

Toxicological profile of *Grewia bicolor* root extract

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Abstract: In recent years, traditional system of medicine has become a topic of global importance. Many of the plant species that provide medicinal herbs have been scientifically evaluated for their possible medicinal, pharmacological and toxicological effects. A number of species of the genus *Grewia* have been used as medicinal herbs to treat several diseases in different parts of the globe. *Grewia bicolor* is the most famous medicinal plant among the genus *Grewia*. Despite the wide use of the plant in traditional medicine, so far no scientific evaluation was carried out on this plant for the preclinical toxicity profile. The present study was conducted to evaluate the safety of *Grewia bicolor* root extract. The plant extract proved that it is relatively safe through the acute and sub-acute studies.

Keywords: Traditional medicine, *Grewia bicolor*, acute toxicity, sub-acute toxicity, *Grewia* spp.

I. Introduction

In folk medicine, the genus *Grewia* spp extract and preparations from various species exhibited various biological effects, e.g. antioxidant, anti-bacterial and analgesic effects. Also, they used as haemostatic agents and in the treatment of kidney diseases [1, 2]. *Grewia bicolor* plant and seeds are considered to be one of the popular medicinal herbs used in tropical Africa, Ethiopia and Somalia; Eastern Africa, Namibia, Botswana, South Africa and Swaziland. It is also available on coastal areas of Benin and Togo, Yemen, Arabian Desert and India [3]. *Grewia bicolor* is a part of Sudanese traditional medicine. The plant is used for treating postulant skin lesions and sometimes as a tranquilizer [4]. The plant also is used to treat and manage diarrhea in many areas of Africa [5]. Mohamed, *et al.*, [6] reported that the intraperitoneal administration of the ethanol extract of *Grewia bicolor* root at a dose of 400 mg/kg to rats in late pregnancy was lethal to all mother rats.

II. Materials and Methods

2.1. Plant material:

Grewia bicolor roots were purchased from its natural homeland at Kurdufan Province, southwest Sudan. The plant was authenticated by Taxonomy Department of Medicinal and Aromatic Plant Research Institute, National Center for Research, Khartoum, Sudan. The roots were then washed and allowed to dry in shade for 7 days. The dried plant material was crushed with a manual grinder into a fine powder. Ethanol extraction process was followed according to [7].

100g of the grounded roots were transferred to a round bottom flask and macerated in 80% ethanol. The flask was stoppered and left for 24 hours at room temperature. The extract then filtered using sterile cotton pieces. The filtrate thus obtained was concentrated under reduced pressure and the solvent recalled was used to extract the mark following the same procedure till exhaustion. The concentrated extract was collected and left to dry at room temperature till constant weight was obtained. The extract was then kept in a refrigerator for experimentation.

2.2. Experimental animals:

Adult male, Wistar Albino Rats (WAR), weighting 110-125 g were housed in standard cages under controlled temperature condition (25°C) and relative humidity (40 %) with a 12 h light cycle beginning at 7 am. The rats were provided with standard diet (laboratory rodent's chow) and tap water. All experiments were carried out between 8 am and 12 noon. This study was approved by the Scientific Research Committee of the College of Pharmacy, Omdurman Islamic University (OIU) in accordance with good clinical practice and international guidelines for animal use in experimentations.

2.3. Acute toxicity study: Determination of the maximum tolerated dose, toxic dose and median lethal dose (LD₅₀) of the tested extract:

Acute toxicity study was designed according to the method adopted by [8,9]. The maximum tolerated dose, the toxic dose and the median lethal dose of the plant's extract was determined by i/p administering of the extract in escalating dose levels of 100, 200, 400, 800, 1600, 3200 and 6400 mg/kg.

40 healthy adult males Wister Albino Rats weighting 120-130g were allotted to 8 groups, 5 animals in each. Group 1 received 10ml/kg, i.p. normal saline and served as negative control.

The extract was given to seven groups as a single dose of the previous tested doses. The animals were observed continuously for 2-24 hours with regard to the behavioral and neurological profiles. The final LD₅₀ value was calculated. Survival and mortality rates were recorded for each dose as well as the behavioral and neurological signs were also recorded. Thus, the required doses were defined.

