Original Article

Long-term continuous administration of a hydro-ethanolic extract of *Synedrella nodiflora* (L) Gaertn in male Sprague-Dawley rats: biochemical, haematological and histopathological changes

Patrick Amoateng[1*], Samuel Adjei[2], Dorcas Osei-Safo[3], Believe Ahedor[2], Seidu A. Mahmood[4], Benoit B. N'guessan[1], Isaac J. Asiedu-Gyekye[1], Alexander K. Nyarko[1]

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[1]Department of Pharmacology and Toxicology, School of Pharmacy, College of Health Sciences, University of Ghana, P.O Box LG 43, Legon, Accra, Ghana [2]Department of Animal Experimentation, Noguchi Memorial Institute for Medical Research, College of Health Sciences, University of Ghana, P. O. Box LG581, Legon, Accra, Ghana [3]Department of Chemistry, University of Ghana, P. O. Box LG56, Legon, Accra, Ghana [4]Pathology Unit, Department of Medical Laboratory Science, School of Biomedical & Allied Health Sciences, College of Health Sciences, University of Ghana, Legon, Accra, Ghana

Corresponding author: Dr Patrick Amoateng **E-mail:** pamoateng@ug.edu.gh

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SUMMARY

Background: Conflicting reports about the toxicity of *Synedrella nodiflora* (L) Gaertn (family Asteraceae), a plant traditionally used in Ghana for the management of epilepsy, abound in literature. The present study evaluates the effect of a 90-day continuous oral administration of a hydro-ethanolic whole plant extract of *Synedrella nodiflora* (SNE) in male Sprague-Dawley rats.

Methods: The toxicological evaluation of the extract (100, 300 and 1000 $mgkg^{-1}$) was focused on haematological, serum biochemical parameters and histopathological changes of some isolated organs.

Results: The extract produced no mortality in the rats treated during the study period. Only SNE 100 $mgkg^{-1}$ produced significant decrease in white blood cell and neutrophil counts and an increase in albumin, globulin, total bilirubin, total protein and potassium levels. The higher doses (SNE 300 and 1000 $mgkg^{-1}$) had no significant effect on all the haematological and biochemical parameters measured. Histopathological assessment of the liver, kidney and heart revealed no abnormalities in rats treated with the extracts. Only the SNE 1000 $mgkg^{-1}$ produced distortions of the branching arrangements of the myocardial fibres and a congested vessel which indicates a healed infarction.

Conclusions: The findings suggest hydro-ethanolic extract of *Synedrella nodiflora* (L) Gaertn generally has a low toxicity profile following a 90-day continuous oral administration in male Sprague-Dawley rats under the present laboratory conditions. However patients with renal or cardiac problems should use the plant with caution.

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INTRODUCTION

Synedrella nodiflora (L.) Gaertn (family Asteraceae), commonly known as the node weed, is a herb found growing indiscriminately along the banks of rivers, streams and roadsides.[1] In Ghana, the whole plant is boiled and the aqueous extract drunk for the treatment of epilepsy whiles the leaves are used for threatened abortion, hiccup, laxative and feed for livestock.[1, 2] It is known that some indigenous tribes in Nigeria use the whole plant for the treatment of cardiac troubles and to stop wound bleeding.[3] In Indonesia the young foliage is eaten as a vegetable and the leaf sap together with other materials, is applied for stomachache, and the plant is used in embrocation for rheumatism.[4] In Malaysia, the leaves are as poultice on leg sores and for the treatment of headache, and the sap instilled into the ear for earache.[4]

The hydro-ethanolic extract of the whole plant has been found to possess anticonvulsant,[5] sedative,[6] *in vitro* antioxidant and free radical scavenging[7-9] and anti-nociceptive properties.[10]

