Lipid profile and haematological effects of ethanolic leaf extract of Gongronema latifolium Benth. against acetaminophen-induced toxicity in albino rats

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Abstract: We examined the lipid profile and haematological effects of ethanolic leaf extract of Gongronema latifolium against acetaminophen-induced toxicity in albino rats. The serum concentrations of total cholesterol, triglycerides, high density lipoprotein and low density lipoprotein were determined using auto-analyzer, while the haematological parameters: RBC, WBC, Hb and PCV were analyzed using haematological auto-analyzer. The haematological parameters reduced in the group administered acetaminophen only, but increased significantly (p<0.05) in all groups administered the leaf extract compared with the negative control. WBC and RBC increased from 4.94 ± 0.14 to 7.83 ± 0.17 (X10⁹/L) and 4.40 ± 0.17 to 6.86 ± 0.22 (X10⁹/L) respectively, while Hb and PCV increased from 151.04 ± 3.69 to 117.81 ± 2.43 (mg/dl) and 25.38 ± 0.52 to 38.37 ± 0.69 (%) respectively in the group administered 600mg/kg of the leaf extract. Total cholesterol, triglycerides and LDL increased, while HDL decreased significantly (p<0.05) in the group administered acetaminophen only. Total cholesterol, triglycerides and LDL reduced significantly (p<0.05), while HDL increased significantly (p<0.05) in all groups administered the leaf extract compared with the negative control. Total cholesterol, triglycerides and LDL reduced from 69.39 ± 2.65 to 51.72 ± 1.57 (mg/dl), 151.04 ± 3.69 to 117.81 ± 2.43 (mg/dl) and 22.69 ± 2.65 to 7.35 ± 1.59 (mg/dl) respectively, while HDL increased from 16.42 ± 0.73 to 20.75 ± 0.64 (mg/dl) in the group administered 600mg/kg of the leaf extract. The result of this study indicate that ethanolic leaf extract of Gongronema latifolium has hypolipidemic and positive haematological effect against acetaminophen-induced toxicity in albino rats. Therefore, Gongronema latifolium leaf may be used in stabilizing the haematological parameters and in the management of conditions of hyperlipidaemia.

Keywords: Acetaminophen, Gongronema latifolium, Haematology, Lipid profile, Medicinal plant.

I. Introduction

There has been a growing interest in the use of various medicinal plants from traditional system of medicine for the treatment of different ailments. Natural compounds from plants are major sources of molecules with medicinal properties. In recent times, developed countries are turning to the use of traditional medicinal systems that involve the use of herbal drugs. Most of the drugs prescribed today come from plants. This is because plant-derived biomolecules make up a significant segment of natural product– based pharmaceuticals. Medicinal plants play vital roles in drug discovery and are very useful for human to cure several ailments. Many medicinal plants have been validated experimentally and have been reported to possess potential anti-inflammatory activity [1]. Tiwari and Rao [2] reported that different composition of the active components in medicinal plants give the plants an edge as better therapeutic agents than chemotherapy in the management of different ailments such as hypertension, atherosclerosis and diabetes.

Paracetamol or acetaminophen is a widely used over-the-counter analgesic (pain reliever) and antipyretic (fever reducer). It is generally safe for use at recommended doses (1,000 mg per single dose and up to 4,000 mg per day for adults). Acute overdose of paracetamol can cause fatal liver damage and the risk is heightened by the consumption of alcohol. Anti-inflammatory drugs like nonsteroidal anti-inflammatory drugs (NSAIDs) are used to reduce the swelling and pain of inflammation. Long-term uses of NSAIDs may cause an adverse side effects to human biological system.

