

The effects of the leaf water extract of *Struchium sparganophora* (Linn.) Ktze asteraceae on the hematopoietic parameters and the organ system of rats

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Abstract: *Struchium sparganophora*, (Linn.) Asteraceae is a culinary herb consumed in the western part of Nigeria and it is known to have a nutritive value. This study was carried out to assess its' effects on the hematopoietic parameters and its toxicity on the organ system of Wistar rats. The animals were randomly divided to five (5) groups. The group 1 (control) received distilled water equivalent to the volume of extract administered while groups, 2, 3, 4 and 5 received the aqueous extract of 0.2g, 0.4g, 0.8g and 1.6g/kg body weight orally respectively for 4 weeks. The rats were anaesthetized with chloroform vapors 24 hours after the last day of extract administration and blood and organ tissues were collected through dissection for hematopoietic as well as histological examinations. Hematological parameters like hemoglobin (Hb) concentration, packed cell volume (PCV), white blood count (WBC) and platelet count were examined. The hematological parameters observed were not significantly different ($P \geq 0.05$) from that of the control except platelet counts that showed significant difference ($P \leq 0.05$). Histological assessment of cerebellum, kidney and trapezoid nuclei of the brain in all groups showed normal cytoarchitecture. However neuropathological damage such as lesions, chromatolysis and necrosis were observed to progress in the liver as the concentration of extract increases. This study shows that the extract at low doses did not cause significant ($P \geq 0.05$) necrohistopathological effects on the brain and the kidney indicating that the plant is safe at low doses but at a high dose of 1.6g/kg and above significant ($P \leq 0.05$) suppression of the platelet level was observed suggesting disturbances of hematopoiesis as even reflected by the necrohistopathological effects on the liver. Thus, the result revealed that the aqueous extract did not possess blood boosting ability and could cause liver damage if consumed at high concentrations.

Keywords: *Struchium sparganophora*, hematopoietic, toxicity.

INTRODUCTION

Plants serve as sources of foods and medicines for humans. It provides protein, carbohydrates, minerals and vitamins for human consumption and medicines for their health needs. Medicinal plants are one of the sources of folk medicines of local people and also provide food, clothing, shelter and medicine (Newall *et al.*, 1996). Many plants like Spinach, Rhubarb, Pea, Okra, Bean, Asparagus, *etcetera* are sources of proteins and other nutrients for mankind (Jian, 2007). Vegetables are an integral part of human nutrition as well as sources of several vitamins such as vitamins A, C and biotins. It also provides minerals, dietary fibers, *etcetera*. These vitamins play a role in red blood cell production. African leafy vegetables contribute micronutrients and bio-active compounds to the diets of populations in Africans (Okochi *et al.*, 2003). Green leafy vegetables are used for food in many countries of the world. However, fresh green leafy vegetables nutrients are higher than those with yellowish or lighter green leaves. Evaluation of epidemiological evidence has revealed that fruits and vegetables, with high contents of natural antioxidants

enhances a reduction in mortality from cardiovascular and cerebrospinal diseases although their protective effect on cancer risk has been observed but more is needed to be done to affirm this claim (Obboh, 2006). Many phenolics found in vegetable plants such as flavonoids have antioxidant capacities that are much stronger than those of vitamins C and E. Flavonols and flavones are flavonoids of particular importance because they have been found to possess antioxidant and free radical scavenging activity in foods. Some evidence has shown that flavonoids could protect membrane lipids from oxidation (Obboh, 2006). Pumpkin seeds are known to contain proteins which are reported to inhibit melanoma proliferation (Xie, 2004). Leaf extracts of *Psidium guajava* have also been reported to have haemopoietic effect (Uboh *et al.*, 2010). *Struchium sparganophora* a culinary herb in Nigeria whose leaves are boiled in water, drained completely, added to soup and consumed as vegetable (Kasim *et al.*, 2011a), has been also reported to be as an antidote to poisons (Akah and Ekekwe, 1995), anti malaria (Madureira *et al.*, 2002), anti measles (Burkill, 1985), anti oxidants (Obboh *et al.*, 2008) and to possess nutritive activities (Obboh, 2006). The crude extract of *Struchium sparganophora* is also reported to possess central nervous

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system depressant activities in mice (Aderibigbe and Agboola, 2011). Some compounds have been isolated from the leaf extracts of this plant which are sesquiterpine lactone (Jakupovic *et al.*, 1987), 3methyl, 2, 6 hexacosadienol, vernodalin and luteolin (Kasim *et al.*, 2011b). In furtherance of our investigation into ethnobotanical uses of this plant and its safety for human consumption the present study reveals the effect of water extract on the hemopoietic activities and its' toxicity in wistar rats.