Extracts of the aerial parts of *S. nodiflora* have been found to possess insecticidal property substantiating the use of the leaves of the plant by farmers in Ghana as post-harvest protectants to control storage pests.[11-13] However, reports from these studies suggest that the plant extract presented no neurotoxicity or neurobehavioural effects in rats.[11] The young foliage of the plant is readily eaten by livestock in Ghana, Cameroon and other African countries whereas in Indonesia, it is eaten as a salad[4,14] with no reported cases of toxicities in both humans and livestock. A number of studies have been conducted to evaluate the nutritive potential of *Synedrella nodiflora* in livestock and in one of these studies it was realized that supplementation of the feed of guinea pigs with the node weed plant protects the animals from copper and lead-induced toxicities.[15] However, a 14-day administration of an aqueous extract from the plant to male rats was found to have some degree of toxicity on the haematological parameters, liver and also caused degenerative changes in the germinal epithelial cells of the seminiferous tubules.[16] Contrary to this report, a current toxicological assessment of a hydro-ethanolic extract from the plant suggests that it possess no observable toxicity in male Sprague-Dawley rats following a 14-day oral administration and the lethal dose of this extract was found to be greater than 6400 $mgkg^{-1}$.[17,18] The present study was conducted with the aim of establishing the toxicological effects of a hydro-ethanolic extract following a 90-day continuous oral administration in male Sprague-Dawley rats and this will provide further information regarding the safety of this plant, which has an enormous potential for drug discovery.

METHODS

Plant collection and extraction

Samples of the whole plant were collected from the Botanical Gardens, University of Ghana, Accra, in August 2012 and authenticated by the Department of Botany, University of Ghana, Legon, Accra where a voucher specimen (PA01/UGSOP/GH12) was kept. The hydro-ethanolic extract was made as previously described.[5] Briefly, the samples of the collected plant were air-dried for seven days and powdered. Suitable amounts of the powder were cold-macerated with 70 % v/v of ethanol in water. The hydro-ethanolic extract was then evaporated to a syrupy mass under reduced pressure, air-dried and kept in a dessicator and the percent yield calculated. The resultant product was subsequently referred to as the extract or SNE.

Phytochemical screening of SNE

The hydro-ethanolic extract was tested qualitatively for the presence of flavonoids tannins, saponins, sterols, alkaloids, cardiac glycosides, coumarins, triterpernoids, anthraquinones and phenolic compounds based on test methods as previously described.[19]

Animals

A total of 20 Male Sprague-Dawley rats (Hsd:SD strain), weighing 150-200 g and 6-8 weeks old, were obtained from and maintained at the Department of Animal Experimentation, Noguchi Memorial Institute for Medical Research (NMIMR), University of Ghana, Legon, where all experimental procedures were performed. All animal procedures and techniques used in these studies were approved by the Scientific and Technical Committee (STC) of the Noguchi Memorial Institute for Medical Research [reference number STC-6 (1) 2012-13] and also by the Noguchi Institutional Animal Care and Use Committee (NIACUC), College of Health Sciences, University of Ghana with protocol number NIACUC-2012-01-1E. It was also ensured that all experiments carried out on animals conformed to the OECD guidelines. The animals were housed in groups of five in stainless steel cages (34 cm x 47 cm x 18 cm) with soft wood shavings as bedding, fed with normal commercial pellet diet (AGRIMAT, Kumasi), were given water *ad libitum* and maintained under laboratory conditions (temperature 22±2 °C, relative humidity 60-70%, and 12 hour light-dark cycle).

Animal Groupings and extract administration

The acclimatized SD rats were randomly grouped into four (five rats/group) namely; vehicle (distilled water 1.667 $mlkg^{-1}$), SNE 100 $mgkg^{-1}$, SNE 300 $mgkg^{-1}$ and SNE 1000 $mgkg^{-1}$. The animals were dosed daily for ninety (90) days with vehicle and SNE by oral gavage in order to mimic the traditional folkloric route of administration. The doses of the extract were selected based on previously reported doses of the extract that were found to be pharmacologically active.[5,6,10] All drug administrations were given at 8:00 GMT each day and the determinations (e.g blood collection and post-mortem examinations) were done before 15:00 GMT.

Clinical Observation and sub-chronic toxicity study

Animals in each group were weighed on the first day before the extract administration and on the 14^{th}, 42^{nd} and 91^{st} day of the experimental period. The animals were monitored daily after the extract/vehicle administration and observed for any clinically observed toxidromes such as changes in movement, salivation, mydriasis, respiratory pattern, piloerection, frequency and consistency of stool and mortality within forty-eight hours.

