Research Article

Acute and Subchronic Evaluation of Aqueous Extracts of *Newbouldia laevis* (Bignoniaceae) and *Nauclea latifolia* (Rubiaceae) Roots used Singly or in Combination in Nigerian Traditional Medicines

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Abstract: This study was aimed at evaluating the acute and subchronic toxicities of aqueous extracts of *Newbouldia laevis* stem bark and *Nauclea latifolia* roots, used extensively, in Nigerian herbal medicine. Acute toxicity study was carried out on Swiss albino mice. The extracts mixed (1:1) in the doses ranging between 1.0 g to 20.0 g/kg body weight (bwt) were administered orally to the mice (22.5 ± 2.5 g) and observed continuously for the first 4 h and hourly for the next 12 h, then 6 hourly for 56 h. In subchronic toxicity study, wistar rats (150 ± 20 g) were fed with 100, 250 and 500 mg/kg bwt daily of the extract mixture (1:1) combination and 500 mg/kg bwt dose of the respective extracts for 30 days. The effects on the biochemical and haematological parameters were evaluated and also the effects on some vital organs were histologically examined. The results showed that all the animals that received 20 g/kg bwt of the extracts (1:1) combination survived beyond 24 h. Significant (p<0.05) decrease in the plasma glucose level occurred. Lipid profile study showed insignificant increase in total cholesterol (T Chol), High Density Lipoprotein (HDL) and Triglycerides (TG) levels compared to the control while low density lipoprotein (LDL) level decreased appreciably. Aspartate Aminotransferases (AST), Alanine Aminotransferases (ALT) and creatinine levels decreased significant (p<0.05). The photomicrographs of hepatic, kidney and testicular tissues treated with 500 mg/kg bwt of the respective extracts and 500 mg/kg bwt of the combination indicated no abnormalities. The extracts could be considered safe at the doses administered since they did not provoke toxic effect on the key organs examined.

Keywords: Acute, *Newbouldia laevis*, *Nauclea latifolia*, subchronic, toxicity

INTRODUCTION

Plants and their derived products from the outset have served as veritable sources of food for humans and animals. The discovery since prehistoric era that plants products, in addition to their nutritive values, could serve as therapeutic weapons against various human, animal and even plant diseases has made plants a sine qua non to human and animal lives (Ogbonnia et al., 2008; 2011a). Plant derived medicines popularly known as “Herbal drug” or “phytomedicine” is currently renowned and is recognized as the most common form of alternative medicine. It is used by about 60% of the world population both in the developing and in the developed countries where modern medicines are predominantly used (Rickert et al., 1999; Ogbonnia et al., 2009).

The use of herbal remedies especially in the form of teas or extracts for the treatment of various diseases is gaining increasing popularity, making them the mainstay of health care system, especially among the rural populace in the developing countries. Their increasing popularity could be attributed to their advantages of being efficacious and a cheap source of medical care. WHO Expert Committee (1985) supports the appropriate use of herbal medicines and encourages the use of herbal drugs that have been proven to be safe and effective. Herbal medicines most often in the developing countries are prepared by the traditional herbalists many without formal education and are simply used on the basis of traditional knowledge and experience to protect, restore or improve health. Only a few of these preparations have been tested and evaluated scientifically for safety. There is therefore the need for a scientific evaluation of herbal medicines’ for safety, in addition to the experience gathered from their traditional use over the years.
Secondly, there is a growing disillusionment with modern medicine and also misconception that herbal remedy being natural may be devoid of adverse and toxic effects often associated with allopathic medicines (Ogbonnia et al., 2011b). Herbal preparations could be contaminated with microbiological and foreign materials, such as heavy metals, pesticide residues or even aflatoxins. Contaminants when present in an herbal preparation may give it the capacity to produce prominent health defects underscoring the claimed safety. An increase in the morbidity and mortality associated with the use of herbal or the so called traditional medicines has raised universal attention in the last few years (Bandaranayake, 2006; Ogbonnia et al., 2011b). Upon exposure, the clinical toxicity may vary from mild to severe and even life threatening making the safety and toxicity evaluations of these preparations imperative.

