Influence of *Nauclea latifolia* Leaf Extracts on Some Hepatic Enzymes of Rats Fed on Coconut Oil and Non-Coconut Oil Meals

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**Abstract**

This work focuses primarily on the comparative response of rat liver enzymes to oral administration of the water-soluble fraction of 95% ethanol extract of *Nauclea latifolia* Sm. (Rubiaceae) leaves with 10% coconut oil meal and normal rat chow fed for 8 weeks. Forty-eight mature male albino rats of the Wistar strain weighing between 200 and 230 g were divided into two experimental groups. In experiment 1, group 1 \((n = 6)\) was fed normal rat chow for 8 weeks, and groups 2, 3, and 4 \((n = 6)\) were on normal rat chow for 8 weeks before treatment with 170, 340, and 510 mg/kg body weight, respectively, of oral dose of the water-soluble fraction of the ethanol extract of *N. latifolia* leaves. In experiment 2, group 1 \((n = 6)\) was fed the 10% coconut oil meal as the experimental control, and groups 2, 3, and 4 \((n = 6)\) were fed the 10% coconut oil meal for 8 weeks before commencing treatment for 2 weeks with the extract of *N. latifolia* leaves. The effects of the *N. latifolia* leaf extract on some marker enzymes were analyzed. There was a significant increase \((p < 0.05)\) of aspartate aminotransferase (AST) activity in all the groups when compared to the control, but the increase was higher in the 10% coconut oil meal fed groups. Alanine aminotransferase (ALT) activity decreased significantly \((p > 0.05)\) in experiment 1 animals when compared with control. Increase in ALT activity was however observed in experiment 2 \((p < 0.05)\). Alkaline phosphatase (ALP) and lactate dehydrogenase (LDH) activities did not change in both experiments. There was no significant \((p > 0.05)\) change in \(\gamma\)-GT activity in experiment 1, but in experiment 2 glutamyl transferase (GGT) decreased in the water-soluble fraction of the ethanol extract. *N. latifolia* leaf extract is capable of reducing the activity of \(\gamma\)-GT if raised by other factors. We also concluded that feeding animals with 10% coconut oil meal predisposes them to more adverse effects by the extract of *N. latifolia* leaves.

**Keywords:** Coconut oil meal, hepatic enzymes, leaf extract, *Nauclea latifolia*.

**Introduction**

Coconut is one of the major saturated fat sources apart from palm kernel, and the two are the only natural sources of lauric oil available to the world market (Berger et al., 1991). The use of this oil is similar to that of other lauric oil sources (Akpanabiatu et al., 2000). Coconut oil was noted in the U.S. Surgeon General’s report (1988) on nutrition and health as an example of vegetable oil rich in saturated fatty acids. Coconut oil is also known to be the principal cholesterol-raising fat because it contains large amounts of lauric (C: 12: O) and myristic (C: 14: 0) acids (Keys et al., 1957). The effect of long-term feeding of rats with vegetable oil as one of the dietary components has been reported to reduce significantly the peroxidation potential of hepatic mitochondria and microsomes (Nesaratnam et al., 1993). Also, the antioxidant effects of vegetable oil and vitamin C have been established in both plasma and tissues (Abdel-Baset et al., 1997). The effects of drug-metabolizing enzymes in rat fed on palm oil have been investigated, and the report suggests that red palm oil offers protection against chemical carcinogens (Manorama et al., 1993). It however follows that the report by other workers on the antidote effect of red palm oil is well founded (Akpanabiatu et al., 2000).
However, we do know that many agents acting concomitantly in the biological system affect the various enzyme systems in the body and plasma. Multiple drug therapy is a common practice in both orthodox and traditional medicines. Whereas coconut oil is hyperlipidemic, *Nauclea latifolia* leaf extract is said to be hypolipidemic, like most other plants extract (Schiff, 1970; Chong, 1991; Udoh, 1998; Eno & Owo, 1999). Scanty literature is available on the medicinal use of *N. latifolia*, but the local uses of the extract of *N. latifolia* leaves are very common in Nigeria. The antihypertensive effect and phytochemical screening of this herb have been documented (Udoh, 1998). We have also observed that the alcohol extract of the leaves of this plant could have high antimicrobial activity in our laboratory. Previously, the effects of *N. latifolia* leaf extract on some enzymes of clinical significance have been investigated in our laboratory. Drug metabolizing enzyme activities are closely related to the level of protein in the body.

