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SUB-CHRONIC TOXICOLOGICAL INVESTIGATION OF *Gmelina arborea* (VERBENACEAE) IN HEALTHY WISTAR RATS

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Gmelina arborea, Wistar rats, Acute toxicological assessment, 30 day sub-chronic toxicological assessment

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ABSTRACT: The study was designed to determine the acute and sub-chronic toxic effects of aqueous extract of *Gmelina arborea* (Roxb.) in healthy male Wistar rats. An aqueous bark extract of *G. arborea* was administered at graded doses (0.25-2.00 g/kg) to Wistar rats in the acute toxicity test. Sub-chronic toxicity was evaluated by daily administration of the extract at 1.00 g/kg to Wistar rats for 30 days. All animals were physically active and no death was observed up to the dose of 2.00 g/kg in the acute toxicity study. The hematological parameters, serum concentration of liver enzymes and relative organ weight did not differ significantly in treated rats as compared to untreated healthy rats ($P > 0.05$). The histopathological study revealed no treatment-related gross cellular changes in vital organs in treated rats. The administration of the bark extract of *G. arborea* (1.00 g/kg) was found to be toxicologically safe in Wistar rats.

INTRODUCTION: Medicinal plants have historically been considered as valuable therapeutic agents for the treatment of diabetes mellitus and/or dietary adjuncts to the existing therapies.

Numerous reports contradict the popular belief that medicinal plants are safe because they are natural in origin. However, the use of medicinal plants for therapeutic purposes may cause adverse toxicological effects to human health¹.

Therefore, it is important to investigate toxicological effects of plant extracts, in order to make certain that they are lack of toxicity.

Gmelina arborea Roxb. (Common name: Et-demata, Family: Verbenaceae) is a large spreading, deciduous tree, distributed in tropical Asia, commonly found in the Southern region of Sri Lanka².

Every part of this plant is valuable in medicine and decoction of the bark of *G. arborea* is successfully employed for the treatment of diabetes mellitus by Ayurvedic physicians in Sri Lanka³.

The investigation of antidiabetic mechanisms of aqueous bark extract is extensively studied by our group; the optimum effective dose was found to be 1.00 g/kg in streptozotocin induced diabetic rats (*unpublished data*). Several other reports also confirmed that the bark extract of *G. arborea* possess antihyperglycemic properties in diabetic animal models⁴⁻⁶. However, there is limited scientific data available on the toxicological effects of *G. arborea* in Wistar rats.

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Therefore, the aim of the this study was to determine the toxicological effects of aqueous bark extract of *G. arborea* in male Wistar rats through biochemical, hematological and histopathological assessments

MATERIALS AND METHODS:

Chemicals: A UV Visible Spectrophotometer (Gallenkamp PLC, UK), automated hematological analyzer (Sysmax KH21, Japan) were used for spectrophotometric and hematological measurements respectively.

Plant material: Bark of *G. arborea* was collected during May–June 2012 from the Southern region of Sri Lanka. Botanical identity was determined by the descriptions given by Jayaweera ², confirmed by comparing the authentic samples at Royal Botanical Gardens, Peredeniya, Sri Lanka. A voucher specimen was preserved at the Department of Biochemistry, Faculty of Medicine, University of Ruhuna, Sri Lanka (Attanayake/2011/ 05).

Preparation of the aqueous plant extract: The bark parts were cut into small pieces, dried at 40°C until a constant weight was reached and coarsely ground. Powdered plant material (50.00 g) was dissolved in 400.0 mL of distilled water and refluxed for 4 h. The mixture was strained through cheese-cloth and the final volume was adjusted to 50.0 mL. A single dose of 0.25, 0.50, 0.75, 1.00, 1.25, and 2.00 g/kg was administered orally to healthy rats in acute toxicity study. The antihyperglycemic therapeutic dose (1.00 g/kg) was administered orally to healthy Wistar rats in sub-chronic toxicity study.

Animals: Healthy male rats of Wistar strain (200 ± 25 g, body weight) were used to carry out experiments. They were housed in standard environmental conditions at the animal house of Faculty of Medicine, University of Ruhuna, Sri Lanka (Tem 25 ± 2°C, relative humidity 55-65% and 12 ± 1 h light/ dark cycle). Rats were fed with standard diet (MRI rat formulae, Sri Lanka) with free access to water before and during the experiment. The rats were randomized into various groups and allowed to acclimatize for a period of seven days under standard environmental conditions before the commencement of the experiment.

