Original Article

Thai Journal of Pharmaceutical Sciences

Acute and sub-acute toxicity profile of methanol leaf extract of *Geophila obvallata* on renal and hepatic indices in Wistar rats

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Received: Apr 16, 2020
Accepted: Jul 29, 2020
Published: Feb 07, 2022

ABSTRACT

Introduction: *Geophila obvallata* (Rubiaceae) is a medicinal herb used for the treatment of diseases associated with oxidative damage. **Objective:** The study investigated the toxic effects of acute and sub-acute oral doses of methanol extracts of *G. obvallata* (GOE) in rats. **Materials and Methods:** In acute toxicity study, extract doses of 1600, 2900, and 5000 mg/kg were orally administered. The rats were observed for signs of toxicity for 2 weeks. In sub-acute toxicity study, the extract was administered to rats at doses of 100, 500, and 1000 mg/kg for 28 days, while control rats received only water. At the end of 28 days, rats were sacrificed. Organ weights, biochemical and histological parameters were determined using standard procedures. **Results:** In acute toxicity studies, the extract did not induce death after single dose administration. Hence, the lethal dose 50 was above 5000 mg/kg. The results of sub-acute toxicity study showed no significant changes in body weights, organ weights, kidney function, and histology. In contrast, there were significant changes in hematology and biochemical indices at higher extract doses of 500 and 1000 mg/kg. **Conclusion:** These results suggest that GOE is non-toxic at a dose of 100 mg/kg and can be used for therapeutic applications at an equivalent dose in humans.

Keywords: Biochemical indices, *Geophila obvallata*, hematology, histology, lethal dose 50

INTRODUCTION

Globally, the consumption of herbal formulations for health care purposes has increased.[1] This is due to the belief that these formulations are organic, harmless and effective in the treatment of diseases.[2] Developing countries in Africa use herbal formulations as alternative treatment for various illnesses due to inadequate medical facilities and unaffordability of conventional medicines.[3,4] It has been reported that herbal formulations are safer and less damaging to biological systems than synthetic drugs.[5] The World Health Organization recommends that complementary medicine should be adopted by member states in developing proactive policies that will strengthen the use of medicinal plants in keeping populations healthy.[6]

One of the many medicinal plants used in Nigerian folkloric medicine is *Geophila obvallata* (GO) which is commonly known as "*avbovbo tor*" and "*ekoro*" in Edo and Yoruba tribes, respectively.[7] In terms of taxonomic classification, it belongs to the kingdom plantae, order Gentianales, family Rubiaceae and genus *Geophila*.[8] It is an edible rainforest plant that grows extensively in the tropical rain forest floors, especially the *Gelegele* forests, Okomu oil palm reserves and Rubber Research Institute at Iyanomo in Edo State, Nigeria.[9] This herb has been used by the rural natives of Edo state as a decoction for the treatment of abdominal problems, headache, hypertension, tooth ache, jaundice, diabetes, stroke, and cardiovascular diseases.[7] The aqueous and methanol extracts of the leaves of this plant have been reported to possess antioxidant qualities as a result of its bioactive components.[10] However, there has been no scientific evaluation of the short or long-term toxicological effects of this plant on biological systems. The kidney and liver tissues are organs usually investigated in toxicological studies because of their roles in excretion and biotransformation of xenobiotics.[11] This study was aimed at investigating the acute and sub-acute toxicity profiles of extracts of GO (GOE) with respect to some renal and hepatic indices in Wistar rats.

MATERIALS AND METHODS

Chemicals

The chemicals were purchased through a local vendor from Randox Ltd (USA) with a high quality.

Collection of Plants and Preparation of the Extract

The fresh leaves of GO were collected by following leads supplied by a local healer at Ugbowo Quarters, Benin City, Nigeria. They were confirmed by Dr. Akinigboso (taxonomist), at the life science Department, University of Benin, Benin City and voucher number UBHa 0312 was assigned to it. GO was then deposited at the Plant Biology and Biotechnology herbarium, University of Benin, Benin City for future references.

