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Effects of Aqueous Extract of *Nauclea latifolia* Stem on Lipid Profile and Some Enzymes of Rat Liver and Kidney

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Abstract: The study was carried out to investigate effects of administration of aqueous extract of *Nauclea latifolia* stem on lipid profile and some liver and kidney parameters of rat. Thirty rats were grouped into five. Each group consisting of six rats each. Group 1 served as the control and was administered 2 mL distilled water. The remaining groups were administered 200, 400, 600 and 800 mg kg^{-1} b.wt. of aqueous extract of *Nauclea latifolia* stem, respectively. Administration lasted for twenty eight days during which three rats from each group were sacrificed twenty four hours after administration on the fourteenth day and the remaining, twenty eighth day. Liver, kidney and blood were collected and alkaline phosphatase, alanine aminotransferase, aspartate amino transferase, urea, creatinine, malondialdehyde level as well as lipid profile evaluated. The results showed a significant ($p<0.05$) concentration dependent decrease in total cholesterol (TC) and high density lipoprotein cholesterol (HDL-C) and increase in low density lipoprotein cholesterol (LDL-C) and triglycerides (TG) at both periods. Similar results were obtained in serum concentration of creatinine and urea with TC and HDL-C. There was significant concentration dependent reduction ($p<0.05$) in liver and kidney alkaline phosphatase, alanine aminotransferase and aspartate aminotransferase activities with a corresponding significant increase ($p<0.05$) in their serum activities. This revealed a time and concentration dependent hepatotoxic and nephrotoxic potentials of the aqueous extract of *Nauclea latifolia* stem.

Key words: *Nauclea latifolia*, lipid profile, liver, kidney

INTRODUCTION

Plants have been in use of man for prevention, treatment and management of diseases for a very long time without even the knowledge of the component and toxicity of the plant (Shafaei *et al*., 2011). The growing interest in herbal medicine demands information on the various plant preparations used in the treatment of diseases (Sarwar *et al*., 2011). Scientific evaluation of medicinal plant is important to the discovery of novel drugs and also helps to assess toxicity risks associated with the use of herbal preparations and other conventional drugs of plant origin.

Nauclea latifolia, "Egbo egbesi" in Yoruba, "Ubulu inu" in Ibo and "Tabasiya" in Hausa is a Rubiaceae commonly known as pin cushion tree. It is a straggling shrub or small tree of about 10 ft high and is a native of the tropical Africa and Asia. The leaves are broadly elliptic to round ovate. It is found in areas like Abuja, Enugu, Akwa Ibom, Cross River, Kontangora, Shaki and some other parts of Nigeria. It has found application in folk medicine for treatment of malaria, hypertension, diarrhea, tuberculosis, dysentery and also as a laxative (Okiemy-Andissa *et al*., 2004). Phytochemical analysis identifies indole-quinolizidine, alkaloids (glycoalkaloids) and saponins as the major components (Karou *et al*., 2011). Gidado *et al*. (2005) reported antidiabetic properties for the root and leaf extract while Taiwe *et al*. (2010) reported anti-depressant and anti-anxiety effect for the root extract of the plant. A decoction of the stem in water has been demonstrated to exhibit a high antiparasitic potential (Benoit-Vical *et al*., 1998). The aqueous extract also showed effectiveness against chloroquine resistance strains of *Plasmodium falciparum* (Benoit-Vical *et al*., 1998). Hot aqueous and ethanolic extract was demonstrated to exhibit strong antibacterial property (Okiei *et al*., 2011). Alkaloid rich extract of *N. latifolia* can react in vitro with mammalian DNA, leading to G2-M cell cycle arrest and heritable DNA-damage. In liver, kidney and blood cells, it induces single-strand breaks (Traore *et al*., 2000). However, despite the acclaimed and documented uses, there appears to be a paucity of information on the safety of repeated and prolonged use of this plant. Therefore, the aim of this study is to evaluate the effect of prolonged and repeated administration of aqueous extract of *Nauclea latifolia* on

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some biochemical parameters of rat liver and kidney and to assess its effect on lipid profile of rats.

