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Evaluation of *Tribulus terrestris* effect on kidney and liver function in healthy mice

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ABSTRACT

This research aims to evaluate the pharmacological efficacy of aqueous spine extracts of *Tribulus terrestris* (TT) on liver and kidney function through physiological, biochemical, haematological, and histological assessments. *Tribulus terrestris* is renowned for its therapeutic benefits in traditional medicine practices in China, India, Pakistan, and Sudan for treating various chronic ailments. The study employed 5 weight per volume percentage (% w/v) of TT aqueous spines extract. BALB/c mice, both male and female, aged 4-6 weeks, were maintained in controlled ecological conditions (temperature: 22-24°C, humidity: 50-70%, light/dark cycle: 12-h). The mice were divided into two groups: the control group received normal tap water throughout the experimental period, while the other group was administered 5% (w/v) TT spines solution for 15 days. The outcomes of BALB/c mice treated with 5% *Tribulus terrestris* were compared with the control group after 15 days, revealing no significant difference in the drug's efficacy. The physiological results indicated reduced body weight, food intake, and adverse effects in the biochemical profile, including urinary and serum electrolyte levels, liver function tests, increased random blood glucosem, aspartate aminotransferase (AST), alkaline phosphatase (ALP), alanine aminotransferase (ALT), bilirubin (BIL), cholesterol (CHO), high-density lipoprotein (HDL), and triglycerides (TRI). Similarly, kidney function tests showed increased Urinary Nitrogen and creatinine plasma levels, with low blood urea nitrogen (BUN), urinary creatinine, and glomerular filtration rate (GFR). However, no significant difference was observed in haematological profile readings. In conclusion, the findings suggest that the aqueous spine extract of *Tribulus terrestris* does not demonstrate beneficial effects on liver and kidney function, indicating that this study supports the notion that the plant cannot be utilized as a medicinal remedy.

1. Introduction

Throughout history, plants have served as the predominant source for healthcare treatments worldwide. Even in the modern era, plant extracts continue to be utilized, primarily due to their comparatively lower incidence of adverse effects in contrast to synthetic chemical drugs (Karimi et al., 2015). Plants contain lesser metabolites such as flavonoids, alkaloids, saponins, tannins, and terpenoids (Jain et al., 2019). Secondary metabolites in medicinal plants exhibit antioxidant properties, enabling them to neutralize free radicals within the human body. Owing to these medicinal capabilities, certain pharmaceutical companies are undertaking the development of herbal medicines to address a wide range of chronic illnesses and combat harmful pathogens (Pan et al., 2013; Lim et al., 2019; Koc and Cengiz, 2020).

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Besides therapeutic effects of the plants, some plants can also show toxicity and few studies are present to report their negative effects and their toxicities on humans. Therefore, before treating human beings with plant extracts requires proper evaluation.

The plant *Tribulus terrestris* (TT) is associated with the family zygophyllaceous, which is a perennial creeping herb distributed worldwide. It is traditionally practiced as a medicine since ancient times. Well known for ethnopharmacology to treat chronic ailments (Tulunay et al., 2015). The enhancement of male fertility has been a well-established practice in China, Pakistan, and India. Additionally, these substances have been reported to exhibit aphrodisiac properties, possibly attributed to their capacity to release nitric oxide, which may underlie their aphrodisiac effects (Chauhan et al., 2014). Administration of TT in goats has increased plasma testosterone (Rogerson et al., 2007), known to possess steroidal saponins (Semerdjieva and Zheljazkov, 2019), to treat infertility, low sex drive, and also erectile dysfunction (Chauhan et al., 2014). Athletes commonly employ these substances to enhance their muscle strength and performance in sports (Dinchev et al., 2008). TT has been found to contain certain alkaloids known to be toxic to sheep. Therefore, comprehensive investigations are warranted to study the toxicological effects and potential adverse consequences following its utilization (Aslani et al., 2003; Ştefănescu et al., 2020). Bulgarian TT increases transaminases via rhabdomyolysis (Chen et al., 2013). Grazing of this plant in sheep causes cholangitis and photosensitization (Glastonbury et al., 1984). Despite its widespread medicinal use, a limited number of studies have reported toxicity associated with the utilization of this plant. Therefore, the present study aims to thoroughly investigate the pharmacological efficacy of aqueous spine extracts concerning physiological, biochemical, haematological, and histopathological parameters relevant to liver and kidney function.