MATERIALS AND METHODS

Plant collection

Fresh leaves of the plant, *Struchium sparganophora* were obtained from a stream at Adiewokotu Street in Sagamu Community of Ogun State in the month of February. Botanical identification of the plant was done at the Herbarium of the Forestry Research Institute of Nigeria (FRIN), Ibadan by Mr. I.K. Idewo, comparing it with voucher specimen 105358. A sample was kept in the Pharmacognosy Department, Faculty of Pharmacy, Olabisi Onabanjo University. The plant was sun-dried for two days and then macerated into dry powder.

Extraction

Fifty (50) g of the powder leaf was soaked in 200mls of water for two weeks shaking occasionally. After two weeks, it was filtered and the filtrate was dried to 28mls (i.e. 1ml of the extract being equal to 1.7g plant material). The extract was preserved in a refrigerator at 4°C before use.

Laboratory animals

Twenty five (25) Wistar rats of both sexes weighing 100-170grams were procured from the animal house, Department of Veterinary Medicine, University of Ibadan, Nigeria. They were bred for weeks in the animal house before relocated to the animal house facility of the Faculty of Pharmacy, Olabisi Onabanjo University, Sagamu, Ogun state, Nigeria. They were handled according to National Institute of Public Health Service (PHS) Policy on Human Care and the Use of Laboratory Animals (2002). They were acclimatized for two weeks before commencement of the study and kept at a temperature of 22±1°C, relative humidity of 55±10%, and fed on a standard pellet (Pfizer, Nig.) and water *ad libitum*. They gained weight of averagely 30-40g after being fed.

Experimental procedure

The animals of weights 150g to 170g were divided randomly to five (5) groups. Group 1 was maintained as the untreated control while groups 2 to 5 were the experimental groups. Each of the rats was marked and put into different cages according to their groups. Administration of the aqueous extract was done orally using orogastric tubes and syringes. The animals received

their doses daily for 4 weeks. The control group received distilled water equivalent to the volume of the extract and groups 2, 3, 4 and 5 received the aqueous extract of *Struchium sparganophora* at doses of 0.2g, 0.4g, 0.8g & 1.6g/kg body weight respectively. After four (4) weeks the animals were anaesthetized with chloroform vapors 24 hours after last day of extract administration and dissected. The blood and organs were isolated for the hemopoietic and histological examination.

Hematological analysis

Packed cell volume (PCV) was read on an Erma particle counter (Model PC 607 Erma Inc., Japan), the hemoglobin concentrations were determined by cyanomethaemoglobin method, described by Alexander and Griffiths (1993). The red blood cells (RBC) count was estimated by the visual method described by Dacie and Lewis (1991) while white blood cell (WBC) and platelets were estimated by differential counts described by Tietz (1985).

Histological analysis

The gross parameters measured included:

1. The normal/abnormal morphological appearance of the brain, liver and kidney.
2. Weight of the brain, liver and kidney using a Kertz' precision weighing balance.
3. The microscopic parameters measured and the methods used included:
4. The normal/abnormal histological appearance of the brain, liver and kidney and Trapezoid Nuclei using Hematoxylin and Eosin staining methods.
5. Identification of centrilobular necrotic (CN) areas, hydropic changes, fatty degeneration, congested sinusoids and hepatocytes with piknotic nucleus using a Leitz' binocular microscope with an eyepiece graticule.
6. Average densities and diameters of Purkinje cells of the brain and the densities of damaged Trapezoid Nuclei using a Leitz' binocular microscope with an eyepiece graticule. The density of damaged Trapezoid Nuclei was estimated in a field of X (0.0 to 1.0 mm) per section and in a total of 5 sections per animal in each of the rats in the control group and the experimental groups. The total calculated density per rat was divided by five in both the control and experimental groups to calculate the mean of the calculated total.

Tissues of the organs (brain, liver, kidney) were dissected passed through the process of fixation, dehydration, clearing, infiltration, embedding, blocking, sectioning and staining. The tissues were each trimmed down to about 3mm thickness, so as to obtain good fixation.

