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Original Research Article

Acute Safety Assessment of Ethanol Leaves Extract of Cemba (*Acacia rugata* L.) on The Kidney in Male Wistar Rats

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ABSTRACT

This study aimed to assess the safety of acute oral administration of leaves extract of cemba (*Acacia rugata* L.) in male rats. The rats were grouped and administered 250, 500, and 750 mg/kg of the extract. The animals were observed for fourteen days for mortality in all treatment groups. Blood samples were taken, and the kidneys were excised for histopathological examination. None of the animals died. A significant ($p<0.05$) increase in the level of creatinine and blood urea nitrogen (BUN) at doses of 500 and 750 mg/kg BW, but the increased level of creatinine at 500 mg/kg BW did not reach the limit level (0.8 mg/dL). However, histopathological results showed no alteration in renal tissues. These results indicate that leaf extract of *A. rugata* may be safely used as a medicinal herb and recommended at doses lower than 250 mg/kg..

Keywords: Safety assessment, *Acacia rugata* L., kidney

Introduction

Indonesia is known as a country that has a rich biodiversity of plants that can be used as traditional medicine, such as cemba (*Acacia rugata* (Lam.) Fawc. Rendle). Belonging to the Fabaceae family, this genus is widespread worldwide, with the most incredible diversity of species in the tropics. In the Enrekang, South Sulawesi, *A. rugata* grows greatly in mountain valleys.[1] Traditionally, *A. rugata* leaves are used as a spice, "Nasu cemba".[2]

Traditionally, the ethnic Duri in Enrekang uses the leaves of *A. rugata* to treat patients with cardiovascular diseases, hypertension, infection, diabetes mellitus, and inflammation.[3] Seeds are usually used in childbirth to facilitate delivery. They are applied externally for leprous patches, prurigo, abscesses, eczema, and buboes.[4] Ismail *et al.* (2018) isolated three fungal endophytes from *A. rugata* with their antibacterial activity against *Escherichia coli* and *Staphylococcus aureus.*[1] Phytochemical screening showed that *A. rugata* contains flavonoids, polyphenols, terpenoids, saponins, and volatile oil. In addition, *A. rugata* leaves have also been reported as antioxidants associated with the presence of phenolic and flavonoid compounds.[4]

Previously, *A. rugata* revealed cytotoxic and thrombolytic attributes.[4] Anticancer drugs should not exert anticancer properties on normal cells, but they are toxic to fast-growing cells.[5,6] failure to achieve hemostasis causes a thrombus to form in the circulatory system, which blocks blood vessels and has catastrophic repercussions in atherothrombotic diseases such as myocardial infarction, which can occasionally result in mortality.[7,8] To ensure safety, it is essential to consider extract toxicity as a crucial component.

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Toxicity is the state of being adversely affected by toxic substances, the severity of which is based on the chemical characteristics of the poisonous compounds and the cell membrane.[9-11]

The present investigation aimed to evaluate the safety of the ethanol extract obtained from the leaves of *A. rugata* in male Wistar rats. Rats are used in this study because they are small, easy-to-handle, calm mammals whose physiology resembles that of humans. Moreover, the Wistar rat has been widely used as a model for the study of the toxic effect of medicinal plants.[12] However, this study focuses on kidney acute safety assessment in male Wistar rats.

Materials and Methods

Experimental animals

Male Wistar rats were purchased from the Sekolah Tinggi Ilmu Farmasi Makassar, Indonesia. They were housed in propylene cages with alternate 12-hour light and dark cycles to maintain a temperature of 22±1 °C. All of the rats were given access to high-quality pelleted feed and water, *ad libitum*. The health research ethics committee at the Muslim University of Indonesia (Voucher No. 0011/A.1/KEPK-UMI/I/2022) approved all the experimental protocols.

Plant materials

The leaves of *A. rugata* were collected in mountain valleys in Enrekang, the Province of South Sulawesi, Indonesia. The leaves collection was carried out in the dry season (June 2020). The leaves were handpicked, washed under running tap water, and drained. The leaves were dried using an oven at temperatures of 40°C (Memmert, Germany) for 48 h, then ground with a food grinder (Philips, Indonesia) to produce a fine powder.