2. Materials and methods

2.1. TT preparation

The TT sample employed in this study was obtained from the natural garden located in Turabah City, Kingdom of Saudi Arabia, and authenticated by a botanical specialist. Active compound quantification of TT was not conducted; instead, the spines were subjected to a meticulous drying and grinding process to obtain a finely powdered form. From this powder, 5% TT powder was derived and combined with distilled water. Specifically, 5 grams of finely ground TT spines powder were mixed with 100 ml of distilled water, heated to 60°C for 5 minutes, allowed to stand overnight, filtered, and subsequently used for the treatment group over a 15-day period.

2.2. Animal selection

BALB/c mice of both genders (males and females), aged 4-6 weeks, were procured from the King Fahd-Medical Research Center at King Abdulaziz University, Kingdom of Saudi Arabia. All experimental procedures were conducted at the Biology department, University College of Turabah. The mice were housed in environmentally controlled conditions, maintaining a temperature range of 22-24°C, humidity levels between 50-70%, and a 12-hour light/dark cycle. Two groups were established for the mice: the first group, the control group (n=10), received normal tap water throughout the entire duration of the experiment, while the second group, the treatment group (n=10), was administered a 5% (w/v) solution of TT spines for a period of 15 days. Each group (control and treatment) included an equal number of male and female mice, i.e., 5 each. All animal experiments were conducted following the approval from the university ethics committee and adhered to the guidelines and regulations for local and international animal welfare and care.

2.3. Metabolic cages experiments

On the 11th day of the experiment, the mice were transferred to metabolic cages for the subsequent four days. The first day was designated for acclimatization to the new environment, while the subsequent three days were allocated for sample collection. Throughout the metabolic cage phase, daily measurements of food and fluid intake were recorded for both the control and treatment groups. Urine samples were collected daily using urine collecting tubes to facilitate biochemical analyses. On the final day of the experiment, blood samples were obtained from all animals by accessing the retro-orbital plexus through the use of diethyl ether (sourced from Germany, by Roth). These blood samples were then collected in blood collecting tubes for subsequent analyses.

2.4. Biochemical analysis

The serum concentrations of Sodium (Na^+) and Potassium (K^+) were determined using flame photometry (AFM 5051, Germany). For the analysis of urinary creatinine and plasma concentrations, a kinetic method was employed, while the UREA colorimetric method was utilized to ascertain urinary urea concentration. Blood cholesterol, ALT, AST, GPT, GOT, LDL, and HDL Cholesterol levels were measured using the direct method, based on the CHOD PAP techniques. Triacylglycerol (TRI) and uric acid levels were assessed using the GoPt and Uricase methods, respectively. Random glucose levels were determined using kits from BIOLABO (France) (Khan et al., 2019). Complete blood counts were analyzed using an electronic hematology particle counter (MDM 905, Germany) equipped with a photometric

unit for hemoglobin quantification. All measurements were performed in accordance with the manufacturers' specifications and guidelines.

2.5. Histological analysis

Histological analysis was conducted on the last day of the experiment, following the sacrifice of all mice to obtain kidney samples for subsequent histological examination (Bancroft and Gamble, 2008). A Nikon Eclipse i80 light microscope was employed to visualize and analyze the stained sections for any pathological alterations. Additionally, a digital camera (Nikon OXM 1200C, Japan) was used to capture images at various magnifications as required.

2.6. Statistical analysis

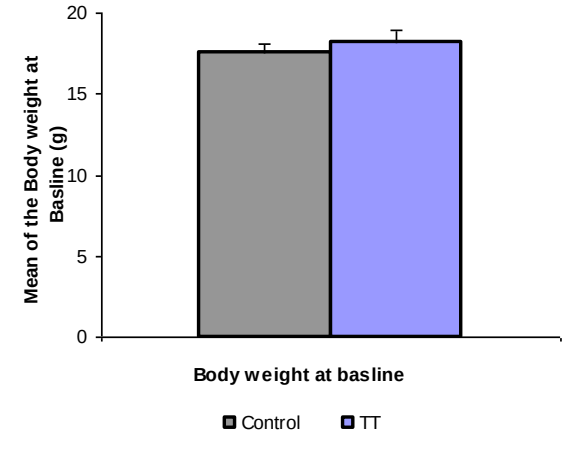
Statistical analysis was conducted to determine the significant difference between the control group and the group of mice treated with 5% TT. The t-test (ANOVA) was performed using GraphPad Prism 8.4.3.686 software (USA). All values were expressed as mean ± standard deviation, following the convention established by Manzoor et al. (2016). Results were considered significant when the probability factor (P-value) was less than 0.05 ($P < 0.05$).