In toxicity studies, the majority of the enzymes measured as indices of drug metabolism are released into the bloodstream when cells are damaged or their functions are disrupted. Cell membrane integrity as assessed by its ability to prevent enzyme leakage is dependent on intracellular energy. The cell membranes are therefore impermeable to enzymes as long as the cells are metabolizing normally (Teitz, 1986; Numakami et al., 1999).

In this study, we are interested in evaluating the effect of the water-soluble fraction of the ethanol extract of *Nauclea latifolia* leaves on the enzyme activities of rats fed on 10% coconut oil meal. This could provide a possible explanation of the toxicity of *Nauclea latifolia* Sm. (Rubiaceae) leaves extract on raised lipid level.

**Materials and Methods**

**Preparation of plant materials and animal care**

Fresh leaves of *Nauclea latifolia* Sm. were obtained from the premises of the Medical School, University of Calabar, Nigeria, in the month of June 2001. The leaves were identified and authenticated by the botanist in the Botanical Garden of the University. Voucher specimen no. MIA 2001 was deposited at the herbarium in the Department of Botany, University of Calabar. The leaves were dried in a Plus 11 oven (Gallenkamp, Leichester, UK) at 55°C and crushed using laboratory blender (National blender, Matsushita Co., Japan). The ground leaf was stored in a glass bottle with a plastic screw cap and kept in the refrigerator (4°C).

Forty-eight mature male albino rats of the Wistar strain, weighing 200–230 g, were obtained from animal stock in the Department of Biochemistry, University of Calabar, Nigeria. The animals were housed in a well ventilated experimental animal house under constant environmental and adequate nutritional conditions throughout the period of the experiment. Feeding of animals was done ad libitum, alongside drinking water. The 48 animals were divided into 8 groups of 6 rats each.

**Experiment 1**

Group 1 (normal control): Rats were subjected to oral administration of 1.5 ml of normal saline as placebo.

Groups 2, 3, and 4: Rats were treated with 170, 340, and 510 mg/kg body weight, respectively, of the water-soluble fraction of the ethanol extract of *Nauclea latifolia* leaves.

**Experiment 2**

Group 1: (coconut oil control): In this group, rats were fed with 10% coconut oil diet for 8 weeks before treatment with normal saline as placebo.

Groups 2, 3, and 4: In these groups, rats were fed with 10% coconut oil diet for 8 weeks before treatment with 170, 340, and 510 mg/kg body weight, respectively, of the water-soluble fraction of the ethanol extract of *Nauclea latifolia* leaves.

**Preparation of coconut oil and coconut oil meal**

Dry coconut fruits were purchased from Akim Market, Calabar Municipality, Cross River State, Nigeria. The nuts were broken manually and the coconut meat was removed and grated using an aluminum grater. The grated meat was washed with sufficient tap water to obtain coconut milk. The milk was filtered and evaporated to dryness using an aluminum pot on a gas cooker. The oil was decanted into a stainless steel cup and dried in a Plus 11 oven at 65°C for 2 h to remove water residues from the oil. The oil was further decanted into a bottle with a plastic screw cap and stored in the refrigerator for use. Ten grams of the oil was mixed and mashed with 90 g of normal rat chow until there was homogeneity. The 10% coconut oil meal was prepared daily on demand.

