WORLD JOURNAL OF PHARMACY AND PHARMACEUTICAL SCIENCES
SJIF Impact Factor 7.421
Volume 7, Issue 12, 1-9 Research Article ISSN 2278 – 4357

HAEMATOLOGICAL AND HISTOPATHOLOGICAL CHANGES IN SWISS ALBINO MICE FOLLOWING ORAL ADMINISTRATION OF CRUDE WATER EXTRACT OF *CASUARINA EQUISTEFOLIA* L. LEAVES

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Article Received on
25 September 2018,

Revised on 16 Oct. 2018,
Accepted on 04 Nov. 2018,

DOI: 10.20959/wjpps201812-12717

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ABSTRACT

The study was aimed at determining the toxicity of extracts of *Casuarina equistefolia* leaves on some vital organs and haematological parameters of mice. Twenty Swiss male mice (15-20g) grouped A, B, C, and D. The mice were orally administered 200mg/kg, 400mg/kg, 600mg/kg of the extracts and normal saline daily for 30 days. The histology and haematological parameters were done using standard methods. The results revealed that all the mice treated with the extracts and the control increased linearly until termination of treatment with week 4 showing a dose dependent in weight increase. Haematologically, there was a general reduction in some of the indices such as the haemoglobin counts, Packed Cell Volume (PCV), Red Blood Cell (RBC) counts, Mean Corpuscular Volume (MCV), Mean Corpuscular Haemoglobin (MCH) counts and Mean Corpuscular Haemoglobin Concentration (MCHC) when compared to the values of the control group. But, the White Blood Cells (WBC) counts showed a dose dependent increase when compared to the control. On differential counts of WBC in mice, the result revealed that the neutrophil, monocytes and esinophils counts increased in a dose dependent fashion when compared with the increases observed in the control groups. In the contrary, the lymphocytes counts reduced with increase in dose when compared with the control and basophil count was nil for all groups. Histopathologically, organs examined were apparently normal at the end of the four weeks of exposure to treatment. However, there was severe haemorrhage and congestion in the lungs for 600mg/kg treated group while minor

haemorrhage was observed in the lungs of mice treated with 400mg/kg. The results of the toxicological experiments indicated that all of the extracts tested were well tolerated in mice at a lower dosage but might not be safe when dosage is high. Hence, the consumption should be with caution.

KEYWORDS: *Casuarina equistefolia*, Crude Water Extracts, Haematological Changes, Histoarchitectural changes.

INTRODUCTION

Casuarina is a shrub native to Australia and Island of the pacific. They are common in tropical and subtropical areas. The tree has delicate, slender ultimate branches and leaves that are no more than scales, making the tree look more like a wispy conifer. Australian pine grows to a height of 12m and width of 7m. The plant does especially well in windswept locations and is widely planted as wind – breaks. In Panama the fruit is mixed with powdered nutmeg used in relieving toothache (Walter and Memory, 1977). Recently Onwuliri and Umezurumba (2003) reported its efficacy against *Salmonella typhi*. The lethal dose concentration of the leaf extract has been determined to be 2884.03 mg/kg in mice (Malann *et al*., 2013) indicative of its relative safety in traditional medicine.

MATERIALS AND METHODS

Study area

The studies on the sub- acute toxicity was carried out in the Animal House Unit of the Department of Pharmacology University of Jos and Haematology laboratory of the College of Veterinary and Medical Laboratory Technology and the Central Diagnostic Laboratory of the National Verterinary Research Institute (NVRI) Vom, near Jos, Plateau state.

Sub-Acute Toxicity

Twenty Swiss albino mice all males weighing between 16g to 24g were divided into four groups per extract. Groups A to C were treated with 200mg/kg, 400mg/kg and 600mg/kg extracts of *C. equistifolia* and group D received normal saline. Thereafter, the animals were sacrificed, blood collected and vital organs examined microscopically.