Herbal recipes are prepared most often from a combination of two or more plant products which many a time may contain active constituents with multiple physiological activities and could be used in the treatment of a variety of disease conditions (Piem et al., 2006). Therefore, warning regarding the potential toxicity of herbal therapies employed in the treatment of diseases over a long period of time demands that the practitioners should be kept abreast of the reported incidence of renal and hepatic toxicity associated with the ingestion of medicinal herbs (Tédong et al., 2007). For a plant or herbal preparation containing active organic principles to be identified for use in the traditional medicine especially for long term treatment, a systemic approach is required for the evaluation of efficacy and safety through experiment and clinical findings (Mythilypriya et al., 2007).

The aim of this study was to evaluate the safety of a Newbouldia laevis (N. laevis) and Nauclea latifolia (N. latifolia) roots teas or extracts used singly or in combination by carrying out the acute and subchronic toxicity studies in animal model. Subchronic toxicity evaluation is required to establish potential adverse effects of these highly valuable herbal products that are now widely consumed for their physiological benefits.

MATERIALS AND METHODS

Plant materials: The plant materials, Newbouldia laevis (P. Beav.) Seem. ex Bureau (Fam. Bignoniaceae) root bark and Nauclea latifolia Smith (Fam. Rubiaceae) root bark were bought from Mushin market in Lagos suburban and were authenticated by Mr. B.O. Daramola of the Department of Botany and Microbiology, University of Lagos. The voucher specimens LUH 3595 and LUH 3756, respectively were deposited at the Department of Botany Herbarium.

Laboratory animal acquisition and maintenance: Male and female Wistar rats (weighing 150±20 g) and Swiss albino mice of both sexes (weighing 22.5±2.5 g) were used for this study. These animals were bred and housed in the Animal Facility Centre of the College of Medicine, University of Lagos, Iidi-Araba, Lagos and were separated and kept for one week to allow for acclimatization in a cage lined with wooden shaves, at room temperature with adequate ventilation, under a naturally illuminated environment with 12 h of light and 12 h of darkness. The animals were fed a certified feed (Feeds Nigeria Limited) and had free access to tap water but fasted overnight prior to oral administration of the extracts.

Preparation of plant extracts: The root barks of the plants were washed to remove foreign materials and then cut into smaller pieces for easy drying and blending. They were dried at an ambient temperature between 45-50°C in an oven for five days and powdered to coarse particles using a commercial blending machine. Four hundred and sixty grams (460 g) of the powdered N. laevis root and four hundred and twenty grams (420 g) of the powdered N. latifolia roots were, respectively boiled with 2.0 L distilled water for 25-30 min and allowed to decoct. After 24 h they were strained with cotton wool; the liquid extracts were then filtered using a filter paper (Whatman’s no. 4). The resulting filtrates were lyophilized yield 24.8 g (5.4 % yields) and 22.2 g (5.3 % yields) for N. laevis and N. latifolia extracts, respectively.

For each dose used, the volume administered was calculated using (Tédong et al., 2007) equation as follows: V (ML) = (D×P)/C. Where D = dose used (g/kg body weight), P = body weight (g), C = concentration (g/mL) and V = volume.

Acute toxicity study of the extracts mixture: Thirty five male and female Swiss albino mice weighing 22.5±2.5 g were used for the acute toxicity study. They were randomly distributed into one control group and six treated groups, containing five animals per group and were maintained on standard animal diet (Feeds Nigeria Limited) and provided with water ad libitum. They were allowed to acclimatize for seven days to the laboratory conditions before the experiment. After fasting the animals’ over-night, the control group received 0.4 mL of 2% w/v acacia suspension orally. Then the solution of the extract was prepared by dispersing 4.38 g of the mixture of the dried plant extracts containing an equal amount of each extract with 5 mL of 2% w/v acacia suspension and each treated group received the extract doses as follows: 1.0, 2.5, 5.0, 10.0, 15.0 and 20.0 mg/g body weight (bwt) orally. The animals were observed continuously for the first 4 h and for every hour for the next 12 h, then 6 hourly for 56 h after administering to observe any changes in general behaviour or other physiological activities (Shah et al., 1997; Bürger et al., 2005).
Subchronic toxicity study: Male and female Wistar albino rats weighing 150±20 g were used and were allowed to acclimatize to the laboratory conditions for seven days, maintained on standard animal feeds and provided with water ad libitum. The animals were weighed and divided into six groups of five animals each. The control group received a dose of 0.5 mL of acacia suspension and other groups received specified doses orally respectively once a day for 30 days (Pieme et al., 2006; Joshi et al., 2007; Mythilypriya et al., 2007).

