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ORIGINAL ARTICLE

Biochemical and haematological changes following an acute toxicity study of a hydro-ethanolic whole plant extract of *Synedrella nodiflora* (L) Gaertn in male Sprague-Dawley rats

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***Synedrella nodiflora* (L) Gaertn (family Asteraceae), a common weed in Ghana, is traditionally used for the management of epilepsy, hiccup and threatened abortion. To further promote the ethnopharmacological uses of the plant, an acute toxicity of a hydro-ethanolic whole plant extract was assessed in male Sprague-Dawley rats. The lethal dose (LD_{50}) and effects of a single oral administration of the extract (1600, 3200 and 6400 mg kg^{-1}) on haematological and serum biochemical parameters were measured. The extract produced no mortality in the rats treated during a 48-hour examination and after a subsequent 12-day assessment. Thus the LD_{50} was indicated as being greater than 6400 mg kg^{-1}. The extract also did not significantly affect any of the haematological and serum biochemical indices. This result suggests that acute oral administration of the hydro-ethanolic extract of *Synedrella nodiflora* is virtually non-toxic in male Sprague-Dawley rats under normal laboratory conditions.**

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INTRODUCTION

Synedrella nodiflora (L.) Gaertn (family Asteraceae) is a common weed of waste places and found along the banks of rivers, streams and also along roadsides (Mshana *et al.,* 2000). 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 (Dalziel, 1931; Mshana *et al.,* 2000). The plant is also used by subsistence farmers of Ghana as post-harvest protectants (Cobbinah *et al.,* 1999). Traditional uses of the plant in other African and some Asian countries have been reported. In Nigeria, it is known that some indigenous tribes use the whole plant for the treatment of cardiac distresses and to stop wound bleeding (Idu and Onyibe, 2007). The foliage is readily eaten by livestock in Cameroon (Irvine, 1961). 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 (Burkill, 1985). In Malaysia, a poultice of the leaves are used for managing sore legs and for the treatment of headache and the sap is instilled into the ear for earache (Burkill, 1985).

The hydro-ethanolic extract of the whole plant has been found to possess anticonvulsant (Amoateng *et al.,* 2012), sedative (Woode *et al.,* 2011), *in vitro* antioxidant and free radical scavenging properties (Amoateng *et al.,* 2011) and anti-nociceptive properties (Woode *et al.,* 2009). To explore the plant for further pre-clinical anti-epileptic drug discovery and development, it is important to investigate the toxicity of the plant. The leaf extract of *S. nodiflora*, among other plants investigated, has been reported

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to control storage pests but had no toxic effect in vertebrates (Belmain *et al.,* 2001). The insecticidal effects of various solvent extracts of the aerial parts of *S. nodiflora* on the fourth instar larvae of *S. litura* has also been reported (Martin and Gopalakrishnan, 2005). To clarify the extent to which the plant is toxic in rodents, the present study reports an acute toxicity of the hydro-ethanolic extract of the whole plant of *Synedrella nodiflora* in male Sprague-Dawley rats.

MATERIALS AND METHODS

Plant collection and extraction

Samples of the plant were collected from the Botanical Gardens, University of Ghana, Accra in August 2012 and were identified and authenticated at Ghana Herbarium, Department of Botany, University of Ghana, Legon, Accra where a voucher specimen (# PA01/UGSOP/GH12) was kept. The hydro-ethanolic extract was prepared as previously described by Woode *et al.,* (2011). 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, 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, triterpenoids, anthraquinones and phenolic compounds based on test methods as previously described by Evans (2001).

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 x 47 cm x 18 cm) with soft wood shavings as bedding, fed with normal commercial pellet diet (AGRIMAT, Kumasi), given water *ad libitum* and maintained under laboratory conditions (temperature 22 ± 2°C, relative humidity 60-70% and 12 hour light-dark cycle) for seven (7) days prior to the acute toxicity study. 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 (NIH, Department of Health Services publication No. 83-23, revised 1985).

Animal Groupings and Acute extract administration

The acclimatized SD rats were randomly grouped into four (five rats/group) namely; vehicle (distilled water 1.667ml kg^{-1}), SNE 1600 mg kg^{-1}, SNE 3200 mg kg^{-1} and SNE 6400 mg kg^{-1}. The vehicle and SNE were administered orally (by gavage) to mimic the traditional folkloric route of administration.