The fixation was carried out in 10% formalin, dehydrated in ethanol (50-100%), cleared in xylene, and embedded in paraffin. Slides were prepared and then stained with hematoxylin and eosin (H-E) stains for Photomicroscopic observation,

STATISTICAL ANALYSIS

All the results were expressed as mean \pm standard error of mean (S.E.M.) Statistical analysis was carried out using student's t-test and differences between means were considered to be significant when $P \leq 0.05$.

RESULT

Hematological analysis

Histological analysis

Histological results obtained after treatment with aqueous extract of *Struchium sparganophora* are shown in table 2.

DISCUSSION

The result of the effect of different doses of aqueous extract of *Struchium sparganophora* on the hematopoietic parameters of rats are shown in table 1, the extract produced non significant effect ($P \geq 0.05$) in the packed cell volume (PCV), haemoglobin (Hb) concentration, red blood corpuscles (RBC), Neutrophil, lymphocyte and eosinophil at the different concentrations of extract used compared with the control. However, the platelets decreased significantly ($P \leq 0.05$) on administration of increasing crude extract concentrations.. Though most researches carried out on plant extract to determine their haemopoietic potential showed that most are haemopoietic in nature (Uboh *et al.*, 2010) but in a recent research to study the effect of aqueous extract of *Polygala fruticosa* (sweet tea bush) on hematopoietic parameters of rats it was observed that the extract showed a significant decrease in platelet count suggesting disturbances of hematopoiesis (James *et al.*, 2009). Previous research on

Vernonia amygdalina (Bitter leaf) has also revealed that the leaf infusion induced haemolysis of human erythrocyte (*in vitro*) (Oboh, 2006). The reduction in platelet level in this present study may have occurred due to lysis of blood cells and probably suppression of blood cell synthesis by saponins found in the leaf extract because saponins are known to be toxic to body system (Watt *et al.*, 1962). Reduction in the number of platelet could cause thrombocytopenia which can result in spontaneous bruising and prolonged bleeding after injury. Thrombocytopenia could as well be caused by decrease in the production of thrombopoietin, a hormone produced by the liver which regulates the production of platelets by the bone marrow. Histological results obtained after treatment with aqueous extract of *Struchium sparganophora* for four (4) weeks are shown in table 2, there were no significant changes ($P \geq 0.05$) in the weight of the brain, kidney and liver though the frequency of lesions was found to steadily increase with increase in dose as shown in the brain, kidney and liver of the treated rats. The relationship between dosage of the extracts and the lesion in brain as well as kidney at low doses were found to be insignificant ($P \geq 0.05$). However, hepatocytic degeneration and vacuolation was observed in the liver of the group that was administered highest dosage of the extract fig. 2 compare to the control fig. 1. Thus, the liver was significantly affected ($P \leq 0.05$). In this study the normal tissues are seen in the control groups figs. 1, 3 and 5 which manifested a normal architecture with a central vein and hepatocytes radiating from it. The portal triad consisting of hepatic artery, portal vein and bile duct which constituted various zones are conspicuously displayed. This same architecture was seen

Tables 1: Effects of different doses of aqueous extract of *Struchium sparganophora* on hematological parameters

Extract Con.(g/kg)	Control	0.2	0.4	0.8	1.6	P value
PCV (%)	35.75 \pm 3.20	155.00 \pm 18.93	35.20 \pm 1.93	35.00 \pm 5.76	26.50 \pm 4.05	≥ 0.05
Hb (g/dl)	11.95 \pm 1.10	12.32 \pm 0.81	11.73 \pm 1.85	11.72 \pm 0.64	11.72 \pm 0.64	≥ 0.05
RBC ($\times 10^5$)	262.50 \pm 23.14	228.25 \pm 36.26	219.20 \pm 19.17	187.20 \pm 62.22	147.25 \pm 42.28	≥ 0.05
WBC ($\times 10^9/l$)	262.50 \pm 23.14	228.25 \pm 36.26	219.20 \pm 19.17	187.20 \pm 62.22	147.25 \pm 42.28	≥ 0.05
Neutrophil(%)	14.00 \pm 0.71	17.80 \pm 8.24	15.60 \pm 4.71	13.25 \pm 4.15	12.59 \pm 3.23	≥ 0.05
Lymphocyte(%)	81.80 \pm 8.21	84.70 \pm 4.71	84.75 \pm 1.70	86.25 \pm 3.75	87.50 \pm 3.25	≥ 0.05
Eosinophil(%)	0.00 \pm 0.00	0.00 \pm 0.00	0.00 \pm 0.00	0.40 \pm 0.40	0.50 \pm 0.50	≥ 0.05
Platelets($\times 10^9/l$)	155.00 \pm 18.93	81.00 \pm 2.45	78.75 \pm 1.25	70.00 \pm 10.80	64.00 \pm 4.00	≤ 0.05