MATERIALS AND METHODS

Plant material, animals and reagents: The plant sample, obtained from herbs sellers at Oja-Oba market, Ilorin, was identified at the Department of Plant Biology, University of Ilorin, Ilorin, Nigeria. Thirty healthy female albino rats (*Rattus norvegicus*) weighing between 210 and 230 g were obtained from the small Animal Holding Unit of the Department of Biochemistry, University of Ilorin, Ilorin, Nigeria. The animals were housed in clean metabolic cages that provided free access to rat pellets (Bendel Feeds and Flour Mills limited, Ewu, Nigeria) and tap water that was free of contaminants. The cages were kept in a well ventilated standard housing condition (Temperature: 28-31°C; Photoperiod: 12 h natural light and 12 h dark; Humidity: 50-55%).

Preparation of extract: Sample of the stem of the plant *Nauclea latifolia* was dried and then pounded in a mortar; it was then further ground to powder and stored in an air tight container until required. Two hundred grams of the powder was mixed with 1000 mL of distilled water and shaken thoroughly at intervals to ensure adequate extraction. It was then soaked for 48 h and then filtered using Muslin cloth after which a filter paper was used to obtain a pure filtrate. The filtrate was collected and then evaporated to dryness in a steam bath to give a brownish black residue (chocolate-like) which was stored in a small plastic container. The resulting residue was reconstituted in distilled water to give the required doses of 200, 400, 600 and 800 mg kg^{-1} b.wt., respectively.

Animal grouping and extract administration: The experimental animals were handled and used in accordance with international guide for the care and use of laboratory animals (National Research Council, 1996). Thirty female albino rats were randomly divided into five groups (A-E) consisting of 6 rats each. Group A served as the control and was orally administered with 2 mL of distilled water daily. Group B, C, D and E were orally administered with 2 mL graded doses of 200, 400, 600 and 800 mg kg^{-1} b.wt. of the extract respectively for twenty eight days.

Preparation of serum and homogenates: At the end of fourteenth day, the first sets of rats (3 from each group) were sacrificed and the remaining rats were kept on the same dose of extract until the twenty eighth day when they were sacrificed twenty four hours after. The rats were anaesthesized in chloroform vapour. When the rats became unconscious, the neck area was quickly cleared of fur and skin to expose the jugular veins. The veins after being slightly displaced (to avoid contamination with interstitial fluid) were then sharply cut with a sharp sterile blade. The blood was collected into clean dry beakers and allowed to clot at room temperature for 60 min. It was then refrigerated for another 45 min (Akanji and Ngaha, 1989). The clear serum was collected with Pasteur pipette into clean, dry sample bottles and kept frozen until required 12 h later.

The animals were, thereafter, quickly dissected and the liver and kidney removed (which are the organs of interest) into ice cold 0.25 M sucrose solution to maintain the integrity of the tissue. The liver and kidney were blotted with tissue paper and then weighed. The liver and kidney were homogenized in cold 0.25 M sucrose solution (1:5 w/v). The homogenates were transferred into special specimen bottles and kept frozen also until required (Ngaha, 1982) for the enzyme assay.

Determination of biochemical parameters: Serum creatinine was determined by the method described by Butler (1975) and serum urea concentration by the method described by Veniamin and Vakirtzi-Lemonias (1970). The activities of specific enzymes were determined in the liver and kidney homogenates and also in the serum. Alkaline phosphatase (EC 3.1.3.1) activity was determined using the method described by Wright *et al.* (1972). Alanine aminotransferase (EC 2.6.1.1) and aspartate amino transferase (EC 2.6.1.2) activities were determined using the method described by Reitman and Frankel (1957). Malondialdehyde was obtained by the method of Varshey and Kale (1990). The total cholesterol concentration was assayed using Cod-PAP method reported by Fredickson *et al.* (1967). HDL-cholesterol concentration was assayed using dextran as described by Albers *et al.* (1978). LDL-cholesterol concentration was assayed by polyvinyl sulphate method (PVS) as described by Assmann *et al.* (1996).

Statistical analysis: All data are presented as mean + standard deviation. The data from the various groups were compared for statistical significance using Duncan Multiple Range test according to Montgomery (1976). In all cases, probability level of 95% was taken as significant.

RESULTS

The effects of fourteen days administration of aqueous extract of *N. latifolia* stem at different concentrations on serum MDA, urea and creatinine levels

are presented in Table 1. There was a significant decrease ($p<0.05$) in the concentration of serum urea and creatinine in all the treatment groups when compared with the control. However, there was no significant difference ($p>0.05$) in the serum urea levels of the treatments groups when compared. Serum malondialdehyde level showed no significant difference ($p>0.05$) when compared at all levels of treatment. Also, the extract at 200 mg kg^{-1} b.wt. caused the highest reduction in the concentration of serum creatinine. All these may be suggesting that the effect of the extract on these parameters is independent of concentration.