A method modified by Agbai *et al*.[12] was adopted. Fresh leaves were washed and air-dried for 7 days. Air-dried leaves were blended by a grinding machine (hammer type) (Meecan, CM/L-2264458, UK) until a smooth texture was obtained, and was later weighed and packaged. About 87.52 g of the blended leaves were extracted in the Soxhlet extractor using methanol (70%) (1:10 w/v)[13] prior to homogenization and continuous agitation for 2 days. Whatman's paper (No. 1) was used to filter the homogenate and the filtrate was concentrated to aridness at 40°C[14] within 24 h to obtain about 46.20 g of methanol extract, and then, dried over anhydrous $CuSO_4$ in a dessicator. The dried residues were stored in airtight containers at 4°C before laboratory experiments.

Experimental Animals

Rats used in this experiment were obtained from the Department of Biochemistry animal house, University of Benin, Benin city. They were caged in a hygienic, conducive habitat with proper lighting. The rats weighed between 130 and 200 g. The rats were fed orally with rat pelleted feed (Agro feeds, Nigeria), they had access to dirt-free drinking water, and they were housed in steel cages, in compliance with the National Research Council guidelines for the care and use of laboratory animals.[15] Ethical principles regulating the use of living animals for research were strictly adhered to as adopted by Ward and Elsea.[16] The research procedures for animal handling were endorsed by the animal use and care committee, National veterinary research institute, Vom, with approval number nvriAUCC F001/19.

Acute Toxicity Study

A slight modification of Lorke's method[17] was employed in this study. Mixed genders of 10 males and 10 female Wistar albino rats (*Rattus norvegicus domestica*) were chosen and organized into four sets of five rats per set. The control rats were given tap water (10 ml/kg/body weight) while the other three sets were orally administered with a single dose of GOE at 1600, 2900, and 5000 mg/kg body weight. Observation for signs of toxicity was carried out 1, 2, and 4 h after treatment and periodically during the first 24 h, then, daily for 2 weeks following treatment. Changes in the skin, eyes and mucus membrane, body weight and behavioral patterns were noted during the test period.[18]

Sub-acute Toxicity

This investigation was completed in 28 days according to the OECD guidelines 407.[19] Experimental animals were divided into four sets of five rats per set of mixed sexes; both sexes were placed in separate cages to prevent mating. Set 1 served as control (i.e. the rats were fed without extract), while the other sets were daily fed by oral administration of GOE at different doses (100, 500, 1000 mg/kg) for 28 days.

On day 28, the rats were anesthetized using isoflurane after fasting for the night while blood samples were taken for biochemical and hematological analyses using both EDTA and plain vials while the kidney and liver were harvested for histological assessment.

Relative Organ and Body Weights Study

The changes in body weights were recorded on a weekly basis, while the organs (the liver, kidneys, brain and heart) were weighed using standard weighing balance to calculate relative organ weight for the different sets on the sacrifice day.

Relative organ weight (%) = [Absolute weight of organ (g)/weight of rat on sacrifice day (g)] × 100

Hematological Analysis

The indices analyzed in the blood samples included hematocrit, corpuscular volume, erythrocyte count, lymphocytes, neutrophils, monocytes, thrombocyte count, basophils, and leukocyte count (White blood cell) were performed by means of an automated analyzer (BiopacBS-1100i, Shanghai, China).

Serum Biochemistry

Dry tubes were used to collect blood samples which were spun at 3000 rpm for 10 min at 5°C to get the serum isolates used for the following experiments.

Liver Function Tests

The activities of liver enzymes (alanine transaminase [ALT], aspartate transaminase [AST], and alkaline phosphatase [ALP]) and the concentrations of total bilirubin, albumin and total proteins were analyzed using specific commercial kits according to the manufacturer's protocol (Alpha Laboratories UK, London).

Kidney Function Tests

The kidney function tests investigated included: Serum creatinine and urea as well as serum electrolytes (HCO_3^-, Na^+, K^+, Cl^-). They were determined using specific commercial kits according to the manufacturer's protocol (Alpha Laboratories UK, London).

Lipid Profile

Total cholesterol (TC), serum triacylglycerol, and other lipid profile indices were evaluated according to the protocols outlined by Tiez.[20]

In vivo Antioxidant Study of GOE

Malondialdehyde (MDA) determination

Lipid peroxidation level was evaluated using spectrophotometry[21] by measuring, MDA which interacts with thiobarbituric acid to produce a colored complex at 532 nm in an acidic medium.

