ORIGINAL ARTICLE

Sub-acute toxicity of a hydro-ethanolic whole plant extract of *Synedrella nodiflora* (L) Gaertn in rats

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Objectives: *Synedrella nodiflora* (L) Gaertn (family Asteraceae) is traditionally used in Ghana for the management of epilepsy, hiccup and threatened abortion. The anticonvulsant and other related neuro-pharmacological effects of a hydro-ethanolic extract in murine models have been established. To this end, we evaluated a sub-acute toxicity of the hydro-ethanolic whole plant extract in rats. **Materials and Methods:** The effects of a continuous 14-day oral administration of the extract (100, 300 and 1000 mg/kg) on haematological and serum biochemical parameters were measured. **Results:** The extract produced no mortality in the rats treated during the study period. The extract also did not significantly affect any of the haematological and serum biochemical indices measured. **Conclusion:** This result suggests that a 14-day oral administration of the hydro-ethanolic extract of *Synedrella nodiflora* is relatively safe in Sprague-Dawley male rats under the present laboratory conditions.

Key words: Male rats, sub-acute toxicity, *Synedrella nodiflora*

INTRODUCTION

Synedrella nodiflora (L.) Gaertn (family Asteraceae) is a common weed of waste places found predominately in Ghana and other west African countries as well as tropical Asia.[1] In Ghana, traditional healers boil the whole plant and administer the aqueous extract for the management of epilepsy. They also use the leaves for threatened abortion, hiccup, laxative and as a feed for livestock.[1,2] Subsistence farmers in Ghana also use the leaves of the plant as post-harvest protectants.[3] In Nigeria, some indigenous tribes traditionally use the whole plant for treating cardiac problems and to stop wound bleeding.[4] The leaves of the plant is eaten by human as vegetable in Indonesia.[5] Traditional healers in Indonesia also use the leaf sap together with other materials for stomachache and rheumatism[6] In Malaysia, the leaves are used for poulticing sore legs and for the treatment of headache, and the sap is instilled into the ear for earache.[6] The leaf extract of *S. nodiflora* have been reported to control storage pests but had no toxic effect in vertebrates.[7] Also the insecticidal effects of various solvent extracts of the aerial parts of *S. nodiflora* on the fourth instar larvae of *Spodoptera litura* has been reported.[8]

We have demonstrated that the hydro-ethanolic extract of the whole plant possess anticonvulsant,[9] sedative,[10] *in vitro* antioxidant and free radical scavenging[11] and antinociceptive properties.[12] There is little scientific information regarding the toxicological effects of the plant especially regarding the doses used in the above mentioned pharmacological effects. The present study therefore, seeks to evaluate the effect of a 14-day administration of the hydro-ethanolic extract in doses established for anticonvulsant effects on haematological and biochemical parameters in Sprague-Dawley rats.

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MATERIALS AND METHODS

Plant Collection and Extraction

The whole plant samples were collected from the Botanical Gardens, University of Ghana, and Accra in August 2012. They were then brought to the Herbarium, Department of Botany, University of Ghana, Legon, Accra for identification and authentication, following which a voucher specimen (#PA01/UGSOP/GH12) was kept. The hydro-ethanolic extract was prepared as previously described.[12] 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

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kept in a desiccator and the percent yield was calculated. The resultant product was subsequently referred to as the extract or SNE.

Animals

Male Sprague-Dawley (SD) rats (150-200 g), 6-8 weeks old were obtained from the Animal Experimentation, Noguchi Memorial Institute for Medical Research (NMIMR), University of Ghana, Legon, Accra. The animals were housed in groups of five in stainless steel cages (34 cm × 47 cm × 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) for 7 days prior to the acute toxicity study.

Animal Groupings and 14-day Extract Administration

The acclimatised SD rats were randomly grouped into four (five rats/group) namely; vehicle (distilled water 1.667 ml/kg), SNE 100 mg/kg, SNE 300 mg/kg and SNE 1000 mg/kg. The vehicle and SNE were administered orally (gavage) to mimic the traditional folkloric route of administration. All animal procedures and techniques used in these studies were in accordance with the Noguchi Institute Animal Care and Use Committee (NIACUC) guidelines as well as the National Institute of Health Guidelines for the Care and Use of Laboratory Animals and other known internationally acceptable guidelines for evaluating safety and efficacy of herbal medicines.[13-16]

A 14-day Clinical Observation and Sub-acute Toxicity Study

Following daily administration of the extract, the animals were monitored and observed daily for any clinical signs of toxicity such as abnormality in the movement, salivation, mydriasis, respiratory pattern, piloerection, frequency and consistency of stool) and mortality. The animals were weighed on the 1st, 8th, 11th and on the 15th day. After weighing the animals on the 15th day of the study period, the rats were euthanised and blood samples were collected from each animal via cardiac puncture into BD microtainer brand tube with ethylenediaminetetraacetic acid (EDTA) (1 ml) and BD vacutainer SST – II Advance (5 ml) for haematological and biochemical analyses, respectively. An automated haematology analyser (KX-2IN, Sysmex Corporation, Japan) was used for the haematological analysis and Selectra Junior version 04 autoanalyser (Vital Scientific Bv, Netherlands) for the biochemical assays.