**Preparation of plant extracts**

Bulk ethanol extraction of the crushed leaf of *Nauclea latifolia* was done by Soxhlet extraction method using an electro-thermal heating mantle. The extract was dried in an oven at 55°C to remove all the ethanol and was stored in the refrigerator until required for use. The ethanol extract was re-extracted in cold water for use in this experiment. The residue was removed by filtration using Whatman no. 1 filter paper. The concentration of the extract was determined gravimetrically by drying a known volume and weighing the dried residue.
Table 1. Serum levels of aspartate and alanine aminotransferase, alkaline phosphatase, \( \gamma \)-glutamyl transferase, and lactate dehydrogenase activities.\(^a\)

<table>
<thead>
<tr>
<th>Enzyme (u/l)(^b)</th>
<th>Group 1 (control)</th>
<th>Group 2</th>
<th>Group 3</th>
<th>Group 4</th>
</tr>
</thead>
<tbody>
<tr>
<td>Aspartate aminotransferase</td>
<td>7.82 ± 0.18</td>
<td>18.10 ± 2.8</td>
<td>14.49 ± 2.02</td>
<td>33.84 ± 8.40</td>
</tr>
<tr>
<td>Alanine aminotransferase</td>
<td>12.66 ± 5.23</td>
<td>6.38 ± 0.96</td>
<td>3.83 ± 0.99</td>
<td>6.99 ± 1.35</td>
</tr>
<tr>
<td>Alkaline phosphatase</td>
<td>226.49 ± 36.98</td>
<td>234.60 ± 28.66</td>
<td>225.42 ± 24.082</td>
<td>275.19 ± 81.41</td>
</tr>
<tr>
<td>( \gamma )-Glutamyl transferase</td>
<td>3.25 ± 1.38</td>
<td>3.976 ± 1.04</td>
<td>4.77 ± 1.83</td>
<td>4.40 ± 0.92</td>
</tr>
<tr>
<td>Lactate dehydrogenase</td>
<td>1195.79 ± 23.64</td>
<td>998.73 ± 20.36</td>
<td>1397.70 ± 13.33</td>
<td>1269.04 ± 0.47</td>
</tr>
</tbody>
</table>

\(^a\)Enzyme activities of normal Wistar albino rats treated with the water-soluble fraction of the ethanol extract of \( N. \) latifolia leaves. 
\(^b\)Mean ± SD of 6 determinations.

in the refrigerator throughout the period of the experiment preserved the prepared extract.

Animal treatment and preparation of serum

Administration of \( N. \) latifolia leaf extract was done by intragastric syringe. In experiment 1, group 1 animals served as the control and received normal saline (1.5 ml). In groups 2, 3, and 4, animals were treated with graded doses of the water-soluble fraction of the ethanol extract corresponding to 170, 340, and 510 mg/kg body weight of animals, respectively. The same procedure was repeated in experiment 2. Administration of the extract was carried out daily for days between the hours of 10 a.m. and noon. Twenty-four hours after the last administration, the animals were anesthetized under chloroform vapor. Blood samples for sera preparation were collected by cardiac puncture into sterile plain tubes. Serum samples were separated from the clot by centrifugation at 3000 \( \times \) g for 5 min using a benchtop centrifuge (MSE Minor, England). Serum samples were separated into sterile plain tubes and stored in the refrigerator for analyses. All analyses were completed within 24 h of sample collection.

Chemicals and reagents

All reagents and chemicals that were used in this work were of analytical grade. Serum aspartate aminotransferase (AST) and alanine aminotransferase (ALT) activities were determined using Biosystems reagent (Barcelona, Spain) and instrument kits. Quimica Clinica Aplicada SA (QCA, Amposta, Spain) reagent kit was used for the determination of lactate dehydrogenase (LDH). Alkaline phosphatase was determined by kinetic procedure of Alpha Diagnostics kit (Alpha Diagnostics Inc., Monterey Park, CA, USA). Biolabo kit was used for the determination of \( \gamma \)-glutamyl transferase (\( \gamma \)-GT). The absorbances of all the tests were determined using spectrophotometer (HAICH, DR 3000, Germany).

Statistical analysis of data was determined with the use of standard Students’ \( t \)-test method, and \( p < 0.05 \) was regarded as significant. The group data are expressed as mean ± SD.