Treatments were as follows:

- **Group I**: Extract A- *N. laevis*, at a dose of 500 mg/kg bwt.
- **Group II**: Extract B- *N. latifolia*, at a dose of 500 mg/kg bwt.
- **Group III**: (1:1) Mixture at a dose of 500 mg/kg b.wt.
- **Group IV**: (1:1) Mixture at a dose of 250 mg/kg b.wt.
- **Group V**: (1:1) Mixture at a dose of 100 mg/kg b.wt.
- **Group VI**: Normal rats were treated with 0.5 mL 2% w/v acacia suspension (positive control).

Evaluation of biochemical parameters: The initial weights of the animals were recorded. Thereafter, weights were taken at five days intervals from the beginning of the treatment (Ogbonnia et al., 2011a; 2011b). On the 31st day, after overnight fast, the animals were sacrificed under mild diethyl ether anaesthesia and blood was obtained via cardiac puncture into fluoride oxalate, heparinized and EDTA containers. The blood collected with fluoride oxalate tube was centrifuged within 5 min of collection at 4000 g for 10 min and plasma obtained was used to determine the blood glucose level. The Total Cholesterol (TC), Triglycerides (TG) and High Density Lipoprotein (HDL-cholesterol) levels and other biochemical parameters were estimated with heparinized blood using precipitation and modified enzymatic procedures from Sigma Diagnostics (Wasan et al., 2001). Low density lipoprotein (LDL-cholesterol) level was calculated using Friedwald equation (Crook, 2006). Plasma was analyzed for Alanine Amino Transferase (ALT) and Aspartate Amino Transferase (AST) and creatinine by standard enzymatic assay method. The Total Plasma Protein (TPP) content was determined using enzymatic spectroscopic methods (Hussain and Eshrat, 2002). The total bilirubin (T. BIL) was determined using Jandrassik and Grof technique as described by Koch and Doumas (1982). Plasma albumin was determined based on its reaction with bromocresol green (Binding method) as described by Spencer and Price (1971). Urea was determined according to Urease-Berthelot method as described by Weatherburn (1967). Alkaline Phosphate (ALP) was analyzed according to Kind technique as described by Koch and Doumas (1982).

Haematological study: The haematological parameter was determined using blood collected with EDTA containers (Ogbonnia et al., 2011a; 2011b). Diethyl ether was used to anaesthetize the animals before blood samples were collected through heart puncture into EDTA tubes for the haematological parameters analysis. The blood samples were analyzed for Red Blood Cells (RBC) by haemocytometric method (Dacie and Lewis, 1984); the haemoglobin (Hb) content was by Cyanmethaemoglobin (Drabkin) method (Dacie and Lewis, 1984); the haemoglobin (Hb) content was by Cyanmethaemoglobin (Drabkin) method (Dacie and Lewis, 1984); the haemoglobin (Hb) content was by Cyanmethaemoglobin (Drabkin) method (Dacie and Lewis, 1984); Packed Cell Volume (PCV) was according to Ekaidem et al. (2006) while White Blood Cells (WBC) and its differentials (neutrophil and lymphocyte) were determined as described by Dacie and Lewis (1984).

Tissue histology: The organs were fixed in 10% formal saline for seven days before embedding in paraffin wax. Each organ tissue of heart, kidney, liver and testis was sectioned at 5 µm and stained with Haematoxylin and Eosin (H and E) stain. The slide specimens were examined under light microscope at high power magnification for changes in organ architecture and photomicrographs were taken.

Data and statistical analysis: All data collected were summarized as mean±sem. The data for the subchronic toxicity test were statistically compared by using student t-test.

**RESULTS**

Acute toxicity test: The acute toxicity study (Table 1) recorded 100% survival beyond 24 h for all the animals that received 20.0 g/kg bwt of the extract. The median acute toxicity (LD₅₀) of the extract should therefore be over 20.0 g/kg bwt.

