Original Article

Toxic Potential of Ethanolic Extract of *Acacia nilotica* (Garad) in rats

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Abstract

Objective: The study aimed to investigate acute and sub-acute toxic potential of ethanolic extract of *Acacia nilotica* (Garad) in rats.

Methods: LD_{50} of Garad was determined. Changes in behavioral response, induced by sub-acute treatments were recorded. Elevated urea, creatinine, ALT (GPT) and AST (GOT) in plasma were taken as evidence for impaired kidney and liver function.

Results: LD_{50} of Garad extract was found to be 215.36 mg/kg (124.84 – 317.47). Regarding sub acute treatment, observations on seventh and fourteenth days showed slight to moderate sedation at various doses. On day 21 no behavioral changes were recorded. However, a treatment for three weeks induced significant elevation in urea and ALT ($p < 0.05$).

Conclusion: It can be concluded that the ethanolic extract of Garad has got some toxicity when administered sub acutely and intraperitoneally in rats, particularly at high dose (60 mg/kg).

Key words: LD50, hepatotoxicity, nephrotoxicity.

Acacia nilotica , known in Sudan as Garad, belongs to the family *fabaceae*. It is a native species of acacia in Africa and the Indian subcontinent. Different parts of the tree are widely used in traditional medicine. *Acacia nilotica* bark is used to treat haemorrhage, diarrhea, dysentery and leprosy. The root is used for the treatment of tuberculosis and impotence. The bruised leaves are used as poultices onto ulcers. *A. nilotica* gum showed low toxicity potential in rats maintained on diets containing 2% and 8% gum for two weeks[1]. It has been reported that Acacia nilotica (Linn.) gum, flower and leaf aqueous extracts have got chemopreventive activity on 7, 12-dimethylbenz (a)anthracene (DMBA) induced skin papillomagenesis in male Swiss albino mice[2]. A steroid 3beta-Acetoxy-17beta-hydroxy-androst-5-ene was isolated from aerial parts of *Acacia nilotica* (L.)[3]. The steroid showed dose-dependent anti-inflammatory activity against TPA (12- O tetradecanoylphorbol-13-acetate) induced mouse ear edema. The antimutagenic and cytotoxic effects of different extracts/fractions of *Acacia nilotica* prepared by maceration method were investigated. It was concluded that these effects, exhibited by acetone extract, may partially be ascribed to the presence of gallic acid and other polyphenols[4]. The methanolic extract of *Acacia nilotica* was observed to cause a dose-dependent (3-30 mg/kg) fall in arterial blood pressure[5]. It has been suggested that this action may be mediated through calcium channel blockade. Survey of the existing knowledge has revealed that the studies on toxicological effects of *Acacia nilotica* were very limited; more studies are needed for evaluation of the toxic potential of *Acacia nilotica*, as it gains special importance to Sudanese patients. The specific objective of this study was to investigate the toxic potential of Garad extract sub-acute treatment on behavior and hepatic / renal functions in rats.

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Materials and Methods

Animals: Wister SWR albino rats, 3-5 months age, weighing 90-150g were used. The animals were housed in groups and kept under controlled conditions of room temperature (25+1˚C) and relative humidity (≈50%). Animals were fed on standard laboratory rodents chow and water.

***Ethanolic extract of Acacia nilotica*:**

Fruits of *Acacia nilotica* were procured from Omdurman local market. Hundred grams of crushed seeds were washed and macerated in 500 ml of absolute ethanol with occasional shaking for 2 hours and then kept overnight. The ethanolic extract was filtered and the filtrate was evaporated at room temperature. 42 grams of the residue was then dissolved in a final volume of 10 ml of distilled water to give a stock solution of 4.2 g/ml which was aliquoted and kept at 4C° until used.

LD_{50} determination:

Karber's method was used[6]. Preliminary experiments were carried out for ethanolic extract of *Acacia nilotica* to determine the approximate doses which produced 0 – 100% lethality. These were found to be 75 mg/Kg and 500 mg/Kg, respectively. Rats were then divided randomly into 7 groups of 5 rats each. Doses of 75, 100, 112.5, 125, 187.5, 250 and 500 mg/Kg were administered to each group separately. Animals were observed over the next 24 hours. The percentage lethality in each group was recorded and the LD_{50} was calculated.

Treatment protocols for behavioral and hepato/ nephrotoxicity studies:

Intraperitoneal doses of one fourth (high toxic dose), one eighth (medium toxic dose) and one sixteenth (low toxic dose) of the LD_{50} of Garad were used as described before[7]. Three sets of animals were used for each of the behavioral and hepato/ nephrotoxicity studies. Each set was formed of four groups (group 1 - 4), five rats each. One ml/ Kg normal saline was administered to group 1. Ethanolic extract of *Acacia nilotica* was administered as 60, 30 and 15 mg/ Kg to group 2, 3 and 4, respectively.

