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Effects of Aqueous Extract of a Combination of Nauclea Latifolia Root and Acalypha Torta Leaves on Liver, Kidney and Heart of Wistar Albino Rat

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Abstract
Aim: *Although herbal remedies are natural, they can cause some serious damaging effects on the vital organs of the body due to deficiencies in standardization and safety regulations. The aim of this study is to investigate the toxicological effects of orally administered aqueous extract of a combination of N. latifolia root and A. torta leaves mixed in the ratio of 5:1 respectively on the liver, kidney and heart of rat.*
Materials and Methods: *The dry root and leaf samples of N. latifolia and A. torta respectively were collected, pulverized and mixed in the ratio of 5:1 respectively. Aqueous extract of the mixture was obtained after soaking in water for 24 hours and evaporating the filtrate to dryness using Rotary Evaporator. Sub-chronic toxicities on the liver, kidney and heart were assessed following oral administration of the extract at the doses of 50.0 and 100.0mg/kg body weight for 90 days in Wistar albino rats. All the indices of liver, kidney and heart functions were measured using Randox Diagnostic Test Kits following the manufacturer's method. A total of twenty-seven rats (120-150g) were used.*
Results: *Results of the sub-chronic toxicity showed no adverse effects on the liver, kidney and heart of the experimental rats since there was no significant differences ($p>0.05$) in the serum levels of alanine aminotransferase (ALT), aspartate aminotransferase (AST), alkaline phosphatase (ALP), total bilirubin(TBill), creatinine, urea and lactate dehydrogenase 1 (LDh1) activity. Histopathological examination of these organs showed no organ-system perturbation with normal architectures in the liver, kidney and heart.* ***Conclusion:*** *These findings may suggest that these plants at the doses tested were well tolerated by the animals with little or no deleterious effects.*

Keywords: *Nauclea latifolia, Acalypha torta, Histopathology, Toxicity*

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I. Introduction

Plant- derived medicines popularly known as herbal drug or phytomedicine is recognized as the most common form of alternative medicine. Almost 65% of the world's populations have incorporated traditional medicine (mainly herbs) into their primary modality of health care (Fabricant and Farnsworth, 2001). Many people in developing countries, particularly those in rural areas, have more access to traditional than modern medicines and use them more frequently for primary healthcare. African Traditional Medicine (ATM) is the mainstay of primary health care for the majority of those in the rural areas in Africa and up to 80% of the population uses traditional medicine for primary healthcare (WHO, 2003). WHO Expert Committee support the appropriate use of herbal medicine and encourages the use of those that have been certified safe and effective. However in rural areas these drug are produced by traditional herbalists who lack the formal education and only use crude knowledge and experience to restore health and only few of these preparations have been tested and evaluated scientifically for safety. Therefore there is a need for a scientific evaluation for safety of these herbal medicines.

II. Materials And Method

PLANT MATERIALS: Roots of *Nauclea latifolia* and leaves of *Acalypha torta* were collected from Nnamdi Azikiwe University school environment and Abagana respectively in Anambra State Nigeria. They were identified and authenticated in the Department of Botany, Nnamdi Azikiwe University, Awka.
ANIMALS: Mature albino rats weighing between 120-150g were purchased from Chris Animal Farm, Mgbakwu, Awka and used for this study. The animals were housed in the animal facility of the Department of Applied Biochemistry, Nnamdi Azikiwe University, Awka, They were allowed to acclimatize for 7days at room

temperature with adequate ventilation. The animals were also fed a certified feed (guinea growers mesh pellet) obtained from Eke-Awka market, Awka and had free access to clean water *ad libitum*.

CHEMICALS: All the chemicals used were of analytical grade and these included chloroform, formalsaline from BDH Limited, Poole, England; Randox kits from USA whereas paraffin, haematoxylin and eosin dyes were made by May and Baker Limited, Dagenham, England.