Variation of weights: The effect of the drug on the body weights of the control and treated animals is shown in Table 2 and the percentage increase in the

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Table 1: Acute toxicity of the (1:1) mixture of the plant extracts in mice

| Groups | Doses (g/kg) | Log dose | Number of animals | 24 h Mortality | % Mortality | Probit |
|--------|--------------|----------|------------------|----------------|-------------|-------|
| I      | 1.00         | 0.0000   | 5                | 0              | 0           | 0     |
| II     | 2.50         | 0.3940   | 5                | 0              | 0           | 0     |
| III    | 5.00         | 0.6990   | 5                | 0              | 0           | 0     |
| IV     | 10.0         | 1.0000   | 5                | 0              | 0           | 0     |
| V      | 15.0         | 1.1760   | 5                | 0              | 0           | 0     |
| VI     | 20.0         | 1.3010   | 5                | 0              | 0           | 0     |

Control received 0.4 mL each of 2% acacia suspension
weight of the treated animals compared to the control is shown in Fig. 1. Generally, there was insignificant (p>0.05) increase in the body weights of the treated animals compared to the control in the early days but from the 20th day a significant increase (p≤0.05) in weight in the group treated with 250 mg and significant decrease in the weight especially in the group treated with the highest dose of the mixture compared to the control was observed.

Biochemical parameters: Table 2 is a summary of the results on the biochemical parameters. The Blood Glucose Level (GLU) showed significant decrease in all the groups with the exception of the group that received the mixture at 250 mg/kg b.wt where the decrease was marginal. In the lipid profile study, there was insignificant increase in total cholesterol (T Chol), High Density Lipoprotein (HDL) and Triglycerides (TG) levels in the treated groups compared to the control while Low Density Lipoprotein (LDL) level decreased markedly in all the groups except for the group that received the extract mixture at doses of 100 and 250 mg/kg b.wt respectively where significant decrease (p<0.01) occurred.

There was significant decrease (p<0.05) in ALT, AST and creatinine levels in the treated animals compared to the control. On the other hand, Total Plasma Protein (TPP) showed marginal increase in all the treated animals. There was however insignificant (p>0.05) decrease in the Total Bilirubin (T. BIL), urea and Albumin (ALB) of the treated groups compared to the control while significant decrease in Alkaline Phosphate (ALP) level occurred in all the treated groups with the exception of the group treated with N. latifolia.

Haematological study: The effects of the extracts on the blood components and white blood cell differentials were presented in Table 3. Significant increases (p<0.05) were observed in the haemoglobin content and Red Blood Cells (RBC) in the groups that received: N. laevis and N. latifolia compared to the control while only marginal increase was noticed in the groups that received the mixtures. Significant increase in Packed Cell Volume (PCV) was noticeable only in the group that received N. laevis while marginal increase was observed in the other treated groups. The White Blood Cells (WBC) increased significantly in the group that received N. laevis while decreasing marginally in the other treated groups. Significant decrease (p<0.05) in the level of neutrophil was observed in the group that received N. latifolia and the mixtures at 250 and 500 mg/kg b.wt, respectively while marginal decrease occurred in the other treated groups. On the other hand, lymphocyte level showed marginal decrease in all the treated groups with the exception of the animal group that received the extract mixture at 250 mg/kg b.wt where marginal increase was observed. The platelet level increased significantly in all the treated groups with the exception of group that received N. laevis where it exhibited marginal decrease.

Histology results: Figure 2a, Normal heart: The photomicrograph of the untreated cardiac muscle in
Table 3: Effect of the individual extracts and their (1:1) mixture on hematological profile in control and treated rats after 30 days

| Groups | WBC×10^3 | RBC×10^6 | Hb (g/dL) | PCV (%) | PLT×10^3 | NEU (%) | LYMP (%) |
|--------|----------|----------|-----------|---------|----------|---------|----------|
| Group I | 16.1±0.7* | 7.3±0.1** | 13.3±0.2** | 41.1±0.5** | 53.8±5.6 | 33.3±1.6 | 55.8±1.6 |
| I group II | 12.7±0.6 | 7.8±0.1** | 13.8±0.1** | 34.6±0.4 | 70.8±2.2* | 21.4±0.4* | 59.5±2.3 |
| I group III | 10.8±0.4 | 6.9±0.1 | 12.9±0.1 | 35.7±0.4 | 56.2±2.3* | 27.5±1.3** | 56.5±1.5 |
| I group IV | 9.9±0.2 | 6.4±0.2 | 12.5±0.1 | 35.6±0.4 | 55.9±3.9** | 21.8±1.1* | 61.7±1.9 |
| I group V | 10.1±0.5 | 6.3±0.1 | 11.2±0.1 | 34.3±0.3 | 68.9±3.7* | 35.5±2.3 | 51.8±1.1 |
| I group VI | 13.0±0.5 | 6.2±0.2 | 11.0±0.1 | 32.5±0.4 | 54.0±2.3 | 36.1±1.7 | 59.9±1.4 |

