td>2.458 ± 0.0099</td>
</tr>
<tr>
<td>Terpenoids</td>
<td>2.415 ± 0.0071</td>
</tr>
<tr>
<td>Flavonoids</td>
<td>2.310 ± 0.014</td>
</tr>
<tr>
<td>Phenol</td>
<td>1.371 ± 0.042</td>
</tr>
<tr>
<td>Cyanogenic glycosides</td>
<td>0.00325 ± 0.00035</td>
</tr>
</tbody>
</table>

Data represented as Mean ± SEM

**Effect of methanolic extract of *Citrus lanatus* Seeds on oxidative stress parameters of acetaminophen intoxicated rats**
As shown in table 2, there was significant ($p<0.05$) increase in the MDA concentration of rats exposed to acetaminophen and 0.5 ml of distilled water compared with the normal control rats while non-significant ($p>0.05$) increase was observed in the MDA level of rats exposed to acetaminophen and treated with varying doses of the extract compared with the normal control rats. The rats in the tested groups showed significant ($p<0.05$) decrease in the level of GSH, vitamin C and catalase activity compared with the normal control rats, while the superoxide dismutase activity in the acetaminophen exposed rats administered 0.5 ml of distilled water was
significantly (p<0.05) reduced compared with the normal control rats. The acetaminophen exposed rats treated with 200 and 400 mg/kg b. w of *C. lanatus* seed extract showed non-significant (p>0.05) reduction in the plasma MDA concentration compared with the rats exposed to acetaminophen and 0.5 ml of distilled water. The rats exposed to acetaminophen and treated with 200 and 400 mg/kg b. w of the seed extract showed significant (p<0.05) increase in the level of GSH, vitamin C and activity of catalase and superoxide dismutase (Table 2)

**Effect of methanolic extract of *Citulus lanatus* seeds on lipid profile of acetaminophen intoxicated rats**

Table 3 showed significant (p<0.05) increase in the levels of total cholesterol among tested groups compared with the normal control rats. There was non-significant (p>0.05) increase in the HDL and LDL levels of rats in the tested groups compared with the normal control rats. The acetaminophen exposed and treated with varying doses of the extract showed significant (p<0.05) increase in the plasma total cholesterol and HDL level compared with the acetaminophen exposed rats while as, the rats exposed to acetaminophen and treated with 200 and 400 mg/kg b. w of the extract showed non-significant (p>0.05) increase in the plasma VLDL and TAG level compared with acetaminophen exposed rats (table 3).

### Table 2: Effect of methanol Extract of *Citulus lanatus* Seed on Oxidative Stress Parameters of acetaminophen intoxicated rats

<table>
<thead>
<tr>
<th>TREATMENT</th>
<th>MDA</th>
<th>GSH</th>
<th>Vit. C</th>
<th>CAT</th>
<th>SOD</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal Control</td>
<td>2.64 ± 0.38</td>
<td>5.61 ± 0.86</td>
<td>2.72 ± 0.07</td>
<td>5.68 ± 0.30</td>
<td>0.99 ± 0.02</td>
</tr>
<tr>
<td>Acetaminophen + 0.5 ml of distilled water</td>
<td>3.22 ± 0.05</td>
<td>4.25 ± 0.18</td>
<td>1.93 ± 0.03</td>
<td>3.03 ± 0.25</td>
<td>0.87 ± 0.04</td>
</tr>
<tr>
<td>Acetaminophen + 5 mg/kg b. w. of silymarin</td>
<td>2.65 ± 0.34</td>
<td>5.02 ± 0.07</td>
<td>2.05 ± 0.05</td>
<td>4.93 ± 0.20</td>
<td>0.92 ± 0.03</td>
</tr>
<tr>
<td>Acetaminophen +200 mg/kg b. w. of extract</td>
<td>2.99 ± 0.09</td>
<td>4.25 ± 0.02</td>
<td>2.28 ± 0.14</td>
<td>3.29 ± 0.04</td>
<td>0.98 ± 0.03</td>
</tr>
<tr>
<td>Acetaminophen +400 mg/kg b. w. of extract</td>
<td>2.84 ± 0.14</td>
<td>4.96 ± 0.92</td>
<td>2.02 ± 0.01</td>
<td>3.48 ± 0.15</td>
<td>0.96 ± 0.05</td>
</tr>
</tbody>
</table>

n = 4; *P<0.05 compared with the control (one way ANOVA; LSD post hoc test); values represented in Mean ± SEM

