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Toxic Effect of *Gardenia ternifolia* Fruit on Rats

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Authors' contributions

This work was contributed between all authors. Author HMF planed, conducted the study, and wrote the first draft of the manuscript. Authors AMEH and HEK guided the work. Author AMEH revised the manuscript. Author HMO managed the histopathological study. All authors read and approved the manuscript.

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ABSTRACT

Aim: This study aims to screen the aqueous extract of *Gardenia ternifolia* fruit for toxicity in Wistar albino rats by determination of morality, Percentage of weight change, hematology, biochemistry and histopathology.
Methodology: Twenty four male Wistar albino rats were divided into four groups, each of 6. Group 1 (control), group 2 and 3 (sub-chronic toxicity) and group 4 (acute toxicity). The aqueous extract was administered orally at a dose of 50 and 500 mg/ kg/ day -for four weeks- to group 2 and 3, respectively. Group 4 received 2000 mg/kg once, and group 1 was kept as a control. Clinical signs and mortality were observed daily. The weights of the animals were recorded weekly at week intervals till the end of the experiment. Blood samples were collected for hematology and biochemistry. Specimens of Liver and kidney were kept in 10% formalin for histopathology.
Results: The results revealed that no clinical signs of toxicity or mortality were recorded during the experiment in all groups. The percentage of weight gain was lowest in group 4 compared with group 1 (control). The hematology and biochemistry of group 1 and 2 were not affected. However,

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both were altered in group 4. White Blood Cells (WBC) were significantly (P<0.05) increased; Red Blood Cells (RBC), Hemoglobin (Hb) and Packed Cell Volume (PCV) were significantly (P<0.05) decreased. Total protein and albumin were significantly (P<0.05) decreased. Cholesterol, urea, creatinine, Alanin Transaminase (ALT), Asparate Transaminase (AST) and Alkaline phosphatase (ALP) were significantly (P<0.05) increased. But, bilirubin was not affected in all groups. Histopathological changes on liver and kidney correlated with the hematological and biochemical alterations.

Conclusion: The aqueous extract of *G. ternifolia* fruit was safe and not lethal to rats at low doses; the highest dose altered the haematology, biochemistry and histology of the tested animals.

Keywords: Gardenia ternifolia; fruit; toxicity; aqueous extract; rats.

1. INTRODUCTION

The objective of plant toxicity is to clarify the toxic properties of the plant part. The toxicity of *G. ternifolia* is necessary since this has not been previously done in depth.

Medicinal plants are often assumed to be efficient and safe; however, there are some reports on poisoning consecutive to plant based-medicine administration [1]. Thus, interest is accorded to toxic effects of plant extracts.

Only scanty information is available regarding the toxicity of *G. ternifolia*. *Gardenia ternifolia* Schum and Thonn., subspecies jovis-tonantis (Welw.) verdec., var. jovis-tonantis (Welw.) Aubrer., F 1.Four. Soud.-Guin 460 (1950). The synonymous name is *G.lutea* Fresen, *Decameria jovis tonantis*. It belongs to the family Rubiaceae, and locally known as Abu Gawie [2].

Gardenia ternifolia fruit contains β-amyrin, steroids, fatty acids, oleonilic acid, an iridoid glucoside (geniposide) [3]. Other species of Gardenia were shown to contain iodol, alkaloids, triterpens, flavonoids, and iridoids. Oral administration of geniposide, and another iridoid glycoside of the fruit of *Gardenia* spp. were found to cause diarrhoea in mice [4]. Genipin, a hydrolysate of geniposide, act as a propulsive agent in the large intestine. Additionally, the fruit was reported as an inhibitor of platelets aggregation [5]. On the other hand, macerated roots administered orally as antihelmintic for the treatment of sheep and goats [6]. Decoction of *G. ternifolia* leaves was used as antifebrile and antimalarial [7], and as a remedy against toxic effects [5], and Diabetes mellitus. Recently, the leaves showed anticancer activity [8].

