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Acute toxicity of the methanolic extracts of *Terminalia brownii* bark in rats

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Abstract

This study was designed to reveal the toxicopathological effects of the methanolic extract of the bark of *Terminalia brownie* in Swiss albino rats. The methanolic extracts of the bark were given at doses rate of 100, 500 and 1000 mg/kg (group 1, 2 and 3 respectively) body weight to Swiss albino rats. Oral administration of the extracts caused symptoms such as dullness, inappetence and decreased activity in group 2 and 3 rats. The mean values of haemoglobin (Hb) concentration, red blood cell counts (RBC) and packed cell volume (PCV) decreased in the three treated groups. Serum analysis indicated no changes in the activity of the enzyme alanine amino transferase (ALT) or the concentration of urea, creatinine, bilirubin, total protein, and total albumin in the sera of all treated rats during the course of the experiment. However, there was an increase in the activity of the enzyme aspartate amino transferase (AST) in the sera of group 3 and 4 rats. The main lesions found were congestion and hemorrhage in the liver, kidney, lung, stomach and small intestine.

Keywords: Bark of *Terminalia brownii*, Rats, Toxicity

Introduction

Terminalia brownii (Combertacaea) is a traditional Sudanese medicinal plant known locally as shagarat elsobag. It is widely spread tree throughout the Sudan. Details of its botanical description were given by Elgaszali et al. (1997) and Omer and Elnima (1999). The maceration of the bark of the plant has been used in traditional medicine for the treatment of cough and bronchitis in the west and Souteast Sudan (Elgazali et al., 1997) and for the treatment of diarrhoea and gonorrhoea (Zakaria et al., 2007).

Screening of active principles in the bark revealed the abundance of tannins, saponins, appreciable amounts of flavonoids and traces of other constituents (Omer and ElNima, 1999). Whereas, *in vitro* studies revealed a high antimicrobial activity residing in the extracts of bark and leaves of the plant against bacterial pathogens (Omer and Elnima, 1999; Zakaria et al., 2007).

The objective of this research was to find the effect of the methanolic extract of the bark of *Terminalia brownii* in albino rats and to correlate the clinical and pathological changes with possible changes in the concentration of certain serum constituents of these animals.

Materials and Methods

Twenty four Wister albino rats weighed 150-200 gm were used in this experiment. They were housed in laboratory cages, fed with pellets and fresh vegetables and were watered *ad libidum* throughout the experimental period. The green bark of the plant was collected, dried in the shade and then coarsely ground using a morter and pestle. About 500 g from the coarsely ground bark was put in soxhlet apparatus (Quick Fex 5183). One liter of 70% methanol was added to the plant and left for 24 hours in the apparatus. The methanolic extract was then filtered. The filtrate was evaporated under vacuum till dryness at 40^{o}C using a rotary evaporators. The obtained solid extract was removed, weight and kept as a stock solution for use.

The rats were allowed one week for adaptation. After the adaptation period, the rats were divided into four groups randomly. One group was kept as control.

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The other groups were given the methanolic extract 100, 500 and 1000 mg/kg body weight respectively for 6 days. All the rats were bled before dosing and then after every two days. Serum was analyzed for the activities of the alanine amino transferase (ALT) and aspartate aminotransferase (AST) by the method described by Reitman et al. (1967). The concentrations of total proteins were detected using the methods of Weichselbaum (1946). For total albumin, the methods of Doumas et al. (1971) and Webster et al. (1974) were used. Total bilirubin was estimated by the method described by Jendarassik and Groph (1938). Creatinine levels were measured by the method of Falling and Hausen (1971) and urea by the method of Varley (1967). For histopathological studies, tissues were fixed in 10% formal saline, and 6-µm paraffin sections were stained with haematoxylin and Eosin (H and E).

Statistical analysis

All data were subjected to the statistical package for social sciences (SPSS). Mean was tested using two way analysis of variance (ANOVA) procedure, according to Mead and Gurnow (1983) and then separate using Duncan's multiple range test (DMRT).