### Table 3: Effect of methanol Extract of *Citulus lanatus* Seed on Lipid Profile of acetaminophen intoxicated rats

<table>
<thead>
<tr>
<th>TREATMENT</th>
<th>T. Chol</th>
<th>HDL</th>
<th>LDL</th>
<th>VLDL</th>
<th>TAG</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal Control</td>
<td>1.63 ± 0.10</td>
<td>0.73 ± 0.10</td>
<td>0.43 ± 010</td>
<td>0.48 ± 0.14</td>
<td>1.00 ± 0.30</td>
</tr>
<tr>
<td>Acetaminophen + 0.5 ml of distilled water</td>
<td>1.68 ± 0.09</td>
<td>0.83 ± 0.16</td>
<td>0.63 ± 0.14</td>
<td>0.23 ± 0.03</td>
<td>0.48 ± 0.05</td>
</tr>
<tr>
<td>Acetaminophen + 5 mg/kg b. w. of silymarin</td>
<td>1.62 ± 0.07</td>
<td>0.79 ± 0.12</td>
<td>0.51 ± 0.12</td>
<td>0.34 ± 0.04</td>
<td>0.65 ± 0.09</td>
</tr>
<tr>
<td>Acetaminophen +200 mg/kg b. w. of extract</td>
<td>2.03 ± 0.09</td>
<td>1.08 ± 0.22</td>
<td>0.53 ± 0.17</td>
<td>0.43 ± 0.03</td>
<td>0.90 ± 0.07</td>
</tr>
<tr>
<td>Acetaminophen +400 mg/kg b. w. of extract</td>
<td>2.00 ± 0.12</td>
<td>0.98 ± 0.22</td>
<td>0.65 ± 0.16</td>
<td>0.38 ± 0.02</td>
<td>0.78 ± 0.45</td>
</tr>
</tbody>
</table>

n = 4; *P<0.05 compared with the control (one way ANOVA; LSD post hoc test); values represented in Mean ± SEM
Discussion

This study demonstrated the effects of methanolic seed extract of *Citrulus lanatus* on some oxidative stress and lipid profile parameters in normal and acetaminophen intoxicated rats. The percentage yield of the methanolic seed extract of *C. lanatus* was found to be 25.7 g (5.5 %). Quantitative phytochemical analysis of the extract as shown in table 1 revealed high concentration of alkaloids and low concentration of steroids, terpenoids, flavonoids, phenol, saponins and tannins while trace levels of cyanogenic glycosides were also observed.

Oral administration of methanolic extract of *Citrulus lanatus* at doses of 200 mg/kg and 400 mg/kg body weight to acetaminophen exposed rats showed significant ($P<0.05$) decrease in MDA concentration compared with the acetaminophen exposed rats administered with 0.5 ml of distilled water as vehicle as shown in table 2. An increase in the level of lipid peroxidation in hepatotoxic rats suggests there is an increased generation of free radicals.

There was non-significant ($p>0.05$) increase in vitamin C concentration in all the acetaminophen exposed hepatotoxic rats treated with the varying doses of the extract compared with hepatotoxic rats. Vitamin C is always the first antioxidant nutrient that counters oxidation at cytoplasmic level and is thus depleted [20]. This action probably spared the availability of vitamin C in the system.

The activities of superoxide dismutase (SOD) showed significant ($p<0.05$) increase in acetaminophen exposed rats administered with varying doses of the extracts compared with the acetaminophen exposed rats. The catalase (CAT) activity also showed significant ($p<0.05$) increase in hepatotoxic rats treated with varying dose of the extract compared with the hepatotoxic rats. Cellular radical scavenging systems include the enzymes such as SOD which scavenges the superoxide anions by catalyzing its dismutation and catalase (CAT) a haem enzyme which removes hydrogen peroxide [21]. Therefore, reduction in the activity of these enzymes (SOD and CAT) results in a number of deleterious effects due to the accumulation of superoxide anion and hydrogen peroxide.

Glutathione (GSH) concentration showed significant ($p>0.05$) increase in all the acetaminophen intoxicated rats administered with varying doses of the extract compared with hepatotoxic rats. Decreased GSH concentrations in hepatotoxic rats was considered to be an indicator of increased oxidative stress. Increase in glutathione concentration may protect the tissues against organ associated injury by reducing the susceptibility to toxic radicals [22].

High density lipoprotein (HDL) acts as a powerful endogenous defense mechanism against atherogenesis. Apolipoprotein A-1 is a central component of HDL that led to the formation of HDL *in vivo*. It has been observed that apolipoprotein A-1 transgenic expression resulted in the reduction of lesion formation in apolipoprotein-E knockout mice [23]. Lahiji and Navab, (2003) [23] observed “seedling molecules” the products of oxidation of linoleic acid and arachidonic acid, essential for the oxidation of LDL by human artery wall cells could be rapidly removed from LDL and from artery wall cells by human apolipoprotein A-1 and synthetic apolipoprotein A-1 mimetic peptides. However, LDL has been described as an anti-inflammatory in the basal state and pro-inflammatory during an acute phase response, suggesting that HDL and LDL derived oxidized phospholipids are involved in non-specific innate immunity [24].

Conclusion

The result from present study showed that *Citrulus lanatus* seeds contains some nutrients possibly antioxidants that are capable of suppressing oxidative stress. The study suggests that the plant extract may be a good antioxidant as its supplementation may decrease lipid peroxidation and increase plasma vitamin C concentration. However, further studies are to be carried out in-order to determine the mechanism of action.

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None declared

Conflict of Interest

Authors declare that there is no conflict of interest to reveal
References