Haematological Test

Blood samples were collected after all animals surviving toxicity studies were killed by anaesthetizing with chloroform in a dessicator and laid on a dissecting board on its back then

dissected using a scissors. Incision was created on the jugular vein around the shoulder and blood was collected using syringes and transferred into EDTA bottles to prevent clotting (Uguru, 2002). The red blood cells, white blood cells, platelets counts, haemoglobin content, PCV and differential counts were determined in the Haematology laboratory of the College of Veterinary and Medical Laboratory Technology Vom using standard procedures (Ibu and Adeniyi, 1989).

Tissue Histology

The mice surviving the sub-acute toxicity test were killed by anaesthetizing with chloroform in a dessicator and laid on a dissecting board on its back then dissected using a scissors and the vital organs including the heart, lungs, liver, spleen, kidney and intestine were carefully dissected out and fixed in 10% formalin. The organs were transported in sample bottles to the Histopathology unit of the Central Diagnostic Laboratory NVRI Vom for further processing and sectioning using standard techniques (Barker and Silverton, 1976). Tissue slice of 3 to 4cm thick were cut and put in the automatic tissue processor where they were further fixed in 10% formalin-saline solution for 2 hours. They were then dehydrated for 2 hours in each of the following ascending grades of alcohol: 85%, 90%, and 100% v/v. the dehydrated tissues were then cleared in toluene for 2 hours, after which the tissue slice were embedded in paraffin wax and are left to cool. The blocks were trimmed at 5 microns. The ribbons of sections were dewaxed in xylene and rehydrated in the following descending grades of alcohol: 100%, 90%, and 70% v/v. They were then stained in haematoxylin for about 5 minutes, differentiated in 1% acid alcohol, blued in scoff's tap water and stained in eosin for 3 minutes. They were later rinsed, dehydrated in ascending grades of alcohol: 70%, 90% and 100% v/v finally cleared in xylene and mounted in a box. The slides were then examined microspically for pathological lesions.

Statistical Analysis

Results are expressed as mean ± standard error of mean (SEM). The student t-test was used to compare means of treated groups and control for any significant difference of mice treated with leaf extracts and the control group using the SPSS version 11.0. All data were analyzed at a 95% confidence interval ($\alpha = 0.05$).

RESULTS

The results presented in table 1 revealed that all the mice treated with 600, 400 and 200mg/kg crude water extract of *C. equistifolia* and the control group increased linearly from day 0

when treatment was initiated to day 28 when treatment terminated. Week 4 showed a dose dependent change in weight 15.43±0.70, 15.31±0.50 and 16.70±0.40g compared to the weights at the commencement of the experiment 15.31±1.68, 15.17±1.27 and 16.40±1.03g respectively for 600, 400 and 200mg/kg, the weight gained increased relative to decrease in dose of administration. The control group gave a weight increase of 15.06±2.45 on day 0 to 15.53±0.87g at the end of week 4.

Table 1: Effect of daily administration of the aqueous extract of *C. equistefolia* on body weight in mice over a period of 28 days (Sub-acute toxicity).

Treatment			Body weight		
	Day 0	Week 1	Week 2	Week 3	Week 4
600mg/kg	15.31±1.68	15.34±0.69	15.46±0.58	15.22±0.57	15.43±0.70
400mg/kg	15.17±1.27	15.18±0.66	15.21±0.43	15.26±0.43	15.31±0.52
200mg/kg	16.40±1.03	16.41±0.39	16.49±0.33	16.60±0.33	16.70±0.40*
Control	15.06±2.45	15.06±0.92	15.21±0.75	15.39±0.74	15.53±0.87