Determination of super oxide dismutase (SOD)

The SOD assay was carried out according to Xin *et al.*[22] Adrenaline solution was formed by dissolving (5 mg) adrenaline in 10 ml of distilled water. Then, 0.10 ml of serum was agitated in potassium buffer at pH 7.8. Buffer was mixed with 0.3 ml of adrenaline solution which was then added to 0.2 ml of the extract inside a cuvette, agitated and read at 450 nm.

Estimation of catalase

The method of Aebi[23] was employed in estimating catalase activity. This is based on the ultraviolet absorption and decomposition of hydrogen peroxide (H_2O_2) by catalase over time. Absorbance is easily measured at 240 nm.

Determination of reduced glutathione (GSH)

The technique of Xifan *et al.*[24] was employed in GSH determination. It is based on the principle that GSH interacts with alloxan and O_2 in alkaline medium at a wavelength (320 nm).

Histopathology

On the 28th day, the liver and kidneys excised from the sets administered with the extracts and the control groups were collected and weighed and quickly set in 10% neutral buffered formalin at pH 7.4 and developed for histological studies. Following fixation, tissues were cleansed in graded series of alcohol, washed in xylene, inserted into paraffin, segmented by a microtome (5-µm thin) and tainted with dye in glass slides. Segments were viewed by a standard microscope (at 100× and 400×) magnification.[25]

Analysis of Data

Analysis of variance (ANOVA) (One- way) was used to analyze data, and data are presented as mean ± SEM. ANOVA was followed by Dunnett's multiple comparison test. A $P < 0.05$ was considered statistically significant. Statistical analysis of data was done using Minitab 16.

RESULTS

Acute Toxicity Analysis

No signs of lethality or morbidity were detected in the rats given different doses up to 5000 mg/kg of GOE for 2 weeks. Therefore, the median lethal dose (LD_{50}) of GOE was higher than 5000 mg/kg.

Sub-acute Oral Toxicity Study

Administration of GOE for 28 days continuously did not induce morphological changes or general behavioral changes in treated rats compared to the control group. No deaths were observed during the period.

Body Weights

The body weight alterations of rats given graded doses of GOE are indicated [Figure 1]. Daily administration of GOE at different doses (100, 500 and 1000 mg/kg) did not result in significant changes in the body weight of GOE-fed rats when compared with the control.

Relative Organ Weights

The weights of rats organs treated with GOE were non-significantly different from the control set [Figure 2].

Biochemical Analysis

Catalase activity

The catalase activity of both control and GOE-fed rats are indicated [Table 1]. The results indicated no significant difference in catalase activity after sub-acute treatment with different doses of GOE for 28 days, when compared to control set.

Superoxide dismutase activity

Superoxide dismutase activities of GOE-treated and control rats are shown [Table 2]. The results indicated significant

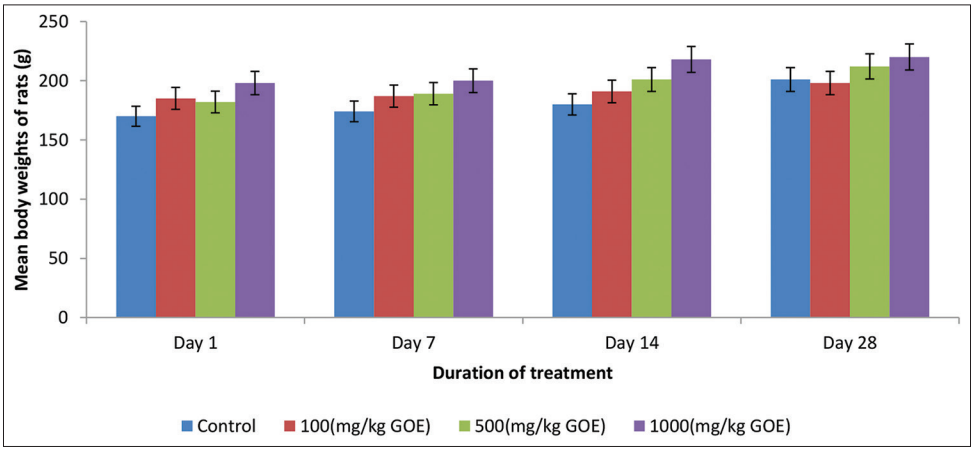


Figure 1: Effect of methanol extract of *Geophila obvallata* (100, 500 and 1000 mg/kg) on mean body weights of rats in sub-acute toxicity study. Values are mean±SEM of five rats. Compared to the control group (one-way analysis of variance followed by Dunnet's *post-hoc* test)