Results

The dosing schedule and time of expiry of rats dosed with *Terminalia brownii* bark methanolic extract are shown in Table 1. Rats of group 2 showed no clinical signs throughout the course of dosing. Rats of group 3 and 4 showed dullness, inappetence and decreased activity after the 4th dose. The post mortem findings of rats of the four groups are summarized in Table 2. The lesions were similar in all treated rats. However, there were variations in severity of lesions between the different doses used. The predominant lesions were congestion and haemorrhages in the lung, liver, kidney, stomach and small intestines. The mean values of haemoglobin concentration (Hb), RBCs and PCV percentages decreased significantly in the three treated groups. The mean values of MCV increased significantly in all treated groups ($P<0.05$), while there was no significant changes in the values of MCHC in all group (Table 3). The biochemical changes associated with the bark extract are shown in Table 4. There are no significant changes in the activity of the enzyme ALT in the serum of any rat of all treated groups, but there was a significant increases in the concentration of the enzyme AST in the sera of groups 3 and 4 rats ($P<0.05$). There were no significant changes in the concentration of total protein, total albumin, urea, creatinine and bilirubin in the sera of all treated rats during the course of the experiment. Histopathological changes were congestion and hemorrhage in the liver kidney, lung and small intestine. In group 4 rats there are local areas of necrosis and infiltration of mononuclear cells in the portal areas of the liver.

Table 1: Dosing schedule and fate of rates given methanolic extract of *Terminalia brownii* bark for 6 days

Group No.	Animal no	Fate of animal	Percentage of mortality
Group (1) control	1	6days slaughtered	0%
	2	"	
	3	"	
	4	"	
	5	"	
	6	"	
Group 2 100 mg/kg body weight	7	Died on day 2	33.3%
	8	6 days slaughter	
	9	"	
	10	Died on day 4	
	11	6 days slaughtered	
	12	"	
Group 3 500 mg/kg body weight	13	Died on day 4	50%
	14	Died on day 3	
	15	6 days slaughtered	
	16	"	
	17	Died on day 2	
	18	6 days slaughtered	
Group 4 1000 mg/kg body weight	19	Died on day 2	66.6%
	20	6 days slaughtered	
	21	Died on day 4	
	22	6 days slaughtered	
	23	Died on day 3	
	24	Died on day 4	

Discussion

In the present study, it was shown that the methanolic extract of the bark of the plant was fatal to many rats of all groups dosed orally with different concentrations of the extract. In the animals congestions and haemorrhages of the liver, lungs, kidneys, stomach and small intestine were the main gross and microscopic lesions observed. This is probably due to the toxic substance present in the plant extract (Adam, 1974; Adam and Magzoub, 1975; Thoria et al., 2001). Similarly, congestion in the internal organs was also reported in experimental animals fed ginger and garlic (Ali and Mohammed, 1986).

The decreased values of RBCs and Hb could be attributed to the liver and renal damage which usually lead to lack of erythropoietin (Erselve, 1995). Similarly, decreased values of RBCs and Hb occurred in intramuscularly treated rats with the methanolic extract of *Ambrosia maritima* (Fatih-ElRahman, 2003).

In this study, there were no significant changes in the activity of the enzyme ALT, but there was a significant increase in the enzyme AST, in the sera of rats belonged to group 3 and 4. An increase in the serum enzymes activity has been utilized as an indicator

Table 2: Necropsy findings in Albino rats dosed with methanolic extract *Terminalia brownii* bark for 6 days

Site	Lesion	Group 1 (Control)	Group 2 (100 mg/kg body weight)	Group 3 (100 mg/kg body weight)	Group 4 (100 mg/kg body weight)
Liver	Congestion	-	+	++	+++
	necrosis	-	-	+	+
	fatty changes	-	-	-	-
Kidneys	Haemorrhages congestion	-	-	+	+
	atrophy of the fat in the renal pelvis	-	+	++	+++
		-	-	-	-
Heart	Congestion	-	+	+	+
	Serous atrophy of the cardiac fat	-	-	-	-
Lungs	Pulmonary congestion and haemorrhage	-	++	+++	+++
	Pulmonary oedema		-	+	+
Stomach	Gasterities	-	+	+	++
Small intestine	Enterilies	-	+	+	++