The animals described as fasting were deprived of food for 12 h. All protocols used in this study were approved by the Ethics Committee of Faculty of Medicine, University of Ruhuna, Sri Lanka guided by the CIOMS international guiding principles of biomedical research involving animals.

Acute toxicity study: Acute toxicity testing was performed for plant extracts following the Organization for Economic Cooperation and Development (OECD) guideline 425, fixed dose procedure. Six groups containing healthy male rats (n=6/group) received aqueous extract of *G. arborea* at dose of 0.25, 0.50, 0.75, 1.00, 1.25, and 2.00 g/kg orally while the untreated healthy control group received distilled water. Animals were observed individually after dosing once during the first 30 min, periodically during the first 24 h and thereafter for two days. Behavioral changes and signs of toxicity were observed.

Sub-chronic toxicity study: Rats were randomly allotted to two groups (n=6/group). The first group served as the untreated healthy control group received distilled water daily. The rats in the second group received the aqueous bark extract of *G. arborea* at the therapeutic dose (1.00 g/kg) daily for 30 days. The body weight of each rat was assessed before the commencement of dosing, during the experimental period at weekly intervals and on the day of sacrifice. The amount of food, water consumed were measured daily from the quantity of food, water supplied and the amount remaining after 24h.

The fasted (12 h) animals were sacrificed on the 30th day of the experiment. Blood samples were collected via cardiac puncture for serum biochemical and hematological analysis respectively. The heart, lung, small intestine, liver, spleen, pancreas and kidney were carefully isolated for relative organ weight assessment and liver, spleen, kidney were fixed in buffered formalin for histopathological examination.

The relative organ weight (ROW) of heart, lung, small intestine, liver, spleen, pancreas and kidney of each animal was calculated as follows.

$$\text{ROW} = \frac{\text{Absolute organ weight (g)}}{\text{Body weight of Wistar rat on the day of sacrifice (g)}} \times 100\%$$

Biochemical assessment: Serum activities of ALT; alanine aminotransferase, AST; aspartate aminotransferase, ALP; alkaline phosphatase were estimated to assess the effects on liver using spectrophotometric enzyme assay kits (Stanbio, USA)⁷.

Hematological assessment: Hematological analysis was performed using a hematological analyzer. Total hemoglobin, total red blood corpuscles, platelet count, red cell indices including mean corpuscular volume, mean corpuscular hemoglobin, mean corpuscular hemoglobin concentration, total white blood corpuscles, percentage of neutrophils, lymphocytes, eosinophils and monocytes of blood samples were recorded.

Histopathological assessment: The liver, spleen and kidney were fixed in 10% formalin in labeled bottles. Tissues were processed routinely and embedded in paraffin wax. Sections were stained with hematoxylin and eosin for light microscopic examination of histopathological changes.

Statistical analysis: Results were expressed as mean \pm SEM. The toxicological data was analyzed by two sample t-test using the Minitab Statistical Software respectively. Results were considered to be significant at $P < 0.05$.

RESULTS: The animals did not show any changes in general appearance during the three day period following a single oral administration at all selected doses of the extract of *G. arborea*. Morphological characteristics (fur, skin, eyes and nose) appeared normal. No tremors, convulsion, salivation, diarrhoea, lethargy or unusual behavior were observed.

Sub-chronic toxicity study: The oral ingestion of the extract of *G. arborea* over 30 days caused no significant changes ($P > 0.05$) in body weights of animals (Figure 1), consumption of food (Figure 2), intake of food (Figure 3), relative weight of the organs in treated rats as compared to the control rats (Figure 4).

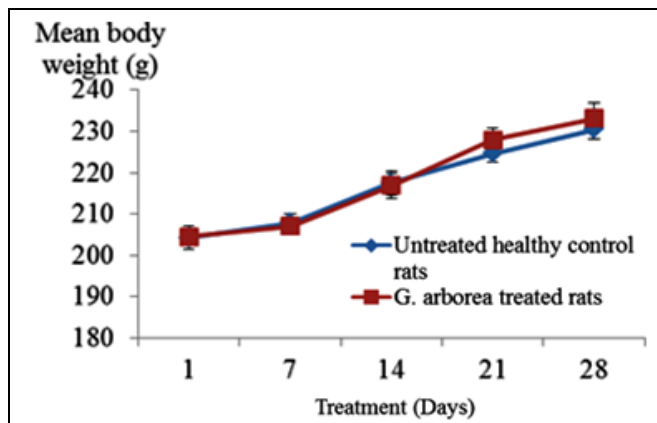


FIGURE 1: EFFECT OF THE *GMELINA ARBOREA* EXTRACT (1.00g/kg) ON MEAN BODY WEIGHT OF RATS FOR 30 DAYS. Each data point represents the mean \pm SEM (n=6/group). The two sample t-test at $\alpha = 0.05$ showed no statistical difference between mean body weight in plant treated rats compared to untreated control rats.

