Atherosgen and Haematologic Indices of Paracetamol-Overdosed Albino Rats Treated With Aqueous Leaf Extracts of *Euphorbia Heterophylla* and *Jatropha Curcas*

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**Abstract**

**Objective:** The protective effect of aqueous leaf extracts of *Euphorbia heterophylla* and *Jatropha curcas* against paracetamol-induced acute changes in lipid, atherosgenic and haematologic parameters of albino rats were studied.

**Methodology:** Twenty-five adult male albino rats weighing 180 to 200 g were randomly assigned into 5 experimental groups (I-V) of five animals each. Group I animals were administered 10 ml of distilled water, while group II rats were given 1000 mg/kg paracetamol. Groups III-V were pretreated with vitamin C (500 mg/kg), *E. heterophylla* (200 mg/kg) and *J. curcas* (1000 mg/kg) respectively, 1 h before administration of 1000 mg/kg paracetamol. The animals were orally administered the extracts/drugs daily for 14 days.

**Result:** Paracetamol administration reduced significantly (p<0.05) the total cholesterol, triglyceride, high density lipoprotein cholesterol (HDL-c), low density lipoprotein cholesterol (LDL-c) and total non HDL-c concentrations as well as white blood cell (WBC) count of the animals when compared with the control. Pre-treatment of the animals with vitamin C non-significantly (p>0.05) countered the observed effects of paracetamol overdose more than the extracts of *E. heterophylla* and *J. curcas*. Acute paracetamol overdose did not significantly (p>0.05) affect most of the atherosgen risk predictor indices and haematologic parameters studied.

**Conclusion:** The results indicate that atherosgenic and haematologic indices were less responsive than lipid parameters to paracetamol-induced toxicity. Furthermore, aqueous leaf extracts of *E. heterophylla* and *J. curcas* had less protective effect than vitamin C against serum lipidemic changes induced by paracetamol.

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**Introduction**

Many medicinal plant species contain both important nutrients and phytochemicals that could be pharmacologically essential [1]. The ethnopharmacological importance and application of plants in the treatment of diseases and ailments in traditional setting have been an age-long practice [2]. Currently, herbal products are preferred by many people because of less testing time, higher safety, efficacy, cultural acceptability and assumed 'lesser' side effects. Furthermore, the phytochemicals present in plant-based products are thought to be compatibility with the human body because they form part of the daily chemicals, humans ingested with food [3]. *Euphorbia heterophylla* and *Jatropha curcas* are among such traditional plants that have been reported to be useful for various purposes.

*E. heterophylla* plants are commonly available in temperate and tropical regions of the world. They occur in nature as herbs, shrubs or trees [1,4]. The plant is commonly called Mexican fire plant, milk weed and Spurge weed [5]. *E. heterophylla* belongs to the family Euphorbiaceae and to the subgenus *Poinsettia*, a group with stipules modified into glands [6]. It has a milky sap that is poisonous. Notwithstanding the poisonous nature of the plant's sap, it has been widely used in the treatment of various tropical diseases such as malaria, asthma, eczema and gonorrhea, as well as wart and respiratory tract infection [5]. In southeastern Nigeria, Igbo traditional doctors use the aqueous leaf extract of *Euphorbia heterophylla* for treatment of bacterial and parasitic infections of the blood and skin, and as a purgative [7]. Many pharmacologically important phytochemicals such as 4-hydroxybenzoic acid, quercetin, beta-amyrin, and stigmastanol were isolated from the leaves. Similarly, essential and non essential amino acids including, but not limited to, alanine, aspartic acid, cysteine, serine, proline, methionine and glutamic acid were also found to be present in the plant's leaf extract [8]. It was also observed that oral administration of aqueous leaf extract of the plant significantly reduced plasma glucose concentration of experimental induced diabetes in rats [9]. Aqueous leaf extract of *E. heterophylla* has also been reported to have good anticoagulant and preservative effect on human whole blood [10].

Like *E. heterophylla*, *J. curcas* L. is a widely grown flowering plant. It is a perennial plant that belongs to the Euphorbiaceae family [11]. The plant grows into a large shrub or small tree of up to 5 m in height. In the tropics, *J. curcas* is commonly grown as a living fence in fields and settlements [12]. All parts of the plant are very poisonous. The seed of *J. curcas* was reported to contain a purgative, phytotoxic oil called curcin, which causes dehydration and cardiovascular collapse as a result of haemorrhagic gastro-enteritis, and central nervous system...
depression [13]. However, it is gaining a lot of economic significance because of its several potentials in industrial application and medicinal values. The leaves of *J. curcas* are traditionally used in different forms in West Africa for the treatment of various ailments like fever, guinea worm sores, joint rheumatism, mouth infections and jaundice [3].