Similar pattern of results were obtained when the rats were administered with the extract for twenty eight days (Table 2). However, the serum urea and creatinine concentrations following twenty eight days of administration were significantly higher ($p<0.05$) compared to that of fourteen days.

Table 1: Effects of daily administration of aqueous extract of *Nauclea latifolia* stem for fourteen days on rat serum urea, creatinine and MDA

Treatments	Urea (mmol L^{-1})	Creatinine (mmol L^{-1})	MDA (μmol L^{-1})
Control	10.30±0.41^{a}	41.00±4.50^{a}	49.70±0.50^{a}
200 mg kg^{-1}	7.80±0.16^{b}	5.50±0.44^{b}	49.50±0.47^{a}
400 mg kg^{-1}	7.25±0.04^{b}	8.50±1.23^{c}	48.90±0.47^{a}
600 mg kg^{-1}	7.65±0.20^{b}	15.50±2.82^{d}	48.89±0.50^{a}
800 mg kg^{-1}	7.80±0.08^{b}	35.00±1.71^{e}	49.12±0.50^{a}

Values are Mean±SD (n = 6), Column with different superscripts are significantly different at $p<0.05$

Table 2: Effects of daily administration of aqueous extract of *Nauclea latifolia* stem for twenty eight days on rat serum urea, creatinine and MDA

Treatments	Urea (mmol L^{-1})	Creatinine (mmol L^{-1})	MDA (μmol L^{-1})
Control	21.00±1.91^{a}	55.00±4.50^{a}	51.70±2.50^{a}
200 mg kg^{-1}	17.60±0.26^{b}	16.50±1.44^{b}	50.00±1.47^{a}
400 mg kg^{-1}	18.23±1.40^{b}	19.00±1.23^{c}	49.00±1.47^{a}
600 mg kg^{-1}	17.55±0.90^{b}	19.50±2.80^{d}	50.89±1.50^{a}
800 mg kg^{-1}	16.80±1.08^{b}	40.11±2.77^{e}	51.12±1.50^{a}

Values are Mean±SD (n = 6), Column with different superscripts are significantly different at $p<0.05$

Table 3 and 4, respectively represent the effects of daily administration of aqueous extract of *Nauclea latifolia* stem for fourteen and twenty eight days at varied concentration on rat serum lipid profile. Table 3 shows that repeated administration of the extract for fourteen days significantly increased ($p<0.05$) LDL-cholesterol and triacylglycerol concentration in the serum but significantly decreased ($p<0.05$) serum level of total cholesterol and HDL-cholesterol when compared to control in a pattern that is concentration dependent.

The trend of the results obtained following the daily administration of aqueous extract of *Nauclea latifolia* stem for twenty eight days (Table 4) is similar to that of fourteen days administration of the extract (Table 3). However, administration of the extract for twenty eight days further increased LDL-cholesterol and triacylglycerol concentration in the serum.

Figure 1-6 reveal the activities of some enzymes in the serum, liver and kidney of rats following the daily administration of aqueous extracts of *Nauclea latifolia* stem at the dose levels of 200, 400, 600 and 800 mg kg^{-1} b.wt. for fourteen and twenty eight days, respectively. Figure 1 illustrates the effect of 14 days administration of the extract on serum, liver and kidney alkaline phosphatase activities. The activity of the enzyme decreased significantly ($p<0.05$) in the liver and kidney with a relative increase ($p<0.05$) in serum activity of the enzyme in a concentration dependent manner.

The activity of the enzyme decreased significantly ($p<0.05$) in the liver and kidney following twenty eight days administration of the extract at 200 and 400 mg kg^{-1} b.wt. and then increased significantly ($p<0.05$) afterwards (Fig. 2). At 800 mg kg^{-1} b.wt., the activity of the enzyme was significantly higher ($p<0.05$) in the liver when compared with the control. The serum activity of the enzyme increased significantly when compared with the control.