For each study, the first set of animals was treated daily for seven days, the second set was treated daily for 14 days and the third set was treated daily for 21 days.

Behavioral toxicity study:

Changes in behavioral response, induced by various treatments were recorded according to a standard method of observation[8]. Effects of acute and sub-acute toxicities were recorded at 30 minutes, 1, 2 and 24 hours.

Assessment of hepato- and nephrotoxicity:

Treated and control groups were sacrificed after 7, 14 and 21 days, respectively, and plasma was obtained and kept at –20°C for biochemical determination of urea, creatinine, ALT and AST. Plasma elevation of urea and/or creatinine and ALT and/ or AST was taken as evidence for impaired kidney and liver functions, respectively[9, 10].

Statistical analysis:

Data were expressed as mean ± SEM. Statistical comparisons among different groups were made using analysis of variance (ANOVA) followed by Mann-Whitney-U test. Significance was accepted at $p < 0.05$. Data obtained from dose/response analysis of acute toxicity test were calculated as described[11].

Results: *Effects of acute doses of ethanolic extract of Garad on survival of rats:*

The LD_{50} value with 95 % CL was found to be: 215.36 mg/kg (124.84 – 317.47 mg/ kg), (Table 1). Various lethal doses caused difficulty in respiration, lethargy, writhing, spastic paralysis and coma.

Table 1: Effects of acute doses of ethanolic extract of Garad on survival of rats:

Dose (mg/kg)	No. of animals	No. of dead animals (%)
500	5	5 (100)
250	5	3 (60)
187.5	5	2 (40)
125	5	0 (0)
112.5	5	0 (0)
100	5	1 (20)
75	5	0 (0)

LD50 = 215.36 mg/kg (124.84 – 317.47)

Behavioral changes induced by Garad extract after acute treatment:

Acute behavioral changes were recorded during the first 24 hours post treatment. No differences were noted in rats after normal saline injection of acute dosing at various time intervals. The lowest dose (15 mg/kg) of Garad extract induced a slight decrease of alertness at half an hour post treatment that was accompanied with a slight decrease in animals' spontaneous activity at 1 and 2 hours post treatment. A slight rise in passivity was also evident at 2 hours. The middle dose (30 mg/kg) had similar effects with increased degree of sedative behavior at the same time intervals. The highest dose (60 mg/kg) gave more intense behavioral responses compared to lower doses. All these effects, regardless of the dose, returned to normal at 24 hours post treatment.

Behavioral changes induced by Garad extract after sub-acute treatment:

Sub-acute behavioral changes observations were taken during day 7, 14 and 21 post treatment at intervals of 1/2, 1 and 2 hours post treatment. A circadian rhythm in behavioral changes was noted after the sub acute treatment. The 15 and 30 mg/kg doses induced slight decrease in locomotion at 1/2 hour post treatment. The 60 mg/kg dose caused moderate inhibition of motor activity at 1/2 and 1 hour post treatment. At day 14, these doses induced less reduction in spontaneous activity compared to day 7. At day 21 there was no evidence of behavioral changes.

Assessment of nephrotoxic and hepatotoxic potential of Garad after sub-acute treatment:

At day 7, there was a slight decrease of urea with increasing dose of Garad compared to the control group. However, creatinine was slightly decreased at low and middle doses but returned to the control level with high doses of Garad. Compared to the control group, the AST level was significantly increased in the high dose group. The ALT showed only a slight increase in the low and middle dose groups but a more pronounced increase in the high dose group, $p < 0.05$ (Table 2).

Table 2: Effect of Garad on urea, creatinine, ALT and AST after 7 days treatment

Group	Urea (mg/dl) M±SD	Creatinine (mg/dl) M±SD	ALT (U /ml)) M±SD	AST (U /ml) M±SD
Control	34.09 ± 2.27	1.196 ± 0.134	13.6 ± 0.927	6 ± 0.837
Low Dose	39.87 ± 1.57	1.464 ± 0.134	14.4 ± 1.661	5.4 ± 1.364
Medium Dose	28.41 ± 1.69	1.332 ± 0.002	14.2 ± 0.374	5 ± 0.707
High Dose	29.54 ± 4.37	1.196 ± 0.134	16 ± 0.894	10.2 ± 4.283

One way ANOVA, Tukey Kramer Multiple comparison.