Student t-test *Significant (p<0.05); **Significant (p<0.01); All values are represented as Mean±sem; Group I: N. laevis, (500 mg/kg b.wt) Group II: N. latifolia, (500 mg/kg b.wt) Group III: Mixture (1:1) (500 mg/kg b.wt), Group IV: Mixture (1:1) (250 mg/kg b.wt), Group V: Mixture (1:1) (100 mg/kg b.wt), Group VI: Control

Fig. 2: Normal heart×400 N. laevis 500 mg/kg ×400 N. latifolia 500 mg/kg×400 (1:1) Mixture 500 mg/kg×400

Fig. 3: Normal Kidney×400 N. laevis (500 mg/kg)×400 N. latifolia (500 mg/kg)×400 Mixture (500 mg/kg)×400

Fig. 4: Normal liver×400 N. laevis (500 mg/kg)×400 N. latifolia (500 mg/kg)×400 Mixture (1:1) (500 mg/kg)×400

longitudinal section demonstrated the normal arrangement of muscle fibres which branched to give the appearance of three dimensional networks. The nuclei, deeply stained were seen centrally situated within the muscle fibres. The photomicrographs Fig. 2b to d of cardiac tissues treated with N. laevis root extract, Nauclea latifolia root extract and the (1:1) mixture (500 mg/kg bwt) respectively showed no abnormal features.

Figure 3a, Normal Kidney: The photomicrograph of the untreated renal tissue showed the cortical area with the renal corpuscles which appeared as a dense rounded mass separated from surrounding structures by Bowman’s space. The photomicrographs Fig. 3b to d of the renal tissue treated with N. laevis root extract, N. latifolia root extract and the (1:1) mixture (500 mg/kg bwt), respectively showed no abnormal features.

Figure 4a, Normal liver: The photomicrograph showed hepatocytes radially arranged in columns converging from the lobular margins towards the central vein with each column interspaced by hepatic
primitive spermatogenic cell series actively differentiating while the tails of matured sperm cells showed wavy appearance at the lumina. Close to the germinal epithelium were more so close to the germinal epithelium were more primitive spermatogenic cell series actively differentiating while the tails of matured sperm cells show wavy appearance at the lumina. The photomicrographs Fig. 4b to d of hepatic tissues treated with N. laevis root extract, N. latifolia root extract and the (1:1) mixture (500 mg/kg bwt), respectively indicated no lesion.

Figure 5a, Normal Testis: The cyto-architecture of a cross section of normal testicular tissue illustrated the seminiferous tubules in transverse plane with distinct boundary. Close to the germinal epithelium were more primitive spermatogenic cell series actively differentiating while the tails of matured sperm cells show wavy appearance at the lumina. The photomicrographs Fig. 5b to d of hepatic tissues treated with N. laevis root extract, Nauclea latifolia root extract and the (1:1) mixture (500 mg/kg bwt), respectively showed no abnormal features in the seminiferous tubules as well as cellular interstitium.

**DISCUSSION**

Herbal medicines are now receiving greater attention as an alternative to clinical therapy leading to increase in their demands (Mythilypriya et al., 2007). In the rural communities of developing countries, the exclusive use of herbal drugs to treat various diseases is still very common and is prepared most often and dispensed by herbalists without formal training. Experimental screening method is therefore important in order to establish the active components present, ascertain the efficacy and safety of the herbal products (Chakarvath, 1993).

In the acute toxicity study of the (1:1) mixture of the extracts, no noticeable changes in the behavior and in the nervous system responses were observed in the treated animals. All the mice that received 20.0 g/kg body weight dose of the mixture survived beyond the 24 hour period of observation, therefore, suggesting the median acute toxicity value (LD50) of the mixture to be above 20.0 g/kg bwt. According to Ghosh (1984) and Klaasen et al. (1995), the mixture can be classified as being non toxic, since the LD50 by oral route was found to be above 15 g/kg body weight, which was also much higher than WHO toxicity index of 2 g/kg (Ogbonnia et al., 2011a, b). The viscera of the sacrificed animals did not show any macroscopic changes and since none of the survived animals convulsed it could be postulated that the mixture did not exert some effects on the nervous system (Ogwale, Okeng et al., 2003).