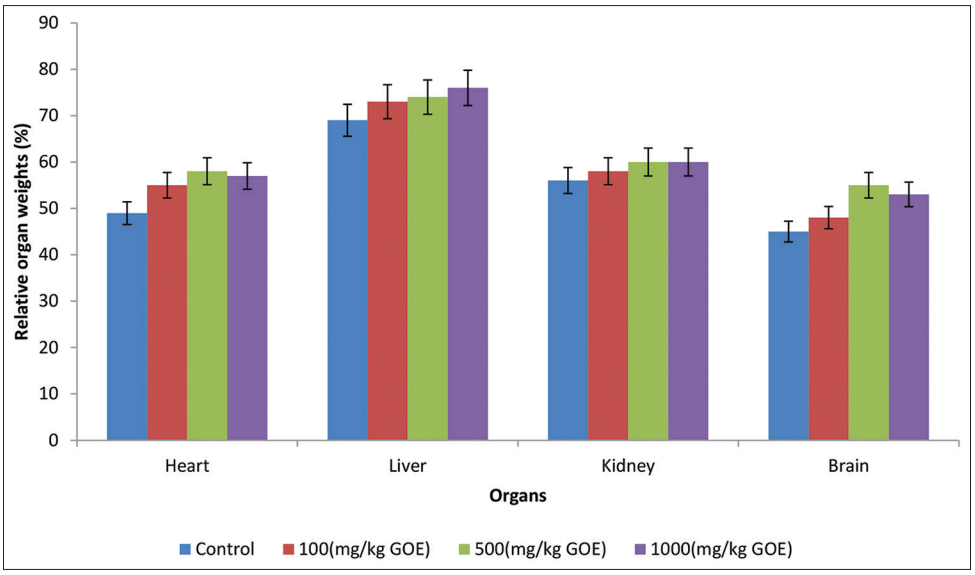


Figure 2: Effect of sub-acute administration of *Geophila obvallata* extract on the relative weight of organs. Values are mean±SEM of five rats. Compared to the control group (one-way analysis of variance followed by Dunnet's *post-hoc* test)

Table 1: Effect of sub-acute administration of *Geophila obvallata* extract on the catalase activity (unit/mg of wet tissue)

Organs	Control	*Geophila obvallata* (mg/kg BW)		
		100	500	1000
Heart	53.01±2.10	48.66±6.37	52.42±1.42	52.45±2.50
Liver	52.82±0.81	52.30±2.73	53.70±1.50	53.35±0.90
Kidneys	50.96±1.72	51.76±0.50	52.17±0.92	53.03±2.06
Brain	54.83±1.51	54.00±3.00	54.48±0.32	54.57±2.63

Values are mean±SEM of five rats. Compared to the control group (one-way ANOVA followed by Dunnet's *post-hoc* test)

Table 2: Effect of sub-acute administration of *Geophila obvallata* extract on the superoxide dismutase activity (unit/mg of wet tissue)

Organs	Control	*Geophila obvallata* (mg/kg BW)		
		100	500	1000
Heart	7.56±0.12	8.82±0.21	8.45±0.10	8.40±0.51
Liver	6.24±1.41	7.08±0.34	11.56±0.83*	15.44±1.70*
Kidneys	8.36±0.57	9.43±0.55	9.52±0.84	9.58±0.13
Brain	6.56±0.37	7.28±0.12	8.54±0.20	8.74±0.50

Values are mean±SEM of five rats. *Significantly different from the control sets ($P<0.05$)

increases ($P < 0.05$) in superoxide dismutase activity in the liver after sub-acute treatment with 500 and 1000 mg/kg bw of GOE for 28 days.

GSH Activity (mmol/GSH of Wet Tissue)

The reduced GSH levels of GOE-treated rats and control rats are shown [Table 3]. The results indicated no significant difference ($P > 0.05$) in reduced GSH levels in the liver after sub-acute treatment with different doses of GOE for 28 days, when compared to control set.

