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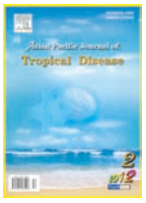

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Toxicological evaluation of ethanolic extract of *Tabernaemontana coronaria* (L) R. Br.

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ABSTRACT

Objective: To investigate the acute and sub acute toxicological evaluation of ethanolic extract of *Tabernaemontana coronaria*. Method: The acute toxicity profile as well as possible haemostatic activity of the ethanolic extract of the plant after sub acute oral administration was studied in male wistar albino rats.The rats were sacrificed 24 h after last treatment. Blood was collected by cervical decapitation for biochemical and haematological assessment. Liver and kidney were also excised, placed in 10% formalin for histopathological evaluation. Results: The ethanolic extract of *Tabernaemontana coronaria* did not produce any significant effect on haematology, lipid profile and renal profile. But only a slight increase in ALP and LDH is seen in group V rats. The histopathological study did not show any abnormalities in the liver and kidney. Conclusion: Although the ethanolic extract of *Tabernaemontana coronaria* has shown only slight increase in ALP and LDH activity in group 5 rats, the histopathological study confirmed the safety nature of the plant.

1. Introduction

An impressive pool of natural pharmacy– A gift provided by the rich floral Biodiversity of India to the herbal health practitioners[1]. Plants have formed the basis of sophisticated traditional medicine (TM) practices that have been used for thousands of years by people in China, India and many other countries[2]. In the recent years research on medicinal plants has attracted a lot of attentions globally. Demand is steadily increasing not only in developing countries but also in the industrialized nations. WHO estimates that approximately 80% of the developing world's population meets their primary health care needs through traditional medicine. Within the last few decades, many plants have been screened for their biological and pharmacological properties by researchers. These efforts are continually being taken to examine the merits of traditional medicine in the light of modern science with a view aimed at adopting effectively beneficial medical practices and discouraging harmful ones[3].

Although many herbs have been used over the centuries and are generally considered as safe, there have been a number of recorded cases of intoxication with certain herbal products. For instance, high administrative doses of common culinary spice herbs such as onion and garlic were found to cause toxic effects on liver and lungs [4]. Prolonged period of consumption of other common traditional herbal preparations such as ginkgo, St. John Wort, ginseng, echinaceae and ephedra was also associated with side effects such as kidney injuries, adverse events of cardiovascular and central nervous systems[5,6]. Therefore, toxicological studies are necessary to assess the safe level of exposure of herbs used as folk medicinal and culinary ingredients.

Toxicity of medicinal plants may be related to the mixtures of active compounds that they contain; this interactions with other herbs and drugs, contaminants, adulterants or their inherent toxicity. Plants have complex mixtures of terpenes, alkaloids, saponins and other chemicals, increasing the risk of adverse reactions to any one of them or to the additive or synergistic effects of chemical interactions[7].

Tabernaemontana coronaria (Apocyanaceae) is a spreading, bushy, many-branched shrub. It bears white, waxy summer flowers and has oblong leaves with wavy margins. Native to parts of India, China, and Thailand, They are rich in alkaloids or glycosides. Some species are valuable sources of medicine, insecticides, fibers, and rubber. The ethanol and aqueous extracts of *T. coronaria* flowers possessed significant antioxidant and

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anti-inflammatory activities[8]. It has a very good local anesthetic and anti cancer activity[9].

Hence, the objective of the present study is to evaluate the toxicity effect of *Tabernaemontana coronaria* in wistar albino rats with focus on haematology, lipid profile, liver profile, renal profile and histopathology analysis.

2. Materials and methods

2.1. Collection and preparation of plant material

The plant material was collected in and around Coimbatore District, Tamil Nadu, India. The plant was authenticated by Dr. G.V. S. Murthy, Joint Director, Botanical Survey of India, Southern Regional Centre, Tamil Nadu Agricultural University Campus, Coimbatore, Tamil Nadu, India. The voucher number is BSI/SRC/5/23/09-10/Tech.- 987.