There were no significant (p≥0.05) changes in the body weight observed in the early days in all the treated animals compared to the control thus signifying that there was no stimulation or depression in the appetite. There was however, a significant decrease in the body weight from the day 20 which might have resulted from the suppression of appetite especially in the group treated with the highest dose of the mixture. A significant increase in weight was observed in the group treated with 250 mg/kg bwt which is attributable to unidentified factors.

The extracts and the mixture exhibited some hypoglycaemic activities having lowered significantly the plasma sugar levels supporting the use of the plants by the local herbalists in the treatment of diabetes. In lipid profile study, though total cholesterol and triglycerides exhibited marginal increase, there were however appreciable decrease in LDL level coupled with an increase in HDL level. Usually, a rise in lipid profile in particular LDL level is known to be predictive for coronary events such as atherosclerosis and coronary heart disease (Blake et al., 2002). In this case, however, where the LDL level decreased considerably, the extract might be said not to be harmful to the cardiovascular system. Moreso, the photomicrograph of cardiac tissue of the treated animals showed normal appearance.

The liver and kidneys have demonstrated to play crucial roles in various metabolic processes and are, therefore, particularly exposed to the toxic effects of exogenous compounds (Bihde and Ghosh, 2004). AST and ALT are common liver enzymes because of their higher concentrations in hepatocytes, but only ALT is remarkably specific for liver function (Crook, 2006). Therefore an elevation in plasma concentration of ALT is an indication of liver damage (Horton et al., 1996). AST is mostly present in the myocardium, skeletal muscle, brain and kidneys (McIntyre and Rosaki, 1987; Withhawaskul et al., 2003). Thus, the liver and heart release AST and ALT and an elevation in plasma concentration are an indicator of liver and heart damage (Wasan et al., 2001; Crook, 2006). In this study, a significant (p≤0.05) decrease in both ALT and AST values were observed in the animals treated with.

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**Fig. 5: Normal Testis×400**

The photomicrographs Fig. 4b to d of hepatic tissues treated with N. laevis root extract, N. latifolia root extract and the (1:1) mixture (500 mg/kg bwt), respectively indicated no lesion.
respective single doses of *N. laevis* extract and *N. latifolia* extracts and also in the animals treated with different doses of the mixture. This clearly demonstrated that the extracts and their mixture had neither nephrotoxic nor deleterious effects on the heart. Serum bilirubin levels could be expressed as total bilirubin comprising of conjugated and non-conjugated or as direct bilirubin comprising only of the conjugated and an increase in bilirubin level could be attributed to three major causes such as hemolysis, biliary obstruction and liver cell necrosis (Tilkian *et al.*, 1979). A decrease in bilirubin level observed in the animals that received *N. latifolia, N. laevis* and the different doses of the mixture was confirmatory that the extracts and their mixtures did not have deleterious effect on the liver. The increase in total protein level in all the treated animal groups could be linked to the protective effect of the extracts against oxidative damage to the liver. An increase in total protein level has been reported to have hepatoprotective effect (Oyagbemi *et al.*, 2008). Creatinine is excreted by glomerular filtration and the clearance is dependent on the rate at which it is removed from the blood by the kidneys, therefore, an increase in the plasma creatinine level suggests kidney damage specifically renal filtration mechanism (Wasan *et al.*, 2001). In this study, marked decrease in creatinine level occurred in all the treated groups indicating that the extracts might have facilitated renal filtration mechanism.

The RBC, Hb and PCV levels increased considerably particularly in the *N. laevis* extract treatment. An increase in the levels of these haematological parameters was indicative that the extracts have the potential to stimulate erythropoietin release in the kidney known to enhance RBC production (erythropoiesis) (Polenakovic and Sikole, 1996; Sanchez-Elsner *et al.*, 2004). Similar observation has been made on a number of plants (Sanchez-Elsner *et al.*, 2004; Mbaka *et al.*, 2010; Mbaka and Owolabi, 2011). The white blood cells serve as scavengers that destroy the microorganisms at infection sites, removing foreign substances and debris that results from dead or injured cells (Guilhermino *et al.*, 1998). Consequently, the level is known to rise as body defence in response to toxic environment (Ngogang, 2005). In this study, WBC count exhibited decrease in all the extract treated groups except in animals’ treatment with *N. laevis*. The decrease suggested that most of the extracts did not exhibit toxic effect even at high doses. The lymphocyte, the main effector cells of the immune system (Mc Knight *et al.*, 1999; Tédong *et al.*, 2007) was also observed to decrease appreciably indicating that the extracts did not exert challenge on the immune system of the treated animals. Marked increase in platelet level was however observed in all the treated animals with the exception of *N. laevis* treated. The reason for the increase could not be established.