Effects of GOE on Hematological Indices

The effects of sub-acute administration of GOE on hematological parameters are shown [Table 4]. Daily administration of GOE for 28 days did not cause any significant difference in most of the hematological parameters when compared with the control group. However, there were significant decreases ($P < 0.05$) in hematocrit and hemoglobin (HB) concentration at 1000 mg/kg.

Effects of GOE on Liver Indices

The effect of sub-acute administration of GOE on liver indices is presented [Table 5]. A significant increase ($P < 0.05$) in ALP activity at 1000 mg/kg was observed while other liver markers showed normal levels.

Effects of GOE on Kidney Function in Rats

Sub-acute administration of GOE in the treated rats caused no significant difference ($P > 0.05$) in the kidney parameters (bicarbonates ion, creatinine, uric acid, sodium ion, potassium ion, urea, and chloride ion levels) investigated [Table 6].

MDA (mg/dl of Wet Tissue) Activity

MDA (mg/dl of wet tissue) levels in both control and GOE-fed rats are indicated [Table 7]. The results revealed significant increases ($P < 0.05$) in MDA levels in the liver and kidney after sub-acute treatment with 500 and 1000 mg/kg bw doses of GOE for 28 days.

Effects of GOE on Lipid Profile in Rats

Effects of sub-acute administration of GOE on the lipid profile of experimental rats are shown [Table 8]. GOE treatment at 100 and 500 mg/kg resulted in significant ($P < 0.05$) decreases in TC, triglyceride (TG) and low-density lipoprotein (LDL)

cholesterol in treated groups. Both doses also showed non-significant increases in high-density lipoprotein (HDL) and very low-density lipoprotein (VLDL) cholesterol in treated rats.

Histology Analysis

Histology results of assessment of GOE effects on the liver after 28 days of administration is shown [Figure 3a-d]. The microscopic examination revealed no significant pathological alterations in the liver for all experimental groups. It revealed unambiguous, observable rows of normal liver cells resulting from (a) central veins (b) hepatic sinusoids after 28 days of extract administration.

The histological effect of GOE on the kidney after 28 days of administration is revealed [Figure 4a-d]. The microscopic examination revealed no significant pathological alterations in the kidney for all experimental groups. It also revealed very clear and visible glomeruli as indicated by (a) renal tubules (b) renal corpuscles (c) medullary ray after 28 days of extract administration.

Table 3: Effect of sub-acute administration of *Geophila obvallata* extract on the activity of GSH (mmol/GSH of wet tissue) of treated rats

Organs	Control	*Geophila obvallata* (mg/kg BW)		
		100	500	1000
Heart	19.97±0.11	21.67±0.16	21.46±0.57	21.68±0.43
Liver	20.72±1.32	22.56±0.22	22.43±0.31	21.88±1.02
Kidneys	19.63±0.55	20.76±0.32	20.61±0.19	21.34±1.20
Brain	21.39±0.25	22.56±0.14	22.52±1.03	22.56±0.91

Values are mean±SEM of five rats. Compared to the control group (one-way ANOVA followed by Dunnet's *post-hoc* test), GSH: Glutathione

DISCUSSION

Information regarding the toxic effects of GO methanol extract in health care does not exist in previous research archives. To guarantee the quality of GOE for human consumption, a methodical toxicity assessment was needed to estimate the dangers of toxicity and to provide a basis for safe dose selection and scientific data in humans.

The acute toxicity study revealed that there were no signs of morbidity or death after 2 weeks of treatment. The rats were able to tolerate higher doses of GOE. Therefore, the LD_{50} of GOE is above 5000 mg/kg body weight when taken orally.

In summary, it can be said that oral treatment with GOE caused no striking negative effects on the body weights and relative organ weights of the fed set in the sub-acute assessment.

The bone marrow is a major location for novel blood cell manufacture and a vulnerable tissue targeted by toxic compounds in the hematopoietic system.[26] In this study, there was a slight decrease in hematocrit and HB concentrations at 1000 mg/kg when compared with the control groups. However, these alterations were considered minor and toxicologically insignificant. This implies that GOE has no lethal implication on the hematopoietic system.