Mortality was also recorded and the cause investigated. On day 91 of the study period, blood samples were collected from each animal via cardiac puncture into BD microtainer brand tube with EDTA (1 ml) and BD vacutainer SST – II Advance (5 ml) for haematological and biochemical analysis, respectively. An automated haematology analyzer (KX-2IN, Sysmex Corporation, Japan) was used for the haematological analysis and Selectra Junior version 04 autoanalyzer (Vital Scientific Bv, Netherlands) for the biochemical assays indicative of renal function (urea, creatinine, potassium and sodium), lipid profile (total cholesterol, triglycerides, high density lipoprotein (HDL), low density lipoprotein (LDL), very low density lipoprotein (VLDL) cholesterol) and liver function (total protein, albumin, globulin, direct, indirect and total bilirubin, alanine aminotransferase (ALT), aspartate aminotransferase (AST) and alkaline phosphatase (ALP) enzyme assays).

The rats were euthanized in a chloroform chamber and immediately autopsied. All visible organs and tissues were macroscopically examined, harvested and stored in formalin. A gross necropsy was performed and post-mortem examinations conducted. All carcasses were incinerated.

Histological Examination of some isolated organs

The isolated hearts, livers and kidneys were preserved in 10% neutral buffered formalin solution for 7 days and washed with water. Tissues were cut with a disposable microtome blade, into approximately 3 mm thick slices. Three slices each were obtained from each of the organs under investigation. Dehydration of the organs was done using ethanol and in xylene. Paraffin wax blocks of the tissues were prepared and tissue blocks were cast using a molten wax dispenser, plastic cassettes and mould boxes. The tissue blocks were sectioned at 4µm using a rotary microtome. They were then mounted onto microscopic slides and then dried overnight. The slides were later observed under a light microscope after being stained with hematoxylin and eosin (H&E) dyes. The slides were identified with codes written on the frosted sides of the slides.

Data Analysis

GraphPad Prism Version 5.0 for Windows (GraphPad Software, San Diego, CA, USA) was used for all statistical analyses. Data was expressed as mean±SEM (Standard Error of Mean). $P < 0.05$ was considered statistically significant when data of test groups were compared with that of vehicle-control in a one-way ANOVA followed by Dunnett's multiple comparison test or a two-way ANOVA followed by a Bonferroni's posthoc test where applicable.

RESULTS

Phytochemical screening of SNE

SNE as screened for the presence of various phytochemical constituents produced evidence for the presence of the following: flavonoids, tannins, saponins, alkaloids, cardiac glycosides, coumarins, triterpenoids, sterols, anthraquinones and phenols.

Clinical Observations

The SNE-treated rats did not show any observable abnormality in the movement, salivation, mydriasis, respiratory pattern, piloerection, frequency and consistency of stool of rats in comparison to the vehicle-treated group as daily observed and throughout the entire study period. No mortality was also recorded during the study period.

Changes in animal weight

There was no significant (P=0.98) difference in the weights of all the animals when measured on the 0, 14th, 42nd and 91st day of the study (Table 1).

Table 1 The effects of SNE (100, 300 and 1000 mgkg^{-1}) on the weights (g) of rats in a 90-day sub-chronic toxicity study.

Day	Body weight (g)			
	Vehicle	SNE 100mgkg^{-1}	SNE 300mgkg^{-1}	SNE 1000mgkg^{-1}
0	218.80 ± 5.43	185.20 ± 10.15	184.00 ± 7.98	183.60 ± 13.30
14	254.60 ± 8.48	241.60 ± 8.23	241.20 ± 11.91	229.20 ± 7.68
42	344.40 ± 0.37	333.20 ± 12.94	328.80 ± 22.52	321.00 ± 12.37
91	368.00 ± 11.95	361.60 ± 13.95	358.80 ± 24.06	364.50 ± 16.38

Post-mortem Observations

The post-mortem examination of the SNE and vehicle-treated rats revealed no visible abnormal effect in all major organs observed.

Organ Weight Variations

There were no significant (p=0.96) variations in the weights of the major organs isolated from vehicle-treated and the SNE-treated groups of rats (Table 2).