Gardenia ternifola was used in East Africa traditional medicine to manage malaria, ulcers, cough, syphilis, stomachache, arthritis, asthma, epilepsy and other mental problems, fever, pain, paludism, and as purgative and astringent, anti-snake venom, laxative [9]. Different parts of the plant exhibited various biological activities from separate studies including antihelmentic, bronchodilator and antiviral activities. *Gardenia* spp. contains flavonoids, alkaloids, iridoids and terpenoids. An ethnobotanical survey in Quémé, Southern Benin showed that *G. ternifolia* was used as antihypertensive [10].

In Sudan, *G. ternifolia* fruit was investigated for molluscicidal, antibacterial [11], mosquito larvicidal [12], toxicological [13], and antitheilerial activity [14]. Accordingly, *G. ternifolia* was investigated for toxicological evaluation in rats.

2. MATERIALS AND METHODS

2.1 The Plant

Gardenia is a glabrous shrub or small tree up to 5 m high. Leaves are opposite, ternate or 4-together, apex obtuse, base cuneate, margin entire. Inflorescences solitary. Fruit berries, ellipsoid, grey-brown [3] (Fig. 1).

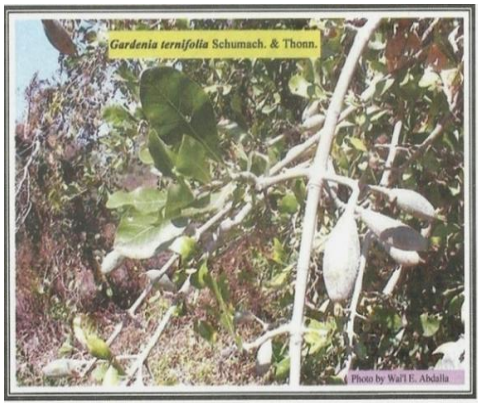

Fig. 1. *Gardenia ternifolia* Schum. and Thonn., Photo by Dr .Wai'l E. Abdalla

2.1.1 Plant collection

The fruits were collected from Eastern Nuba Mountains. The plant part was identified and authenticated at the Medicinal and Aromatic Plants Research Institute, Khartoum, Sudan. The voucher specimen has been deposited in the herbarium museum of the Institute. The fruit was cut into slices; air dried in the shade, coarsely powdered and kept in polyethene bags at room temperature.

2.2 Animals

Twenty four male Wistar albino rats were brought from Medicinal and Aromatic Plants Research Institute, Khartoum, Sudan, and kept in metal cages. They were left to acclimatize for a period of one week prior to the start of the experiment. The rats were fed with a standard diet which is manufactured commercially for poultry (Layers) and vegetables. Feed and water were provided *ad libitum*. This work was carried out according to the international regulation for the use of laboratory animals.

2.3 Preparation of Aqueous Extract

The aqueous extract of the plant was prepared by simple maceration of 100 g of the powdered sample in 500 ml of hot distilled water; maintained at room temperature with continuous stirring till cooled down. The extract was then filtered through Whatman No. 1 filter paper and transferred to the freeze drier (Trivac, U.S.A.). The yield percentage of the extract was calculated as below:

Yield percentage = (Weight of extract obtained) / (Weight of plant sample) × 100

The required weight of the aqueous extract administered to each group was calculated according to the dose; dissolved in 6 ml of distilled water. The volume of the extract administered orally to each animal based on the body weight.

2.4 Experimental Designs

Twenty four male Wistar albino rats weighing (95-114 g) were divided into four groups, each of 6 rats. Group 1(control), groups 2 and 3 (sub-chronic toxicity), and group 4 (acute toxicity). The extract was given at one of the fixed dose level (50, 500 and 2000 mg/kg).

2.4.1 Screening of aqueous extract of *G. ternifolia* fruit for toxicity

A single dose of 2000 mg/kg aqueous extract of *G. ternifolia* fruit was administered orally to the rats in group 4 for investigation of acute toxicity. For subchronic toxicity, the extract was administered orally to group 2 and 3 at a dose of 50 and 500 mg/ kg/ day, respectively, for four weeks. Group 1(control) was kept as a control.

Clinical observations and mortality were reported on a daily basis. Weights of rats were recorded at the day of dosing, at weekly intervals thereafter, and at the time of death or when the animals were sacrificed.