Extraction of A. rugata

Five hundred grams of powdered leaves were macerated using 1.000 mL of 70% ethanol. Three separate 24-hour extractions with periodic stirring generated filtrate and residue, which were then separated by filtering. The filtrate obtained was evaporated using a rotary evaporator to produce a thick extract, then freeze-dried to get a dry extract.

Qualitative screening
The screening of the main groups of chemical constituents (alkaloid, flavonoid, saponin, tannin, terpenoid, and steroid) of the extract was carried out qualitatively.

Alkaloid
Ten milligrams of the extract were heated and filtered after being dissolved in 10 mL of acidified alcohol. Then, divided into three reaction tubes, one portion, and another portion were added Dragendorf and Mayer reagent, respectively. For an alkaloids test, the appearance of a cream (when using Mayer reagent) or reddish-brown precipitate (when using Dragendorf reagent) was considered positive.[13]

Flavonoid
The Shinoda test was performed to assess the presence of the flavonoid. In reaction tubes, 1 mL of extract was dropped with concentered hydrochloric acid, and added a bit of magnesium. After adding reagents, a few pink hues, scarlet or crimson red hues, or green to blue hues were thought to indicate the presence of flavonoids.[13]

Saponin
Foaming formed after shaking the extract in 2 mL of water indicates the presence of saponin.[13]

Tannin
The previously boiled and filtered 0.1 g of extract received a few drops of 0.1% of ferric chloride. Then, a brownish green or a blue-black coloring was looked for.[13]

Terpenoid and steroid
Salkowski tests to determine the presence of terpenoids. One milliliter of concentrated sulfuric acid was added after 0.1 g of the extract had been added, along with 0.4 mL of chloroform. Then, it was observed for reddish brown coloration.[13]

Acute toxicity assay
The acute toxicity study was conducted according to the OECD Guidelines for the Testing of Chemicals standard guidelines for using animals in scientific research, Guideline No. 425,[14] with minor modifications. Twelve rats were divided randomly into four groups of three rats each, with the administered leaves extract of *A. rugata* as follows: Group 1 (negative control); II-IV (250, 500, and 750 mg/kg BW, respectively). Following a 24-hour fast (during which only water was provided), each rat from groups II to IV received a single dose orally. Group I rat received distilled water. For the first 24 hours after dosing, the animals were observed. After that, the animals were permitted unrestricted access to food and water, and daily observation was carried out for 14 days. The rats from each group were fasted overnight and anesthetized. A heart puncture was done on all groups, and blood samples were obtained for biochemical evaluations. Following a cervical dislocation, the kidneys were excised and prepared for histopathological examination.

Determination of creatinine and BUN
Dry tubes containing the drawn blood samples were centrifuged at 3000 rpm for 15 minutes to get the serum. An automated serum analyzer (HumaStar Analyzers) was used to evaluate the following parameters serum creatinine, and blood urea nitrogen (BUN).[15]

Histopathological study
The kidneys from each rat were dehydrated using increasing concentrations of isopropyl alcohol (70–100%) after being promptly fixed in 10% v/v of formalin in phosphate-buffered saline (PBS). A Leica rotary microtome cut paraffin slices of 5 µm thickness from the paraffin-embedded organs (Bright B5143 Huntington, England). The slides were stained with hematoxylin and eosin (H&E). Photomicrographs were obtained using a Leica DM750 Camera Microscope, and the slides were examined under a light microscope.[16]

Statistical analysis
The results were presented as mean ± SEM. Comparisons between groups were done using a paired T-test. $P \leq 0.05$ was considered statistically significant.