AQUEOUS EXTRACTS: Eight hundred and fifty grams (850g) of *Nauclea latifolia* and seven hundred and fifty grams (750g) of *Acalypha torta* were mixed and soaked with 4litres of distilled water and this was allowed to stand for 24hours with intermittent occasional stirring, after which Whatman No.1 filter paper was used to filter and then placed in a rotary evaporator under reduced pressure and temperature below 40°C to obtain the aqueous extract as a semi-solid residue. The yield was then calculated and the extract stored in the freezer at -2°C until used

Sub-Chronic Toxicity: Twenty seven (27) male albino rats weighing between 120g-150g were used and allowed to acclimatize to the laboratory condition for 7days and maintained on standard animal feed. The animals were weighed and divided into 3 groups of 9 rats each. The control group was fed the normal feed only while the test groups (2 and 3) were given 50mg/kg and 100mg/kg respectively of the aqueous extract. The extract was adminstered orally via a canular once a day for 3 months (90 days).

At the end of each month, 3 animals from each group were anaesthetized using chloroform swab. Blood was collected by heart puncture into plain sample tubes and allowed to clot. After centrifugation, the serum obtained was used for biochemical parameters determinations. The organs were collected carefully and fixed in 10% formalsaline. The organ sections were trimmed, processed and stained with haematoxylin and eosin (HE) stains for microscopic examination.

ANIMAL GROUPING

Group 1 = Normal feed
Groups 2 = 50mg/kg extract
Groups 3 = 100mg/kg extract

BIOCHEMICAL ASSAYS

Assay kits used for the biochemical assays were obtained from Randox Laboratories Ltd., Admore Diamond Road, Crumlin, Co., Antrim, UK Qt 94QY. Aspartate aminotransferase (AST), and Alanine aminotransferase (ALT) were determined using Reitman and Frankel method, (1956). Blood urea content was determined by the method described by Fawcett and Scott (1960). Creatinine was determined by the colorimetric method described by Bartels and Bohmer (1972).Total bilirubin was determined by the colorimetric method described by Jendrassik and Grof (1938). Alkaline phosphatase (ALP) and Lactate dehydrogenase 1 (LDH1) were both determined by the method of Rec (1972).

STATISTICAL ANALYSIS: Results of the study were expressed as mean± standard deviation. Differences between mean of the treated groups and their control in this animal studies were analyzed using Analysis of Variance(ANOVA) of SPSS 16.0 spread sheet statistical package. Values were taken to be significant at ($p<0.05$).

III. Results

BIOCHEMICAL STUDY

The results in Table 1 showed that alanine aminotransferase (ALT) activity decreased significantly ($p<0.05$) only in the first month between the control and treatment groups, No difference was observed in the remaining months. However, on the AST activity a significant decrease was observed between the treatment groups on the third month($P<0.05$). A significant increase ($P<0.05$) was seen in the Alkaline phosphatase activity in Group B when compared to the Control. A significant decrease was seen in Group C only in the second month when compared to Group B but no significant difference was observed in the third month. There was a significant decrease ($p<0.05$) in bilirubin level in group C compared to the control and between the Groups in both the first and second month, however, no significant difference ($p>0.05$) was observed in the third month. Data obtained from the kidney functions shows a significant increase ($p<0.05$) in urea level in the first and second month only in Group C as compared to the control but no significant difference was observed in the third month while the creatinine level was seen to have a significant decrease ($p<0.05$) in the second month as compared to the control in Group C, and not significant in the third month**.** The evaluation of the effects of the aqueous combination of the extract on the Heart function enzyme indicated that the lactate dehydrogenase 1 showed a significant increase ($P<0.05$) between the treatment groups and the control group but no significant difference existed between the treatment groups.This is summarized in the table 1 below.

Table1: Effects of extract on liver, kidney and heart indices.

Photomicrographs of the histopathological analysis of the liver, kidney and Heart of experimental animals are shown in FIG 1 to 3 below. The results of the histopathological analysis of the organs of the experimental animals showed no organ system perturbations for both the treatment and control groups.