Gongronema latifolium is commonly called “utazi” and “arokeke” in South Eastern and South Western Nigeria respectively. The leaf is bitter and it is primarily used as spice and vegetable in traditional folk medicine [3, 4]. The presence of phytochemicals (tannins, saponins, alkaloids, flavonoids and hydrocyanide), proximate (crude fat, ash, fat and protein), mineral elements (Cr, Cu, Se, Zn and Fe) and vitamins (A, C, riboflavin, niacin and thiamine) in the root, bark and twig extracts of Gongronema latifolium were reported by
Egburg et al. [5]. However, the concentration of these phytochemicals vary among these plant parts. The reported vitamins have variously been shown to possess antioxidant activities particularly A and C [6].

Imo and Uhegbu [7] reported the presence of different alkaloids, flavonoids, phenolic compounds, lignans, terpenes, phytosterols, allicins, hydroxycinnamic acids, saponins and carotenoids in Gongronema latifolium leaf. Some of the phytochemicals detected in high quantities among the different groups of phytochemicals reported in 100g of Gongronema latifolium leaf include Cinchonidine 52.47mg, Oxassianine 43.51mg, Lupanine 35.65mg and Buphanidine 33.33mg (Alkaloids), Hyperoside 37.54mg, Quercetin 31.03mg and Kaemferol 24.80mg (Flavonoids), Tannic acid 116.60mg, Ferulic acid 82.26mg and Vanillic acid 64.17mg (Total phenolic compounds), Retusin 4.40mg and Galgravin 4.33mg (Lignans), Nerol (geraniol) 33.05mg and Beta pinene 32.79mg (Terpenes), 5-avenasterol 9.42mg and Stigmasterol 4.89mg (Phytosterols), Chlorogenic acid 48.87mg and Caffeic acid 23.01mg (Hydroxycinnamic acids), Saponine 59.11mg and Sapogenin 50.79mg (Saponins) and Beta-cryptoxanthin 433.14mg, Xanthophylls 311.36mg and Carotene 158.36mg (Carotenoids).

The aim of this study was to examine the lipid profile and haematological effects of ethanolic leaf extract of Gongronema latifolium against acetalinophen-induced toxicity in albino rats.

II. Materials And Methods

2.1 Drug

Acetaminophen (product of Emzor pharmaceutical industries Ltd., Nigeria) was purchased from a pharmacy shop (Ndukwe Family Chemist Nig. Ltd.) in Umuahia, Abia State, Nigeria.

2.2 Plant Material and Extraction

The leaf of Gongronema latifolium was harvested at Itaja-Amaegbu Olokoro in Umuahia, Abia State, Nigeria. The plant was identified at the Department of Plant Science and Biotechnology, Abia State University, Uturu and voucher specimen deposited at the herbarium of Plant Science and Biotechnology department. The plant material was sun-dried. The dried leaf of Gongronema latifolium was milled to powder. 250g of the powder was extracted with 800 ml of 70% ethanol by cold maceration for 48 hours and filtered. The filtrate was evaporated to dryness using a soxhlet extractor. Different concentrations of the extract was prepared for the experiment by appropriate dilution using normal saline.

2.3 Experimental Animals

Fifty male albino rats aged seven (7) weeks were used in this study. The rats were kept in the animal house, Department of Biochemistry, Faculty of Biological and Physical Science, Abia State University, Uturu. The animals were allowed to acclimatize for 7 days under standard laboratory conditions with free access to commercial rat feed and water.

2.4 Experimental Design

The animals weighing between 134g-160g were randomly placed into five (5) groups with ten (10) rats in each group. Group 1 served as the normal control (it received a placebo of normal saline). Group 2 received acetalinophen (1000 mg/kg b.w.) only as negative control. Group 3 received 200 mg/kg of leaf extract of G. latifolium and acetalinophen (1000 mg/kg b.w.). Group 4 received 400 mg/kg of leaf extract of G. latifolium and acetalinophen (1000 mg/kg b.w.). Group 5 received 600 mg/kg of leaf extract of G. latifolium and acetalinophen (1000 mg/kg b.w.).