Haematological Parameters

The haematological analysis revealed a significant (p<0.01) decrease in the white blood cell and neutrophil counts for the SNE 100 mgkg^{-1} treated group (Table 3). There was, however, no significant difference (p=0.10-0.56) between the vehicle and extract treated group (100, 300 and 1000 mgkg^{-1}) with respect to the other haematological parameters measured (Table 3)

Table 2 The effects of SNE (100, 300 and 1000 mgkg^{-1}) on the weights of major organs (g) isolated from rats in a 90-day sub-chronic toxicity study.

Organs	Organ weight(g)			
	Vehicle	SNE 100mgkg^{-1}	SNE 300mgkg^{-1}	SNE 1000mgkg^{-1}
Heart	1.10 ± 0.16	1.42 ± 0.05	1.42 ± 0.22	1.23 ± 0.08
Lungs	1.96 ± 0.14	2.36 ± 0.15	1.94 ± 0.15	1.95 ± 0.16
Kidneys	2.22 ± 0.10	2.50 ± 0.09	2.14 ± 0.20	2.08 ± 0.05
Liver	12.90± 0.22	13.90 ± 0.52	11.90 ± 1.29	12.28 ± 0.37
Brain	1.58 ± 0.07	1.68 ± 0.05	1.66 ± 0.05	1.58 ± 0.05
Spleen	0.74 ± 0.05	0.84 ± 0.05	0.70 ± 0.05	0.73 ± 0.05
Intestines	23.32± 0.71	20.96 ± 1.76	21.60 ± 1.76	21.15 ± 0.55
Stomach	2.54 ± 0.08	2.63 ± 0.14	2.36 ± 0.20	2.58 ± 0.16
Testicles	15.08± 1.20	14.68 ± 1.14	14.22 ± 0.98	14.08 ± 1.50

Biochemical Parameters

The serum biochemical markers as measured were grouped as those indicating the kidney function, lipid profile and liver function assays as indicated above. There was no significant difference (P=0.14-0.54) between the vehicle-treated and SNE (100, 300, 1000 mgkg^{-1})-treated rats regarding the serum concentrations of urea, creatinine, and sodium (kidney function) of the rat subjects (Table 4). However, there was a significant (P<0.01) increase in potassium concentration in the SNE 100 mgkg^{-1}-treated group.

There was a significant difference (P=0.03) between the treatment groups with regards to HDL levels but this difference was not significant for any of the SNE-treated group in comparison to the control (Table 4). The other parameters measured for the lipid profile were not significantly different (P=0.08-0.99) for the SNE-treated animals in comparison to those treated with the vehicle (Table 4). Regarding the liver function profile of the rats used in this study, there were significant difference between the vehicle-treated and the SNE-treated rats for albumin (P=0.04), globulin (P=0.01), total bilirubin (P=0.03) and total protein (P=0.003), and these parameters were only significantly high in the SNE 100 mg/kg in comparison with the vehicle-treated groups. The other liver function parameters measured were not significantly different (P=0.28-0.83) for the SNE-treated animals in comparison to the vehicle-treated rats (Table 4).

Table 3 Haematological analysis of SNE (100, 300 and 1000 mgkg^{-1}) after a 90-day observation period