2.5 Blood Collection

Blood samples were collected weekly-starting from week zero (Control) -from the orbital sinus of rat eye- in Ethylene diamine tetraacetic acid (EDTA) vacutainers for haematological examination using Sysmex Haematology System KN-21N/Germany, and plain vacutainers for serum analysis using Sysmex Biochemistry System / Germany). The procedures were carried out as described in the manuals of automated machines.

2.6 Pathological Examination

Three rats from each group were sacrificed at the end of the experiment. Necropsy was performed; specimens of liver and kidney with pathological lesions were fixed in 10% neutral buffered formalin and processed for histopathology.

2.7 Statistical Analysis

The data generated during the study were analyzed using the statistical package, Basica-Epistat. Chi^2 tests were employed to detect heterogeneity/homogeneity between groups. The data are expressed as mean ±SD. The results with $P<0.05$ were considered significant.

3. RESULTS

3.1 Yield Percentage

The yield percentage (w/w) of aqueous extract of *G. ternifolia* fruit was 8.44%.

3.2 Effect of *G. ternifolia* Extract on Signs of Toxicity and Mortality of Rats

Signs of toxicity and/or mortality were not observed after oral administration of the doses

50, 500 and 2000 mg/kg body weight to group 2, 3, and 4, respectively.

3.3 Effect of the Extract on Weight

The body weights of rats in group 1, 2, 3, and 4 were significantly (P<0.05) increased. The highest percentage of weight gain in group 1 whilst the lowest in group 4 (Table 1).

3.4 Effect of the Extract on Hematology

The haematological changes on the blood of rats given an aqueous extract of *G. ternifolia* fruit were presented (Table 2). WBC, RBC, Hb and PCV were not affected in group 2 and 3, but significantly (P< 0.05) changed in group 4 compared with group 1.

3.5 Effect of the Extract on Biochemical Parameters

The results of the toxicological effects of the aqueous extract of *G. ternifolia* fruit on the biochemical parameters were summarized (Table 3). Oral administration of the extract at doses of 50 mg/ kg (group 2) and 500 mg / kg (group 3) had no effect on the blood biochemistry. However, a dose of 2000 mg/ kg significantly (P< 0.05) altered all the biochemical parameters except billirubin.

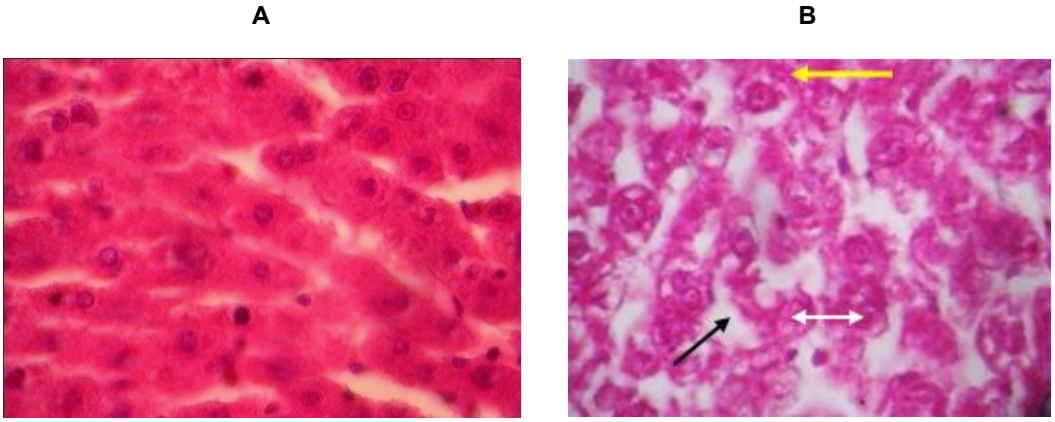

Fig. 2. Section of rat Liver (A) Normal control in group 1. (B) After given aqueous extract of *G.ternifolia* fruit at a dose of 2000 mg/ kg (group 4) showed accumulation of cytoplasm at boundaries of hepatocytes (yellow arrow), vesicular nuclei (white arrow), dilatation of sinusoid, H&E (× 40)