3. Results

The physiological profile of the body weight at baseline was recorded lower among the control group mice in comparison to the TT group mice (see Fig. 1A), however, after 15 days of treatment with 5% spines of TT weight of the body was considerably reduced in TT group mice in comparisons to control group mice as seen in Fig. 1B. Intake of food was found higher in control group mice and lower in TT group mice (Fig. 1C), whereas, fluid intake was recorded vice versa (Fig. 1D). Both wet fecal (Fig. 1E) and dry fecal (Fig. 1F) was measured lowered among the TT group mice and higher for control group mice. Fig. 1G shows that urine output was slightly more in TT group mice in comparison to control group mice.

Fig. 2, illustrates the urinary electrolytes, where Na^+ (Fig. 2A) and K^+ (Fig. 2C) excretion was recorded higher among control group mice and lower for TT control group mice. Plasma Na^+ conc. (Fig. 2B) was found to be significantly low for the control group mice when compared to TT group mice, in contrast, $Ca2^+$ excretion (Fig. 2E) was measured to be significantly greater for the control group mice when compared to TT group mice. However, K^+ conc. (Fig. 2D) and $Ca2^+$ conc. (Fig. 2F) was found to be slightly lower in control group mice in comparison to TT group mice. Kidney function results showed higher values of BUN (Fig. 2G) and plasma creatinine (Fig. 2J) for TT group mice in comparison to control group mice. Whereas, lower levels of urinary nitrogen (Fig. 2H), urinary creatinine (Fig. 2I), GFR (Fig. 2K), and normalized 24-h creatinine clearance (Fig. 2L) were measured for control group mice when compared to TT control mice.

Table 1 presents the impact of administering 5% TT spines on various parameters in the TT group mice compared to the control group mice. Notably, the TT group exhibited elevated levels of Random Blood Glucose, ALP, ALT, AST, BIL, CHO, HDL, TRI, and Uric acid concentration; however, these differences were not statistically significant. Regarding White Blood Cells, the TT group mice displayed a slight increase compared to the control group mice (Table 1). Furthermore, a more substantial increase in Lymphocyte counts per 10^3/ml and Granulocytes as a percentage was observed in the TT group mice. Conversely, the control group mice demonstrated higher values for Lymphocyte percentage, Granulocytes count per 10^3/ml, Monocytes count per 10^3/ml, Monocytes percentage, Hemoglobin concentration (g/l), and Mean Corpuscular Volume (fl) when compared to the TT group mice. Histopathological examination revealed degeneration of renal tubules in the TT group mice, characterized by cytoplasmic vacuolation and pyknosis of the nuclei, as depicted in Fig. 3. It is worth noting that the degeneration of cortical tubule epithelial cells appears to be a nonspecific entity, encompassing diverse morphological changes and variable cellular features.



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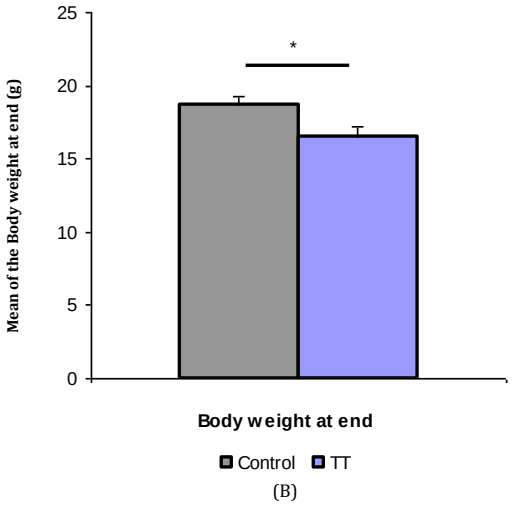

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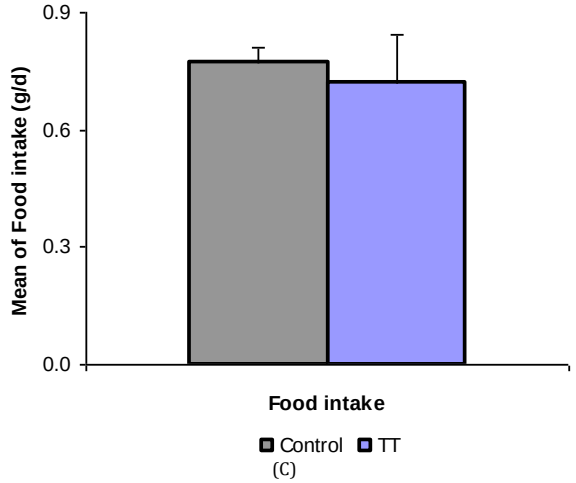