Table 3: Effects of daily administration of aqueous extract of *Nauclea latifolia* stem for fourteen days on rat serum lipid profile

Treatments	Total-cholesterol (mmol L^{-1})	HDL-cholesterol (mmol L^{-1})	LDL-cholesterol (mmol L^{-1})	TAG (μmol L^{-1})
Control	120.11±10.11^{a}	96.30±8.80^{a}	20.50±1.22^{a}	111.10±10.50^{a}
200 mg kg^{-1}	81.10±6.50^{b}	70.11±6.20^{b}	30.11±2.11^{b}	120.00±10.47^{b}
400 mg kg^{-1}	64.56±4.91^{c}	70.55±6.00^{b}	33.12±2.00^{b}	123.50±11.07^{b}
600 mg kg^{-1}	58.10±4.21^{d}	50.21±4.21^{c}	42.19±2.78^{c}	136.99±10.59^{c}
800 mg kg^{-1}	33.49±2.33^{e}	26.35±1.33^{d}	53.22±3.44^{d}	141.12±10.50^{d}

Values are Mean±SD (n = 6), Column with different superscripts are significantly different at $p<0.05$

Table 4: Effects of daily administration of aqueous extract of *Nauclea latifolia* stem for twenty eight days on rat serum lipid profile

Treatments	Total-cholesterol (mmol L^{-1})	HDL-cholesterol (mmol L^{-1})	LDL-cholesterol (mmol L^{-1})	TAG (μmol L^{-1})
Control	135.11±10.11^{a}	106.30±8.80^{a}	31.50±1.22^{a}	121.10±11.50^{a}
200 mg kg^{-1}	96.10±6.50^{b}	84.11±6.20^{b}	43.11±2.11^{b}	130.00±9.47^{b}
400 mg kg^{-1}	77.56±4.91^{c}	83.55±6.00^{b}	44.12±2.00^{b}	133.50±10.07^{b}
600 mg kg^{-1}	68.10±4.21^{d}	61.21±4.21^{c}	55.19±2.78^{c}	148.89±10.39^{c}
800 mg kg^{-1}	44.49±2.33^{e}	35.35±1.33^{d}	66.22±3.44^{d}	155.10±11.51^{d}

Values are Mean±SD (n = 6), Column with different superscripts are significantly different at $p<0.05$

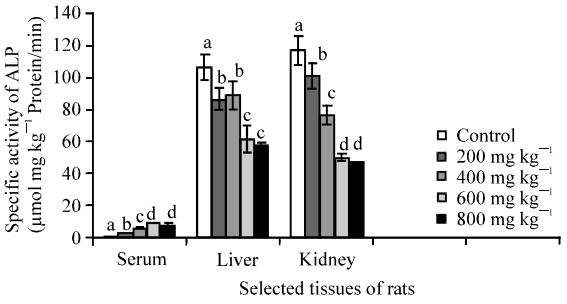

Fig. 1: Specific activity of alkaline phosphatase in selected rat tissues following the daily administration of aqueous extract of *Nauclea latifolia* stem for 14 days, Values are Mean±SD (n = 6) (bars with different superscripts are significantly different at p<0.05)

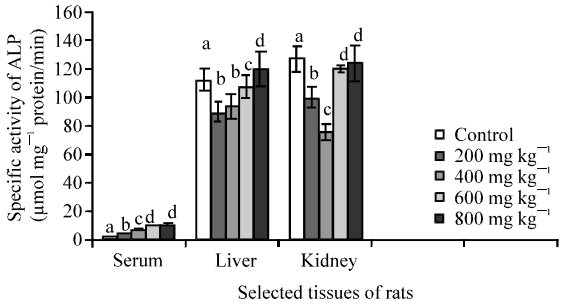

Fig. 2: Specific activity of alkaline phosphatase in selected rat tissues following the daily administration of aqueous extract of *Nauclea latifolia* stem for 28 days, Values are Mean ±SD (n = 6) (bars with different superscripts are significantly different at p<0.05)

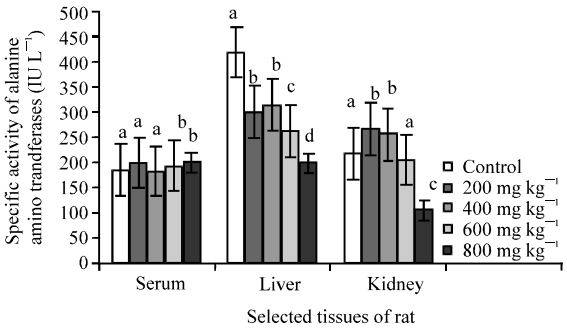

Fig. 3: Specific activity of alanine amino transferase in selected rat tissues following the daily administration of aqueous extract of *Nauclea latifolia* stem for 14 days, Values are Mean±SD (n = 6) (bars with different superscripts are significantly different at p<0.05)