**CONCLUSION**

The extracts were relatively safe with LD$_{50}$ over 20 g/kg bwt. The study showed that the different combinations of the extracts exhibited hypoglycaemic activity. They were also observed not to be harmful to the cardiovascular system due to considerable decrease in LDL level. The study also revealed that the extracts at doses administered did not provoke toxic effects to the animals’ liver instead they exhibited hepatoprotective effect. There was also no indication of kidney damage which was confirmatory from the renal tissue histology.

**REFERENCES**

Bandaranayake, W.M., 2006. Modern Phytomedicine: Turning Medicinal Plants into Drugs. In: Ahmad, I., Aqil F. and M. Owais (Eds.), Wiley-VCH Verlag GmbH and Co. KGaA, Weinheim.

Bihde, R.M. and S. Ghosh, 2004. Acute and subchronic (28-day) oral toxicity study in rats fed with novel surfactants. AAPS Pharm. Sci., 6(2): 1-10.

Blake, G.J., J.D. Otvos, N. Rifai and P.M. Ridker, 2002. Low-density lipoprotein particle concentration and size as determined by nuclear magnetic resonance spectroscopy as predictors of cardiovascular disease in women. Circulation, 106: 1930-1937.

Bürger, C., D.R. Fischer, D.A. Cordenunzzi, A.P. Batschauer de Borba, V.C. Filho and A.R. Soares dos Santos, 2005. Acute and subacute toxicity of the hydroalcoholic extract from Wedelia paludosa (Acmela brasiliensis) (Asteraceae) in mice. J. Pharm. Sci., 8(2): 370-3.

Chakarvath, B.K., 1993. Herbal medicines safety and efficacy guidelines. Regul. Affairs J., 4: 699-701.

Crook, M.A., 2006. Clinical Chemistry and Metabolic Medicine. 7th Edn., Hodder Arnold, London, pp: 426.

Dacie, J.C. and S.M. Lewis, 1984. Practical Hematology. 5th Edn., Churchill Livingstone, London.

Ekaidem, I.S., M.I. Akpanabiatu, F.E. Ubong and O.U. Eka, 2006. Vitamin B$_{12}$ supplementation: Effects on some biochemical and hematological indices of rats on phenytoin administration. Biochemistry, 1(18): 13-37.

Ghosh, M.N., 1984. Toxicity Studies. In: Fundamentals of Environmental Pharmacology. 2nd Edn., Scientific Book Agency, Culcutta, pp: 153-158.

Guilhermino, L., A.M. Soares., A.P. Carvalho and M.C. Lopes, 1998. Acute effects of 3, 4- Dichloroaniline on blood of male wistar rats. J. Chemosphere, 37: 619-632.

Horton, R., L.A. Moran, R. Ochs, J.D. Rawn and K.G. Scrimgeour, 1996. Principles of Biochemistry. 2nd Edn., Prentice Hall.
Ngogang, J.Y., A. Hussain and H.M. Eshrat, 2002. Hypoglycemic, hypolipidemic and antioxidant properties of combination of curcumin from *Curcuma longa* Linn and partially purified product from *Abroma augusta* Linn in streptozotocin induced diabetes. Indian J. Clin. Biochem., 17(2): 33-43.

Joshi, C.S., E.S. Priya and E.S. Venkataraman, 2007. Acute and subacute studies on the polyherbal antidiabetic formulation Diakyur in experimental animal model. J. Health Sci., 53(2): 245-249.

Klaasen, C.D., M.O. Amdur and J. Doull, 1995. *Methods of Clinical Chemistry*. American Ass. Clin. Chem., Washington, DC, 9: 113.

Mbaka, G.O., O.O. Adeyemi and A.A. Oremosu, 2010. Acute and sub-chronic toxicity studies of the ethanol extract of the leaves of *Sphenocentrum jollyanum* (menispermaceae). Agric. Biol. J. N. Am., 1(3): 265-272.