The liver biomarkers are specific tools in examining liver toxicity during drug biotransformation.[27] The assessment of liver and kidney functions in this research revealed that GOE consumption at graded doses, had no effects on the ALT and AST liver indices, although, ALP levels increased significantly ($P < 0.05$) at 1000 mg/kg as an indication of biliary duct obstruction or cholestatic disease at higher doses.[28]

The notable potential of the liver to rejuvenate its cells makes it exceptional in overcoming various forms of necrosis

Table 4: Effect of sub-acute administration of *Geophila obvallata* extract on hematological profiles

Parameters	Control	*Geophila obvallata* (mg/kg B.W)		
		100	500	1000
WBC (10^3/mm^3)	7.11±0.23	6.62±0.12	6.78±1.23	5.59±0.88
RBC (10^6/mm^3)	7.01±0.83	5.05±0.49	6.49±0.50	6.63±0.23
Hematocrit (%)	41.45±0.16	40.02±1.40	39.94±0.15	24.68±0.14*
Hemoglobin (%)	15.98±0.50	14.15±0.43	13.80±1.6	9.73±0.48*
MCV (μm^3/red cell)	59.50±0.45	60.75±0.21	60.25±0.42	59.25±1.50
MCH (pg/red cell)	19.46±1.52	20.95±1.80	19.98±1.00	19.30±0.50
MCHC (g/dL)	32.68±0.90	34.53±2.80	32.90±0.70	32.08±1.10
Platelets (10^3 cells/mm^3)	391.80±160.1	388.80±169.30	382.00±83.10	397.20±122.60
Lymphocytes (%)	58.52±1.40	50.08±1.23	52.10±0.50	53.47±0.25
Neutrophils (%)	34.80±0.11	35.70±1.20	36.90±±0.55	37.20±0.32
Monocytes (%)	3.30±0.32	3.47±2.00	2.33±1.02	3.34±0.20
Basophils (%)	0.04±1.11	0.05±0.48	0.06±0.42	0.03±1.50
Eosinophils (%)	1.07±0.05	1.15±0.23	1.09±0.98	1.71±0.01

Values are mean±SEM of five rats. *Significantly different from the control sets ($P<0.05$). WBC: White blood cell, RBC: Red blood cell, MCV: Mean corpuscular volume, MCH: Mean corpuscular hemoglobin, MCHC: Mean corpuscular hemoglobin concentration

Table 5: Effect of sub-acute administration of *Geophila obvallata* extract on liver indices

Parameters	Control	*Geophila obvallata* (mg/kg B.W)		
		100	500	1000
AST (IU/L)	19.06±1.77	20.92±1.78	20.88±0.50	21.66±1.54
ALT (IU/L)	14.68±1.45	16.50±1.23	16.25±0.76	15.48±0.06
ALP (IU/L)	65.17±1.63	61.46±1.09	64.63±0.02	89.12±0.07*
Total bilirubin (mg/dL)	0.70±0.54	0.98±0.10	1.01±0.61	1.13±0.59
Total protein (g/dL)	7.57±1.70	7.41±2.23	7.61±0.87	8.69±0.96
Albumin (mg/dL)	3.49±0.50	3.63±0.5	3.74±0.99	3.81±0.81

Values are mean±SEM of five rats. *Significantly different from the control sets ($P<0.05$). ALT: Alanine transaminase, AST: Aspartate transaminase, ALP: Alkaline phosphatase

Table 6: Effect of sub-acute administration of *Geophila obvallata* extract on kidney indices

Parameters	Control	*Geophila obvallata* (mg/kg B.W)		
		100	500	1000
Urea (mg/dL)	44.81±2.50	41.81±9.09	40.84±0.63	45.16±3.30
Creatinine (mg/dL)	0.69±0.27	0.62±0.09	0.65±0.39	0.75±0.11
Uric acid (mg/dL)	11.25±0.42	12.14±0.25	14.09±0.37	13.27±0.12
Na^+ (mEq/L)	140.44±2.93	143.41±6.88	143.57±8.53	144.62±5.34
K^+ (mEq/L)	2.44±0.67	2.48±0.45	2.38±0.97	2.43±0.39
Cl- (mEq/L)	77.42±2.50	76.77±1.20	71.94±1.38	71.24±0.25
HCo_3-(mEq/L)	39.11±2.29	43.13±0.54	37.15±0.90	42.25±0.61

Values are mean±SEM of five rats. Compared to the control group (one-way ANOVA followed by Dunnet's *post-hoc* test).