Paracetamol, also known as acetaminophen, is a widely used over-the-counter non-steroidal anti-inflammatory drug (NSAID) for treatment of pains and fever [14]. It is not a very strong analgesic, but can be used in conjunction with opioid analgesics in the management of more severe pains such as post-surgical and cancer pains [15]. It is generally safe when used at recommended doses even when taken for a long time. However, when taken in overdose paracetamol can be very toxic and fatal [16]. Paracetamol toxicity has been ascribed to the formation of N-acetylbenzoquinoneimine (NAPQI), a toxic metabolite by hepatic cytochrome P450 [17]. NAPQI oxidizes the lipids and proteins of tissues, depletes glutathione and alters calcium homeostasis [18]. The oxidation of lipids and damage to liver hepatocytes could lead to derangements in lipid profile. The role of derangement in lipid profile in the progression of cardiovascular diseases (CVD) has been well established, with primary interest in CVD therapy focused on deranged low density lipoprotein cholesterol (LDL-c) levels [19]. High plasma triglyceride and cholesterol levels are both independent and synergistic risk factors for cardiovascular diseases and are often correlated with hypertension, obesity and diabetes mellitus [20]. Significant elevations in plasma low density lipoprotein (LDL) and very low density lipoprotein (VLDL) cholesterol concentrations are known risk factors for cardiovascular disease and are also often seen in hypertension and obesity [21]. Meanwhile, different combinations or ratios of these lipid profile parameters have been reported to be better indices for identification of high risk individuals to CVDs than direct use of the lipid parameters themselves [19]. Atherogenic index of plasma (AIP), atherogenic coefficient (AC) and Castelli’s risk indices (CRIs) are the ratios of such lipid profile parameters that have been studied as markers of lipid atherogenic risk [22]. They are calculated lipid fraction ratios which are suggestively gaining attention in clinical setting for assessing the risk of CVD, over and above the routinely used lipid profile parameters [23]. In this study, we investigated the protective effect of the aqueous leaf extracts of *J. curcas* and *E. heterophylla* against paracetamol-induced changes in atherogenic and haematologic indices of albino rats.

**Materials and Methods**

**Plants collection and extract preparation**

The leaves of *Jatropha curcas* and *Euphorbia heterophylla* were collected from bushes around Ezibobo in Owerri West Local Government Area of Imo State. They were authenticated by a botanist, Mr Francis Nwanze of the Department of Forestry and Wildlife, Federal University of Technology Owerri, Nigeria.

Apparently healthy leaves of the two plants were separately air-dried for 2 weeks and then oven-dried at a temperature of 50°C for 8 h to a constant weight. Using pestle and mortar, the dried leaves were ground into powder form. The powders were separately stored in labeled airtight containers. Five hundred grams (500 g) of each leaf powder was suspended in 100 ml of 0.5% tween 80 distilled water only. The powders were reconstituted and administered as 1000 mg/kg of paracetamol. Group IV animals were pretreated with single oral dose of 200 mg/kg of *Euphorbia heterophylla* leaf extract one hour before administration of 1000 mg/kg of paracetamol. This served as test group 1. Group V animals were pretreated with daily oral dose of 1000 mg/kg of *Jatropha curcas* leaf extract 1 h before administration of 1000 mg/kg of paracetamol. This served as test group 2. The chosen dose ranges for the paracetamol and aqueous plant extracts used were based on result of earlier toxicity studies [1,24,25]. The oral administration of the drugs and plant extracts were for a period of fourteen days.

**Sample collection**

At the end of the 14 days of treatment, the animals were fasted overnight, anaesthetized with diethyl ether and sacrificed. Then, 5 mL of whole blood was collected by cardiac puncture. About 3 mL of each blood sample was gently dispensed into a well labeled 10 ml capacity plain sample bottle, while the rest was dispensed into ethylene diamine tetra acetic acid (EDTA) bottle. The blood samples in the plain bottles were allowed to clot and centrifuged at 2000 rpm for 15 minutes to separate sera from the clot. The sera were carefully separated into fresh solutions. Then, respective doses of 200 mg/kg and 1000 mg/kg for *E. heterophylla* and *J. curcas* were prepared from the stocks. Each concentrate was transferred into labeled glassware and refrigerated at 4°C until needed.

