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Effect of an Aqueous Extract of a Combination of *Nauclea latifolia* Root and *Acalypha torta* Leaves on Heamatological Parameters.

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Abstract
Aim: *The safety of several commercially available herbs has recently come into question due to reports of adverse effects and potential interaction with prescribed drugs. This study was aimed at evaluating the sub-chronc toxicity of aqueous extract of a combination of Nauclea latifolia root and Acalypha torta leaves on some heamatological parameters of albino rats.*
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. The Acute toxicity was assessed according to Lorke's method to determine the Lethal Dose (LD_{50}). Sub – chronic toxicological effects of 50.0 and 100.0 mg/kg body weight of the extract on heamatological indices were ascertained for 3 months at one month intervals. Thirty nine rats (120-150g) were used for the study and the extract was administered orally throughout the period of studies. Parameters were measured using standard methods.*
Result: *Results showed that the LD_{50} was above 5000mg/kg body weight and no behavioural changes were observed within the 24 hours. Sub-chronic toxicity evaluation following administration of 50 and 100mg/kg body weight doses of the extracts showed non-significant changes ($p>0.05$) in the heamatological parameters when compared to the control. The results indicated no significant changes ($p>0.05$) in the packed cell volume (PCV), heamoglobin concentration (HB), total white blood cell count (TWBC) and white blood cell differential count when compared to the control.*
Conclusion: *Even though further investigations are still needed, the present findings suggested that these doses of the aqueous extract of the mixture of N. latifolia root and A. torta leaf (5:1) do not have deleterious effects on the blood and blood forming tissues in rat.*
Keywords: *Nauclia latifolia, Acalypha torta, Heamatology, Toxicology*

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I. Introduction

Plants and their derived products either as extracts, pure compounds have served as a veritable source of food for humans and animals and also offer an unlimited opportunity for the discovery of new drugs. The discovery since prehistoric era that plants help in the prevention, management and treatment of communicable and non-communicable diseases has over the years attracted the attention of scientist worldwide (Falodun *et al.*,2006). Nowadays, plants are increasingly being used in most parts of the world as hypolipidemic, contraceptive, antihypertensive, treatment of skin disease, antimicrobial and hypoglycemic agents.

The safety of several commercially available herbs has recently come into question due to reports of adverse effects and potential interactions with prescribed drugs (Popata *et al*., 2001. The real drawback in traditional medicine stems mostly from lack of precision in dosage and the imprecise nature of diagnosis, especially of chronic and complicated conditions (Dawit and Ahadu, 1993). However, significant achievement can be made in alleviating these problems through standardization of dosage and quality control of the total or partially purified extracts of widely used remedies that are proven safe and effective on the basis of in vivo and/or in vitro laboratory investigations. Moreover, the concept of side effect is more probably elaborated in the traditional medicine (Gilani and Rahman, 2005). Inappropriate use of dosage results in chronic damage to blood composition and tissue of various organs.

II. Materials And Methods

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, Formolsaline 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 EXTRACT: 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

ACUTE TOXCITY: Twelve test (12) animals were randomly divided into 3 groups of three rats each in the first grouping and one each in the remaining groups. Graded doses of 10mg, 100mg,and 1000mg per kg body weight of the extract were given to the first 3 groups of 3 rats respectively, while graded doses of 1600mg, 2900mg, and 5000mg were given to the last 3 groups of one rat each respectively, The extract was administered orally via a canular and monitored for 24 hours for changes in behavior and mortality(Lorkes, 1980).

SUB-ACUTE 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 were given 50mg/kg and 100mg/kg of the extracts respectively. of the combined extract. The extract was adminstered orally via a canular once a day for 3 months(90 days).

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

EVALUATION OF HAEMATOLOGICAL PARMATERS The haematological parameters were determined using blood collected in EDTA containers (Ogbonnia et al., 2011). Chloroform was used to anaesthetize the animals before blood samples were collected through heart puncture into EDTA tubes for the haematological parameters analyses. The blood samples were analyzed for the haemoglobin (Hb) concentration by the Sahli-Hellige method according to Cheesbrough (2000). Packed Cell Volume (PCV) was according to the microhaematocrit method of Cheesbrough (2000), while White Blood Cells (WBC) and its differentials (neutrophil and lymphocyte) were determined as described also by cheesbrough(2000).

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

Acute toxicity test: Oral administration of a single dose of the extract at doses of 10,100,1000,1600,2900 and 5000mg/kg body weight did not cause any mortality during the 24 hours of observation period. The calculated LD_{50} for aqueous extract at the end of the 24 hours acute toxicity test was >5000 mg/kg. As shown in Table 1 below.