Table 2: Results of histological examination of the brain, kidney and liver

Organ	Concentration of extract (g/kg)						Total lesions	P value
	Control	0.2	0.4	0.8	1.6			
Brain lesion	0	0	0	1	2	3	≥ 0.05	
Kidney lesion	0	1	1	1	2	5	≥ 0.05	
Liver lesion	0	2	3	4	5	14	≤ 0.05	

n = 5, No significant difference $P \geq 0.05$ was observed in the brain and kidney of the control and treated rats. However hepatocytic degeneration and vacuolation were observed in the liver of the treated rats particularly the group administered highest dosage of the extract. Thus, the liver was significantly affected ($P \leq 0.05$).

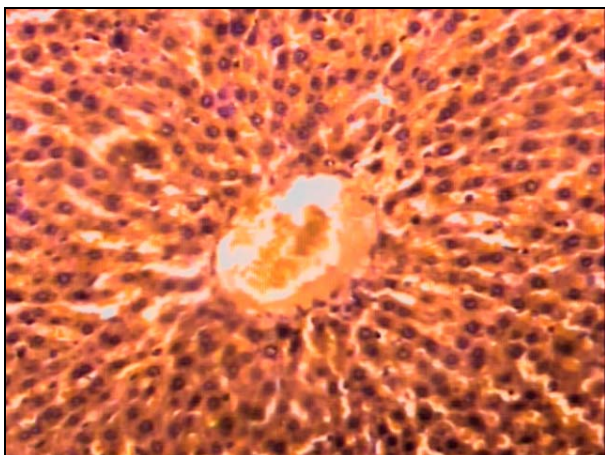


Fig. 1: Photomicrograph of the liver tissue of rat of the control group, hematoxylin and eosin (H& E) stains, 100 X, showing normal histological architecture, with central vein (CV) from which chords of hepatocytes are radiating with portal vein(PV), bile duct (BD).

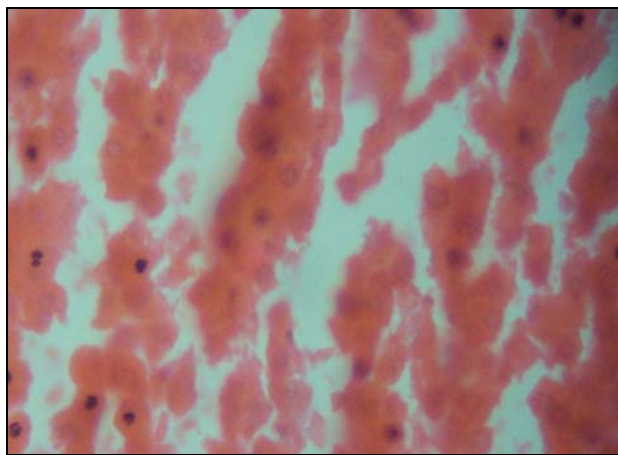


Fig. 2: Photomicrograph of the liver of maximum dose treated rats group, hematoxylin and eosin (H& E) stains, 100 X, showing centrilobular necrotic (CN) areas, hydropic changes, fatty degeneration, congested sinusoids and hepatocytes with pyknotic nucleus.

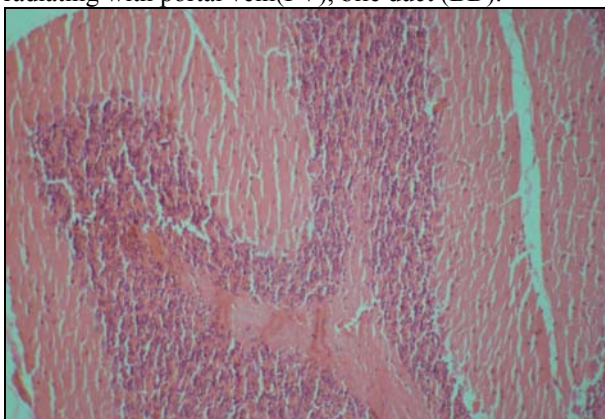


Fig. 3: Photomicrograph of the brain of normal control group, hematoxylin and eosin (H& E) stains, 100 X, showing normal histological architecture, with central vein (CV) from which chords of hepatocytes are radiating with portal vein(PV), bile duct (BD).