The whole plant was cut into pieces, cleaned, air dried and powdered in to a coarse powder. The powder (50 g) was extracted by continuous extraction using soxhlet apparatus with the solvent ethanol for 72 h. The resultant extracts were concentrated to dryness under reduced pressure below 40 °C in rotary evaporator and stored at 4 °C for further use.

2.2. Laboratory animals

Young adult male wistar rats weighing 120-140 g were obtained from the animal house of Karpagam University, Coimbatore and used for the study. Rats were housed at constant temperature of (22±5) °C with a 12 h light, 12 h dark cycle and fed on pellets with free access to tap water. All the experiments were carried out according to the guidelines recommended by the committee for the purpose of control and supervision of Experiments on Animals (CPCSEA) and approved by IAEC, Government of India.

2.3. Experimental design

2.3.1. Acute toxicity study

Different doses of the ethanolic extract corresponding to 500, 1 000 and 2 000 mg/kg body weight were administered orally to three groups of five animals each. Another group of five rats were served as control and this received 1 mL of physiological saline. Clinical signs and symptoms of toxic effects and mortality were observed for 72 h.

2.3.2. Sub-acute toxicity study

The male rats were randomly divided into six groups of five animals each. Five groups were given 100, 200, 300, 400 and 500 mg extract /kg body weight respectively orally through intragastric tube daily for 14 days. The last group received normal saline and served as the control. The rats were given feed and water *ad libitum* throughout the period of the experiment. 24 h after the last treatment the animals were sacrificed by cervical dislocation under chloroform anaesthesia. Part of the blood was collected from the rats into anticoagulant (EDTA) containing bottles for estimation of hematological parameters. The remaining blood was collected and allowed to clot for 20 min at room temperature and then centrifuged at 1 500 r/min for 5 min. The serum was separated for further analysis. The rats were thereafter quickly dissected; the liver and kidney were excised, washed with cold water and immersed in 10%formalin for histopathological analysis.

2.3.3. Estimation of haematological parameters

Packed cell volume (PCV), total white blood cell (WBC) count, total red blood cell (RBC) count, Mean cell volume (MCV), mean cell haemoglobin (MCH), mean cell haemoglobin concentration (MCHC), haemoglobin content, platelet count were carried out according to the method of Tietz[10].

2.3.4. Assessment of lipid profile

Total cholesterol, HDL cholesterol concentrations were estimated by using the commercial kits of span diagnostics using the method of Wybenga and Pileggi,[11]. The triglycerides concentration was assessed by GPO-PAP, End point assay.

2.3.5. Assessment of renal function

Renal function was assessed by measuring urea in serum by DAM method, uric acid concentration was measured by uricase method and creatinine in serum by alkaline picrate method using commercial assay kits.

2.3.6. Assessment of hepatic function

Liver function was assessed by measuring the activities of AST, ALT, ALP and LDH in serum by using commercial kits. AST and ALT activities were determined based on the method of Reitman and Frankel[12] method. ALP activities in serum were assayed by the method of King and King's[13].

2.3.7. Assessment of glucose and mineral content

Assay of glucose in serum was done by GOD-POD assay. sodium, potassium and chloride were analysed in serum by Thiocyanate method using assay kits. The inorganic phosphorus content was analysed in serum by using the method of UV molybdate.

2.3.8. Histopathological examination

The histopathological examination of the liver and kidney were carried out by staining the tissue sections using haematoxylin and eosin (H&E) and were examined and photographed by a histopathologist using a light microscope.

Table 1
Effect of *Tabernaemontana coronaria* on hematological values in male wistar albino rats.