TABLE 1: LD_{50} For The Aqueous Extract

DOSE g/kg	NOS OF DEATH	% MORTALITY	SYMPTOMS
0.01	0/3	0	NONE
0.1	0/3	0	NONE
1	0/3	0	NONE
1.6	0/1	0	NONE
2.9	0/1	0	NONE
5	0/1	0	NONE

EFFECTS OF THE EXTRACT ON GENERAL BODY WEIGHT: The sub-chronic effects of the aqueous extract of the mixture of *Nauclea latifolia* root and *Acalypha torta* leaves on general body weights are summarized In Table 2. The result showed significant ($P<0.05$) increase in body weight in both treated and control groups but no significant ($P>0.05$) difference between the treatment groups was observed.

HEMATOLOGICAL STUDY:

Packed Cell Volume: The result on Packed cell volume (PCV) showed a slight decrease in the treatment groups but statistically not significant, however in the second month a significant decrease ($P< 0.05$) was observed in the treatment groups when compared to the control but not significant in the third month as summarized in Table 3 below.

Table 2: Average body weight of rats in the control Group A,50mg/kg treatment Group B, and 100mg/kg treatment Group C.

Groups	Average initial weight ± S.D.	Average weight for first month ± S.D.	Average weight for 2nd month ± S.D.	Average weight for 3rd month ± S.D.
Group A	134.55±7.33	163.33±8.97	190.5±5.24	213.67±7.09
Group B	110.44±4.90	159.44±18.20	176.33±17.73	190.33±16.77
Group C	123±7.28	176.11±16.62	189.17±18.28	221±10.53

Heamoglobin Level: The Heamoglobin (Hb) levels were seen to slightly decrease in the treatment groups but statistically not significant compared to the control group, however in the second month, a significant decrease($P< 0.05$) was observed in Group C when compared to the control but not significant in the third month as summarized in Table 3 below**.**

The TWBC: The TWBC levels were seen to decrease in the treatment groups but statistically not significant compared to the control group, however the TWBC levels in the second month showed a significant decrease ($P< 0.05$) in Group C when compared to the control but not significant in the third month as summarized in Table 3 below

The WBC Differentials: The WBC Differentials in the treatment groups were seen to decrease when compared to the control but statistically not significant ($P>0.05$). The lymphocyte and Neutrophils levels of the treatment groups were seen to have a significant difference ($P< 0.05$) when compared to the control only in the third month, while the levels of Monocytes, Eosinophils and Basophils were not significant throughout the periods of study as summarized in Table 4 below.

TABLE 3: Effects of the extract on haemoglobin concentration (g/dl).

	GROUPA	GROUP B	GROUP C
1 MONTH	14.825± 1.24	14.4±1.67	14.77±3.74
2 MONTH	15.87±2.2	15.9±2.52	9.67±2.47
3 MONTH	12.67±1.78	12.57±1.91	14.9±3.55

Values are Mean ± S.D

Table 4: Effect of extract on packed cell volume (%).

	GROUP A	GROUPB	GROUPC
MONTH1	44.33±3.05	38.54±13.80	37.90±13.93
2MONTH	48.33±5.50	43.98±15.37	38.47±15.29
3MONTH	38±5.56	33.82±11.39	34.59±12.75

Values are Mean ± S.D

Table 5: Effect of extract on white blood cell count (µl x 10^3)

	GROUP A	GROUP B	GROUP C
1 MONTH	6.65±1.11	5.5±1.22	5.67±1.07
2 MONTH	10.37±0.74	10.83±0.74	12.03±0.35
3MONTH	19.2±10.35	11.63±1.65	7.9±3.51

Values are Mean ± S.D

TABLE 5: Effect of the extract on white blood cell differentials (%).

NEUTROPHILS	**GROUP A**	**GROUP B**	**GROUP C**
1 MONTH	57±2.16	55.67±6.03	57±1.73
2 MONTH	59.33±5.51	57.33±4.16	65.33±8.33
3 MONTH	61.67±6.51	50.33±10.02	42.67±7.02
LYMPHOCYTES			
1 MONTH	43±10.23	39.67±6.11	38.33±2.08
2 MONTH	35±5.29	37±2.65	29.67±6.43