48 hour Clinical Observations and LD_{50} determination

After administration of the extract/distilled water, the animals in each group were observed every seven hours for clinical signs of toxidromes such as changes in movement, salivation, mydriasis, respiratory pattern, piloerection, frequency and consistency of stool and mortality within forty-eight hours. Mortality after twenty-four and forty-eight hour post treatment were recorded and the LD_{50} (the lethal dose) was determined.

A 12-day Clinical Observation and acute toxicity study

The animals were then monitored and observed daily during the next 12 days for any clinically observed toxidromes and mortality. On the 14th day of the study period the rats were euthanized and 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, Nether-

lands) for the biochemical assays (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 test (total protein, albumin, globulin, direct, indirect and total bilirubin, alanine aminotransferase (ALT), aspartate aminotransferase (AST) and alkaline phosphatase (ALP) enzyme 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

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, triterpenes, sterols, anthraquinones and phenols.

Clinical Observations
The single oral administration of SNE (1600, 3200 and 6400 mg kg^{-1}) did not produce observable abnormality in the movement, salivation, mydriasis, respiratory pattern, piloerection, frequency and consistency of stool of rats in comparison to the vehicle-treated group within the first 48-hours and daily for the rest of the 14-day of the study period .

LD_{50}
After monitoring the animals for 48 hours and a further 12 days, SNE (1600-6400 mg kg^{-1}, *p.o*) yielded no deaths. Hence it can be said that the LD_{50} of the extract when orally administered is greater than 6400 mg kg^{-1}.

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

Haematological and Biochemical Analysis
There was no significant difference (P=0.26-0.56) between the vehicle-treated group and the extract (1600, 3200 and 6400 mg kg^{-1}) regarding all the haematological indices measured (Table 1).

The serum biochemical markers were grouped as: renal function (urea, creatinine, potassium and sodium), lipid profile (total cholesterol, triglycerides, high density lipoprotein (HDL), low density lipoprotein (LDL) and very low density lipoprotein (VLDL) cholesterol) and liver function test (total protein, albumin, globulin, direct, indirect and total bilirubin, alanine aminotransferase (ALT), aspartate aminotransferase (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=0.10-0.97) between the vehicle-treated and SNE (1600, 3200, 6400 mg kg^{-1})-treated rats. Similarly there was no significant (P=0.25-0.61) changes in the lipid profile of SNE-treated rats in comparison to vehicle-treated rats. With respect to the liver function assessment of the rats used in the study, there was no significant difference (P=0.28-0.83) between the vehicle-treated and the SNE-treated rats for all parameters measured.

DISCUSSION

The present study presents an acute toxicity of a hydro-ethanolic extract of *Synedrella nodiflora* in male SD rats. The single oral administration of increasing doses of the extract (1600, 3200 and 6400 mg kg^{-1}) was generally found to be less toxic and produced no mortality in the rats during the entire study period. The absence of any statistically significant changes in haematological, biochemical and gross organ assessments of rats treated with the extract provides support for the safety of the extract.

Table 1: Haematological analysis of a single administration of SNE (1600, 3200 and 6400 mg kg^{-1}) after a 14-day observation period in SD male rats