2.4. Sub-acute toxicity:

Sub-acute toxicity study protocol was adapted from [10]. 10 healthy males Wister Albino Rats weighting 120-130g were allotted to 2 groups, 5 animal in each. Group 1 received 10ml/kg, i.p normal saline for 28 days and served as negative control, groups 2 received *G. bicolor* root extract at dose level of 800mg/kg i.p for 28 days. The animals were sacrificed on day 29. Body weights were recorded at day 0, 14, 21 and 28 post the dose administration for the three groups.

Blood samples were collected from the common carotid artery into a heparinized tubes for hematological studies. Parameters such as hemoglobin (Hb) red blood cell (RBC) count, packed cell volume (PCV) and then the values of mean corpuscular volume (MCV), mean cell hemoglobin (MCH) and the mean corpuscular hemoglobin concentration (MCHC), white blood cells (WBC), Neutrophils, Eosinophils, Basophils, Lymphocytes and Monocytes were calculated.

The serum was separated for determining the concentrations of biochemical parameters like blood urea nitrogen (BUN), creatinine, sodium, potassium, total protein, albumin, alanine aminotransferase (ALT) and alkaline phosphatase (ALP). Variations in body weight were monitored during the test period.

After the blood collection livers and kidneys were removed from all rats for detection of gross lesions. All tissues were fixed in 10% buffered formalin solution. After routine processing, the paraffin sections of each tissue were cut at 5µm thickness and stained with haematoxylin and eosin for a microscopic examination.

2.5. Statistical analysis:

The values are expressed as mean ± SEM (Standard Error of Mean) and the data were analyzed using one way ANOVA followed by Tukey-Kramer test. The level of significance was set at p < 0.05. Median anticonvulsant dose (ED₅₀) was calculated according to the method of [11]. A computer program was used to calculate 95% confidence limit (C.L 95%) of ED₅₀.

III. Results

3.1. Acute toxicity study: Determination of the maximum tolerated dose, toxic dose and median lethal dose (LD₅₀) of the tested extract:

The data revealed that the plant extract was well tolerated in rats up to the dose of 800mg/kg, i.p without lethality or toxic signs during 24 h post the dose administration. No mortality was observed during 14 days post the treatment with the tolerated dose. Behavioral signs of toxicity were exhibited by rats that received 1600 mg/kg and above such as increase in respiratory rate, increased abdominal contractions, deep depression and indifference. Also, neurological signs were observed such as paralysis of the hind limbs, writhing, abnormal gait and deformity in shape. These behavioral and neurological signs disappeared with in 24 h of the extract administration. 60% mortality occurred post 60-240 minutes of the administration of the dose 3200 mg/kg while 100 % mortality occurred 10-15 minutes post administration of the dose 6400mg/kg, i.p. The LD₅₀ (median lethal dose) was calculated to be 2663.92 mg/kg. (Table 3.1.1 & 3.1.2)

(Table 3.1.1) Acute toxicity study: Determination of the maximum tolerated dose, toxic dose and median lethal dose (LD₅₀) of the tested extract:

Extract Dose (mg/kg)	Mortality rate	Time of death post administration/ minutes	Toxic dose mg/kg	Lethal dose mg/kg	LD ₅₀ (mg/kg)	C.L 95%
200-1600	0%	0	1600	3200	2663.92	1582.6 – 4475.5
3200	60%	60-240				
6400	100%	10-15				

(Table 3.1.2) Acute toxicity: Description of the toxicity reflected by behavioral and neurological signs of the tested plant extract:

Behavioral signs at the toxic dose	Neurological signs at the toxic dose
Deep depression, indifference, increase in respiratory rate, increase in abdominal contractions.	Paralysis at the hind limbs, writhing, abnormal gait

3.2. Sub -acute toxicity study:

The dose of 800 mg/ kg i.p for 28 days was considered to be the sub toxic dose.

No significant differences either in control group or treated group of plant extract were noticed in body weight as summarized in (Table 3.2.1). The tested dose of extract didn't show any significant changes neither in

hematological nor in biochemical parameters (hepatic and renal function tests) compared to the control group (Tables 3.2.2 and 3.2.3).

The histopathological sections of livers and kidneys of the tested group exhibited normal architecture on comparison to the control group (Figures 1&2).