3 MONTH	33.67±6.35	48.67±11.24	56±5.29
MONOCYTES			
1 MONTH	3±0.82	3.33±1.15	2.33±0.58
2 MONTH	3.67±1.15	3±1.0	3.33±1.15
3 MONTH	4.67±3.05	0.75±1.5	1.33±2.31
EOSINOPHILS			
1 MONTH	1.25±0.5	1.33±0.58	1.67±0.58
2 MONTH	1.67±1.15	1.67±0.58	1.67±1.15
3 MONTH	0	0	0
BASOPHILS			
1 MONTH	0.25±0.5	0	0.67±0.58
2 MONTH	0.33±0.58	1	0.33±0.58
3 MONTH	0	0	0

IV. Discussion

In the acute toxicity study of the extract, no noticeable changes in the behavior and in the nervous system responses were observed in the treated animals. All the rats that received the various graded doses of the extracts survived beyond the 24hours monitoring period according to the methods of Lorkes 1985,suggesting that the median lethal dose LD_{50} is above >5000mg/kg body weight. These findings support the extract under investigation to be a candidate for the category of non-toxic substances according to the Hodge and Sterner (1956) scale for toxicity and classified under the category of substances with low toxicity according to OECD.

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, an increase in the body weight of Group C rats treated with 100mg/kg compared to control but was statistically not significant, this increase could be as a result of appetite boasting effect of the extract.The HB, TWBC and PCV levels were seen to decrease in the treatment groups but statistically not significant compared to the control group, however in the second month a significant increase($p<0.05$) was seen in both the PCV,TWBC and HB level but not significant in the third month. An increase in the levels of these haematological parameters is indicative that the extracts have the potential to stimulate erythropoietin release in the kidney known to enhance RBC production (erythropoiesis) (Sanchez-Elsner *et al.,* 2004).

There was however a reduction in the WBC Differentials in the treatment groups considered during this research work when comparing the results obtained from rats in control group though this decrease was statistically not significant ($p>0.05$), The lymphocyte and neutrophil levels were seen to have a significant difference ($p< 0.05$) only in the third month, while the levels of monocytes, eosinophils and basophils were not significant throughout the period of study. The hematopoietic system is very sensitive to toxic compounds and serves as an important index of the physiological and pathological status in both animals and humans (Adeneye et al.,2006). The above observations indicate that this extract of *Nauclea latifolia* root and *Acalypha torta* leaves do not have any adverse effects on the hematopoietic system but however boasted the body defence system. Detailed toxicity studies including the effects of chronic treatment are recommended to conclude the toxicity profile of this herbal combination.

References

[1] Adeneye, A.A., Ajagbonna,O.P., Adeleke,T.I. and Bello,S.O.(2006). Preliminary toxicity and phytochemical studies of the stem bark aqueous extract of Musanga cecropioides in rats. Journal of Ethnopharmacol, 105:374-379.

[2] Cheesbrough, M. (2000). District Laboratory Practice in Tropical Countries.(1st Edn).vol 2.Pp 301-305

[3] Dawit, A. and Ahadu, A. (1993). Medicinal plants and enigmatic health practices of Northern Ethiopia. Berhanena Selam Printing Enterprise. Addis Ababa, Ethiopia.15-35.

[4] Falodun,A., Okenrobnal,L.O. and Uzoama,N. (2006). phytochemical screening and anti-inflammatory evaluation of methanolic and acqueous extracts of euphobia heterophylia. African Journal of Biotechnology **5 (**6**):**529-531.

[5] Gilani,H. and Rahman,U. (2005). Trends in ethno pharmacology. Journal of Ethno pharmacology, **100**:41-50.

[6] Hodge, H.C. and Sterner, J.H.(1956). Combined tabulation of toxicity classes. Handbook of toxicology, WB Saunders.

[7] Lorke, D.A. (1980). A new approach to acute toxicity testing.Archives of toxicology.54:275-287.

[8] Ogbonnia, S.O., Mbaka,G.O., Anyika,E.N., Emordi,J.E. and Nwakakwa.N.(2011). An evaluation of acute and subchronic toxicities of a Nigerian polyherbal tea remedy. Pakistan Journal of Nutrition, **10** (11): 1022-1028.

[9] Popata, A., Sherara, H. N.and Malkiewieta. (2001). The toxicity of Calliepis Laureola,aSouth african traditional herbal medicine. Journal of Clinical Biochemistry, **34**(2001) 229–236.

[10] Sanchez-Elsner, T., Ramirez, J.R., Rodriguez-Sanz, F., Varela, E., Bernabew, C. and Botella, L.M.(2004). A cross talk between hypoxia and TGF-beta orchestrates erythropoietin gene regulation through SPI and SMADS. Journal Molecular Biology, **36**: 9-24

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