Mbaka, G.O. and M.A. Owolabi, 2011. Evaluation of haematonic activity and subchronic toxicity of *Sphenocentrum jollyanum* (menispermaceae) seed oil. Eur. J. Med. Plants, 1(4): 140-152.

McIntyre, N. and S. Rosaki, 1987. Investigations biochimiques des affections hépatiques. Pharmacie, pp: 294-309.

Mc Knight, D.C., R.G. Mills, J.J. Bray and P.A. Craig, 1999. *Human Physiology*. 4th Edn., Churchill, Livingstone, pp: 290-294.

Mythilypriya, R., P. Shanthi and P. Sachdanandam, 2007. Oral acute and subacute toxicity studies with Kalpaamruthaa a modified indigenous preparation on rats. J. Health Sc., 53(4): 351-358.

Ngogang, J.Y., 2005. Haematonic activity of *Hibiscus cannabinus*. Afr. J. Biotech., 4: 833-837.

Ogbonnia, S.O., G.O. Mbaka, E.N. Anyika, J.E. Emordi and N. Nwakakwa, 2011b. An evaluation of acute and subchronic toxicities of a Nigerian polyherbal tea remedy. Pak. J. Nutr., 10 (11): 1022-1028.

Ogil-Okeng, W.J., C. Obua and W.W. Anokobonggo, 2003. Acute toxicity effects of the methanolic extract of *Fagara zanthoxyloides* (Lam.) root-bark. Afr. Health Sci., 3: 124-126.

Olayebi, A.A., A.B. Saba and R.O.A. Arowolo, 2008. Safety evaluation of prolonged administration of stresroak in grower cockerels. Inter. J. Poultry Sci., 7(6): 1-5.

Pieme, C.A., V.N. Penlap, B. Nkengou, C.L. Taziebou, E.M. Tekwu, F.X. Etoa and J. Ngongang, 2006. Evaluation of acute and subacute toxicities of aqueous ethanolic extract of leaves of *Senna alata* (L) Roxb (Cesalpiniaceae). Afr. J. Biotech., 5(3): 283-289.

Polenackovic, M. and Sikole, 1996. Is erythropoietin a survival factor for red blood cells? J. Am. Soc. Nephrol., 7: 1178-1182.

Rickert, K., R.R. Martinez and T.T. Martinez, 1999. Pharmacist knowledge of common herbal preparations. Proc. West. Pharmacol. Soc., 42: 1-2.

Sanchez-Elsner, T., J.R. Ramirez, F. Rodriguez-Sanz, E. Varela, C. Bernabew and L.M. Botella, 2004. A cross talk between hypoxia and TGF-beta orchestrates erythropoietin gene regulation through SPI and SMADS. J. Mol. Biol., 36: 9-24.

Shah, A.M.A., S.K. Garg and K.M. Garg, 1997. Subacute toxicity studies on Pendimethalin in rats. Indian J. Pharmacol., 29: 322-324.

Spencer, K. and C.P. Price, 1971. Determination of serum albumin using Bromscresol techniques. Ann. Clin. Bioch., 14: 105-115.

Tédong, L., P.D.D. Dzeufiet, T. Dimo, E.A. Asongalem, S.N. Sokeng, J.F. Eleou, P. Callard and P. Kamtchouing, 2007. Acute and subchronic toxicity of *Anacardium occidentale* Linn (Anacardiaceae) leaves hexane extract in mice. Afr. J. Trad. Complement. Altern. Med., 4(2): 140-147.

Tilkian, M., C.B.M. Sarko and G.A. Tilkian, 1979. Clinical Implication of Laboratory Tests. 2nd Edn., The C.V Mosby Co., St Louis, Missouri, USA.

Wasan, K.M., S. Najafi, J. Wong and M. Kwong, 2001. Determination of ACPI and SPI and SMADS. J. Mol. Biol., 36: 9-24.

WHO Expert Committee, 1985. In WHO Technical Report Series of Diabetes Mellitus, pp: 727.

Withawaskul, P., P. Ampai, D. Kanjanapothi and L. Taeosothikul, 2003. Acute and subchronic toxicities of saponin mixture isolated from *Schefflera leucantha* Viguer. J. Ethnopharmacol., 89: 115-121.