Parameters	Total WBC (cells/cmm)	Haemoglobin (g/dl)	Total RBC (mill/cmm)	PCV (%)	MCV(fl)	MCH(pg)	MCHC(%)	Platelets (cells/cmm)
Control	10,200±22.4	11.7±0.4	4.10±0.27	35.4±0.46	90.1±0.54	27.8±0.5	32.5±0.1	350000±7
Group I (100mg/kg)	9,396±11.4	11.2±0.5	3.75±0.21	33.6±0.5	84.3±0.51	26.3±0.4	31.8±0.2	330000±10
Group II (200mg/kg)	9,567±27	11.2±0.5	3.89±0.23	33.6±0.51	89.6±0.54	29.6±0.7	33.3±0.6	360000±8
Group III (300mg/kg)	9,884±18.7	11.5±0.5	4.10±0.27	34.9±0.39	89.7±0.84	28.9±0.6	33.7±0.6	380000±8
Group IV (400mg/kg)	10,600±7.9	11.8±0.2	4.20±0.45	36.9±0.39	90.0±0.88	30.2±0.3	34.3±0.3	387000±7
Group V (500mg/kg)	10,884±18.2	12.5±0.2	4.20±0.42	37.5±0.64	89.3±0.74	29.8±0.22	34.3±0.3	390000±7

Values are expressed as mean±SD for five samples.

2.4. Statistical analysis

Data were expressed as means of five replicates±SD. Data were subjected to one-way analysis of variance (ANOVA). Significant levels were tested at $P<0.05$.

3. Results

3.1. Acute toxicity study

No mortality was recorded in the rats treated with the extract orally at all the doses used. The oral LD_{50} is therefore greater than 2000 mg/kg. This high oral LD_{50} (2000 mg/kg) obtained suggested that the extract is practically non-toxic through this route and is therefore safe in the rats and in its traditional use orally for treatment of the diseases it is indicated for[18].

3.2. Sub-acute toxicity study

3.2.1. Effect on haematological parameters

Table 1 shows the haematological values in blood of control and plant extract treated groups. The extract produced the dose specific effects on haematological parameters such as total RBC, total WBC, haemoglobin, platelet count, PCV, MCH, MCV, MCHC at all the doses compared favorably with the control values and no clinical abnormalities were observed at the end of 14th day. Blood coagulation requires that platelets be in sufficient size, number and function, in the absence of which a satisfactory plug may not occur and haemorrhage may continue following a breach in the vascular endothelium. Flavonoids like quercetin and rutin are used as effective constituents of several pharmaceuticals in the treatment of capillary fragility and phlebosclerosis[19]. Thus the flavonoid and tannin content in the extract may be responsible for the haemostatic property of the ethanolic extract of *T. coronaria*.

Since none of the rats were died in the 14 days study, it suggests that the plant can also be safely used in the treatment of diseases requiring long term drug administration.

3.2.2. Effect on glucose, hepatic and renal profile

Serum concentrations of glucose are shown in Figure 1. Treatment with ethanolic extract of *Tabernaemontana coronaria* did not significantly change glucose concentrations in the experimental rats when compared with control. The effect of the plant extract on renal function is shown in Figure 2. Serum concentrations of urea, uric acid and creatinine concentrations were almost similar to the control values. The effect of the plant extract on hepatic function is shown in Figure 3. The activities of the amino transferases in the plant extract treated groups were not shown any difference when compared to control. Whereas the level of alkaline phosphatases and LDH in group V showed somewhat higher value when compared to control.

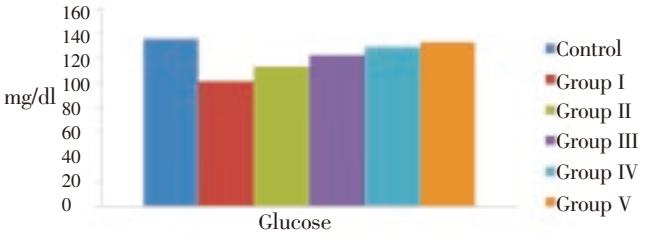

Figure 1. Effect of *T. coronaria* serum gluose level in male wistar albino rats (n=5).