Parameter	Vehicle	SNE 1600	SNE 3200	SNE 6400	P value
WBC (10^3 µL^{-1})	11.98 ± 1.41	10.14 ± 1.22	11.00 ± 0.57	10.28 ± 1.52	0.71
RBC (10^6 µL^{-1})	6.99 ± 0.31	7.40 ± 0.15	7.28 ± 0.22	7.50 ± 0.18	0.44
HGB (g dL^{-1})	13.24 ± 0.66	14.00 ± 0.29	13.94 ± 0.50	14.00 ± 0.10	0.56
HCT (%)	42.26 ± 2.02	44.98 ± 1.10	44.08 ± 1.77	44.80 ± 0.60	0.56
MCV (fl)	60.40 ± 0.62	60.78 ± 0.49	60.52 ± 1.2	59.86± 1.24	0.92
MCH (pg)	18.94 ± 0.20	18.94 ± 0.08	19.14 ± 0.22	18.72± 0.39	0.71
MCHC (gdL^{-1})	31.34 ± 0.13	31.12 ± 0.14	31.64 ± 0.28	31.26± 0.35	0.51
PLT (10^3 µL^{-1})	813.60 ± 171.70	687.20 ± 224.80	816.40 ± 181.60	790.60 ± 103.80	0.95
LYM (%)	88.14 ± 1.37	90.18 ± 0.76	91.42 ± 1.141	88.62± 1.73	0.30
NEUT (%)	6.380 ± 0.47	6.34 ± 0.97	5.22 ±0.83	7.28± 0.95	0.41
LYM (10^3 µL^{-1})	11.44 ± 1.32	9.12 ± 1.09	9.00 ±1.14	7.84± 1.73	0.32
NEUT (10^3 µL^{-1})	0.82 ± 0.10	0.64 ± 0.13	0.56± 0.11	0.62± 0.12	0.45
RDW_SD (fl)	28.62 ± 0.39	28.66 ± 0.54	28.72 ± 0.70	28.30 ± 0.51	0.95
RDW_CV (%)	10.04 ± 0.09	10.04 ± 0.44	10.24 ± 0.32	9.98 ±0.28	0.94
PDW (fl)	7.68 ± 0.21	7.60 ± 0.27	8.18 ±0.32	7.90 ±0.33	0.52
MPV (fl)	6.64 ± 0.11	6.60 ± 0.19	6.86 ±0.16	6.76 ±0.18	0.67
P_LCR (%)	5.38 ± 0.67	4.90 ± 0.91	6.06 ±0.98	5.44 ±0.83	0.83

Table 2: Biochemical analysis of a single administration of SNE (1600, 3200 and 6400 mg kg^{-1}) after a 14-day observation period in SD male rats

Parameters	Vehicle	SNE 1600	SNE 3200	SNE 6400	P value
Renal function test (mmol L^{-1})					
Urea	8.74 ± 0.22	8.08 ± 0.34	9.00 ± 0.23	8.52 ± 0.20	0.10
Creatinine	57.38 ± 5.07	58.48±3.99	51.48±4.23	59.24 ± 2.69	0.54
Potassium	3.34 ± 0.11	3.65 ± 0.12	3.20 ± 0.02	3.56 ± 0.16	0.15
Sodium	141.4 ± 0.24	141.4 ± 1.81	141.40 ± 0.24	140.8 ± 1.02	0.97
Lipid Profile (mmol L^{-1})					
Total Cholesterol	2.11 ± 0.14	2.02 ± 0.08	2.22 ± 0.13	2.20 ± 0.11	0.61
Triglycerides	0.65 ± 0.10	0.46 ± 0.09	0.62 ± 0.09	0.71 ± 0.16	0.48
HDL	1.03 ± 0.04	1.02 ± 0.04	1.01 ± 0.04	1.02 ± 0.04	0.99
LDL	0.82 ± 0.07	0.85 ± 0.02	1.17 ± 0.24	0.86 ± 0.11	0.25
VLDL	0.29 ± 0.05	0.21 ± 0.04	0.28 ± 0.04	0.32 ± 0.07	0.49
Liver function test					
Total Protein (g L^{-1})	63.50 ± 1.50	63.08 ± 2.14	63.85 ± 0.55	65.44 ± 1.18	0.71
Albumin (gL^{-1})	34.68 ± 0.58	33.88 ± 0.99	33.86 ± 0.62	34.96 ± 0.63	0.62
Globulin (gL^{-1})	28.80 ± 1.05	29.16 ± 1.22	16.48 ± 13.74	30.50 ± 0.95	0.47
D. Bilirubin (µmolL^{-1})	1.66 ± 0.10	2.62 ± 0.73	1.66 ± 0.09	1.84 ± 0.20	0.28
Ind. Bilirubin (µmolL^{-1})	0.52 ± 0.15	1.20 ± 0.62	2.02 ± 1.73	0.66 ± 0.18	0.66
T. Bilirubin (µmolL^{-1})	2.18 ± 0.22	3.14 ± 0.71	3.93 ± 0.16	2.50 ± 0.38	0.05
ALT (UL^{-1})	33.55 ± 1.65	33.20 ± 1.23	30.30 ± 0.40	34.43 ± 2.87	0.60
AST (IUL^{-1})	18.08± 2.95	11.18± 2.76	22.58± 2.53	12.43± 1.93	0.03
ALP (UL^{-1})	51.34 ± 4.17	46.53 ± 4.16	38.90 ± 2.90	51.02 ± 6.99	0.26