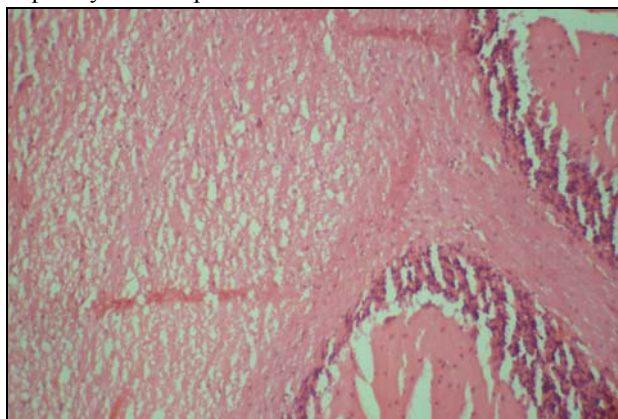


Fig. 4: Photomicrograph of the brain cells of treated rats. The brain cells of treated rats showing normal histological architecture, with central vein (CV) from which chords of hepatocytes are radiating. H&E stain 100 X.

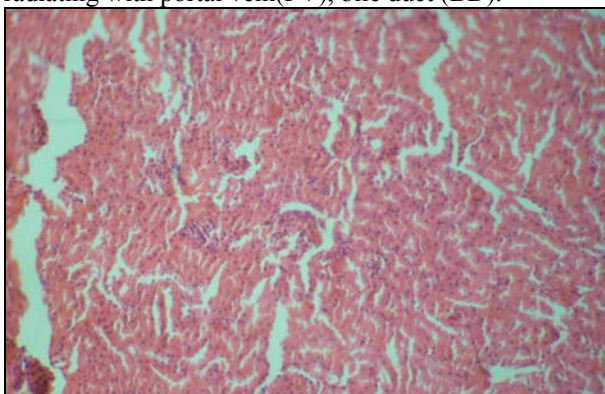


Fig. 5: Photomicrograph of normal kidney of control group, hematoxylin and eosin (H& E) stains, 100 X. Showing normal histological architecture, with central vein (CV) from which chords of hepatocytes are radiating. portal vein (PV), bile duct (BD)

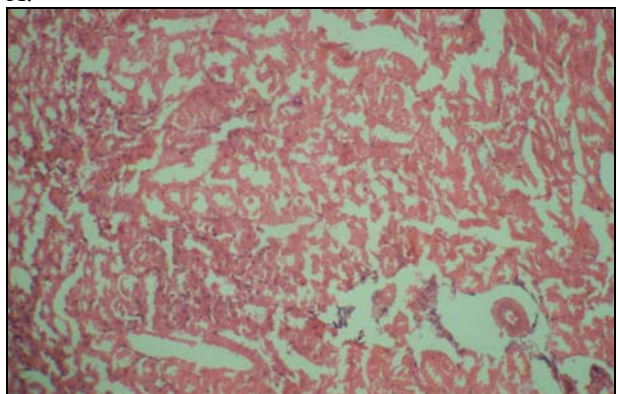


Fig. 6: Photomicrograph of the kidney of maximum dose treated rats group, hematoxylin and eosin (H& E) stains, 100 X., showing slight histological architecture variation from that of the control group, with central vein (CV) from which chords of hepatocytes are radiating.

in the treated kidney and brain as showed in figs. 4 and 6 which portrayed the extract to be non toxic to the brain and kidney at the dose range of the experiment. Reports from medicinal plant research indicates that extracts from some plants are both hepatotoxic and hematotoxic in action while others on the other hand are reported to be hepatoprotective and hematopoietic in nature (Uboh *et al*, 2010). It has also been known that some herbal formulation could restore the hepatic architecture and protect the liver tissue from fatty and degenerative changes, by preventing the toxic chemical reaction, oxidative stress, lipid peroxidation, molecular changes in the liver tissues, micro and macro vesicular fatty changes that lead to necrosis (Koul and Kapil, 1994).

CONCLUSION

This study shows that the leaf extract of *Struchium sparganophora* administered at the doses used and for the duration of the experiment suppressed the platelet level suggesting disturbances of hematopoiesis. Thus, the result of this study indicated that the aqueous extract of *Struchium sparganophora* leaves may not possibly serve as an acceptable blood booster and could cause liver damage if consumed at high concentration.

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