The animals were immediately autopsied and all visible organs and tissues were macroscopically examined, harvested, and stored in formalin. A gross necropsy was performed and post-mortem examinations conducted.

Data Analysis

GraphPad Prism Version 5.0 for Windows (GraphPad Software, San Diego, CA, USA) was used for all statistical analyses. $P \leq 0.05$ was considered statistically significant in all analysis.

RESULTS

Clinical Observations

The oral administration of SNE (100, 300 and 1000 mg/kg) did not produce any observable abnormality in the movement, salivation, mydriasis, respiratory pattern, piloerection, frequency and consistency of stool of the rats in comparison with the vehicle-treated group, in the entire study period.

Post-mortem Observations

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

Haematological and Biochemical Analyses

There was no significant difference (P = 0.06-0.85) between the vehicle-treated group and the extract (100, 300 and 1000 mg/kg) regarding all the hematological indices measured (such as white and red blood counts, haemoglobin concentration and mean corpuscular haemoglobin concentration etc.) [Table 1].

The serum biochemical markers as measured were grouped as those indicating the renal function (urea, creatinine, potassium and sodium electrolytes), lipid profile (total cholesterol, triglycerides, high density lipoprotein (HDL), low density lipoprotein (LDL) and very low density lipoprotein (VLDL) cholesterol) and for liver function (total protein, albumin, globulin, direct, indirect, and total bilirubin, alanine amino transferase (ALT), aspartate amino transferase (AST) and alkaline phosphatase (ALP) enzyme assays) and presented in Table 2. Regarding the renal function of the rat subjects, there was no significant difference (P = 0.15-0.90) between the vehicle-treated and SNE-treated rats (100, 300, 1000mg/kg b.wt). There was also no significant difference (P = 0.07-0.99) in the lipid profile of vehicle-treated rats in comparison with SNE-treated rats. With respect to the liver function assessment of the rats used in the study, there was no significant difference (P = 0.20-0.99) between the vehicle-treated and the SNE-treated rats for all parameters measured.

Animal Weights

There was no significant difference (P = 1.00) between the rats treated with the extract and the vehicle over the 15-day study period [Figure 1].

Table 1: Haematological analysis of 14-day oral administration of SNE (100, 300 and 1000 mg/kg) in male SD rats. Data are mean±SEM (n=5). $P \leq 0.05$ was considered significant when compared to vehicle group (one-way ANOVA followed by a Dunnett's multiple comparison test)

Parameter	Vehicle	SNE 100 mg/kg	SNE 300 mg/kg	SNE 1000 mg/kg	*P* value
WBC (10^3/µl)	11.40±1.55	12.46±2.16	11.16±1.95	9.54±1.23	0.71
RBC (10^6/µl)	7.85±1.04	7.93±0.10	7.67±0.27	7.69±0.14	0.65
HGB (g/dl)	14.58±0.16	14.48±0.27	14.18±0.47	14.30±0.18	0.78
HCT (%)	46.12±0.62	46.24±0.74	44.66±1.61	44.62±0.34	0.48
MCV (fl)	58.72±0.50	58.32±1.17	58.24±1.48	57.96±0.86	0.97
MCH (pg)	18.56±0.17	18.26±0.43	18.52±0.50	18.50±0.45	0.29
MCHC (g/dl)	31.62±0.27	31.32±0.25	31.76±0.16	32.04±0.28	0.75
PLT (10^3/µl)	666.80±127.90	829.20±55.32	797.60±213.40	640.60±150.50	0.75
LYM (%)	84.40±0.78	86.74±1.46	86.08±1.91	86.92±2.20	0.30
NEUT (%)	11.04±0.80	10.54±1.31	10.58±1.75	9.32±1.34	0.56
LYM# (10^3/µl)	9.62±1.33	10.86±1.90	9.58±1.68	8.26±1.04	0.70
NEUT# (10^3/µl)	1.24±0.15	1.30±0.30	1.22±0.31	0.90±0.20	0.68
RDW_SD (fl)	28.62±0.20	28.38±0.27	28.56±0.17	28.24±0.07	0.50
RDW_CV (%)	10.52±0.22	10.26±0.08	10.50±0.34	10.46±0.21	0.85
PDW (fl)	8.36±0.43	7.52±0.18	7.48±0.25	7.44±0.16	0.09
MPV (fl)	6.92±0.13	6.58±0.07	6.53±0.13	6.54±0.10	0.06
P_LCR (%)	6.38±1.05	4.38±0.32	4.25±0.58	4.80±0.65	0.17