ALT	**GROUP A**	**GROUP B**	**GROUP C**
1 MONTH	28.06±1.97	16.13±3.55	17.73±1.527
2 MONTH	47.86±6.43	46.33±12.36	45.46±12.39
3 MONTH	78.13±9.79	80.46±100.90	63.0±10.13
AST			
1 MONTH	81.66±5.48	79.33±6.82	77.7±17.088
2 MONTH	78.33±11.29	122.83±29.76	107±29.46
3 MONTH	111.5±26.83	127.5±6.06	103.3±2.08
ALP			
1 MONTH	617.3±56.55	623.73±54.38	758.77±81.76
2 MONTH	605.37±40.2	797.65±25.28	669.53±52.75
3 MONTH	680.8±23.49	793.7±33.52	785.7±145.05
TBILL			
1 MONTH	1.14±0.006	1.18±0.099	0.652±0.04
2 MONTH	1.139±0.03	1.37±0.211	0.569±0.112
3 MONTH	1.04±0.20	0.84±0.76	0.731±0.104
UREA			
1 MONTH	56.32±5.34	71.95±8.06	74.64±5.75
2 MONTH	60.4±3.88	71.14±18.37	80.47±7.51
3 MONTH	63.48±4.13	65.97±17.18	79.62±7.78
CREATININE			
1 MONTH	0.84±0.07	0.84±0.04	0.87±0.11
2 MONTH	0.763±0.05	0.811±0.03	0.39±0.07
3 MONTH	0.81±0.14	1.103±0.71	1.103±0.71
LDH1			
1 MONTH	167.30±12.37	283.34±21.39	369.67±99.98
2 MONTH	180.77±12.42	175.2±16.60	140.58±38.57
3 MONTH	179.77±10.80	110.73±17.01	132.35±4.565

HISTOPATHOLOGY

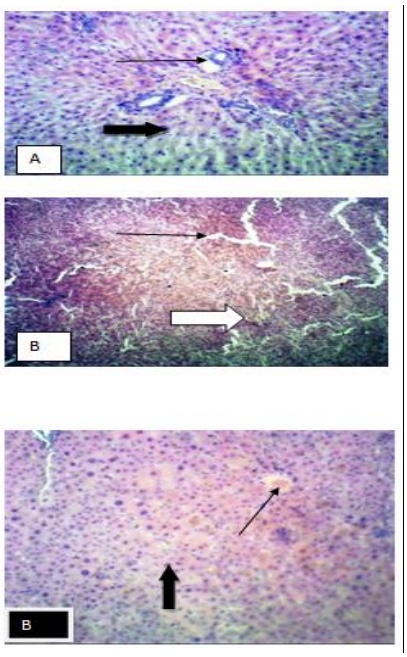

FIG1: Histological features showing normal liver tissue. A (control), B (group B) and C(GroupC) (H and E,staining;x300).The arrow( ) indicates a portal tracts.( ) shows an arrow pointing at one terminal hepatic venule. With a well preserved lobular architecture, normal hepatocytes, normal central vein, capsules with no indication of adhesion or inflammation.

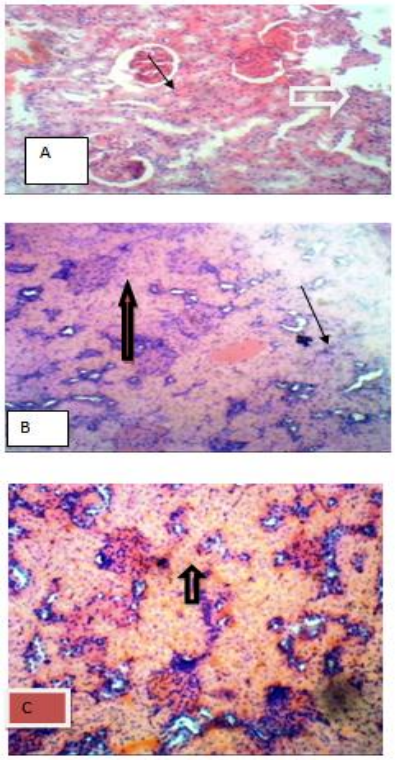

FIG 2. Histological features showing a well-developed kidney A(control), B(group B) and C(Group C) (H and E,staining;x300). ( ) showing Renal cortex harboring healthy-looking glomeruli surrounded by inconspicuous renal tubules.( )shows normal tubular structures with a focal vascular congestion.