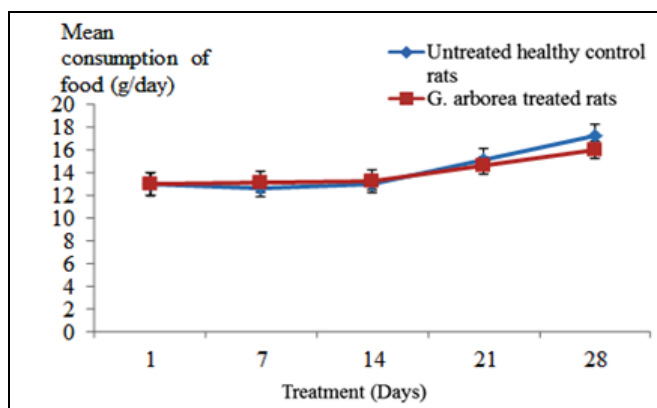


FIGURE 2: EFFECT OF THE *GMELINA ARBOREA* EXTRACT (1.00 g/kg) ON MEAN CONSUMPTION OF FOOD IN RATS FOR 30 DAYS. Each data point represents the mean \pm SEM (n=6/group). The two sample t-test at $\alpha = 0.05$ showed no statistical difference between mean food intake in treated rats compared to untreated healthy control rats.

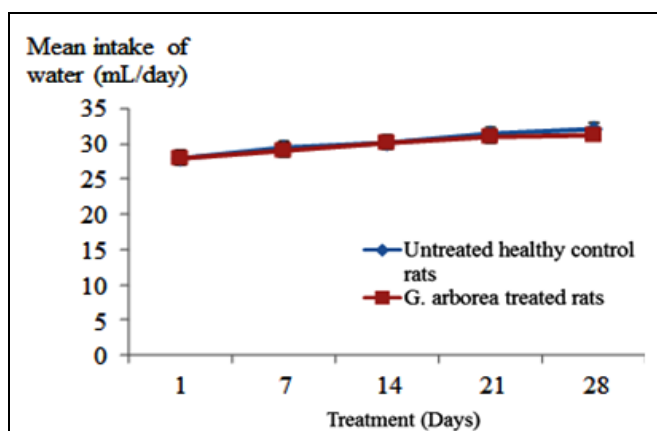


FIGURE 3: EFFECT OF THE *GMELINA ARBOREA* EXTRACT (1.00 G/KG) ON INTAKE OF WATER IN RATS FOR 30 DAYS. Each data point represents the mean \pm SEM (n=6/group). The two sample t-test at $\alpha = 0.05$ showed no statistical difference between mean water intake in treated rats compared to untreated healthy control rats.

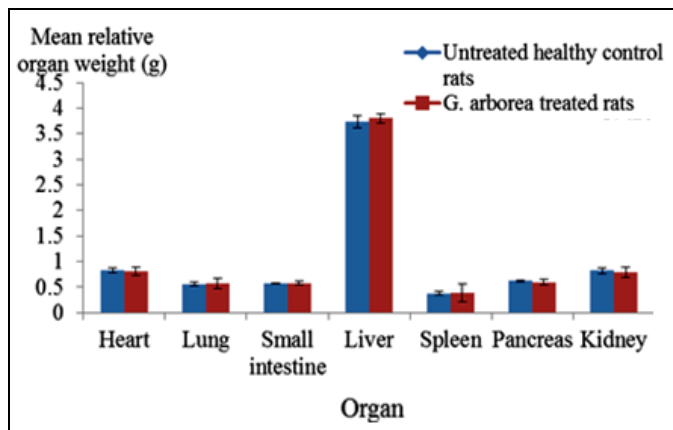


FIGURE 4: EFFECT OF GMELINA ARBOREA EXTRACT (1.00 g/kg) ON MEAN RELATIVE WEIGHT OF ORGANS IN RATS FOR 30 DAYS. Each column represents the mean ± SEM (n=6/group). The two sample t-test at α = 0.05 showed no statistical difference between mean relative weight of organs in treated rats compared to untreated healthy control rats

The results of biochemical and hematological analysis are shown in **Table 1**. There was no statistical difference in the parameters listed in plant treated rats compared to the control (P > 0.05).