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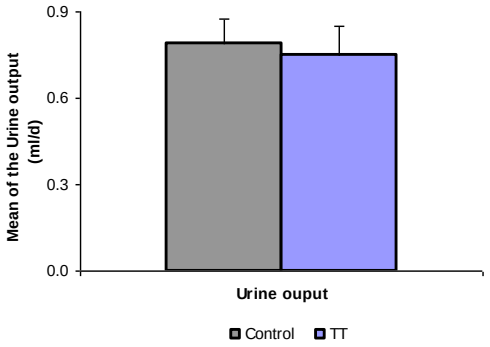
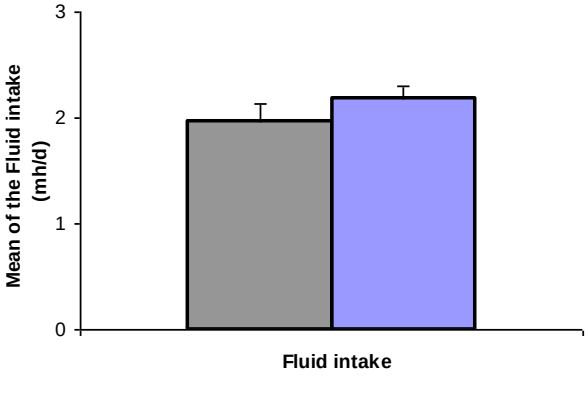


(G)

*: Significant difference between the groups

Fig. 1: Effect of 5% of TT after 15 days of treatment on (A) baseline weight of the body, (B) weight of the body at end, (C) intake of food, (D) intake of the fluid, (E) wet weight of the fecal, (F) dry weight of the fecal and (G) urine output



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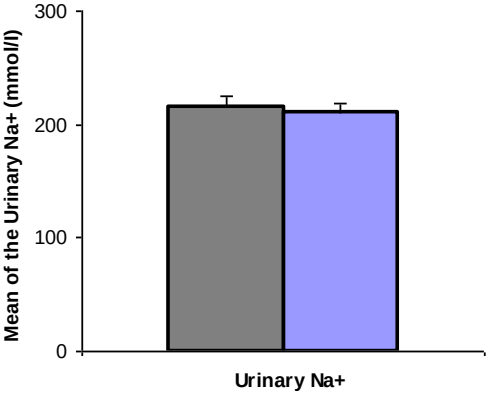

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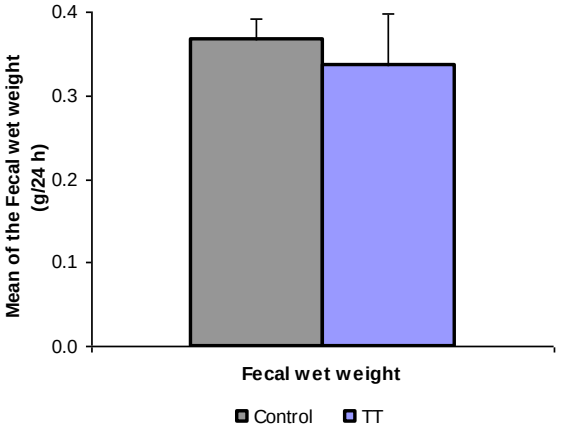

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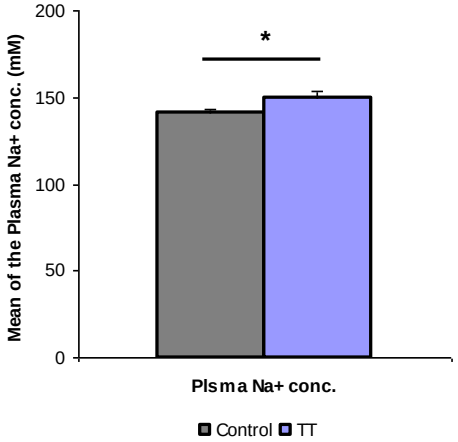