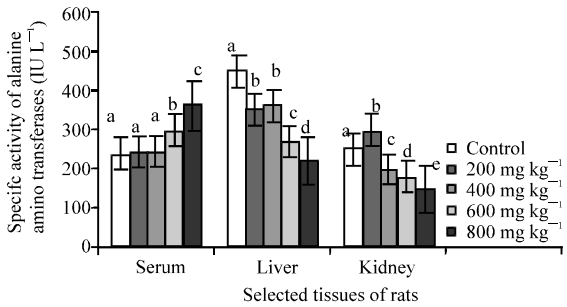

Fig. 4: Specific activity of alanine amino transferase in selected rat tissues following the daily administration of aqueous extract of *Nauclea latifolia* stem for 28 days, Values are Mean ±SD (n = 6) (bars with different superscripts are significantly different at p<0.05)

Figure 3 represents the effect of 14 days administration of the extract on alanine aminotransferase activity of the tissues studied. The liver activity of the enzyme decreased significantly (p<0.05) at all levels of treatment when compared with the control. The activity of the enzyme increased significantly (p<0.05) following the administration of the extract at 200 and 400 mg kg^{-1} b.wt. but dropped significantly (p<0.05) at a concentration of 800 mg kg^{-1} b.wt. when compared with the control. There was no significant difference (p>0.05) in the serum ALT activity of all the treatment groups except at 800 mg kg^{-1} b.wt. where the activity increased significantly (p<0.05).

The effect of twenty eight days administration of the extract on alanine aminotransferase activity of liver and kidney (Fig. 4) gave similar patterns of results to that of 14 days administration (Fig. 3). At 200 mg kg^{-1} b.wt. of the extract, the kidney ALT activity increased significantly (p<0.05) but decreased significantly (p<0.05) afterward when compared with the control. Serum activity of the enzyme increased significantly (p<0.05) following administration of 400 and 800 mg kg^{-1} b.wt. of the extract for twenty eight days.

The liver and kidney aspartate aminotransferase activity decreased significantly (p<0.05) at all levels of treatment except for kidney at 200 mg kg^{-1} b.wt. which increased significantly (p<0.05) when compared with the control following 14 days administration of the aqueous extract of *N. latifolia* (Fig. 5). The serum AST activity was significantly elevated only at 600 and 800 mg kg^{-1} b. wt. of the extract following the 14 days administration.

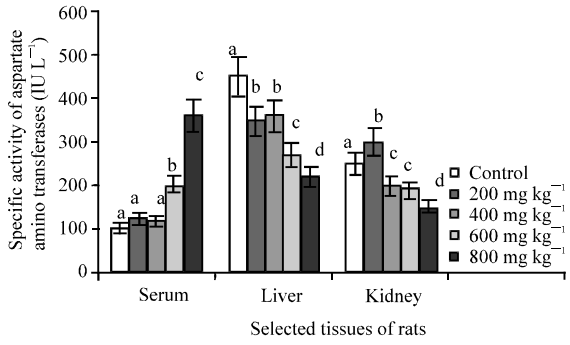

Fig. 5: Specific activity of aspartate amino transferase in selected rat tissues following the daily administration of aqueous extract of *Nauclea latifolia* stem for 14 days, Values are Mean±SD (n = 6) (bars with different superscripts are significantly different at $p<0.05$)

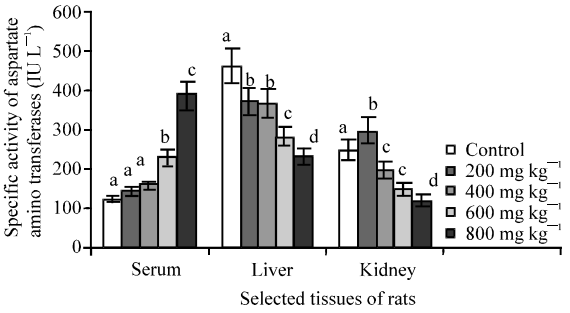

Fig. 6: Specific activity of aspartate amino transferase in selected rat tissues following the daily administration of aqueous extract of *Nauclea latifolia* stem for 28 days, Values are Mean±SD (n = 6) (bars with different superscripts are significantly different at $p<0.05$)

Figure 6 represents the effect of 28 days administration of the extract at varied concentrations on serum, liver and kidney AST activities. Administration of the extracts for 28 days gave a similar pattern of results for liver, kidney and serum AST activity as obtained in Fig. 5.