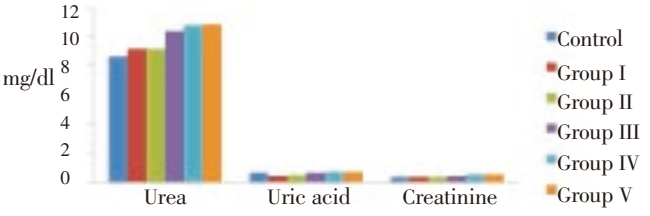

Figure 2. Effect of *T. coronaria* on serum urea,uric acid and creatinine(renal profile)activities in male wistar albino rats (n=5).

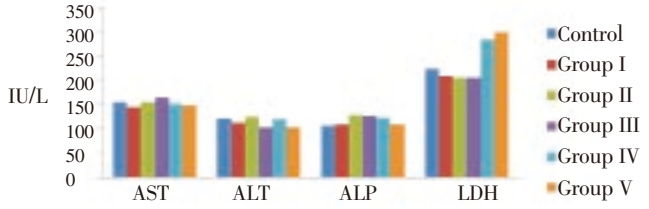

Figure 3. Effect of *T. coronaria* on serum enzyme activities (Liver markers) in male wistar albino rats (n=5).

The biochemical indices of liver and kidney damage monitored in the serum in this study are useful markers for assessing the functional capabilities of the organs[20].

Renal glomerular functions are assessable by measuring the plasma creatinine and urea concentrations[21]. Creatinine is a byproduct from muscle as its concentration in blood will be affected by any changes in muscle mass. Urea is synthesized in the liver as the primary by-product of deamination of amino acids[22]. Hence, increase in plasma creatinine or urea concentration would indicate renal damages. There were no significant differences in urea concentrations between all groups of rats. This shows the safety nature of the medicinal plant.

Biochemical indices of organ function, if altered, will impair the normal functioning of the organs[23]. Therefore, the absence of significant effect on the liver and kidney function indices by the extract of *T. coronaria* is an indication that the normal functioning of these organs were not affected. It further indicates that the normal functioning of the nephron at the tubular and globular levels was not altered.

There are many enzymes found in the serum that did not originally originate from the serum. During tissue damage, some of these enzymes find their way into the serum, probably by leakage[24]. Serum enzyme measurements are therefore a valuable tool in clinical diagnosis, providing information on the effect and nature of pathological damage.

3.3. Effect on lipid profile

Total cholesterol, triglycerides and HDL concentrations in rat serum were depicted in Figure 4. Treatment with the ethanolic extract of *Tabernaemontana coronaria* did not show any abnormalities in lipid profile as far as the control is considered.

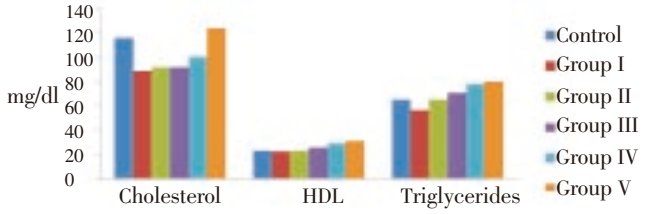

Figure 4. Effect of *T, coronaria* on serum Cholesterol, HDL and Triglycerides (Lipid profile) in male wistar albino rats (*n*=5).

Alterations in the concentration of major lipids like cholesterol, high and low-density lipoprotein cholesterols and triacylglycerol can give useful information on the lipid metabolism as well as predisposition of the animals to atherosclerosis and its associated coronary heart diseases[26]. Elevated levels of all lipids except the HDL-C are associated with increased risk of atherosclerosis. The absence of any effect on all the serum lipid parameters investigated in this study suggests that lipid metabolism in the animals were not altered. This might be an indication that the extract may not likely predispose the animals to atherosclerosis and its associated coronary heart diseases.

3.4. Effect on mineral contents

Serum concentrations of mineral elements are shown in Figure 5. Treatment with ethanolic extract of *Tabernaemontana coronaria* did not significantly change mineral concentrations in the rats when compared with control.