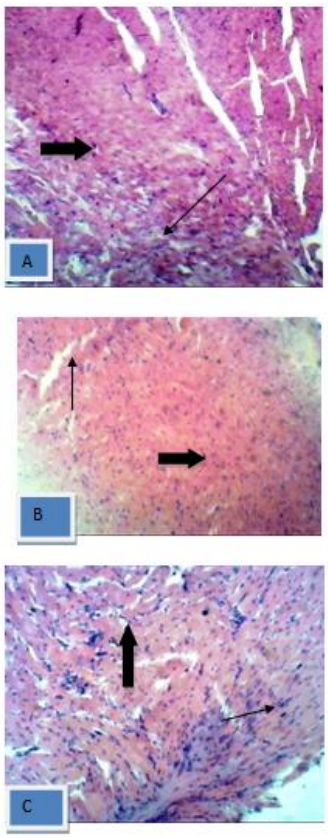

FIG 3 Histological features showing a well-developed Heart section through the right ventricular wall A(control) B(group B) and C(Group C) (H and E,staining;x300). ( ) showing normal collagen distribution in the myocardium.( )shows plump ovoid nuclei

IV. Discussion

The liver plays a key role in the metabolic process of itself as well as other tissues in maintaining the internal environment. Hepatic injury due to some toxic phytochemicals found in medicinal plants and failure to eliminate this metabolic product by the liver often results in marked distortion of the normal function of the liver (Geidam et al.,2004).AST and ALT in the serum are often associated with hepatocellular damage(Lyoussi et al.,2004) however ALT is the major marker for liver function because it is found in higher concentration in the hepatocyte (crook, 2006). One may suggest that the extract after the 3rd month did not have any marked effect on the Liver.

ALP is a sensitive detector in billiary cirrhosis, hepatitis and in disease characterized by inflammation, regeneration, intrahepatic and extrahepatic bile obstruction (Mayne,1994). A significant increase in the alkaline phosphatase activity was observed in Group B administered with 50mg/kg in both the second and third month and in Group C administered with 100mg/kg in the second month, the reason for the increase in ALP is not yet certain, One can suggest that the combined extract might cause hepatic obstruction.

Serum bilirubin levels could be expressed as Total bilirubin comprising of conjugated and non-conjugated or as direct bilirubin comprising only of the conjugated bilirubin. An increase in bilirubin level could be attributed to three major causes such as hemolysis, biliary obstruction and liver cell necrosis resulting to jaundice and its occurs in toxic or infectious diseases of the liver such as hepatitis or bile obstruction (Edem and Usoh,2009).The increase in total bilirubin level observed in Group C in the First and second month could be dose dependent and might not have deleterious effect on the liver integrity. The kidneys are highly vascularized compound tubular glands that function to maintain the composition of body fluids at a constant level and to remove excretory wastes (William and Linda, 2000). The kidney function assessed by evaluating the level of creatinine and urea in the serum, the result indicate no significant difference ($p>0.05$) in the third month compared to the control, suggesting that the aqueous combination of these extract did not have any deleterious effects on the kidney function and filtration mechanism.The heart function was assessed by monitoring the activity of lactate dehydrogenase 1 and aspartate aminotransaminase activity. A significant increase ($p<0.05$) was seen between the treatment groups and control in the third month on the LDH1, this could be dose dependent and may be of no clinical consequence. No significant difference ($p>0.05$) was seen on the AST activity in the treatment groups when compared to the control, but a significant decrease was seen to exist in Group C as compared to Group B in the third month. The histological examination is the golden standard for evaluating treatment related pathological changes in tissues and organs (OECD, 2005). In the present study, histopathological evaluation of organs obtained from rats orally administered with an aqueous combination of *Nauclea latifolia* root and *Acalypha torta* leaves extract indicated that the extract did not distort neither the morphology, texture or appearance of the rats organs.50mg/kg and 100mg/kg of the extracts was well tolerated by the treated rats and no sign of toxicity towards the organs as there were no organ system perturbations.

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