n=5, each data is mean±SEM, * $P \leq 0.05$

The results presented on table 2 generally showed that the 600, 400 and 200mg/kg crude water extract produced a reduction in some of the haematological indices such as the haemoglobin counts 12.26±1.09, 11.30±1.36, 12.00±0.65g/dl respectively compared to the control 12.77±0.41g/dl. The PCV values reduced to 37.20±2.51, 34.00±3.89 and 36.33±1.86% for 600, 400 and 200mg/kg treated groups respectively when compared with the control group 38.00±1.53%. The RBC counts of the treated groups gave a reduction of 4.23±0.28, 3.95±0.38 and 4.16±0.22 x 10^{12}/L compared with the control group 4.31±0.15 x 10^{12}/L. Similarly the MCV also showed lower values 8.78±0.03, 8.56±0.19 and 8.73±0.02 compared to 8.81±0.04cuμ control group, the MCH counts of treated mice gave lower values 2.88±0.09, 2.84±0.08 and 2.88±0.02 while the control was 2.96±0.02pg and MCHC 32.73±1.00, 33.16±0.25 and 33.02±0.22 compared to the higher value 33.59±0.13% of the control group. Conversely, the WBC counts showed a dose dependent increase 5.17±0.52, 6.38±1.54 and 6.56±1.44 x 10^{9}/L for 200, 400 and 600mg/kg respectively compared to the normal saline (control) treated group 4.40±0.90 x 10^{9}/L.

Table 2: Effects of daily administration of the aqueous extracts of *C. equistifolia* on haematological indices in mice after 28 days.

Treatment				Haematological indices			
	Hb g/dl	PCV (%)	RBC $\times 10^{12}$/L	WBC $\times 10^{9}$/L	MCV cuμ	MCH pg	MCHC %
600mg/kg	12.26±1.09	37.20±2.51	4.23±0.28	6.56±1.44	8.78±0.03	2.88±0.09	32.73±1.00
400mg/kg	11.30±1.36	34.00±3.89	3.95±0.38	6.38±1.54	8.56±0.19	2.84±0.08	33.16±0.25
200mg/kg	12.00±0.65	36.33±1.86	4.16±0.22	5.17±0.52	8.73±0.02	2.88±0.02	33.02±0.22
Control	12.77±0.41	38.00±1.53	4.31±0.15	4.40±0.90	8.81±0.04	2.96±0.02	33.59±0.13

n= 5, each data mean±SEM, $p \geq 0.05$

Table 3 shows the effect of daily administration of *C. equistifolia* on differential counts of WBC in mice after 28days. The result revealed that the neutrophil counts increased in a dose dependent fashion 54.33±2.34, 55.75±3.71 and 56.40±3.13 for 200, 400 and 600mg/kg respectively compared with the control group 53.33±1.67. Similarly, the monocytes and esinophils counts also increased 1.33±0.33, 1.00±0.41, 0.80±0.20 and 1.33±0.88, 1.75±0.25, 0.80±0.37 for 200, 400 and 600mg/kg respectively compared to their controls 0.67±0.67 and 0.33±0.33. In the contrary, the lymphocytes counts reduced with increase in dose when compared with the control 39.67±4.92, 41.50±4.09, 41.80±3.51 and 45.67±2.34 respectively for 200, 400, 600mg/kg and normal saline (control) group. The basophil count was nil for all the experimental groups.

Table 3: Effect of daily administration of the aqueous extract of *C. equistifolia* on WBC differential count's in mice after 28 days.

Treatment			Differential counts		
	Neutrophils	Lymphocytes	Monocytes	Esinophils	Basophils
600mg/kg	56.40±3.13	41.80±3.51	0.80±0.20	0.80±0.37	0 NSD
400mg/kg	55.75±3.71	41.50±4.09	1.00±0.41	1.75±0.25	0 NSD
200mg/kg	54.33±2.34	39.67±4.92	1.33±0.33	1.33±0.88	0 NSD
Control	53.33±1.67	45.67±2.34	0.67±0.67	0.33±0.33	0 NSD

n=5, NSD (no significant difference) $p \geq 0.05$

Table 4 shows that the heart, kidney and liver of treated mice and the control group, histopathologically, were apparently normal at the end of the four weeks of exposure to treatment with *C. equistifolia* crude leaf extract. However, there was severe haemorrhage and congestion in the lungs for 600mg/kg treated group while minor haemorrhage was observed in the lungs of mice treated with 400mg/kg *C. equistifoli*a respectively. Meanwhile the lungs of the 200mg/kg and the control group were all normal.