Table 3.2.1. Body weights of rats in sub- acute toxicity of *G.bicolor* extract:

Treated groups	Body weight (g)			
	Day 0	Day 7	Day 14	Day 28
Control	123.00 ± 4.36	136.00 ± 4.30	142.67 ± 6.64	153.00 ± 4.00
<i>G.bicolor</i>	125.00 ± 6.52	140.00 ± 1.30	145.67 ± 3.24	149.00 ± 4.30

Values are expressed as mean ± S.E.M., n = 5.

Table 3.2 .2. Effects of 28 days administration of *G.bicolor* extract in rats

Parameters	Control group	<i>G. bicolor</i> group
RBC (x10 ⁶ /µl)	6.86±0.32	6.84±0.16
Hb (g/dl)	11.80±0.30	11.77±0.34
PCV (%)	36.23±1.07 66	36.70±0.86
MCV (fl)*	53.96±1.43	53.61±0.72
MCH (pg) **	17.60±0.01	17.22±0.19
MCHC (g/dl)	32.56±0.27	32.10±0.59
WBC (x10 ³ /µl)	7.27±1.34	7.59±1.53
Neutrophils (%)	23.61±2.13	22.93±3.74
Eosinophils (%)	1.47±0.55	1.65±0.62
Basophils (%)	0.00±0.00	0.00±0.00
Lymphocytes (%)	69.82± 6.53	67.96±6.54
Monocytes (%)	2.00±0.48	1.46±0.39

Values are expressed as Mean ± S.E.M., n = 5. *fl : femtoliters (10⁻¹⁵L), ** pg : pictograms (= 0.06207 femtomol).

Table 3.2.3. Effects of 28 days administration of *G.bicolor* root extract on kidney and liver functions in rats:

Parameters	Control group	<i>G. bicolor</i> group
Blood urea (mg/dl)	37.67±1.45	34.33±2.96
Serum creatinine (mg/dl)	0.77±0.03	0.80±0.03
Na+ (mmol/L)	147.66±2.84	144.70±6.50
K+ (mmol/L)	6.30±0.20	6.40±0.05
Total protein (g/dl)	7.83±0.49	7.80±0.20
Albumin (g/dl)	4.26±0.13	4.20±0.12
ALT (U/L)	30.33±4.33	26.33±4.76
ALP (U/L)	104.10±8.00	92.33±16.44

Values are expressed as Mean ± S.E.M., n = 5

Fig.(1): Photomicroscope of liver tissue post 28 days: A. control (N. saline, 10ml/kg,i.p/day) ; B. *G. bicolor* (800mg/kg,i.p/day. H&E X 20.

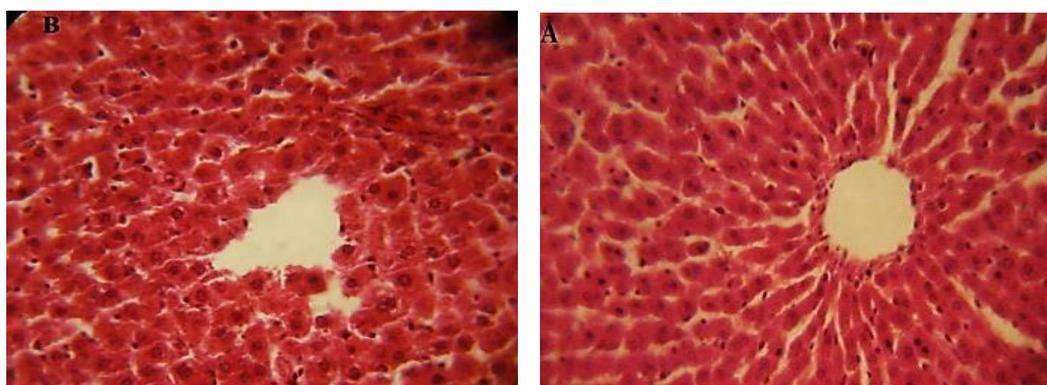
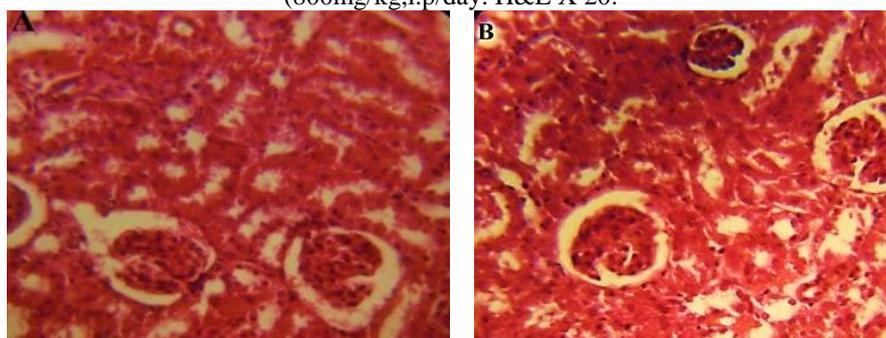