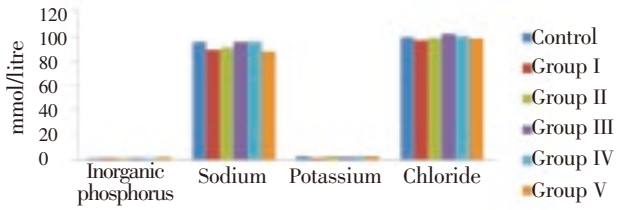

Figure 5. Effect of *Tabernaemontana coronaria* on serum mineral concentrations in male wistar albino rats (n=5)

3.5. Histopathological examinations

Histopathological examinations of kidney and liver of the control and treated rats were examined after the experimental period and presented in Figures 6 and 7 respectively. All the histopathological results of liver showed the hepatocytes in all the 3 zones, the portal triads and central veins appeared normal and of kidney, the glomeruli, the tubules, the blood vessels and interstitium also appeared normal.

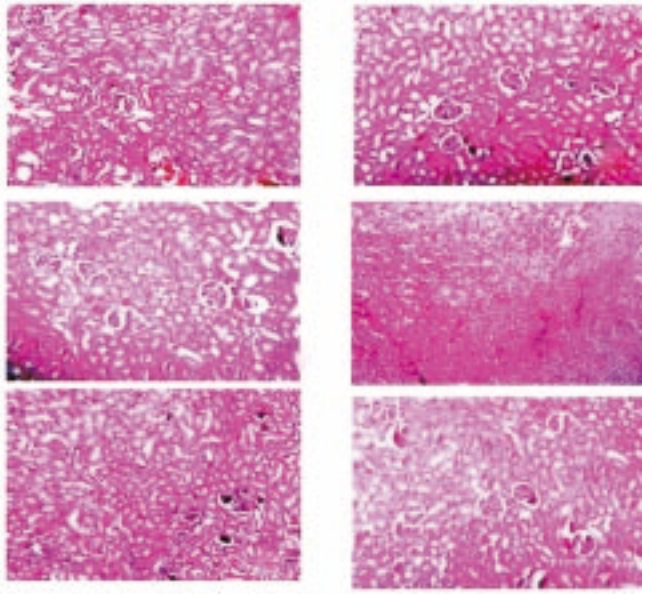

Figure 6. Histopathology of kidney.
A, Kidney of control rat showing normal cortex; showing normal histological structure; C, Kidney of normal rat treated with plant extract *T.coronaria* (200 mg/kg) showing normal glomeruli and tubules; D, Kidney of normal rat treated with plant extract *T.coronaria* (300 mg/kg) showing normal histological structure; E, kidney of normal rat treated with plant extract *T.coronaria* (400 mg/kg) showing normal histological structure; F, Kidney of normal rat treated with plant extract *T.coronaria* (500 mg/kg) showing normal histological structure.
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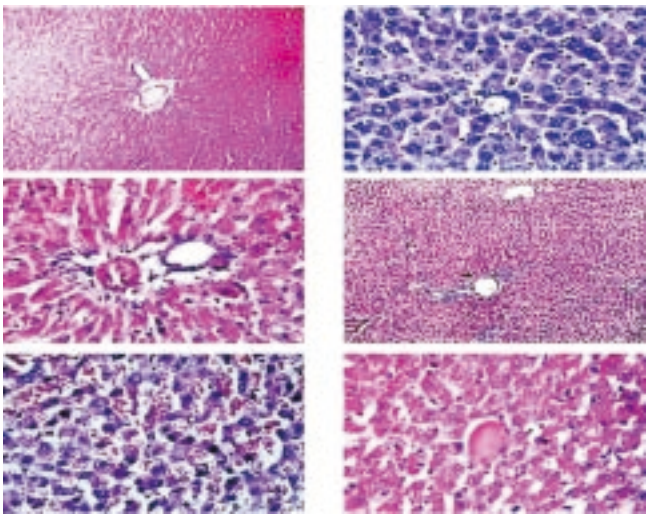

Figure 7. Histopathology of liver. A – Liver of control rat showing normal portal triad and hepatocytes.B – Liver of normal rat treated with plant extract *T.coronaria* (100 mg/kg) showing normal histological structure.C – Liver of normal rat treated with plant extract *T.coronaria* (200 mg/kg) showing normal portal triad and hepatocytes.D – Liver of normal rat treated with plant extract *T.coronaria* (300 mg/kg) showing normal histological structure.E – Liver of normal rat treated with plant extract *T.coronaria* (400 mg/kg) showing normal histological structure. F – Liver of normal rat treated with plant extract *T.coronaria* (500 mg/kg) showing normal histological structure.