Table 7: Effect of sub-acute administration of *Geophila obvallata* extract on the activity of MDA (mg/dl of wet tissue

Geophila obvallata (mg/kg B.W)	Organs			
	Heart	Liver	Kidney	Brain
Control	1.33±0.16	1.64±0.28	1.63±0.20	0.80±0.14
100	1.37±0.51	1.65±1.03	1.65±0.06	0.87±0.01
500	1.39±0.03	1.82±0.09*	1.69±0.12	0.89±0.03
1000	1.41±0.14	1.98±0.10*	2.20±0.01*	0.91±0.19

Values are mean±SEM of five rats. *Significantly different from the control sets ($P<0.05$). MDA: Malondialdehyde

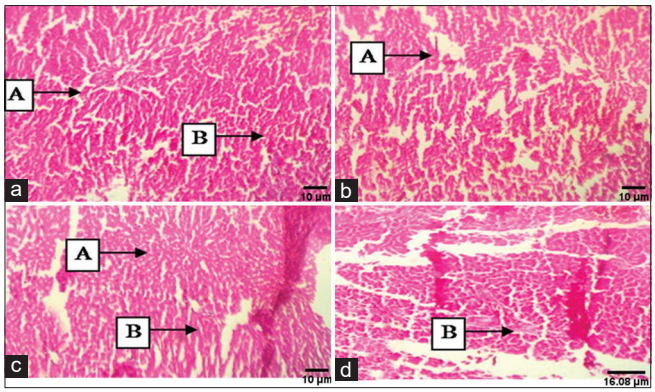
Table 8: Effect of sub-acute administration of *Geophila obvallata* extract on the lipid profile in rats

Geophila obvallata (mg/kg B.W)	Lipid Profile				
	TC	TG	HDL	LDL	VLDL
Control	0.40±0.12	0.43±0.20	0.63±0.04	0.35±0.22	0.29±0.14
100	0.22±0.01*	0.25±0.03*	0.72±0.07	0.31±0.18	0.30±0.01
500	0.31±0.02*	0.30±0.02*	0.78±0.11	0.39±0.30	0.31±0.03
1000	1.20±0.10	1.98±0.10	0.32±0.08*	0.51±0.19*	0.25±0.19

Values are mean±SEM of five rats. *Significantly different from the control sets ($P<0.05$). TC: Total cholesterol, TG: Triglyceride, HDL: High-density lipoprotein, LDL: Low-density lipoprotein, VLDL: Very low-density lipoprotein

and perturbations.[29] An increase in ALP was observed implying that hepato-biliary damage can be an effect of GOE consumption at higher doses thereby causing destruction of the liver cells. Illnesses such as cholestasis of the liver and biliary cirrhosis are associated with elevated liver indices.[30]

Kidney disease can be detected by measurements of kidney indices such as creatinine, uric acid, urea, bicarbonates, potassium, sodium, and chlorides and their normal levels reflect a reduced likelihood of renal problems.[31] In the present study, no significant alterations in plasma creatinine, uric acid, urea, bicarbonates, potassium, sodium, and chlorides levels in GO extract fed rats when compared to the control was observed. This indicates that the functional integrity of the kidney was not compromised after treatment with graded doses of the extract.



Figures 3: (a-d) Effect of *Geophila obvallata* on histology of the liver of rats after 28 days of extract administration (Hematoxylin and eosin staining; 100×). Key: a=Normal control showing normal hepatic histology; b=Administered with 100 mg/kg body weight of methanol extracts of *Geophila obvallata* (GOE), showing normal hepatic structure; c=Administered with 500 mg/kg body weight of GOE, showing normal hepatocytes; d=Administered with 1000 mg/kg body weight of GOE, showing abnormal hepatic structure (a) central veins (b) hepatic sinusoids