As shown in **Figure 5**, the histopathological assessment of liver revealed few lymphocytic infiltrates around the central vein, in the portal tract and in the parenchyma in plant treated rats. The histopathological examination of the tissues of kidney and spleen showed no changes in cellular architecture in treated rats.

However, the histopathological findings were generally consistent with the expected pattern for Wistar rats of the particular age.

TABLE 1: RESULTS OF BIOCHEMICAL AND HEMATOLOGICAL ANALYSIS

Parameters studied	Untreated healthy rats	G. arborea treated rats
Alanine aminotransferase (U/L)	12.39 ± 0.79	11.65 ± 0.80
Aspartate aminotransferase(U/L)	44.21 ± 1.75	42.58 ± 1.02
Alkaline phosphatase(U/L)	61.48 ± 1.50	61.20 ± 2.00
Total hemoglobin (g/dL)	15.25 ± 0.80	14.78 ± 0.98
Red blood corpuscles (10 ⁶ /mm ³)	8.27 ± 1.31	8.00 ± 1.21
Platelet count(10 ³ /mm ³)	1096± 93	1049± 65
Pack cell volume (%)	47.95 ± 2.38	46.14 ± 1.11
Mean corpuscular volume (fL)	61.45 ± 1.28	61.23 ± 1.12
Mean corpuscular hemoglobin (pg)	19.53 ± 0.60	18.90 ± 0.99
Mean corpuscular hemoglobin concentration (g/dL)	31.77 ± 0.90	31.00 ± 0.99
White blood corpuscles (10 ³ /mm ³)	4.82 ± 1.60	4.89 ± 1.67
Neutrophils (%)	42.00 ± 2.77	41.34. ± 1.78
Lymphocytes (%)	55.5 ± 7.73	56.36 ± 4,39
Eosinophils (%)	1.80 ± 0.40	1.60 ± 0.50
Monocytes (%)	0.70 ± 0	0.70 ± 0

Each value represents the mean ± SEM (n=6/ group). The two sample t-test at α = 0.05 showed no statistically difference between the parameters studied in treated healthy rats compared to untreated healthy control rats

DISCUSSION: The acute and sub-chronic toxicological effects of the *G. arborea* extract were investigated in healthy Wistar rats in order to make certain that therapeutic dose (1.00g/kg) is toxicologically safe. Estimation of safety of drugs and plant products is usually performed in animals. It is reported that sub-chronic and chronic effects of herbal extracts including the doses potentially usable in humans are tested usually in rats ⁸. Accordingly rats with Wistar strain were used in the present investigation. Furthermore, a good correlation has been reported between toxicological results in rats and humans than the correlation between mice and humans ⁹.

Based on historical evidence, oral administration is the most convenient and commonly used route when screening for toxicological effects in laboratory animals. The rate of absorption might be slow, but this method costs less and is painless to animals. Further, the same route was used for the administration of herbal remedies as aqueous extracts to patients by physicians since time immemorial ¹⁰. Acute toxicity study was conducted with a range of six doses of 0.25 - 2.00 g/kg including the therapeutic dose of *G. arborea* (1.00 g/kg). The human therapeutic dose was extrapolated to compute the range of doses according to the standard guidelines ¹¹.