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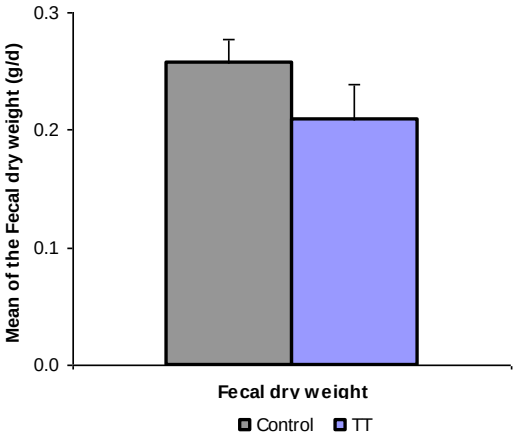

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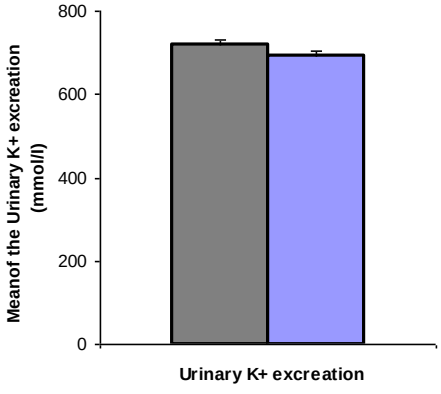

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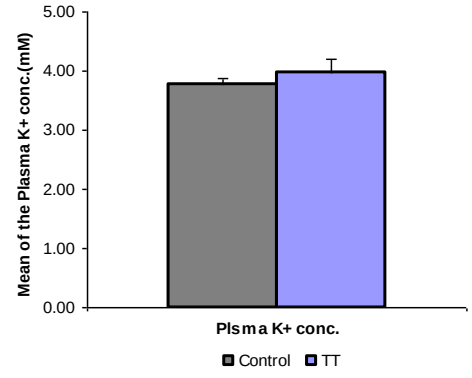

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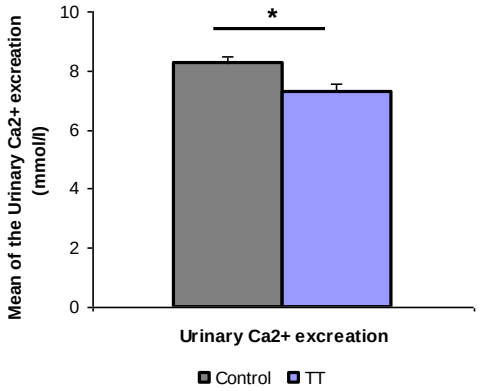

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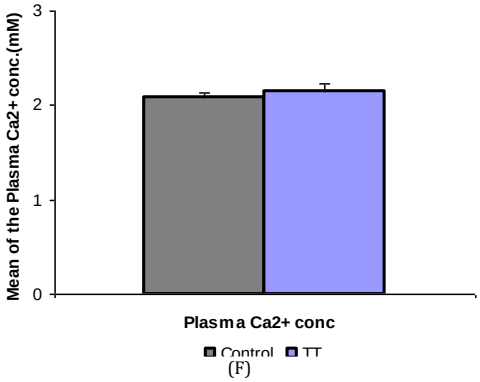

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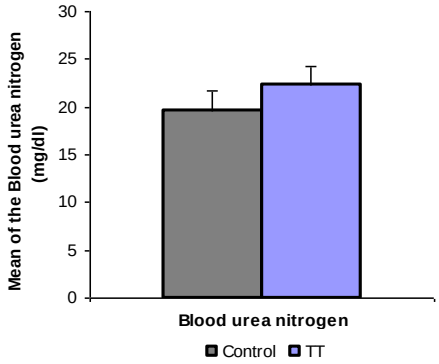

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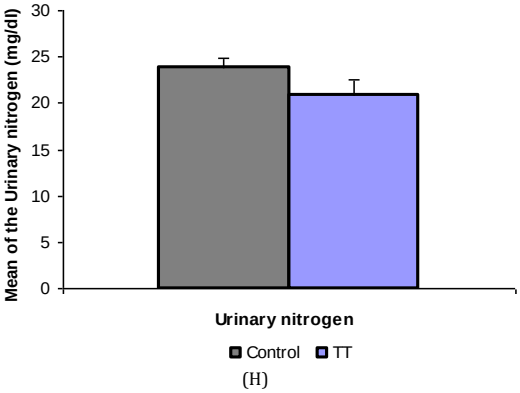