The determination of the lethal dose (LD_{50}) of an oral administration of the hydro-ethanolic extract of *Synedrella nodiflora* (SNE) in this study was paramount. The LD_{50} as determined to be greater than 6400 mg kg^{-1} indicates that SNE is relatively safe. The highest therapeutic dose of the hydro-ethanolic extract demonstrating anticonvulsant and related neuropharmacological properties is lower (i.e. 1000 mg kg^{-1}) (Woode *et al.,* 2009, Amoateng *et al.,* 2011, Amoateng *et al.,* 2012), than the doses used in this study (which are 1600, 3200 and 6400 mg kg^{-1}). Thus this present study only demonstrates the effects of acute administration of high doses of the extract beyond 1000 mg kg^{-1}. Also the extract produced no untoward change in the metabolic and physical behaviour of rats treated in comparison to the vehicle-treated rats. This confirms previous reports that the plant extract is relatively safe in rodents (Belmain *et al.,* 2001). More so, since the leaves of the plant is eaten as food with no known documented or reported adverse effects, the plant extract can also be said as being less toxic in humans as well.

Haemotoxicants such as paracetamol and some phytochemicals are known to reduce red blood cell counts and also affect the haemoglobin concentration and subsequently produce anaemia in experimental animals (Mullick *et al.*, 1973; Patrick-Iwuanyanwu *et al.*, 2007). The haematological parameters as measured in the various doses of SNE in the SD rats failed to produce any significant difference in comparison to vehicle-treated rats, suggesting that the extract possess no deleterious effects on blood and blood-forming cells. Moreso, it may be inferred that the extract may have no haematinic, immunosuppressive or stimulatory properties in rats.

On the renal function of the SD rats, the extract did not produce any significant effect on the biochemical parameters (urea, creatinine, sodium and potassium). These suggest the extract may be devoid of any effect whether therapeutic or adverse confirm again that the extract is less toxic in rodent (Belmain *et al.,* 2001). Furthermore, the extract did not significantly affect the lipid profile (total cholesterol, HDL, LDL and VLDL cholesterols) of the SD rats. This suggests that the extract may not have any potential therapeutic or adverse effect on lipid metabolism.

Liver function was determined by the measurement of well-known liver enzyme markers (ALT, ALP and AST) as well as direct, indirect and total bilirubin, elevations of which may suggest a liver and/ bile duct damage as well as enhanced haemoglobin breakdown (Corns, 2003; Arneson and Brickell, 2007; Odetola, 2012). These measurements were done primarily to detect possible hepatic dysfunction, tissue damage or changes in biliary excretion induced by a single oral administration of high doses of SNE. Since there were no significant changes in liver enzymes following the administration of SNE, it can be inferred that the extract did not induce any hepatic damage (Chalasani *et al.,* 2012). Similarly, since there was no significant change in direct, indirect and total bilirubin after treatment with SNE, it also indicates that the extract did not alter hepatic metabolism or biliary secretions. An overall assessment of the effect of the extract on the liver coupled with no visible macroscopic abnormalities generally suggests that the extract may have no liver toxicity much especially when used below 1600 mg kg^{-1} for therapeutic purposes. However, a sub-acute, sub-chronic or chronic assessment of the therapeutic doses of the extract should be performed to conclude this assertion.

The hydro-ethanolic extract was found to possess flavonoids, tannins, saponins, alkaloids, cardiac glycosides, coumarins, triterpenes, sterols, anthraquinones and phenols confirming previous reports (Amoateng *et al.,* 2012). Since no untoward effects of the extract in rats were found, it could be said that these phytoconstituents as found in the extract may be relatively nontoxic in rodents. However this assertion should be further clarified by other toxicological assessments of the whole extract as well as isolation and toxicological characterization of these phytoconstituents.

CONCLUSION

The hydro-ethanolic extract of *Synedrella nodiflora* (L) Gaertn has an oral LD_{50} being greater than 6400 mg kg^{-1} and no significant effect on the haematological and biochemical parameters in male SD rats.