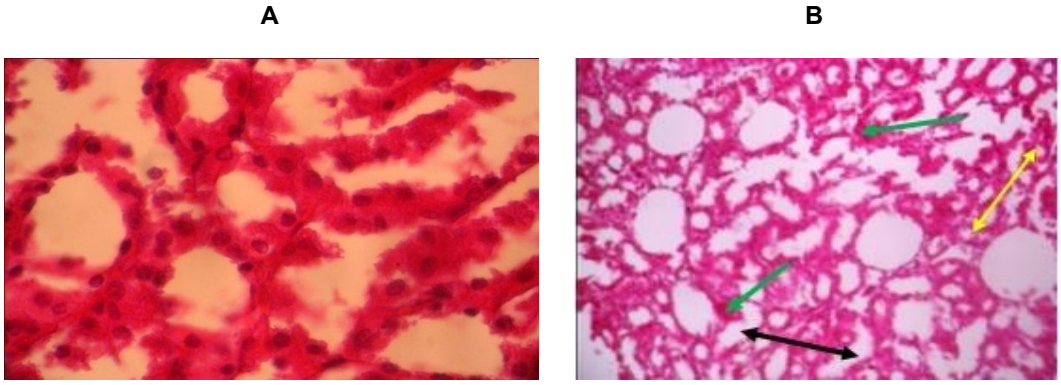

Fig. 3. Section of rat Kidney (A) Normal control in group 1. (B) After dosing of 2000 mg/ kg aqueous extract of *G.ternifolia* fruit (group 4) showed granular eosinophilic cytoplasm (green arrow), degeneration and necrosis of tubules (Black arrow), vacuolation of glomuli (yellow arrow), H & E (× 40)

Table 1. Percentage of weight gain of rats given aqueous extract of *Gardenia ternifolia* fruit

Group no.	Dose (mg/kg)	Weight of rats per week (g)					Wt. gain (g)	Wt. gain (%)
		W 0	W 1	W2	W 3	W 4		
1	0	114.52±2.64	124.03±2.16*	133.19±2.59*	142.19±1.78*	153.70±2.24	39.18	34.21
2	50	111.50±1.87	119.42±4.41*	1277.72±4.71*	135.58±5.66*	143.27±5.81	31.67	28.40
3	500	114.50±1.87	122.25±4.12*	129.83±5.12*	138.72±5.54*	146.81±7.35	32.31	28.22
4	2000	95.50±3.02	108.42±7.51*	118.25±7.17*	119.00±2.19*	-	23.5	24.61

*The data presented as Mean ± SD, *P < 0.05 is significantly different from the control, n= 6*

Table 2. Haematological changes on the blood of rats given aqueous extract of *Gardenia ternifolia* fruit

Group no.	Week No.	Dose (mg/kg)	WBC ($\times10^3 mm^3$)	RBC ($\times10^6 mm^3$)	Hb (g/dl)	PCV (%)
1	0	0	5.90±0.14	6.32±0.08	11.68±0.19	37.50±0.40
	1		5.90±0.09	6.35±0.10	11.72±0.21	37.53±0.39
	2		5.92±0.08	6.34±0.07	11.72±0.21	37.55±0.39
	3		5.93±0.10	6.38±0.07	11.73±0.15	37.57±0.42
	4		5.92±0.15	6.37±0.08	11.73±0.15	37.57±0.43
2	0	50	6.00±0.14	6.52±0.15	11.82±0.25	37.87±0.20
	1		6.00±0.14	6.52±0.15	11.82±0.25	37.87±0.18
	2		6.03±0.12	6.50±0.14	11.80±0.23	37.83±0.20
	3		6.03±0.12	6.50±0.14	11.80±0.23	37.83±0.20
	4		6.03±0.12	6.52±0.15	11.82±0.25	37.87±0.20
3	0	500	6.58±0.32	6.90±0.14	11.92±0.20	38.00±0.14
	1		6.75±0.15	6.90±0.14	11.92±0.20	37.97±0.12
			6.75±0.15	6.83±0.12	11.83±0.18	38.03±0.39
	3		6.77±0.12	6.80±0.13	11.82±0.15	37.67±0.16
	4		6.77±0.12	6.80±0.09	11.82±0.15	37.48±0.20
4	0	2000	6.75±0.19	6.93±0.56	12.85±0.37	39.02±0.15
	1		10.00±0.14	3.15±0.21	9.23±0.34	35.17±0.16
	2		10.12±0.12	3.02±0.20	9.03±0.34	34.97±0.19
	3		10.20±0.89	3.02±0.20	9.02±0.33	34.82±0.29
	4		-	-	-	-