DISCUSSION

The kidney plays a very important role in removal of metabolic wastes from the blood stream. Its functionality therefore can be assessed among many others by determining the serum concentration of excretory constituents (Spencer *et al*., 2011). The significant decrease in serum urea and creatinine levels in all the groups for both fourteen and twenty eight days period of treatment may be suggestive of an alteration in the secretory and excretory functions of the kidney (Stroev and Makarova, 1989) caused by the extract of *Nauclea latifolia* stem. However, the extract appears to have little or no effect on lipid peroxidation of the kidney, as was observed by its effect on malondialdehyde level. The reduction in serum urea concentration by the extract may be suggestive of liver dysfunction rather than kidney dysfunction. This may result from the interference of the components of the extract with the process of getting rid of the ammonia produced from amino acid catabolism in the liver (Whealton *et al*., 1994). Another possible explanation is that urea may be converted to other products undetectable by the direct method of urea determination used in this study.

LDL cholesterol is often designated "bad" cholesterol since high levels of it in the plasma are linked with increased deposition of cholesterol in the arterial walls (Vander *et al*., 1998). HDLs serve as acceptors of cholesterol from various tissues. They promote the removal of cholesterol from cells and its secretion into the bile by the liver (Vander *et al*., 1998). The best single indicator of the likelihood of developing atherosclerotic heart disease is not total plasma cholesterol but rather the ratio of plasma LDL cholesterol to plasma HDL-cholesterol. The reduction in serum total cholesterol concentration following the repeated and prolonged administration of the extract is in agreement with the report of Obianime and Aprioku (2009). This will normally suggest a beneficial effect in pathological conditions. The increase in TAG and LDL cholesterol may predispose the liver to pathological risk (Belal, 2011). Increase in TAG following administration of the extract could be due to decrease lipolysis caused by the extract. Low density lipoprotein (LDLs) transport cholesterol from its site of synthesis in the liver to the various tissues and body cells where it is separated and used by the cells. HDLs on the other hand, transport excess or unused cholesterol from the tissues back to the liver, where it is broken down to bile acids and then excreted this make HDL beneficial to health. The significant reduction in HDL concentration therefore may impact negatively on the function of the liver. The lower the ratio, the lower the risk (Udoh, 1998). The higher ratio obtained here may be suggesting that the extract may not be safe for patients with heart related diseases.

The significant increase in serum alkaline phosphatase (ALP) activity of all treatment groups for fourteen and twenty eight days is suggestive of a possible damage to tissue cell plasma membrane by the aqueous extract of *Nauclea latifolia* stem, thus leading to leakage of membrane component into extracellular fluid (Akanji *et al*., 1993). This is further supported by the

reduction in alkaline phosphatase (ALP) activity in the liver and kidney. This may imply loss of the enzyme to extracellular fluids or the inactivation of the enzyme *in situ* by the components of the extract and their metabolites. The membrane damage done to the kidney and liver by the extract had started manifesting through the serum elevation of cellular enzymes (AST and ALT).

Alanine amino transferase (ALT) activities in the blood are increased in conditions in which cells are damaged or dead (Jimoh and Odutuga, 2001). Elevation of alanine amino transferase and aspartate amino transferase (ALT and AST) activities in the serum and reduction in the liver and kidney of treatment groups following fourteen and twenty eight days administration may indicate derangement of hepatic cytosolic content and interference with kidney amino acid metabolism (Malomo *et al*., 1995). This coupled with reduction in serum urea and creatinine concentration, still point to the fact that the extract may adversely interfere with amino acid metabolism of the liver in a time and concentration dependent manner.

In conclusion, the study revealed that the repeated administration of the extract of *N. latifolia* at varying doses brought about decrease in urea and cholesterol levels implying liver dysfunction and compromise of secretory and excretory functions of the kidney. The significant changes in Alkaline Phosphatase (ALP), alanine aminotransferase (ALT) and aspartate aminotransferase (AST) activities in various tissues studied suggested that prolonged administration of the extract at these doses may cause damage to hepatic and renal cells as well as disrupt amino acid metabolism. It is evident from this investigation that repeated administration of aqueous extract of *Nauclea latifolia* stem may lead to damage in the kidney and liver.

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