Parameter	Vehicle	SNE 100mgkg^{-1}	SNE 300mgkg^{-1}	SNE 1000mgkg^{-1}	*P value*
WBC (10^3 µL^{-1})	6.50± 0.73	2.42 ± 0.52**	5.92 ± 1.02	6.50 ± 0.25	**0.03**
RBC (10^6 µL^{-1})	7.34 ± 0.24	7.90 ± 0.15	7.80 ± 0.18	8.05 ± 0.20	**0.10**
HGB (g dL^{-1})	13.50 ± 0.37	12.06 ± 2.27	14.04 ± 0.37	14.48 ± 0.08	**0.56**
HCT (%)	42.56± 1.10	45.00 ± 1.34	44.26 ± 1.36	46.30 ± 0.56	**0.21**
MCV (fl)	58.08± 0.58	57.90 ± 0.68	56.66 ± 0.5	57.53 ± 0.72	**0.38**
MCH (pg)	18.42 ± 0.18	15.52 ± 2.88	18.00 ± 0.16	18.03 ± 0.48	**0.53**
MCHC (gdL^{-1})	31.68 ± 0.16	26.62 ± 4.86	31.76 ± 0.29	31.28± 0.45	**0.43**
PLT (10^3 µL^{-1})	792.4 ± 19.88	523.0 ± 188.50	729.0 ± 100.80	756.50±114.90	**0.38**
LYM (%)	82.52 ± 1.48	68.34 ± 17.25	76.14 ± 5.05	80.28 ± 2.94	**0.73**
NEUT (%)	14.04 ± 1.25	26.64 ± 15.73	19.14 ± 3.96	15.45 ± 2.00	**0.73**
LYM# (10^3 µL^{-1})	5.36 ± 0.67	2.63 ± 0.49	4.64 ± 0.97	5.25 ± 0.40	**0.13**
NEUT# (10^3 µL^{-1})	0.94 ± 0.09	0.42 ± 0.13*	1.00 ± 0.17	0.98± 0.11	**0.02**
RDW_SD (fl)	30.00 ± 0.28	30.22 ± 0.66	29.88 ± 0.41	29.80 ± 0.39	**0.93**
RDW_CV (%)	11.50 ± 0.23	11.86 ± 0.37	11.82 ± 0.18	12.15 ± 0.55	**0.63**
PDW (fl)	7.88 ± 0.16	9.47 ± 1.42	7.82 ± 0.14	7.90 ± 0.27	**0.16**
MPV (fl)	6.76 ± 0.09	7.23 ± 0.48	6.22 ±0.10	6.87 ±0.18	**0.27**
P_LCR (%)	4.94 ± 0.55	8.37 ± 3.23	4.36 ±0.43	5.80 ±0.81	**0.20**

Table 4 Biochemical analysis of a single administration of SNE (100, 300 and 1000 mgkg^{-1}) after a 90-day observation period in SD male rats

Parameters	Vehicle	SNE 100mgkg^{-1}	SNE 300mgkg^{-1}	SNE 1000mgkg^{-1}	*P value*
Renal function test (mmolL^{-1})					
Urea	8.95 ± 0.39	9.85 ± 0.82	9.01 ± 0.25	8.72 ± 0.31	**0.45**
Creatinine	57.62 ± 2.01	50.14 ± 5.75	66.08 ± 6.91	63.55 ± 1.90	**0.14**
Potassium	5.406 ± 0.42	15.04 ± 2.51**	7.30 ± 0.86	7.66 ± 0.87	**0.001**
Sodium	145.00 ± 1.05	147.80 ± 3.15	144.60 ± 0.68	144.50 ± 0.65	**0.53**
Lipid Profile (mmolL^{-1})					
Total Cholesterol	2.56 ± 0.15	2.62 ± 0.18	2.44 ± 0.09	2.56 ± 0.11	**0.79**
Triglycerides	0.78 ± 0.07	1.36 ± 0.50	0.72 ± 0.14	0.74 ± 0.16	**0.20**
HDL	1.06 ± 0.03	0.97 ± 0.02	0.98 ± 0.03	1.07 ± 0.03	**0.04**
LDL	1.15 ± 0.14	0.35 ± 0.43	1.12 ± 0.06	1.15 ± 0.12	**0.08**
VLDL	0.35 ± 0.03	0.37 ± 0.20	0.33 ± 0.07	0.34 ± 0.07	**0.99**
Liver function test					
Total Protein (gL^{-1})	68.18 ± 1.20	82.56 ± 4.16**	67.22 ± 1.46	70.63 ± 1.92	**0.003**
Albumin (gL^{-1})	34.12 ± 0.89	37.90 ± 1.80*	33.50 ± 0.66	35.18 ± 0.46	**0.04**
Globulin (gL^{-1})	34.06 ± 1.13	59.90 ± 10.74*	33.70 ± 1.18	35.48 ± 1.56	**0.01**
D. Bilirubin (µmolL^{-1})	1.66 ± 0.08	3.60 ± 2.07	1.56 ± 0.13	1.48 ± 0.10	**0.36**
Ind. Bilirubin (µmolL^{-1})	0.24 ± 0.06	1.13 ± 1.00	0.30 ± 0.22	0.20 ± 0.04	**0.48**
T. Bilirubin (µmolL^{-1})	1.90 ± 0.11	4.63 ± 1.35*	1.86 ± 0.16	1.68 ± 0.10	**0.03**
ALT (UL^{-1})	66.66 ± 4.84	54.30 ± 22.81	83.68 ± 12.37	74.45 ± 4.92	**0.38**
AST (IUL^{-1})	6.83 ± 3.05	3.10 ± 1.90	12.15 ± 4.47	16.40 ± 4.10	**0.20**
ALP (UL^{-1})	66.00 ± 24.92	53.70 ± 28.67	45.60 ± 40.42	54.30 ± 53.28	**0.49**