**Drugs used**

The paracetamol and vitamin C tablets used were products of Emzor Pharmaceuticals Ltd, Nigeria. They were sourced from Orchad Pharmacy in Owerri, Nigeria. Each tablet containing 1000 mg of paracetamol was dissolved in 10 ml of distilled water, while each tablet of vitamin C containing 100 mg of ascorbic acid was dissolved in 1 ml of distilled water. They were reconstituted and administered as 1000 mg/kg and 500 mg/kg body weight of the animals respectively.

**Laboratory animals**

Laboratory animals used were made up of 25 adult male albino rats (*Rattus novergicus*) weighing between 180 g and 200 g, obtained from the Department of Verteinary Medicine, University of Nigeria, Nsukka. The animals were kept in stainless steel cages under good laboratory conditions of humidity (60 ± 0.2 %), temperature (30 ± 1˚C) and a 12 h light/dark cycle. They were kept for 14 days to acclimatize to laboratory conditions in the Animal House Unit of Department of Biochemistry, Federal University of Technology Owerri. During the acclimatization period, they were provided with clean water and standard feed (Growers marsh, Vital Feeds Ltd.) *ad libitum*. Ethical approval was obtained for the study protocol from the University ethical committee. Principles of Laboratory Animal Care (NIH Publication, 1985-1993) were fully adopted in all the experimental procedures involving the use and handling of the laboratory animals.

**Grouping of animals**

After acclimatization period, the albino rats were randomly allotted into 5 groups of 5 rats each. Group I served as the negative control and were orally administered daily dose of 10 ml/kg body weight of distilled water only. Group II served as the positive control and were orally administered daily dose of 1000 mg/kg of paracetamol. Group III animals (the standard group) were pretreated with oral dose of 500 mg/kg of vitamin C solution one hour before the oral administration of 1000 mg/kg of paracetamol. Group IV animals were pretreated with single oral dose of 200 mg/kg of *Euphorbia heterophylla* leaf extract one hour before administration of 1000 mg/kg of paracetamol. This served as test group 1. Group V animals were pretreated with daily oral dose of 1000 mg/kg of *Jatropha curcas* leaf extract 1 h before administration of 1000 mg/kg of paracetamol. This served as test group 2. The chosen dose ranges for the paracetamol and aqueous plant extracts used were based on result of earlier toxicity studies [1,24,25]. The oral administration of the drugs and plant extracts were for a period of fourteen days.
labeled sample bottles and were used for lipid profile analysis, while the EDTA containing whole blood was used for haematological parameters.

Lipid profile analyses
Serum triglyceride (TG), total cholesterol (TC) and high density lipoprotein cholesterol (HDL-c) concentrations were determined using enzymatic colorimetric method [26], phosphotungstate precipitation and enzymatic endpoint methods [27] with the aid of commercial reagent kits (Randox Laboratories Ltd, UK). Calculation of the concentration of low density lipoprotein cholesterol (LDL-c) in the serum was as previously described [28].

Calculations of the atherogenic indices
Serum total non-HDL cholesterol (TnHDL-c) concentration was calculated as TC – HDL-c [29], while Castelli’s Risk Index I (CRI-I) and Castelli’s Risk Index II (CRI-II) were determined as TC / HDL-c and LDL-c / HDL-c respectively [22,30]. Atherogenic index of plasma (AIP) and atherogenic coefficient (AC) levels were calculated as log (TG / HDL-c) and (TC - HDL-c) / HDL-c respectively [31,32].

Haematological analyses
Haemoglobin concentration of the anticoagulated blood was determined using Cyan-methaemoglobin method. Heamatocrit (Hct) values and red blood cell (RBC) counts of the animals were determined using Cyan-methaemoglobin method. Heamatocrit (Hct) values and red blood cell (RBC) counts of the animals in comparism to the control animals. Treatment of the paracetamol overdosed animals with vitamin C and extracts of E. heterophylla and J. curcas increased significantly (p<0.05) the TC and total non-HDL cholesterol concentrations of the animals in comparism to the control animals. Treatment of the paracetamol overdosed animals with vitamin C and extracts of E. heterophylla and J. curcas increased non-significantly (p>0.05) the concentrations of the lipids and lipoproteins of the treated groups. Paracetamol overdose did not change significantly (p>0.05) the Castelli’s Risk Index I (TC/HDL-c ratio), Castelli’s Risk Index II (LDL-c/HDL-c ratio), atherogenic coefficient [(TC - HDL-c)/HDL-c ratio] and atherogenic index of plasma (Log [TG/HDL-c]) of the animals when compared with the control, vitamin C, E. heterophylla and J. curcas groups.