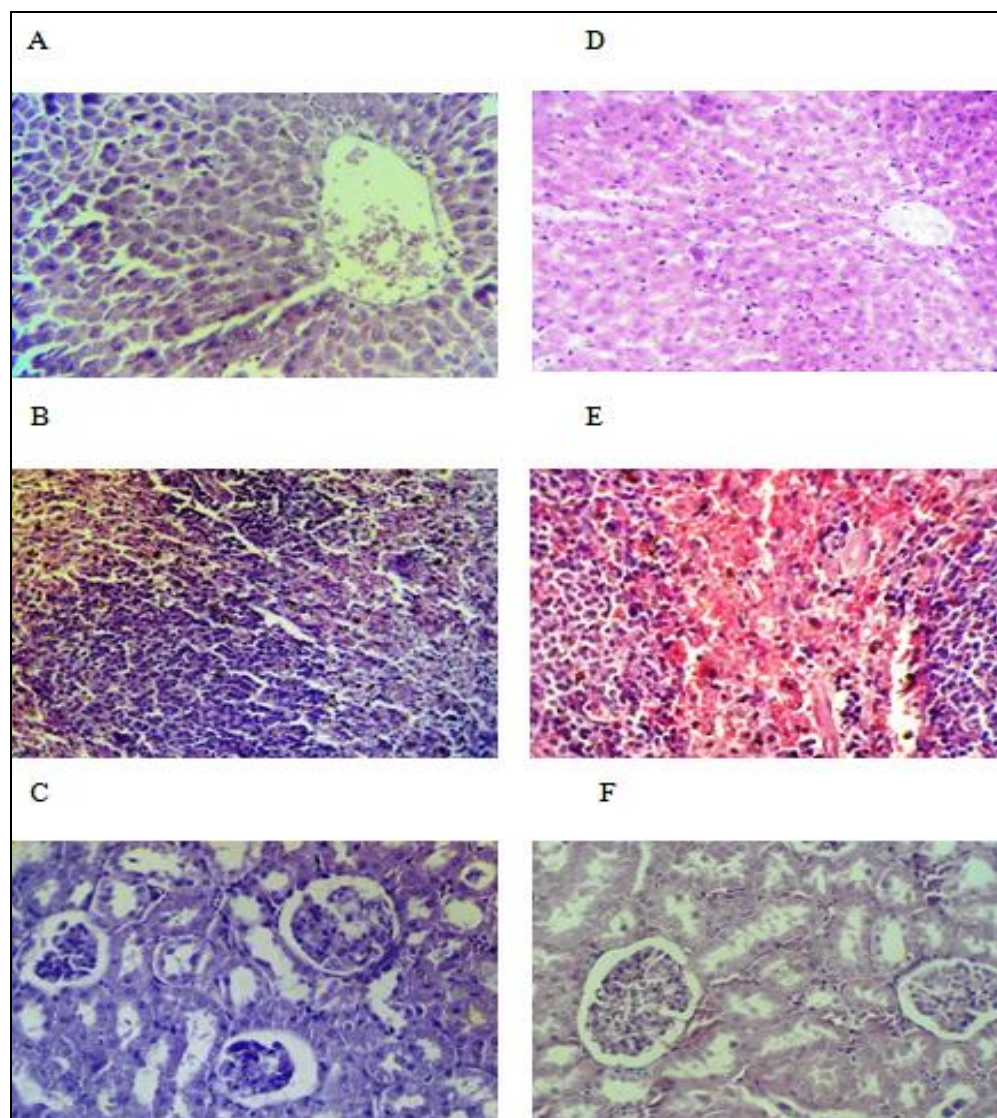


FIGURE 5: PHOTOMICROGRAPHS (X 400) OF LIVER, SPLEEN, KIDNEY HISTOPATHOLOGY FROM UNTREATED HEALTHY RATS (A, B, C), *GMELINA ARBOREA* EXTRACT TREATED RATS (1.0 g/kg) (D, E, F)

The acute toxicity study indicated that treatment of *G. arborea* with selected doses was well tolerated by all test animals, suggesting its safety as an antihyperglycemic agent.

In the present study, the aqueous bark extract of *G. arborea* did not significantly alter body or relative weight of organs in treated rats as compared to untreated rats, which suggests that the extract did not hinder the growth of Wistar rats. In this study, the consumption of food and intake of water were not altered, suggesting that it does not induce or suppress appetite.

The hematopoietic system is very sensitive to toxic compounds and serves as an important index of the physiological and pathological status¹². Such toxicity testing is relevant for changes in the hematological system which has a higher

predictive value for human toxicity, when extrapolated from animal studies. Sub-chronic exposure of Wistar rats to the bark extract of *G. arborea* produced non-significant changes in hematological parameters at $P > 0.05$. The sub-chronic administration of the extract did not alter the hepatocytes as evident by the liver enzyme concentrations. Furthermore the histopathological assessment of organs did not exhibit any abnormalities in rats treated with the extract and corroborated biochemical findings.

The changes in body weights of animals, consumption of food, intake of water, concentrations of liver enzymes, haematological values for the extract of *G. arborea* (1.00g/kg) are in accordance with previously published reports of aqueous and methanol bark extract of *G. arborea* in the same experimental model^{12,13}.

Studies are in progress to elucidate mechanisms of antidiabetic activity using the therapeutic dose of the bark extract of *G. arborea*.

CONCLUSION: The acute toxicity study suggests that aqueous bark extract of *G. arborea* is safe in healthy Wistar rats up to a dose of 2.00 g/kg. The sub-chronic administration of the *G. arborea* at a dose of 1.00 g/kg to rats was found to be toxicologically safe as a potential therapeutic agent in rats.

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