The test animals (groups 3, 4 and 5) received the leaf extract daily as stated above for twenty one days. Groups 2, 3, 4 and 5 animals received acetalinophen one hour after each administration of the leaf extract.

In the test groups, the drug and extract were administered through oral route using gavage intubation. All animals were allowed free access to feed and water ad libitum throughout the study.

2.5 Blood Collection

Twenty four hours after administration of the leaf extract and acetalinophen, the animals were starved overnight, anaesthetized with chloroform and sacrificed. Blood was collected by cardiac puncture and blood samples from each animal collected into dry test tubes. The blood sample was divided into two. The first part was dispensed in heparinized tubes for haematological analysis. The second part of the blood sample was allowed to stand for about 15 minutes to clot and further spun in a centrifuge. Serum was separated from the clot with Pasteur pipette into sterile sample tubes for the measurement of lipid profile.

2.6 Biochemical Analysis

The serum concentrations of Total cholesterol, Triglycerides, High density lipoprotein and Low density lipoprotein were determined using auto-analizer (Biosystem A25 Random Access Analyzer).
Haematological parameters: Red blood cell, White blood cell, Haemoglobin and Packed Cell Volume were analyzed using haematological auto-analyzer (Abacus 380).

2.7 Statistical Analysis
The results were subjected to statistical analysis using Analysis of Variance (ANOVA) and standard student-T-distribution-test: using Statistical package for Social Sciences (SPSS) version 20. Group means were compared for significance at p<0.05. Data were represented as mean ± standard deviation.

III. Results

The results of the study are presented in the tables below

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Group 1 (Normal control)</th>
<th>Group 2 (APAP 1000mg/kg bw)</th>
<th>Group 3 (200mg/kg bw of G. lat. + APAP 1000mg/kg bw)</th>
<th>Group 4 (400mg/kg bw of G. lat. + APAP 1000mg/kg bw)</th>
<th>Group 5 (600mg/kg bw of G. lat. + APAP 1000mg/kg bw)</th>
</tr>
</thead>
<tbody>
<tr>
<td>WBC (X10^3/L)</td>
<td>7.98 ± 0.18</td>
<td>4.94 ± 0.14</td>
<td>7.79 ± 0.14</td>
<td>7.89 ± 0.24</td>
<td>7.83 ± 0.17</td>
</tr>
<tr>
<td>RBC (X10^6/L)</td>
<td>6.38 ± 0.36</td>
<td>4.40 ± 0.17</td>
<td>5.44 ± 0.08</td>
<td>5.98 ± 0.38</td>
<td>6.86 ± 0.22</td>
</tr>
<tr>
<td>Hb (g/dl)</td>
<td>12.39 ± 0.29</td>
<td>8.58 ± 0.14</td>
<td>10.45 ± 0.25</td>
<td>12.17 ± 0.31</td>
<td>12.67 ± 0.28</td>
</tr>
<tr>
<td>PCV (%)</td>
<td>38.15 ± 2.59</td>
<td>25.58 ± 0.52</td>
<td>31.07 ± 0.62</td>
<td>36.19 ± 1.27</td>
<td>38.37 ± 0.69</td>
</tr>
</tbody>
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Results represent mean ± standard deviation of group results obtained (n=10).

Mean in the same row, having different letters of the alphabet are statistically significant (p<0.05) compared with the negative control (group two).

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<td>Total Cholesterol (mg/dl)</td>
<td>47.84 ± 0.26</td>
<td>69.39 ± 2.65</td>
<td>60.65 ± 1.78</td>
<td>56.03 ± 0.37</td>
<td>51.72 ± 1.57</td>
</tr>
<tr>
<td>Triglycerides (mg/dl)</td>
<td>120.11 ± 10.95</td>
<td>151.04 ± 3.69</td>
<td>126.65 ± 0.96</td>
<td>124.26 ± 1.75</td>
<td>117.81 ± 2.43</td>
</tr>
<tr>
<td>LDL (mg/dl)</td>
<td>3.58 ± 0.21</td>
<td>22.69 ± 2.65</td>
<td>18.18 ± 2.00</td>
<td>12.14 ± 0.85</td>
<td>7.35 ± 1.59</td>
</tr>
<tr>
<td>HDL (mg/dl)</td>
<td>21.02 ± 0.17</td>
<td>16.42 ± 0.73</td>
<td>17.61 ± 0.73</td>
<td>19.07 ± 0.52</td>
<td>20.75 ± 0.64</td>
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Results represent mean ± standard deviation of group serum results obtained (n=10).