SNE – Synedrella nodiflora extract; SD – Standard deviation; WBC – White blood cells; RBC – Red blood cells; HGB – Haemoglobin; HCT – Haematocrit; MCV – Mean corpuscular volume; MCH – Mean corpuscular haemaglobin; MCHC – Mean corpuscular haemoglobin concentration; PLT – Platelet; ANOVA – Analysis of variance; LYM – Lympohocyte; NEUT – Neutrophil; RDW – Red cell distribution width; PDW – Platelet distribution width; MPV – Mean platelet volume; P_LCR – Platelet larger cell ratio

Table 2: Biochemical analysis of 14-day oral administration of SNE (100, 300 and 1000 mg/kg) in male SD rats. Data are mean±SEM (n=5). $P \leq 0.05$*; $P \leq 0.01$ compared to the vehicle group (one-way ANOVA followed by a Dunnett's multiple comparison test)**

Parameters	Vehicle	SNE 100 mg/kg	SNE 300 mg/kg	SNE 1000 mg/kg	*P* value
Renal function test (mmol/l)					
Urea	8.02±0.26	7.31±0.37	7.12±0.40	7.87±0.39	0.26
Creatinine	65.46±3.27	57.90±3.37	62.70±1.69	66.16±1.57	0.15
Potassium	7.25±1.88	5.37±0.33	4.92±0.23	5.63±0.40	0.39
Sodium	143.40±0.81	142.60±0.10	142.80±1.02	142.60±0.60	0.90
Lipid Profile					
Total cholesterol	2.47±0.05	2.50±0.09	2.41±0.08	2.35±0.10	0.57
Triglycerides	0.78±0.03	0.83±0.09	0.78±0.08	0.54±0.08	0.06
HDL	0.99±0.04	1.01±0.03	0.97±0.02	0.99±0.04	0.82
LDL	1.11±0.04	1.12±0.11	1.09±0.03	1.12±0.07	0.99
VLDL	0.35±0.02	0.38±0.04	0.35±0.03	0.25±0.04	0.07
Liver function test					
Total protein (g/dl)	68.76±1.74	68.68±0.65	69.36±2.50	66.06±1.24	0.53
Albumin (g/dl)	34.20±0.24	34.28±0.54	34.06±0.34	33.78±0.79	0.91
Globulin (g/dl)	34.58±1.66	34.42±0.77	35.28±2.27	32.30±1.14	0.58
D. Bilirubin (µmol/l)	1.68±0.12	3.40±1.07	3.06±0.54	3.02±0.59	0.31
Ind. Bilirubin (µmol/l)	−0.73±0.03	−0.79±0.07	−0.64±0.03	−0.68±0.06	0.20
T. Bilirubin (µmol/l)	0.95±0.14	2.77±1.12	2.42±0.54	2.34±0.55	0.29
ALT (U/l)	91.34±18.50	91.88±23.94	108.40±44.78	79.04±10.86	0.90
AST (U/l)	10.76±8.46	8.00±5.13	8.24±5.33	10.04±5.91	0.99
ALP (U/l)	318.50±22.73	353.60±24.43	359.0±31.83	309.3±13.42	0.39

SNE – Synedrella nodiflora extract; SD – Standard deviation; ANOVA – Analysis of variance; HDL – High density lipoprotein; LDL – Low density lipoprotein; VLDL – Very low density lipoprotein; ALT – Alanine amino transferase; AST – Aspartate amino transferase; ALP – Alkaline phosphatase

DISCUSSION

The study presents the sub-acute toxicity of a hydro-ethanolic extract of *Synedrella nodiflora* in male SD rats. The 14-day administration of increasing doses of the extract (100, 300 and 1000 mg/kg) did not significantly affect haematological, biochemical and gross organ assessments of the rat subjects used.