+ ⟶ +++, increasing severity of lesions; (-), Absence of lesions

Table 3: Mean haematological values in blood of rats dosed with methanolic extract *Terminalia brownii* bark for 6 days

Hg (g/dL)				
Days	G1 (Control)	G2 (100 mg/kg)	G3 (500 mg/kg)	G4 (1000 mg/kg)
0	13.08±0.98^{a}	12.97±0.97^{a}	12.97±0.60^{a}	13.00±0.38^{a}
2	13.10±0.94^{a}	12.70±0.85^{a}	11.18±0.87^{b}	11.22±0.81^{b}
4	13.10±0.92^{a}	8.82±0.75^{c}	8.28±0.96^{c}	8.63±0.39^{c}
6	13.07±0.93^{a}	8.12±1.24^{c}	8.23±0.95^{c}	8.47±0.97^{c}
RBCs/µl				
0	6.99x10^{6}±0.67^{a}	6.65x10^{6}±0.77^{a}	6.93x10^{6}±0.53^{a}	6.88x10^{6}±0.27^{a}
2	6.98x10^{6}±0.67^{a}	65.6x10^{6}±0.63^{b}	6.06x10^{6}±0.76^{b}	5.62x10^{6}±0.45^{b}
4	6.96x10^{6}±0.67^{a}	4.47x10^{6}±0.35^{c}	4.17x10^{6}±0.48^{e}	4.25x10^{6}±0.12^{d}
6	6.95x10^{6}±0.67^{a}	3.74x10^{6}±0.64^{h}	3.98x10^{6}±0.47^{f}	4.22x10^{6}±0.20^{d}
PCV (%)				
0	41.02±3.05^{a}	40.40±3.57^{a}	40.02±2.26^{a}	39.88±1.63^{a}
2	41.03±3.02^{a}	39.12±4.26^{a}	38.48±3.76^{a}	34.58±2.11^{b}
4	41.02±3.05^{a}	29.20±1.96^{c}	28.13±1.69^{c}	29.88±1.49^{c}
6	41.00±2.97^{a}	26.28±4.40^{c}	27.23±3.36^{c}	28.43±2.53^{c}
MCV (f/L)				
0	58.63±2.31^{b}	58.03±2.48^{b}	59.35±3.58^{b}	57.83±1.13^{d}
2	58.58±2.24^{b}	58.78±3.43^{b}	62.20±5.64^{b}	60.87±2.09^{c}
4	58.60±2.31^{b}	59.70±4.12^{b}	63.20±2.35^{b}	62.33±3.86^{c}
6	58.65±2.31^{b}	71.07±7.74^{a}	73.58±2.87^{a}	71.60±7.64^{a}
MCHC (%)				
0	31.63±0.45^{c}	32.53±1.84^{c}	32.65±1.10^{c}	32.62±0.71^{c}
2	31.65±0.44^{c}	32.60±0.59^{c}	31.07±4.39^{c}	32.42±0.89^{c}
4	31.70±0.40^{c}	32.15±2.01^{c}	32.13±2.24^{c}	32.35±2.02^{c}
6	31.65±0.34^{c}	32.77±5.97^{c}	32.18±1.67^{c}	32.50±1.18^{c}

Mean values (±SD) having different superscript letters in columns and rows (between groups and days) are significantly different ($P<0.05$)

of liver injury caused by poisonous plants and by trematode parasites in domestic animals (Gopinath and Ford, 1969).

In our study, there were no significant changes in total protein or albumin concentrations. This may indicate that the ability of the liver to synthesize protein was not affected. It is documented that over 90% of serum protein including albumin, are synthesized by the liver (Mohamoud, 1977). The absence of bilirubinaemia and splenic haemosiderosis in these experimental animals suggested that the animals did not suffer from haemolytic anaemia (Ford et al., 1968; Adam and Magzoub, 1975). Dosing of the bark extract to albino rats caused no significant changes in the non protein, nitrogen constituents of serum, urea and creatinine, which usually increase when severe glomerular damage occurs (Mahmoud, 1977).

It seems reasonable, therefore to conclude that the methanolic extract of *Terminalia brownii* bark is toxic in the doses orally to the albino rats.