Results and Discussion

Utilizing natural remedies as an alternative therapy for treating various illnesses has received much attention recently. Phytochemicals are bioactive plant substances with diverse biological functions and toxicological effects. Phytochemical investigations showed the presence of alkaloids, flavonoids, saponins, tannins, and terpenoids as major phytochemical constituents. The flavonoids were detected with the highest intensity (Table 1).
The presence of significant amounts of flavonoid, flavonoid, saponin, tannin, and terpenoid justified the traditional usage of *A. rugata* for cardiovascular diseases, infection, diabetes mellitus, and inflammation. In addition, the flavonoids identified in *A. rugata* have been reported for antioxidant, cytotoxic, and thrombolytic attributes.[4] Despite being widely accepted and used in the treatment of many diseases, medicinal plant toxicity must not be disregarded.
Toxicological research on substances, including plant extracts, requires acute toxicity testing, which examines the negative consequences of a single dose of material. This study focused on acute kidney toxicity. Acute oral administration of single doses of the leaves extract of *A. rugate* 250 mg/kg to 750 mg/kg were studied. Animals exposed to up to 5000 mg/kg neither died nor had their behavior altered, implying that the extracts are of relatively low acute toxicity.
To assess the toxicity of *A. rugate* in the kidney, biochemical parameters, including creatinine and BUN as well as histopathological evaluation, were carried out. Creatinine is often produced by the nonenzymatic breakdown of creatine, a protein that is typically delivered in the liver and the muscle, with the rate of formation varying with muscle mass. The kidney then excretes this creatinine from the body through urine. Serum creatinine levels rise above normal if renal clearance is compromised. As a result, creatinine is a crucial biomarker of renal health.[17-20]
The creatinine concentrations were significantly higher ($p > 0.05$) in the serum of rats treated with 500 and 750 mg/kg BW when compared with before the experiment. In contrast, those treated with 250 mg/kg BW were non-significantly ($p < 0.05$) differences as well as control throughout the 14 days (Figure 1). Although there was an increase in the dose of 500 mg/kg BW, the increase did not exceed the normal creatinine levels of the Wistar rat male. Rat serum creatinine levels typically range from 0.4 to 0.8 mg/dL.[21] Increased serum creatinine levels are typically linked to renal damage. Especially on the dose of 500 mg/kg, the elevation reached the normal limit of creatinine. This suggests that leaf extract of *A. rugate* at high doses may be associated with kidney toxicity.
The BUN test quantifies the urea nitrogen levels in the blood. The kidneys eliminate urea nitrogen from the blood as waste. Generally, there were no significant ($p > 0.05$) variations in the relative level of the BUN of the rats among the doses of 350 mg/kg BW as well as control compared to before treatment (Figure 2).

Table 1: Phytochemical constituents present in leaves of *A. rugata*

Phytochemicals	Presence
Alkaloid	+
Mayer	+
Dragendorf	+
Flavonoid	+
Saponin	+
Tannin	+
Terpenoid and steroid	+

Note: + present

However, the relative BUN level significantly increased in rats treated with 500 and 750 mg/kg BW of leaf extract of *A. rugata* (Figure 2). The increased level of 500 and 750 mg/kg BW exceeds normal limits in the Wistar rat male.

Rat BUN typically ranges from 15 to 22 mg/dL and is resistant to age and gender-related variations.[21] This study found that BUN levels significantly increased with graded doses of 500 and 750 mg *A. rugate* leaves extract. Additionally, there is a strong correlation between elevated BUN levels and unfavorable outcomes in acute kidney failure. BUN levels should therefore be considered when evaluating a patient's prognosis for acute renal failure. This implies kidney damage may be linked to large doses of *A. rugate* leaf extract.

Histopathological examination of kidney tissue in both the control and treated group revealed a normal histological morphology (Figure 3). The control and treated groups do not show necrosis, hemorrhage, fatty kidney, or inflammation. However, no deleterious effects were observed during a histopathological examination of the kidneys treated with the extract and the control groups. The signs of kidney tissue damage, such as necrosis, hemorrhage, fatty kidney, and inflammation, were not found both before and after treatment. This is the first-time leaves extract of *A. rugate* toxicity was reported.

Conclusion

In conclusion, the serum renal biochemical parameters, including creatinine and BUN, were considerably changed after acute administration of leaves extract of *A. rugate* at doses of 500 and 750 mg/kg BW. The extracts at 250 mg/kg show no biochemical abnormalities. As a result, this study advised using *A. rugate* at doses of 250 mg/kg or less.

Conflict of Interest

The authors declare no conflict of interest.