Results

Tables 1 and 2 show the effects of the water-soluble fraction of the ethanol extract of \( N. \) latifolia leaves on some enzymes of biochemical importance in normal experimental rats and rats fed 10% coconut oil meal for 8 weeks, respectively. The enzymes of interest were aminotransferases (AST and ALT), alkaline phosphatase (ALP), \( \gamma \)-glutamyl transferase, and lactate dehydrogenase.

The activity of AST increased significantly (\( p < 0.05 \)) in all the groups, but the increment was much higher in the 10% coconut oil meal–fed animals. ALT activity decreased significantly in experiment 1 animals when compared with the control animals, but in experiment 2 the ALT activity increased significantly (\( p > 0.05 \)). There is no significant in ALP and LDH activities in Tables 1 and 2. There was, however, no significant (\( p > 0.05 \)) difference in \( \gamma \)-GT activity of rats fed 10% coconut oil meal, but therapy with \( N. \) latifolia leaf extract to normal rats that did not consume the 10% coconut oil meal indicated significant increase in \( \gamma \)-GT activity, which was dose-dependent.

Discussion

An attempt was made in this investigation to study not only the effects of \( N. \) latifolia on normal Wistar albino rats but also rats fed 10% coconut oil meal. There are, however, several reports on the effect of crude extract on the activity of enzymes such as AST, ALT, ALP, LDH, \( \gamma \)-GT, and many more (Udosen & Ojong, 1998; Premalatha et al., 1999; Akpanabiatu et al., 2003).

The activities of ALT, ALP, LDH, and \( \gamma \)-GT were determined in serum of experimental rats because analyses of liver function enzymes are used as indicators of biochemical changes in response to treatment. There was no significant alteration in the serum levels of ALT and LDH of treated rats at all doses relative to control. However, AST activity shows significant increase in Tables 1 and 2, except in group 3 of Table 2. The significant change in AST and insignificant change in ALT as reported in this work are in line with reports on animals
treated with phytochemical extracts (Nada et al., 1997; Akpanabiatu et al., 2003). This is to say that the extract exerts differing effects on both mitochondria and cytosol of the experimental animals.

Lactate dehydrogenase, a sensitive indicator of liver injury, was not affected. Also, ALP was not raised in the two experiments. A nonsignificant deviation from normal in the levels of ALP, LDH, and γ-GT activities in Table 1 coupled with significant reduction in ALT and γ-GT in Tables 1 and 2, respectively, is an obvious indication of nontoxic and protective activity of this herb. Damage to liver cells with necrosis causes the release of intracellular constituents into the bloodstream, and lactate dehydrogenase is a sensitive indicator (Zilva & Pannal, 1984). At the level of insults reported in this work, alkaline phosphatase did not change, probably because there was no serious damage to the animals’ organs.

Alkaline phosphatase has been reported to be involved in the transport of metabolites across the cell membranes, protein synthesis, secretary activities, and glycogen metabolism (Sharma et al., 1996). Therefore, the gradual rise in the serum alkaline phosphatase activity may be due to disturbance in secretary activity of cell or in the transport of metabolites or may be due to altered synthesis of certain enzymes as in other hepatotoxic conditions. Table 1 shows a nonsignificant increase in the activity of γ-GT. It is important to note that the control group fed 10% coconut oil meal had a significantly higher γ-GT activity compared to the normal control. This suggests that a raised γ-GT activity in experiment 2 is due to coconut oil meal (Manorama et al., 1993). In this work, we observed that the water-soluble fraction of the ethanol extract of Nauclea latifolia leaf was capable of reducing the activity of γ-GT if raised by other agent(s).