Table 4: Mean concentration of serum constituents in rats given methanolic extract of *Terminalia brownii* bark for 6 days

AST (µ/L)				
Days	G_1(Control)	G_2(100 mg/kg)	G_3(500 mg/kg)	G_4(1000 mg/kg)
0	8.00±1.55^{c}	8.33±2.16^{b}	8.00±1.55^{c}	8.50±1.64^{c}
2	8.52±1.55^{b}	8.50±1.64^{b}	8.50±1.64^{b}	9.00±1.64^{b}
4	8.46±1.55^{b}	8.40±1.47^{b}	9.17±1.64^{a}	9.17±1.64^{a}
6	8.55±1.55^{b}	8.55±1.47^{b}	9.83±1.64^{a}	9.35±1.47^{a}
ALT (µ/L)				
0	6.667±2.07^{b}	6.667±3.27^{b}	7.333±1.63^{a}	7.333±1.63^{a}
2	6.721±2.07^{b}	6.668±3.27^{b}	7.346±1.63^{a}	7.333±3.01^{a}
4	6.665±2.07^{b}	6.665±3.27^{b}	7.400±1.67^{a}	7.345±3.01^{a}
6	6.675±2.07^{b}	6.668± 3.20^{b}	7.421±1.63^{a}	7.200±2.99^{a}
Total protein (g/100 ml)				
0	5.600±0.14^{a}	5.517±0.16^{a}	5.500±0.09^{a}	5.583±0.15^{a}
2	5.583±0.13^{a}	5.517±0.12^{a}	5.550±0.23^{a}	5.533±0.16^{a}
4	5.610±0.14^{a}	5.500±0.11^{a}	5.517±0.15^{a}	5.533±0.08^{a}
6	5.605±0.14^{a}	5.517±0.12^{a}	5.520±0.15^{a}	5.600±0.08^{a}
Total albumin (g/100 ml)				
0	3.433±0.14^{a}	3.350±0.19^{a}	3.417±0.08^{a}	3.350±0.19^{a}
2	3.433±0.12^{a}	3.383±0.12^{a}	3.433±0.12^{a}	3.367±0.15^{a}
4	3.440±0.14^{a}	3.400±0.06^{a}	3.433±0.08^{a}	3.450±0.14^{a}
6	3.442±0.14^{a}	3.417±0.08^{a}	3.450±0.10^{a}	3.450±0.14^{a}
Total bilirubin (mg/100 ml)				
0	0.1317±0.03^{a}	0.1467±0.01^{a}	0.1317±0.03^{a}	0.1350±0.03^{a}
2	0.1319±0.03^{a}	0.1400±0.01^{a}	0.1318±0.03^{a}	0.1300±0.02^{a}
4	0.1420±0.01^{a}	0.1420±0.01^{a}	0.1300±0.02^{a}	0.1360±0.01^{a}
6	0.1316±0.03^{a}	0.14407±0.01^{a}	0.1267±0.02^{a}	0.1340±0.01^{a}
Creatinine (mg/100g)				
0	1.333±0.12^{a}	1.333±0.16^{a}	1.333±0.12^{a}	1.350±0.10^{a}
2	1.350±0.10^{a}	1.333±0.08^{a}	1.333±0.08^{a}	1.333±0.10^{a}
4	1.345±0.12^{a}	1.333±0.12^{a}	1.317±0.08^{a}	1.367±0.08^{a}
6	1.352±0.10^{a}	1.317±0.10^{a}	1.318±0.08^{a}	1.367±0.08^{a}
Urea (mg/100 ml)				
0	16.52±0.84^{a}	16.68±0.70^{a}	16.30±0.79^{a}	16.00±0.75^{a}
2	16.50±0.84^{a}	16.62±0.58^{a}	16.30±0.67^{a}	15.98±0.78^{a}
4	16.52±0.79^{a}	16.63±0.55^{a}	16.27±0.69^{a}	15.95±0.84^{a}
6	16.55±0.74^{a}	16.58±0.66^{a}	16.22±0.67^{a}	15.98±0.69^{a}

Mean values (±SD) having different superscript in columns and rows (between groups and days) are significantly different ($P<0.05$)

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