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Negative Control</th>
<th>Positive (Paracetamol)</th>
<th>Control (Vitamin C)</th>
<th>Group Test (E.heterophylla)</th>
<th>Group Test (E.curcas)</th>
<th>1</th>
<th>Test Group 2 (J.curcas)</th>
</tr>
</thead>
<tbody>
<tr>
<td>TG (mg/dl)</td>
<td>150.74 ± 6.90</td>
<td>137.64 ± 7.60</td>
<td>145.09 ± 6.30</td>
<td>ab</td>
<td>140.80 ± 4.70</td>
<td>143.37 ± 4.90</td>
<td>ab</td>
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<tr>
<td>TC (mg/dl)</td>
<td>188.51 ± 4.60</td>
<td>182.30 ± 5.00</td>
<td>189.79 ± 6.80</td>
<td>ab</td>
<td>186.80 ± 4.60</td>
<td>187.56 ± 7.30</td>
<td>ab</td>
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<tr>
<td>HDL-c (mg/dl)</td>
<td>40.34 ± 0.26</td>
<td>36.94 ± 0.16</td>
<td>36.72 ± 0.26</td>
<td>ab</td>
<td>37.57 ± 0.16</td>
<td>37.84 ± 0.13</td>
<td>b</td>
</tr>
<tr>
<td>LDL-c (mg/dl)</td>
<td>82.66 ± 0.39</td>
<td>89.80 ± 0.42</td>
<td>85.12 ± 0.70</td>
<td>ab</td>
<td>84.83 ± 0.48</td>
<td>84.55 ± 0.83</td>
<td>c</td>
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<tr>
<td>CRI-I</td>
<td>4.92 ± 0.64</td>
<td>4.94 ± 0.70</td>
<td>4.90 ± 0.61</td>
<td>a</td>
<td>4.92 ± 0.40</td>
<td>4.96 ± 0.41</td>
<td>a</td>
</tr>
<tr>
<td>CRI-II</td>
<td>2.22 ± 0.09</td>
<td>2.24 ± 0.06</td>
<td>2.20 ± 0.02</td>
<td>a</td>
<td>2.23 ± 0.01</td>
<td>2.23 ± 0.03</td>
<td>a</td>
</tr>
<tr>
<td>TnHDL-c(mg/dl)</td>
<td>158.18 ± 4.80</td>
<td>165.36 ± 5.20</td>
<td>151.07 ± 6.60</td>
<td>ab</td>
<td>148.83 ± 4.80</td>
<td>149.72 ± 7.00</td>
<td>ab</td>
</tr>
<tr>
<td>AC</td>
<td>3.92 ± 0.03</td>
<td>3.94 ± 0.08</td>
<td>3.90 ± 0.04</td>
<td>ab</td>
<td>3.92 ± 0.09</td>
<td>3.96 ± 0.05</td>
<td>ab</td>
</tr>
<tr>
<td>AIP</td>
<td>0.57 ± 0.02</td>
<td>0.57 ± 0.01</td>
<td>0.57 ± 0.02</td>
<td>ab</td>
<td>0.57 ± 0.01</td>
<td>0.58 ± 0.01</td>
<td>ab</td>
</tr>
</tbody>
</table>

Table 1: Lipid Profile and Atherogenic Index Parameters of Paracetamol-overdosed Albino Rats Treated with E. heterophylla and J. curcas. Values are mean ± standard deviation. Values with different alphabet letters per row are statistically significant (p<0.05). TC: Total Cholesterol; TG: Triglyceride; HDL-c: High Density Lipoprotein Cholesterol; LDL-c: Low Density Lipoprotein Cholesterol; TnHDL-c: Total Non-HDL-c; CRI-I: Castelli’s Risk Index I; CRI-II: Castelli’s Risk Index II; AC: Atherogenic Coefficient; AIP: Atherogenic Index of Plasma.

Table 2 shows the haematologic indices of the paracetamol overdosed animals. Paracetamol treatment did not change significantly (p>0.05) the blood haemoglobin (Hb) concentration, packed cell volume (PCV) values and red blood cell (RBC) counts of the animals in comparism with the control and those of the vitamin C, J. curcas and E. heterophylla treated groups. However, there was a significant (p<0.05) reduction in the white blood cell (WBC) count of the animals after treatment with paracetamol overdose in comparism with the control. This was significantly (p<0.05) attenuated after treatment of the paracetamol overdosed animals with vitamin C, E. heterophylla and J. curcas.