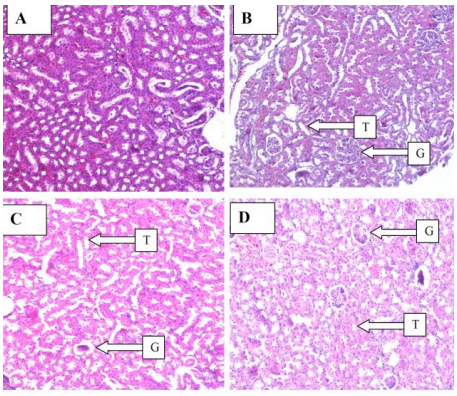

Figure 1 Photomicrographs of kidneys isolated from rats after 90-day continuous administration of (A) Vehicle, (B) SNE 100 mgkg^{-1}, (C) SNE 300 mgkg^{-1} and (D) SNE 1000 mgkg^{-1} showing normal renal tubule (T) and glomeruli (G) (H&E staining, 100×).

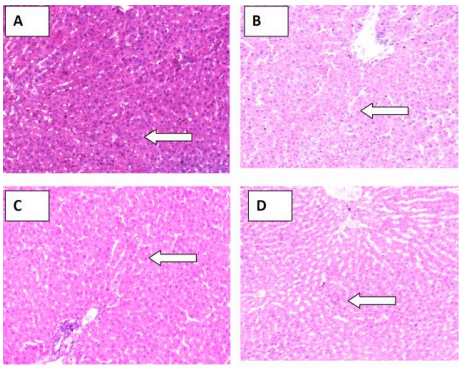

Figure 2 Photomicrographs of livers isolated from rats after 90-day continuous administration of (A) Vehicle, (B) SNE 100 mgkg^{-1}, (C) SNE 300 mgkg^{-1} and (D) SNE 1000 mgkg^{-1} (H&E staining, 100×). The arrow shows the hepatocyte which are evenly distributed.

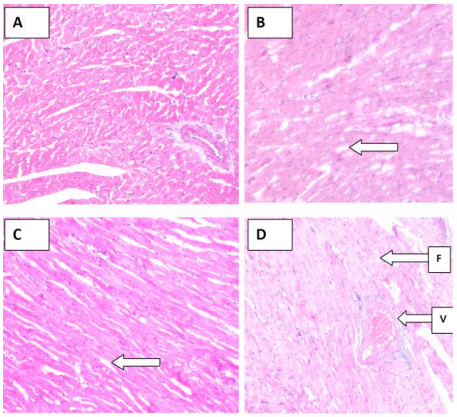

Figure 3 Photomicrographs of hearts isolated from rats after 90-day continuous administration of (A) Vehicle, (B) SNE 100 mgkg^{-1}, (C) SNE 300 mgkg^{-1} showing normal myocardial fibres with characteristic central nuclei and branching arrangement as indicated by the arrows and (D) SNE 1000 mgkg^{-1} showing obvious distortion of the branching arrangements of the myocardial fibres (F) and a congested vessel (V) with little or no inflammatory cell infiltration indicating a healed infarction (H&E staining, 100×).