Table 4: Histopathological observation of tissue of mice administered with aqueous extract of *C. equistifolia* after 28 days of administration.

Treatment		Organs		
	Heart	**Kidney**	**Liver**	**Lungs**
600mg/kg	Normal	Normal	Normal	Severe haemorrhage & congestion
400mg/kg	Normal	Normal	Normal	minor haemorrhage
200mg/kg	Normal	Normal	Normal	Normal
Control	Normal	Normal	Normal	Normal

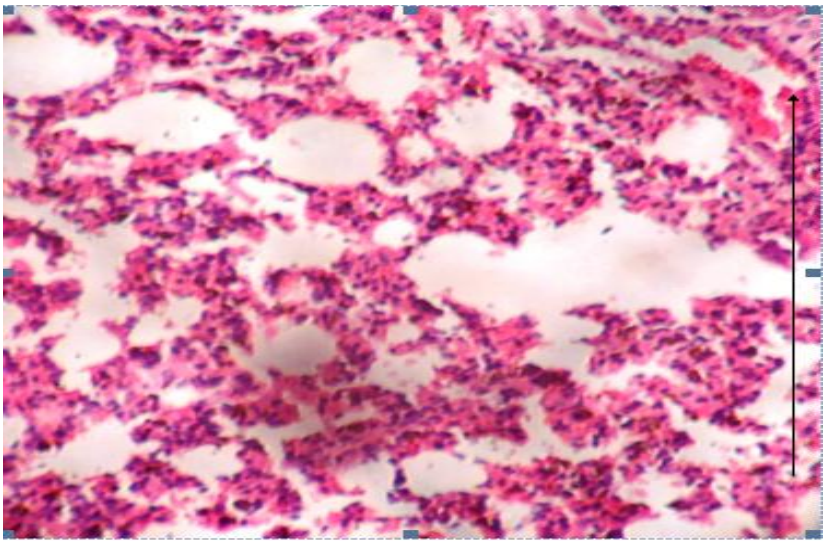

Plate 1: Lung tissue biopsy showing minor haemorrhages stained with H&E X10, treated with 400mg/kg *C. equistifolia.*

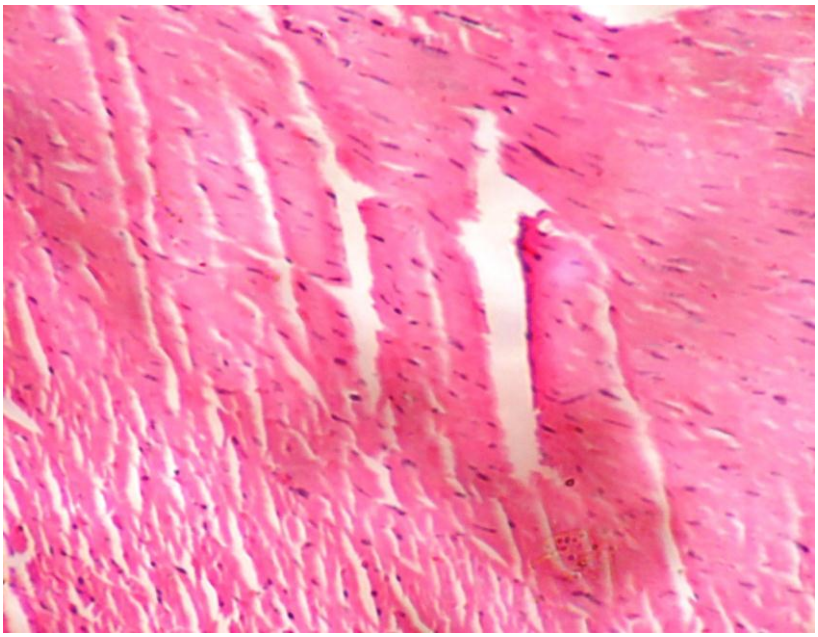

Plate 2: Normal Lung tissue biopsy stained with H&E X10 of control group using *C. equistifolia* extracts.