Fig.(2): Photomicroscope of kidney tissue post 28 days: A. control (N. saline, 10ml/kg,i.p/day); B. *G. bicolor* (800mg/kg,i.p/day. H&E X 20.



IV. Discussion

The tolerated dose of *G. bicolor* extract was found to be 800mg/kg, ip while the toxic dose was 1600mg/kg, ip, no deaths were observed but behavioral and neurological toxic signs were recorded such as increase of respiration and abdominal contraction post the treatment with the toxic dose of *G. bicolor*. This might be related to the presence of serotonin which stimulates the respiration and the abdominal contraction [11, 12]. Regarding to the neurological signs, paralysis at the hind limbs and abnormal gait were observed, these neurological signs suggested to be due to the excessive GABA-ergic chloride channels activation which found to be a strategy for central synaptic neurotransmitter block to induce transient and focal leg paralysis in its host [13]. Writhing also was observed and may be due to the abdominal contraction.

The lethal dose of extract was found to be 3200mg/kg, i.p. The calculated LD₅₀ value was in the range of 2663.92 mg/kg. These values suggested relatively low toxicity of the extract. Similarly, Ukwuani, *et al.*, [14] reported that the toxic evaluation of the methanolic leaves extracts of *Grewia crenata* in rats revealed that the lethal dose of this plant was estimated to be more than 5000 mg/kg. Another similar result was reported by [15] who found that the methanolic leaves extract of *Grewia hirsuta* proved to be safe up to 2000mg/kg when orally administered to rats.

In the sub-acute toxicity study, the extract was administered at a dose of 800mg/kg, i.p for 28 days. No deaths were observed during the study period. The effect of 28 days of administration extract showed no significant change on the animal's body weights. This reveals that it did not adversely affect the basic metabolic processes of the experimental rats.

These results are identical with Wilson, *et al.*, [16] who found that the rats' weights didn't show any significant change after feeding of *Grewia mollis* stem bark with the normal diet at 10% for 4 weeks. Also, [17] reported that no significant changes in body weight were observed after the ingestion of *Grewia asiatica* fruit extract to mice at dose rate of 700 mg /kg daily for 15 days.

The treated group didn't show any significant changes in hematological and biochemical parameters (hepatic and renal function tests) compared to the control group. Assessment of hematological parameters can be used to determine the extent of the deleterious effect of extracts on the animal blood. It can also be used to explain blood relating functions of a plant extract or its products [14]. Heamogram was estimated for both the treated and the control groups. The results showed no unusual effect on RBCs count, hemoglobin, PCV, MCV, MCH, MCHC, WBCs count, neutrophils, eosinophils, basophils, lymphocytes and monocytes in treated animals compared with the control group. This indicates that the extract did not affect blood cells and their production.

A similar result reported about the leaf extract of another *Grewia spp.*, *G. crenata* by Ukwuani, *et al.*, [14] who found that the plant extract didn't show any significant changes in the hemogram of rats that administered a dose range of 900-3600 mg/kg orally for 28 days.

V. Conclusion and Recommendations

- The acute toxicity study showed that the *G. bicolor* extract is relatively safe.
- Heamatological, biochemical and histological results obtained from the liver and kidney function parameters indicated that the ingestion of *G. bicolor* root 800 mg/kg i.p for 28 days had no observable toxic effects on these organs at the tested dose. It was therefore suggested that these results could form the basis for clinical trial in human.
- These results were obtained from experimental animals' models, so these herbal plant should be used in appropriate formulations for further clinical trials in human.

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