Histological Examination of some isolated organs

The histopathological examination of the kidneys and liver did not reveal any abnormalities (Figures 1 and 2). The renal tubules and glomeruli were normal. The heart isolated from rat treated with the vehicle control, SNE 100 mgkg^{-1} and SNE 300 mgkg^{-1} showed normal myocardial fibres with characteristic central nuclei and branching arrangement (Figure 3 A, B and C). However, the isolated hearts from rats treated with SNE 1000 mgkg^{-1} revealed distortion of the branching arrangements of the myocardial fibres and a congested vessel with little or no inflammatory cell infiltration (Figure 3 D).

DISCUSSION

The present study was conducted to assess the toxicity of a hydro-ethanolic extract of *Synedrella nodiflora* (L) Gaertn following a 90-day continuous administration of increasing doses in male Sprague-Dawley rats.

This was done to provide further information regarding the toxicological effects of the hydro-ethanolic extract of the whole plant.

In general, the extract was found to possess low toxicity on haematological and biochemical parameters and very little histopathological changes in the isolated organs from the rats treated with the extract.

The extract, SNE, did not alter the weight of the animals during the 90-day continuous administration since there was no significant difference between the weights of the animals treated with the vehicle and that treated with SNE. This indicates that the extract may not possess any ability to cause an abnormality in carbohydrate or fat metabolisms. [20-22]

Haematological parameters were measured in this study to determine the effect of the extract on blood and blood forming tissues. Measurement of full blood count including the RBC count, haemoglobin concentration, and haematocrit, can establish the presence of anaemia whiles the mean corpuscular volume (MCV) and the reticulocyte count parameters are useful in classifying the anaemia.[23-25]

The leucocytes, or white blood cells, including granulocytes, may be subdivided into neutrophils, eosinophils, and basophils; monocytes; and lymphocytes. Granulocytes and monocytes are nucleated amoeboid cells that are phagocytic and play a central role in the inflammatory response and host defence.[23] Decreased amounts of leucocytes denote leucopenia [24,26] and raised amounts suggest bacterial infections or blood disorders.[27] Only SNE 100 $mgkg^{-1}$ caused a decrease in the white blood cell count as well as the neutrophil counts whiles the other parameters did not significantly differ from the vehicle-control group, thus this low dose can cause leucopenia and neutropenia (decreased levels of neutrophils).[28] Neutropenia predisposes patients to many bacterial and fungal infections.[29] Decreased neutrophil counts may be immune-mediated (where antigen-antibody reactions lead to the destruction of peripheral neutrophils, granulocyte precursors, or both) or non-immune-mediated which may indicate a genetic predisposition that results in direct neutrophil damage leading to inhibition of granulopoiesis or neutrophil function.[30-32] Further investigations into the mechanism(s) by which this leucopenia or agranulocytopenia as caused by SNE 100 $mgkg^{-1}$ during a continuous 90-day administration in SD rats is warranted. The other higher doses of SNE (300 and 1000 $mgkg^{-1}$) did not significantly affect the entire haematological parameters measured in comparison to the vehicle-control group, thus devoid of either enhancing haematopoiesis or causing haematoxicities.

On the effect of the extract on the mammalian renal system, only the low dose, SNE 100 $mgkg^{-1}$, produced a significant increase in the levels of serum potassium (in comparison to the vehicle-control group). This increase in potassium denotes hyperkalaemia which may develop in patients with underlying disorders affecting potassium handling, such as chronic renal failure, and those taking a combination of drugs.[33] Drug-induced hyperkalaemia may also result when there is a reduction in renal potassium excretion due to hypoaldosteronism or an increase in extracellular potassium shifts or an increase in potassium supply.[34] Severe hyperkalaemia can cause life-threatening cardiac dysrhythmias.[35] Given that the vast majority of body potassium is intracellular, the extracellular potassium concentration is significantly dependent on both the total amount of potassium in the body and the distribution of this potassium between the extracellular and intracellular fluid compartments.[36] Furthermore, the extracellular value does not necessarily reflect total-body potassium and thus most drug-induced hyperkalaemia may not produce any obvious excitation in the myocardial cells. [36]

Thus it is possible to have patients presenting with hyperkalaemia and yet at the same time be depleted of total-body potassium. Since the higher doses of the extract (300 and 1000 $mgkg^{-1}$) produced no significant effect on the renal function indices, it may suggest the high doses of the extract are devoid of any effect whether therapeutic or adverse on the renal functions of SD male rats. [37] Moreover, histological assessment of the kidney revealed no observable abnormalities, thus buttressing the assertion that SNE may be devoid of any potentially threatening renal damage or impairment.