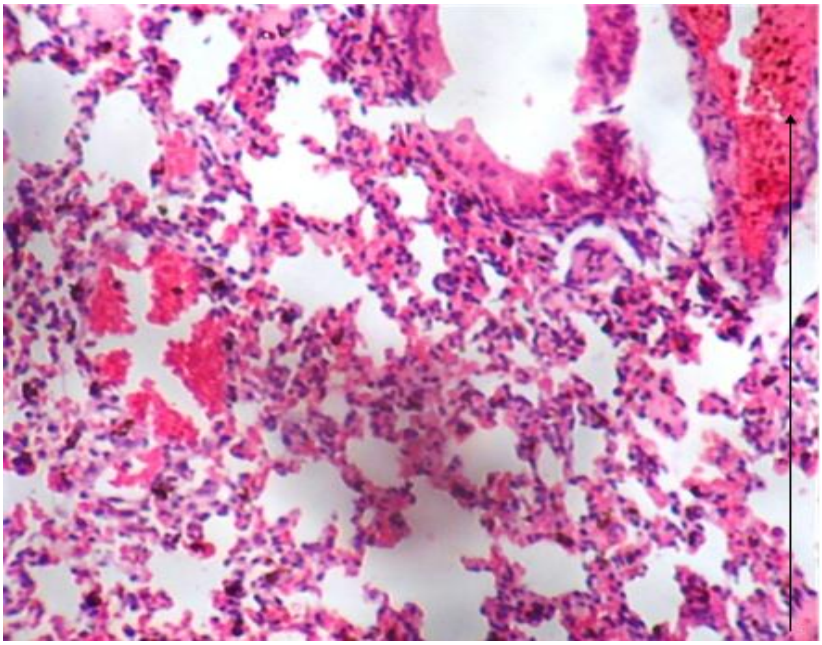

Plate 3: Lung tissue biopsy showing Severe haemorrhage and Congestion when stained with haematoxylin and eosin (H&E) X10, treated with 600mg/kg *C. equistifolia.*

DISCUSSION

The use of *C. equistifolia* have been reported in ethnobotany to posses antimicrobial properties, relieve of pain, treatment of chills, fever, diarrhoe, skin rashes, sores in west Africa (Adesegun and Coker, 2001; Onwuliri and Umeruzumba, 2003 and Ajaiyeoba *et al*., 2004). The stampeding use of medicinal plants for treatment of various ailments necessitates the evaluation of these plants for their safety.

The result of the sub-acute study showed that the extract did not produce any significant ($p > 0.05$) change in the body weight. The results indicated that mice gained weights significantly ($p < 0.05$) with administration of 200mg/kg crude extracts of *C. equistifolia* , but a general decline in week four was observed for all the mice treated with 400 and 600mg/kg of extracts of *C. equistifolia*. This strongly suggests that the extract may have no significant effect on metabolism.

The haematological indices were generally normal and within the normal limits relative to the control group, for mice administered crude extracts of *C. equistifolia* extracts with no significant difference ($p > 0.05$). This suggests that the extracts have no harmful effects on the blood indices such as haemaglobin (Hb), packed cell volume (PCV) count, white blood cell (WBC) count, mean cell volume (MCV), mean cell haemoglobin (MCH), mean cell haemoglobin concentration (MCHC). The haematological indices after 28 days of extract

administration revealed a decrease in haemoglobin, packed cell volume and red blood cell counts but increase in the white blood cell counts that is dose-dependent for mice treated with *C. equistifolia* and no significant changes ($p > 0.05$) in these parameters for mice treated

The administration of the extracts to the mice for 4 weeks resulted into visible pathological changes in the liver, kidney, lung, heart and intestine except for severe haemorrhage and congestion and minor haemorrhage in the lungs of mice administered with 600mg/kg and 400mg/kg of *C. equistefolia* this is in close agreement to the findings of Azuibike *et al.*, 2015 and Yakubu and Salimon (2016) who reported normal histoarchitecure of all organs examined both macroscopically and microscopically but some effect on the organs as a result of high dosage.

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