4. Discussion

Blood coagulation requires that platelets be in sufficient size, number and function, in the absence of which a satisfactory plug may not occur and haemorrhage may continue following a breach in the vascular endothelium. Flavonoids like quercetin and rutin are used as effective constituents of several pharmaceuticals in the treatment of capillary fragility and phlebosclerosis[15]. Thus the flavonoid and tannin content in the extract may be responsible for the haemostatic property of the ethanolic extract of *T.coronaria*. Since none of the rats were died in the 14 days study, it suggests that the plant can also be safely used in the treatment of diseases requiring long term drug administration.

Serum concentrations of urea, uric acid and creatinine concentrations were almost similar to the control values. The effect of the plant extract on hepatic function is shown in figure 4.The activities of the amino transferases in the plant extract treated groups were not shown any difference when compared to control. Whereas the level of alkaline phosphatases and LDH in group V showed somewhat higher value when compared to control.

The biochemical indices of liver and kidney damage monitored in the serum in this study are useful markers for assessing the functional capabilities of the organs[16].

Renal glomerular functions are assessable by measuring the plasma creatinine and urea concentration[17]. Creatinine is a byproduct from muscle as its concentration in blood will be affected by any changes in muscle mass. Urea is synthesized in the liver as the primary by-product of deamination of amino acids[18]. Hence, increase in plasma creatinine or urea concentration would indicate renal damages. There were no significant differences in urea concentrations between all groups of rats. This shows the safety nature of the medicinal plant.

Biochemical indices of organ function, if altered, will impair the normal functioning of the organs. Therefore, the absence of significant effect on the liver and kidney function indices by the extract of *T. coronaria* is an indication that the normal functioning of these organs were not affected. It further indicates that the normal functioning of the nephron at the tubular and globular levels was not altered.

There are many enzymes found in the serum that did not originally originate from the serum. During tissue damage, some of these enzymes find their way into the serum, probably by leakage[19]. Serum enzyme measurements are therefore a valuable tool in clinical diagnosis, providing information on the effect and nature of pathological damage to any tissue[20]. In the present study the ethanolic extract of *Tabernaemontana coronaria* has shown no abnormal values as for as the liver and kidney markers are concerned. Only slight increase in LDH and ALP concentrations in group V rats. But through histopathological evaluation it is confirmed that no abnormalities is seen in both liver and kidney.

Alterations in the concentration of major lipids like cholesterol, high and low-density lipoprotein cholesterols and triacylglycerol can give useful information on the lipid metabolism as well as predisposition of the animals to atherosclerosis and its associated coronary heart diseases [26]. Elevated levels of all lipids except the HDL-C are associated with increased risk of atherosclerosis. The absence of any effect on all the serum lipid parameters investigated in this study suggests that lipid metabolism in the animals were not altered. This might be an indication that the extract may not likely predispose the animals to atherosclerosis and its associated coronary heart diseases.

All the histopathological results of liver showed the hepatocytes in all the 3 zones, the portal triads and central veins appeared normal and of kidney, the glomeruli, the tubules, the blood vessels and interstitium also appeared normal.

In conclusion the ethanolic extract of *Tabernaemontana coronaria* is not acutely toxic to the rats thereby providing a support to the use of *Tabernaemontana coronaria* in indigenous system of medicine. However further long term toxicological studies (chronic study) are needed in order to establish it as medicine for human consumption.

Conflict of interest statement

We declare that we have no conflict of interest.

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