Mean in the same row, having different letters of the alphabet are statistically significant (p<0.05) compared with the negative control (group two).

IV. Discussion
The haematological result of this study showed a significant increase in white blood cells (WBC), red blood cells (RBC), packet cell volume and haemoglobin concentration in the groups administered the leaf extract when compared with the group administered acetaminophen only (negative control). The low level of RBC, PCV and Hb in group two when compared with the normal control (group 1) is believed to be as a result of the excess dose of acetaminophen administered. It is possible that the toxic metabolites of the acetaminophen administered to the negative control must have caused the alterations in the haematological parameters. Administration of excess dose of acetaminophen has been reported to cause an increase in ALT, AST and ALP concentrations which are indicative of liver damage [8]. This will cause serum protein synthesis in the liver to be suppressed. Therefore, the bone marrow may not have enough proteins that may be required to synthesize red blood cell, thereby reducing the concentration of haemoglobin and packed cell volume. The consumption of plant foods causes increase in the synthesis of haemoglobin due to their high content of vitamins and minerals [9] that may stimulate the synthesis of globin component of haemoglobin as was observed in this study.

The concentration of white blood cells increased significantly (p<0.05) in all groups administered the ethanolic leaf extract of Gongronema latifolium when compared with the negative control (administered acetaminophen only). This result shows that the leaf extract administered before the administration of acetaminophen prevented the alteration of the parameters measured compared with the group administered acetaminophen only. It is believed that the phytochemicals present in Gongronema latifolium leaf is responsible for the prevention of the alteration effected in the negative control. Imo and Uhegbu [7] reported that Gongronema latifolium leaf possess an appreciable level of phytochemicals and could be a good raw material
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for the production of some medicinal drugs and can be used in folk medicine for the treatment of some diseases. Some of the phytochemicals may have possibly modulated or influenced the processes involved in the production of red blood cells and white blood cells.

Serum lipid concentrations following the 21-day administration of the leaf extract in this investigation are shown in table 2. Serum total cholesterol, triglycerides and LDL-cholesterol concentrations increased significantly (p<0.05) in the group administered acetaminophen only: compared to the normal control, but was reduced in all groups administered the leaf extract (compared with the negative control). The HDL-cholesterol decreased significantly (p<0.05) in the group administered acetaminophen only when compared with the normal control, but was increased in all groups administered the leaf extract (compared with the negative control). These effects of ethanolic leaf extract of Gongronema latifolium on serum lipid profile parameters is believed to be as a result of the presence of phytochemicals present in Gongronema latifolium leaf. Phytosterols and saponin possesses the ability to reduce blood cholesterol levels in hyper- and normocholesterolemic subjects, while phenolics are also believe to decrease the serum lipid concentration and modulate immunoresponses. In this study, it is believed that polyphenolics and phytosterols are involved in the reduction of the total cholesterol, triglycerides, LDL concentrations and increase in HDL concentration in the groups administered the leaf extract (compared with the negative control), thereby preventing the incidence that may lead to hyperlipidaemia or atherosclerosis.

V. Conclusion

The result of this study indicate that the ethanolic leaf extract of Gongronema latifolium has hypolipidaemic and positive haematological effect against acetaminophen-induced toxicity in the male albino rats. Therefore, Gongronema latifolium leaf may be used in stabilizing haematological parameters and in the management of conditions of hyperlipidaemia.

References