Authors' Declaration

The authors hereby declare that the work presented in this article is original and that any liability for claims relating to the content of this article will be borne by them

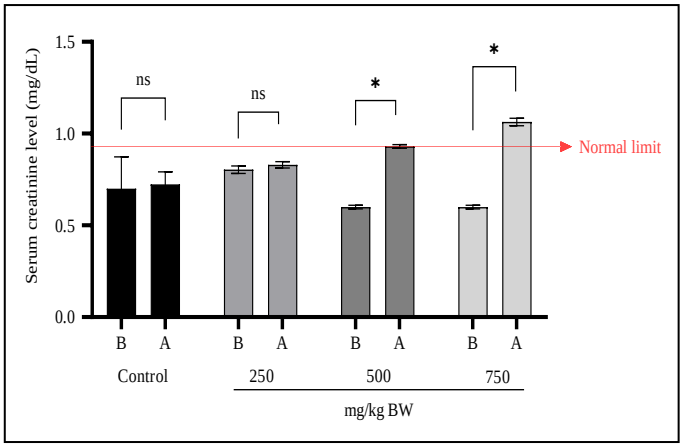

Figure 1: Paired sample T-test on the serum BUN level before (B) and after (A) the administration of different doses of leaves extract of *A. rugata*; (*) significant; (ns) non-significant

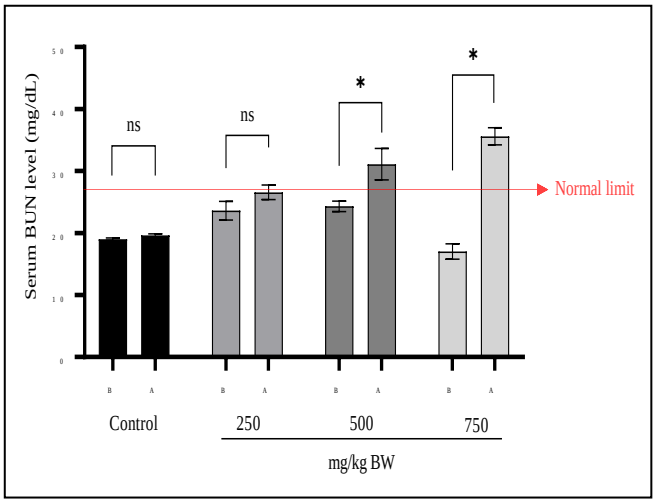

Figure 2: Paired sample T-test on the serum BUN level before (B) and after (A) the administration of different doses of leaves extract of *A. rugata*; (*) significant; (ns) non-significant

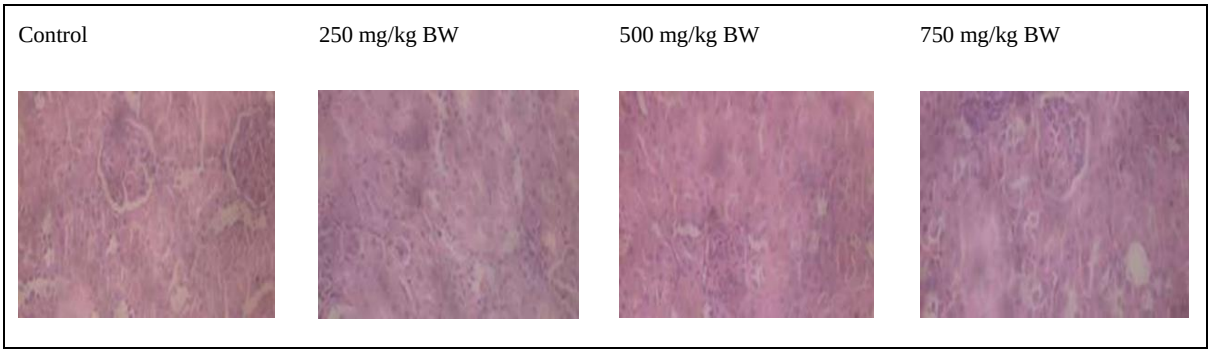

Figure 3: Photomicrograph of sections of rat kidney in control and treated groups. All the kidney-treated group sections were similar to the control (40x)

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