Statistical analysis
Data obtained from the analyses were presented as mean ± standard deviation. The data generated were statistically analysed using one way Analysis of Variance (ANOVA) and Tukey Post HOC test with the aid of GraphPad Prism Version 5.3 (GraphPad, USA). Values that gave p ≤ 0.05 were taken to be statistically significant.

Results
Table 1 shows the lipid profile and atherogenic predictor indices of the paracetamol overdosed animals. Paracetamol treatment reduced significantly (p<0.05) the TG, TC, and HDL-c concentrations, but increased the LDL-c and total non-HDL cholesterol concentrations of the animals in comparism to the control animals. Treatment of the paracetamol overdosed animals with vitamin C and extracts of E. heterophylla and J. curcas increased non-significantly (p>0.05) the concentrations of the lipids and lipoproteins of the treated groups. Paracetamol overdose did not change significantly (p>0.05) the Castelli’s Risk Index I (TC/HDL-c ratio), Castelli’s Risk Index II (LDL-c/HDL-c ratio), atherogenic coefficient [(TC - HDL-c)/HDL-c ratio] and atherogenic index of plasma (Log [TG/HDL-c]) of the animals when compared with the control, vitamin C, E. heterophylla and J. curcas groups.

standard deviation. Values with attenuation in serum TC concentration observed in the extracts-reported to be the most important mechanisms involved in the

treated rats may be attributed to increase in the serum HDL-c
decreasing or reducing the formation of atherosclerotic plaque.

glutahtione (GSH) and induction of oxidative stress have been

direct related with mortality and morbidity from coronary artery
disease when associated with intravascular haemolysis [24]. These observations may be due to the understanding that paracetamol metabolism and toxicity occur mainly in the liver. However, there was observed reduction in white blood cell (WBC) count of the paracetamol-overdosed rats while administration of vitamin C, E. heterophylla and J. curcas extracts led to a significant (p<0.05) increase in the WBC count of the rats, an observation which corroborates previous report that WBC counts are significantly decreased by paracetamol administration [40].

Conclusion

In the light of the results obtained in this study, it may be concluded that atherogenic and haematologic indices were less responsive than lipid parameters to paracetamol-induced toxicity. Furthermore, aqueous leaf extracts of E. heterophylla and J. curcas have protective effect, although less than vitamin C, against serum lipidaemic changes induced by paracetamol toxicity. The observations reported herein may explain the widespread use of the extracts of these plants in ethnotraditional medicine practice.

References


<table>
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<tr>
<th>Parameters</th>
<th>Negative Control</th>
<th>Positive (Paracetamol)</th>
<th>Control (Vitamin C)</th>
<th>Test Group 1 (E. heterophylla)</th>
<th>Test Group 2 (J. curcas)</th>
</tr>
</thead>
<tbody>
<tr>
<td>HB (g/dl)</td>
<td>11.30 ± 1.59a</td>
<td>11.87 ± 1.60a</td>
<td>12.65 ± 1.11a</td>
<td>11.95 ± 1.00a</td>
<td>12.07 ± 0.90a</td>
</tr>
<tr>
<td>PCV (%)</td>
<td>33.93 ± 4.71a</td>
<td>35.60 ± 4.81a</td>
<td>37.95 ± 3.34a</td>
<td>35.85 ± 3.00a</td>
<td>36.07 ± 2.90a</td>
</tr>
<tr>
<td>WBC (x1012/L)</td>
<td>5.45 ± 0.10a</td>
<td>4.20 ± 0.17a</td>
<td>6.68 ± 0.23c</td>
<td>6.45 ± 0.30c</td>
<td>4.77 ± 0.25c</td>
</tr>
<tr>
<td>RBC (x1012/L)</td>
<td>35.65 ± 4.37a</td>
<td>37.70 ± 4.86a</td>
<td>40.05 ± 3.41ab</td>
<td>37.80 ± 2.93ab</td>
<td>38.10 ± 3.05ab</td>
</tr>
</tbody>
</table>

Table 2: Haematological Parameters of Paracetamol-overdosed Albino Rats Treated with E. heterophylla and J. curcas. Values are mean ± standard deviation. Values with different alphabet letters are statistically significant (p<0.05). HB: Haemoglobin; PCV: Packed cell volume; WBC: white blood cell count; RBC: Red blood cell count.