*The data expressed as Mean ± SD, *P < 0.05 is significantly different from control by Chi^2, n= 6*

Table 3. Biochemical changes on blood of rats after oral administration of the aqueous extract of *Gardenia ternifolia* fruit

Group No. / Dose (mg/kg)	Week No.	Total protein (g/dl)	Albumin (g/dl)	Cholesterol (mg/dl)	Billirubin (mg/dl)	Urea (mg/dl)	Creatinine (mg/dl)	ALT (U/L)	AST (U/L)	ALP (U/L)
1 (0)	0	6.15±0.18	3.53±0.16	43.00±0.42	0.10±0.00	14.00±0.07	0.58±0.08	18.33±0.82	13.00±0.89	53.00±1.41
	1	6.39±0.18	3.55±0.31	43.00±1.10	0.10±0.00	14.68±0.23	0.53±0.05	18.33±0.82	13.05±0.90	53.00±1.41
	2	6.46±0.19	3.65±0.23	43.17±0.75	0.10±0.00	14.68±0.32	0.53±0.05	18.42±0.83	13.05±0.90	53.00±1.67
	3	6.57±0.26	3.73±0.23	43.17±0.75	0.10±0.00	14.70±0.28	0.58±0.00	18.42±0.83	13.08±0.94	53.17±1.67
	4	6.67±0.16	3.80±0.17	43.33±0..52	0.10±0.00	14.70±0.26	0.60±0.00	18.43±0.80	13.08±0.94	53.17±1.67
2(50)	0	6.50±0.14	3.85±0.11	41.17±0.98	0.15±0.06	15.12±0.12	0.23±0.05	19.33±0.82	23.50±1.23	58.00±0.89
	1	6.50±0.14	3.85±0.11	41.17±0.98	0.15±0.06	15.12±0.12	o.23±0.05	19.33±0.82	23.50±1.23	58.00±0.89
	2	6.47±0.15	3.82±0.08	41.33±1.21	0.15±0.06	15.22±0.10	0.25±0.06	19.47±0.73	23.52±1.33	58.00±0.89
	3	6.47±0.15	3.82±0.08	41.33±1.21	0.15±0.06	15.22±0.10	0.25±0.06	19.47±0.73	23.52±1.33	58.33±0.82
	4	6.47±0.15	3.82±0.08	41.33±1.21	0.01±0.06	15.22±0.10	0.25±0.06	19.47±0.73	23.52±1.33	58.33±0.82
3 (500)	0	6.22±0.12	3.85±0.11	42.00±0.63	0.15±0.06	16.80±0.21	0.25±0.06	19.50±1.38	26.00±0.89	57.17±1.17
	1	6.17±0.16	3,82±0.08	42.00±0.63	0.15±0.06	16.80±0.21	0.25±0.06	19.50±1.38	26.00±0.89	57.17±1.17
	2	6,17±0.16	3.80±0.06	42.33±0.52	0.15±0.06	16.83±0.16	0.25±0.06	19.62±1.27	26.17±0.75	57.50±1.38
	3	6.10±0.13	3.77±0.05	42.50±0.55	0.15±0.06	16.83±0.16	0.27±0.05	19.62±1.27	26.23±0.74	57.67±1.21
	4	6.17±0.16	3.77±0.05	42.67±0.52	0.15±0.06	16.83±0.16	0.27±0.05	19.62±1.27	26.23±0.71	57.67±1.21
4 (2000)	0	6.15±0.19	3.90±0.09	45.67±0.82	0.23±0.05	16.00±0.80	0.23±0.05	18.30±0.83	25.88±0.17	58.00±0.89
	1	4.12±0.12*	2,22±0.15*	48.50±1.05*	0.25±0.06	19.95±0.89*	0.80±0.06*	25.78±0.23*	32.62±0.26*	63.50±1,05*
	2	4.02±0.04*	2.10±0.09*	48.50±1.05*	0.25±0.06	20.55±0.89*	0.88±0.10*	26.00±0.32*	33.08±0.39*	63.83±0.75*
	3	3.98±0.04*	2.00±0.06*	48.50±1.05*	0.25±0.06	20.93±0.74*	1.02±0.10*	26.25±0.27*	33.15±0.33*	64.00±0.63*
	4	-	-	-	-	-	-	-	-	-