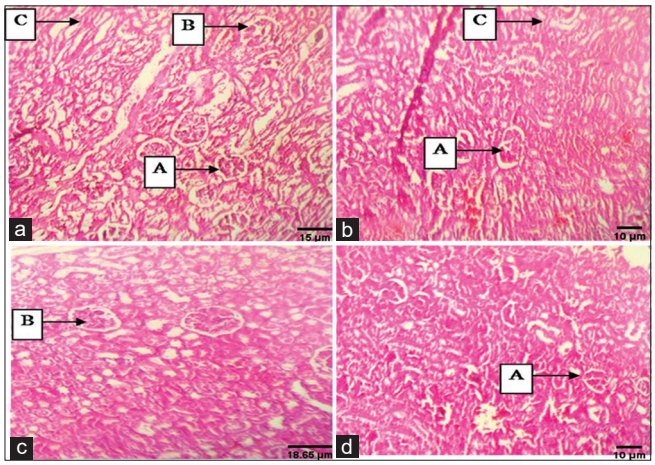


Figure 4: (a-d) Effect of *Geophila obvallata* on histology of the kidney of rats after 28 days of extract administration (Hematoxylin and eosin staining; 100×) Key: a=Normal control showing intact renal tubules, corpuscles and medullary rays; b=Administered with 100 mg/kg body weight of methanol extracts of *Geophila obvallata* (GOE), showing normal renal architecture and medullary rays; c=Administered with 500 mg/kg body weight of GOE, showing normal renal corpuscles; d=Administered with 1000 mg/kg body weight of GOE, showing abnormal renal architecture (a) Renal tubules (b) Renal corpuscles (c) Medullary rays

GOE effects on lipid peroxidation were evaluated by measuring MDA levels, GSH levels, and SOD and catalase enzyme activities. Reduction in catalase, GSH, SOD activities and increases in MDA levels connotes an elevation in oxidative stress in biological entities thereby interfering with the system's antioxidant defence mechanisms.[32] However, in this study, GOE administration at 500 and 1000 mg/kg bw significantly increased ($P < 0.05$) the MDA and SOD levels, especially in the liver of fed sets in comparison to the control. Furthermore, GSH and catalase levels remained within the normal ranges. The potential toxicity of GOE administration at 1000 mg/kg bw could be of concern regarding its chronic use in man. These could be associated with the presence of toxic chemical constituents in the extract that are amplified at this dose.

Cardiovascular dysfunctions as well as other coronary heart diseases are majorly implications resulting from high level of lipids in the blood. In this study, GOE treatment at 100 and 500 mg/kg resulted in significant ($P < 0.05$) decreases in TC, TG and LDL cholesterol in treated groups. Both doses also showed non-significant increases in HDL and VLDL cholesterol in treated rats. It is possible that the extract has the potentials to mobilize lipids from the circulatory system directly into tissues via the activation or inhibition of enzymes in the lipid pathway. This is in agreement with Moller's[33] research which recommended that plant extract administration may prove effective in the management of cardiovascular ailments, diabetes as well as deregulated blood pressure. This information is particularly relevant to the trado-medical practitioners who prepare the leaves of this plant in a decoction for its anti-hypertensive and cardiovascular potentials.

Histological observations of kidney and liver sections from the experimental animals demonstrated no significant pathological conditions in the test group as the liver and kidney tissues of the test groups were consistent with the normal histology of the control.

Preliminary phytochemical analysis carried out on GOE revealed that, the methanol extract had the highest concentration of total flavonoid, tannins, alkaloids and phenolic compounds as well as the best antioxidant activity via DPPH, ABTS and hydroxyl scavenging assays compared to its aqueous solvent.[10] These qualities are responsible for the definite pharmacological effects observed in this study. This suggests that GOE possesses beneficial properties due to its content of phytochemicals, in boosting the body's defence.

CONCLUSIONS

Oral doses of GO leaf extracts can be considered non-toxic especially at 100 mg/kg, as the extract did not elicit lethality in the acute and sub-acute toxicity studies in rats. The findings of this study give credence to the application of GO in folkloric traditional medicine. However, further pre-clinical assessments should be carried out to validate its effectiveness and long-term toxicological safety.

ACKNOWLEDGMENT

The author(s) hereby assert that no support was received from any sources for the publication or research of this article.

CONFLICTS OF INTEREST

The authors have declared that there is no conflict of interest.

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