Subacute toxicity tests are generally done to obtain information on the toxicity of a substance after repeated administration typically for 14 days and as an aid

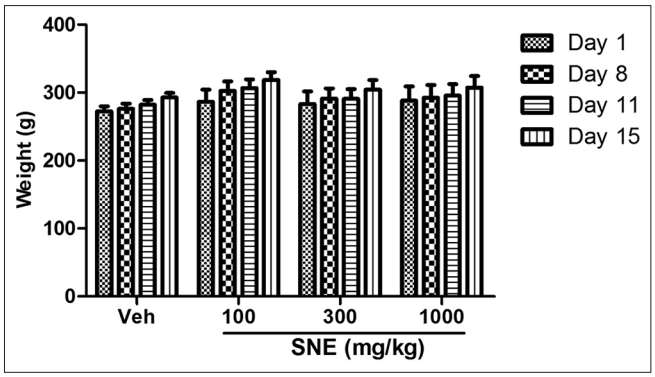

Figure 1: Weights (g) of rats on 1st, 8th, 11th and 15th day following a 14-day administration of SNE (100, 300 and 1000 mg/kg) or distilled water (Veh). Data are mean ± SEM. $P \leq 0.05$ considered significant (two-way ANOVA followed by a Bonferroni's *posthoc* test)

in establishing doses for subchronic studies.[17] The sub-acute toxicity in this study was done using doses of the hydro-ethanolic extract that has demonstrated anticonvulsant and other related neuro-pharmacological effects.[9] The rationale was to determine how toxic these doses of the extract will be, following a short-term administration in laboratory rats.

The hematological parameters measured were not significantly different between the vehicle-treated rats and the various doses of the extract [Table 1]. Haematological parameters such as complete or full blood count including the RBC count, haemoglobin concentration, and haematocrit, can establish the presence of anaemia. Other additional parameters that are helpful in classifying anaemia are the mean corpuscular volume (MCV) and the reticulocyte count.[18-20]

Increased amount of red cell count, haemoglobin concentration and haematocrit suggest erythropoietic or haematinic effects, whereas a reduction connotes the presence of anaemia.[18] The leukocytes, or white blood cells, including granulocytes, may be subdivided into neutrophils, eosinophils, and basophils; monocytes; and lymphocytes. Granulocytes and monocytes are nucleated ameboid cells that are phagocytic and play a central role in the inflammatory response and host defence.[20] Decreased amounts of leucocytes denote leucopenia[18] and raised amounts suggest bacterial infections or blood disorders.[21] The major constituents of the haemostatic system include circulating platelets, a variety of plasma proteins, and vascular endothelial cells. Alterations in these components or systemic activation of this system can lead to the clinical manifestations of deranged haemostasis, including excessive bleeding and thrombosis.[20,22] Since the extract produced no significant changes in all the haematological indices measured, it suggests that SNE may not have any haematotoxic effects in the SD male rats.[23]

On the renal function of the male SD rats, the extract did not produce any significant effect on the biochemical parameters (urea, creatinine, sodium and potassium) measured in comparison with the vehicle-treated group. Elevated serum creatinine and urea suggest kidney or other diseases such as urinary obstruction, muscle disease, arthritis, and hyperthyroidism.[24,25] Since the extract produced no significant effect on the renal function indices, it may suggest the extract is devoid of any effect whether therapeutic or adverse on the renal functions of SD male rats.[26]

Furthermore, the extract did not significantly affect the lipid profile (total cholesterol, HDL, LDL and VLDL cholesterols) of the male SD rats. An increase in the total cholesterol LDL and VLDL cholesterols by an administration of a substance suggests that substance might predispose organisms to possible risk of atherosclerosis and cardiovascular diseases.[27,28] This also suggests that SNE may not have any potential therapeutic or adverse effect on lipid metabolism of SD male rats.

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), the extract did not produce any significant changes in comparison to the vehicle-treated group in all the parameters. Thus, SNE may also be safe in relation to the liver function of SD rats.[29]

An overall assessment of the effect of the extract on the major organs coupled with no visible macroscopic abnormalities generally suggests that the extract is relatively safe when administered orally for a short period of 14 days.[30] These results confirm earlier reports of extracts from the plant where no toxicity in vertebrates was observed.[7] Moreover, there are reports of the leaves of the plant being eaten as food by livestock and humans without any reported toxicities. Thus the extract is safe with its therapeutic doses of 100, 300, and 1000 mg/kg.

CONCLUSION

The hydro-ethanolic extract of *Synedrella nodiflora* (L) Gaertn produced no-observed-adverse-effect-level (NOAEL) significant effect on the haematological and biochemical parameters in male SD rats under the present experimental conditions.

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