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COMPETING INTERESTS

The authors declare that they have no competing interests.

REFERENCES

Amoateng, P., Assumeng Koffuor, G., Sarpong, K., Oteng Agyapong, K.(2011). Free Radical Scavenging and Anti-lipid Peroxidative Effects of a Hydro-ethanolic Extract of the Whole Plant of Synedrella nodiflora (L.) Gaertn (Asteraceae). *Free Rad Antiox*,1:70-8.

Amoateng, P., Woode, E., Kombian, S.B.(2012). Anticonvulsant and related neuropharmacological effects of the whole plant extract of Synedrella nodiflora (L.) Gaertn (Asteraceae). *J Pharm Bioall Sci*,4:140-8.

Arneson, W., Brickell, J.(2007). Assessment of liver function, Clinical Chemistry: A Laboratory Perspective. 1st ed. Philadelphia: F.A. Davis Davis Company.

Belmain, S.R., Neal, G.E., Ray, D.E., Golob, P. (2001) Insecticidal and vertebrate toxicity associated with ethnobotanicals used as post-harvest protectants in Ghana. *Food Chem Toxicol*, 39:287-91.

Burkill, H.M.(1985). The Useful Plants of West Tropical Africa. 2 ed: Royal Botanical Gardens Kew.

Chalasani, N., Younossi, Z., Lavine, J.E.(2012). The diagnosis and management of non-alcoholic fatty liver disease practice guidelines by the American Gastroenterological Association, American Association for the study of Liver Diseases, and American College of Gastroenterology. *Gastroenterol*, 142:1592.

Cobbinah, J.R., Moss, C., Golob, P., Belmain, S.R. (1999). Conducting ethnobotanical surveys; an example from Ghana on plants used for the protection of stored cereals and pulses. NRI Bulletin.

Corns, C.M.(2003). Herbal remedies and clinical biochemistry. *Ann Clin Biochem*, 40:489-507.

Dalziel, J.M.(1931). The hairs lining the loculi of fruits of species of Parinarium. *Proc Linn Soc London*:99.

Evans, W.C. (2001). Trease and Evan's Pharmacognosy. 15 ed. London: Bailliere Tindall.

Idu, M., Onyibe, H.I.(2007). Medicinal Plants of Edo State,Nigeria. *Res J Med Plant*,1:32-41.

Irvine, F.R. (1961). Woody plants of Ghana. London: Oxford University Press.

Martin, R.J., Gopalakrishnan, S.(2005). Insecticidal Activity of Aerial Parts of Synedrella Nodiflora Gaertn (Compositae) on Spodoptera Litura (Fab.). *J Central Eur Agric*, 6:223-8.

Mshana, N.R., Abbiw, D.K., Addae-Mensah, I., Adjanohoun, E., Ahyi, M.R.A., Enow-Orock, E.G., et al.(2000). Traditional Medicine and Pharmacopoeia. Contribution to the revision of ethnobotanical and floristic studies in Ghana. Scientific, Technical and Research Commission of the Organization of African Unity.

Mullick, F.G., Delage, C., Irey, N.S. (1973). Sickle cell crisis associated with drugs. *Arch Environmental Health*; 26:221-222.

Odetola, M.O., Ewuola, E.O., Adu, O.A.(2012). Haematology, serum biochemistry and organ histopathology of rabbits fed graded levels of whole Kenaf (*Hibiscus canabinus*) seed meal. *Int J Agric Res*, 7:86-92.

Patrick-Iwuanyanwu, K.C., Wegwu, M.O., Ayalogu, E.O. (2007). Prevention of CCl4-induced liver damage by ginger, garlic and vitamin E. *Pakistan J Biol Sci*, 10:617-621.

Woode, E., Amoateng, P., Abotsi, W.M.K. (2011). Ethopharmacological analysis of the effects of the whole plant extract of Synedrella nodiflora (L.) Gaertn (Asteraceae) in murine models. *Der Pharmacia Sinica*, 2:54-67.

Woode, E., Amoateng, P., Ansah, C., Duwiejua, M. (2009). Anti-Nociceptive Effects of an Ethanolic Extract of the Whole Plant of Synedrella nodiflora (L.) Gaertn in Mice: Involvement of Adenosinergic Mechanisms. *J Pharm Toxicol*, 4:17-29.

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