*The data presented as Means ± SD, *P<0.05: significantly different from control, n=6*

3.6 Effect of the Extract on Histopathological Findings

Gross anatomy of rats in group 2 and 3 showed normal liver and kidney as compared with the control (group 1). Acute toxicity revealed histopathological changes in the liver and kidney of rats in group 4. The liver was characterized by the accumulation of cytoplasm at boundaries of hepatocytes, vesicular nuclei, and dilatation of sinusoid (Fig. 2B) compared with the control (Fig. 2A). The kidney showed granular eosinophilic cytoplasm, degeneration and necrosis of tubules, vacuolation of glomuli (Fig. 3B) compared with the control (Fig. 3A).

4. DISCUSSION

Herbal drugs are widely used often contain high active pharmacological compounds. Recently, reports have mounted about hepatotoxicity of herbal remedies which ranges from mild liver enzyme alterations to chronic liver disease and liver failure [15].

In this study, Oral administration of *G. ternifolia* aqueous extract at a dose of 50 and 500 mg/ kg for four weeks to group 2 and 3, respectively, had no toxicological effects. This is online with the findings of previous study which mentioned that the aqueous extract of *G. lutea* fruit at a concentration of 75 µg/ml was not toxic to rabbit [11]. In addition, intravenous administration of geniposide- isolated from an alcoholic extract of the fruit to rat at a dose of 2.5 g/ kg was safe and cause no mortality. Moreover, the aqueous extract of *G. lutea* fruit was lethal to fish at a concentration of 75 µg/ ml [11]; however it was safety at the molluscicidal concentration (25-30 µg/ml). The present study confirmed by [16] who found that ingestion of *Gardenia* yellow containing geniposide (2.73%) at a dose of 60 mg/kg /day for 3 months did not cause any severe toxic effects.

On the other hand, a dose of 2000 mg/ kg given once to the rats in group 4 altered the hematology, biochemistry and histology of the liver and kidney. This inconsistent with [17] who found that oral administration of *Gardenia* yellow at doses of 800 mg/ kg up to 5000 mg/ kg to rats increased the activity of transaminases; and a dose of 2000 mg/kg caused histopathological changes in the liver.

Significant Increase of WBC in group 4 indicated inflammation which confirmed by the histopathological findings. Decreased RBC, Hb and PCV indicated a decrease of RBC production or increase of RBC destruction.

The decrease of serum albumin could be indicative of impaired liver excretory and synthetic function. Elevation of ALT and AST correlated with histopathological changes. These findings were supported by [18] who reported that primary and secondary hepatic disease can cause an elevation of both ALT and AST. Elevated transaminases are suggestive of liver necrosis [19]. The increase of serum urea and creatinine in group 4 suggested renal malfunction [20].

5. CONCLUSION

The results revealed that the aqueous extract of *G. ternifolia* fruit at low doses was safety, but the high dose may cause hepatorenal toxicity. A further work is needed for determination of LD_{50} and LD_{99}. The phytochemical analysis is recommended to define the toxic compounds that may exist.

CONSENT

It is not applicable.

ETHICAL APPROVAL

All authors hereby declare that “Principles of laboratory animal care” (NIH publication No. 85-23, revised 1985) were followed in the experiment, as well as specific national laws where applicable. The study has been approved by the Ethical Approval No. EA /0019/ 2018, The Sudan Veterinary Council, Ministry of Cabinet, Republic of Sudan.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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