The complexity of crude extract of medicinal plants is a major factor in the variability of enzyme activities. The multiple mechanisms whereby enzymes are regulated include increased transcription and post-transcriptional modulation, which are apparently mediated through generation of reactive oxygen species and reduced glutathione (GSH) conjugate formation, respectively. Antioxidant protection of cells and tissues prevent free-radical injury and facilitates repair of damaged tissues (Chakraborty et al., 1994; Cadena et al., 1995; Feher et al., 1997). Plant materials contain many antioxidants, including vitamins E, C, and beta-carotene, while serum contains, in addition to the above, antioxidants such as transferrin, uric acid, protein thiol, and ceruloplasmin (Vervaat & Knight, 1996; Abdel-Baset et al., 1997). Antioxidants act protectively to oxidative stress, hence there is likelihood that Nauclea latifolia leaf extract may not pose serious problem to the users. γ-GT is essential to the metabolism of antioxidant glutathione and as such is believed to be important in protecting against oxidative stress (Liu et al., 1998; Rahman et al., 1998).

Hacroft (1987) and Stroeve and Makarowa (1989) reported the use of the AST and ALT ratio in the evaluation of heart and liver disease. In this work, we observed from the tables the possibility of having increase or near unity in AST and ALT ratio in almost all the treatment groups. This observation may be signaling pathology of the heart. The heart therefore may be the source of increase in AST observed in this work. Also, the increase in γ-GT observed in the 10% coconut oil meal–fed control is due to the coconut oil meal, as the treatment groups without 10% coconut oil meal did not change significantly compared to the normal control (without coconut oil meal). This suggests that drug-metabolizing enzymes may be induced by coconut oil meal.

In summary, this study on the influence of Nauclea latifolia leaf extract on some hepatic enzyme activities of rats fed 10% coconut oil meal and non–coconut oil meals show that the 10% coconut oil meal is capable of raising the level of serum γ-GT. Also, we demonstrated that increase in AST was much higher in the 10% coconut oil meal groups (experiment 2) than non–coconut oil meal fed groups (experiment 1). This suggests that raising the lipid level of animals predisposes them to more adverse effects, which may not be due to the extract of Nauclea latifolia leaves. Furthermore, we summit that the water-soluble fraction of the ethanol extract of Nauclea latifolia leaf is capable of reducing the activity of

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**Table 2. Serum levels of aspartate and alanine aminotransferase, alkaline phosphatase, γ-glutamyl transferase, and lactate dehydrogenase activities.**

<table>
<thead>
<tr>
<th>Enzyme (u/l)</th>
<th>Group 1 (control)</th>
<th>Group 2</th>
<th>Group 3</th>
<th>Group 4</th>
</tr>
</thead>
<tbody>
<tr>
<td>Aspartate aminotransferase</td>
<td>5.36 ± 2.05</td>
<td>9.55 ± 1.45</td>
<td>7.99 ± 1.92</td>
<td>12.55 ± 3.57</td>
</tr>
<tr>
<td>Alanine aminotransferase</td>
<td>6.58 ± 2.44</td>
<td>9.41 ± 2.72</td>
<td>7.65 ± 0.41</td>
<td>8.66 ± 2.71</td>
</tr>
<tr>
<td>Alkaline phosphatase</td>
<td>276.06 ± 55.08</td>
<td>242.33 ± 87.08</td>
<td>275.40 ± 56.36</td>
<td>270.98 ± 75.45</td>
</tr>
<tr>
<td>γ-Glutamyl transferase</td>
<td>9.10 ± 0.09</td>
<td>3.09 ± 0.33</td>
<td>3.97 ± 0.81</td>
<td>2.38 ± 0.53</td>
</tr>
<tr>
<td>Lactate dehydrogenase</td>
<td>1040.83 ± 326.70</td>
<td>1354.0 ± 190.95</td>
<td>1184.45 ± 133.13</td>
<td>1225.50 ± 97.01</td>
</tr>
</tbody>
</table>

*aEnzyme activities of normal Wistar albino rats fed on 10% coconut oil meal before treatment with the water-soluble fraction of the ethanol extract of Nauclea latifolia leaves.

bMean ± SD of 6 determinations.
γ-GT if raised by other factors, and that the *N. latifolia* leaf extract contains antihepatotoxic agent(s) that could be used to suppress the effect of some hepatotoxin. Further work on the effect of this herbal extract on some specific hepatotoxin such as aflatoxin B₁ is being considered.

**References**