Regarding the effect of the extract on the lipid profile, the data obtained shows that the extract did not significantly affect the lipid profile (total cholesterol, HDL, LDL and VLDL cholesterols) of the male SD rats. Significant increase in the total cholesterol LDL and VLDL cholesterols by an administration of a substance are consistently associated with possible risk of atherosclerosis and cardiovascular diseases.[38,39] Thus a long-term administration of SNE may not have any potential therapeutic or adverse effect on lipid metabolism of SD male rats. However, a chronic toxicity study with similar data and also in a non-rodent model animal would be needed to confirm this assertion.

Regarding the biochemical parameters as measured to reflect the liver function of the SD rats, namely (total protein, albumin, globulin, direct bilirubin, indirect bilirubin, total bilirubin, ALT, AST and ALP), albumin, globulin total bilirubin and total protein were significantly elevated by SNE 100 $mgkg^{-1}$. Increased levels of albumin may result from dehydration or high protein diet.[40] Elevation in globulin and total protein levels has been associated with chronic infections (parasites, some cases of viral and bacterial infection), liver disease (biliary cirrhosis, obstructive jaundice), carcinoid syndrome, rheumatoid arthritis, ulcerative colitis, multiple myelomas, leukemias, Waldenstrom's macroglobulinaemia, autoimmunity (systemic lupus), collagen diseases and kidney dysfunction (nephrosis).[41] The present data makes it difficult to ascertain the exact cause of this high level of serum globulin in the SNE 100 $mgkg^{-1}$ treated rats. However, since globulins are proteins that include gamma globulins (antibodies) and a variety of enzymes and carrier/transport proteins, specific profile of the globulins needs to be determined by protein electrophoresis to ascertain the exact group of globulins where the abnormality lies.[24] Elevation in bilirubin levels may suggest a liver and/ bile duct damage as well as enhanced haemoglobin breakdown.[42-44] Since a 14-day continuous administration of SNE 100 $mgkg^{-1}$ did not produce such results in male SD rats,[18] it can be suggested that a long-term administration of this dose beyond 14 days may present hyperbiluribinaemia. In contrast, higher doses of the extract (300 and 1000 $mgkg^{-1}$) may not present any liver or bile duct damage during short-or long-term administration. This assertion is further supported by the histological assessment of the liver from the rats pre-treated with SNE (100, 300 and 1000 $mgkg^{-1}$) showing no abnormalities.

Histopathological assessment of the liver and kidneys revealed no organ abnormalities for SNE 100, 300 and 1000 $mgkg^{-1}$. However, only SNE 1000 $mgkg^{-1}$ showed some distortion of the branching arrangements of the myocardial fibres and a congested vessel which suggests a healed infarction.[45,46] Though at this point it is unclear how this infarction resulted, it will be prudent to advice the traditional healer who uses the plant to be cautious when using it in patients with cardiac disorders. The lower doses of SNE (100 and 300 $mgkg^{-1}$) did not produce any observable abnormality thus may not have any cardiotoxicity in rats.

The foliage from the plant is being eaten by livestock and humans as well with no reported or documented toxicities.[4,14] Thus it can be said that toxicities as discovered in the male SD rats can also occur in humans and such studies in patients who use this plant is warranted.

Future research evaluating the effects of the hydro-ethanolic extract in female SD rats, non-vertebrates and even in primates should be considered to provide a holistic overview of the toxicology of the plant.

In conclusion, the hydro-ethanolic extract of *Synedrella nodiflora* (L) Gaertn general has a low toxicity profile following a 90-day continuous oral administration in male Sprague-Dawley rats. However, a low dose of 100$mgkg^{-1}$ may cause leucopenia (and/neutropenia), hyperbilirubinaemia and hyperkalaemia, whereas a high dose of 1000 $mgkg^{-1}$ may result in some degree of cardiotoxicity.

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