Table 3: Effect of ethanolic extract of Garad on urea, creatinine, ALT and AST after 14 days treatment.

Group	Urea (mg/dl) M±SD	Creatinine (mg/dl) M±SD	ALT (IU /ml)) M±SD	AST (IU /ml) M±SD
Control	31.82± 4.37	1.062 ± 0.164	16 ± 1.483	7 ± 1.342
Low Dose	28.73± 1.97	1.062 ± 0.164	13 ± 1.975	5 ± 0.837
Medium Dose	26.36 ± 2.11	1.062 ± 0.164	15.2 ± 1.20	7 ± 1.14
High Dose	33.18 ± 2.75	1.332 ± 0.002	19 ± 1.549	7.6 ± 0.678

One way ANOVA, Tukey Kramer Multiple comparison

Table 4: Effect of ethanolic extract of Garad on urea, creatinine, ALT and AST after 21 days treatment.

Group	Urea (mg/dl) M±SD	Creatinine (mg/dl) M±SD	ALT (U /ml)) M±SD	AST (U /ml) M±SD
Control	17.21 ± 1.95	1.163 ± 0.168	12 ± 0.913	56.5 ± 24.85
Low Dose	31.82 ± 9.14	1.333 ± 0.003	14 ± 0.408	28.5 ± 13.23
Medium Dose	28.18 ± 3.1	1.196 ± 0.251	13.6 ± 0.374	29.2 ± 11.79
High Dose	39.88 ± 1.93*	1.498 ± 0.168	15.25 ± 0.947*	44.75 ± 11.28

One way ANOVA, Tukey Kramer Multiple comparison, * P<0.05, as compared to the control

At day 14, the effects of Garad doses on the levels of urea, creatinine, ALT and AST were less evident compared to day 7. However, there was an increased level of ALT in the high dose group compared to the control group, $p < 0.05$ (Table 3). At day 21, there was a highly significant increased level of urea and ALT, particularly with the high dose, $p < 0.05$ (Table 4).

Discussion

Acacia nilotica is a medicinal plant that contains flavonoids as a major constituent. Flavonoids are benzo-γ-pyrone derivatives widespread in plants. About 4000 individual flavonoids have been isolated. Flavonoid constituents of the diet were first identified as vitamin P and Vitamin C, and were found to be important in the maintenance of capillary wall integrity and capillary resistance[12, 13]. In the normal diet, consumption of flavonoids is estimated as1g/day[14]. *Acacia nilotica* is an indigenous medicinal plant widely used in Sudan and is also common in the Mediterranean region[15, 16]. It has been shown to have significant hypoglycemic effects in rats [17, 18], and antioxidant and cytotoxic activities against large cell carcinoma (COR-L23) cell line[19]. With all these therapeutic potentialities, Garad toxicity worths investigation. The aim of this study was to assess the safety of ethanolic extract of Garad when injected acutely intraperitoneally to rats. The median lethal dose (LD_{50}) studies can provide valuable information on the toxicity of Garad. This type of study is required before any new compound is approved for testing in man. Acute treatment of rats with doses of 50 - 500 mg/kg ethanolic extract of Garad caused 20 – 100% mortality. According to the toxicity rating[20] to our data, it appeared that Garad extract could be considered as very toxic in rats at doses of 50 to 500 mg/kg.

Acute dosing of Garad extract induced sedative effects which appeared as decreased spontaneous activity, decreased alertness and passivity. These effects were dose dependant and disappeared 24 hours later. After sub acute treatment, observations on day 7 and 14 showed slight to moderate behavioral changes at various doses. On day 21 no behavioral changes were recorded. There might be an unknown adaptive mechanism by which behavioral toxicity of Garad ethanolic extract disappears on prolonged administration.

Numerous herbal products and herb derived products could cause hepatic or renal toxicity[21]. Plasma elevations of urea and creatinine have proved to be the most helpful tool in diagnosis of renal impairment, while elevation of ALT and AST are good indicator of hepatotoxic potential. In the present study the four parameters mentioned above did not show any significant change at the end of first and second weeks of treatment. However, urea and ALT increased significantly at the end of 21 days treatment. ALT is present moderately in liver but low in cardiac and skeletal muscle and other tissues. On the basis of these findings, the results of the present study demonstrate that Garad may have got sub acute toxicity. Locally, the wide use of Garad fruits for the traditional treatment of various conditions might be life threatening since we do not know the quantities being

administered in terms of mg/ kg body weight and the potential toxicity of this plant in human. We recommend further investigations that might offer better understanding of the underlying mechanisms and thus better use of Garad.

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