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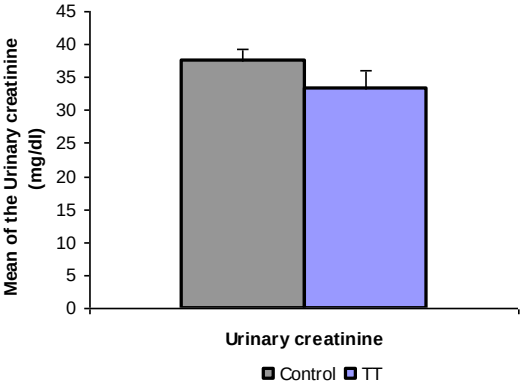

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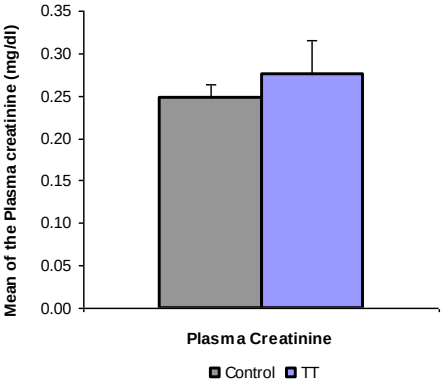

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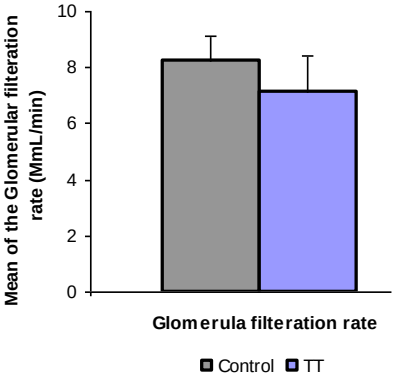

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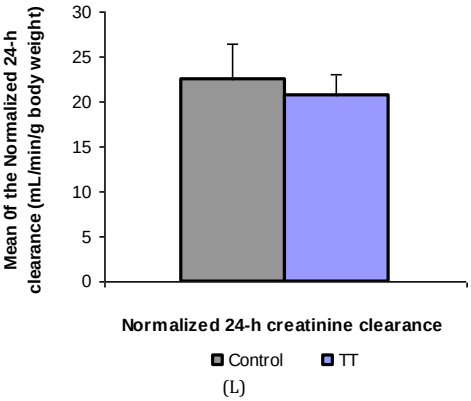

(L)

*: Significant difference between the groups

Fig. 2: Effect of 5% of TT after 15 days of treatment on (A) urinary Na+, (B) plasma Na+ concentration, (C) urinary K+ excretion, (D) plasma K+ concentration, (E) urinary Ca2+ excretion, (F) plasma Ca2+ concentration, (G) blood urea nitrogen, (H) urinary nitrogen, (I) urinary creatinine, (J) plasma creatinine, (K) glomerular filtration rate and (L) normalized 24-h creatinine clearance

Table 1: Effect of 5% of TT on blood glucose level, liver functions, serum uric acid concentration, and complete blood count

Parameters	Control	5% of TT	P-value
Random blood glucose, mg/dL	84.33±7.4 7	94.10±8.67	0.4030
Alkaline phosphatase, U/L	70.58±2.63	72.78±11.17	0.8502
Alanine aminotransferase, U/L	39.45±4.68	40.04±8.10	0.9500
Bilirubin, mg/dl	0.22±0.01	0.25±0.02	0.2361
Aspartate aminotransferase, U/L	43.35±4.23	44.82±7.09	0.8608
High-density lipoproteins, mg/dl	20.10±1.20	23.50±3.92	0.4176
Cholesterol, mg/dl	26.83±1.74	32.17±4.15	0.2513
Triglycerides, mg/dl	36.50±8.19	41.00±7.31	0.6867
White blood cells, 10^3/ml	7.60±0.32	7.96±0.42	0.5055
Uric acid conc., mg/dl	1.70±0.22	2.09±0.44	0.4376
Lymphocyte, %	92.16±0.43	89.29±1.15*	0.0310
Lymphocyte, 10^3/ml	5.71±0.45	6.57±0.31	0.1368
Granulocytes, %	3.04±0.16	3.46±0.32	0.2510
Granulocytes, 10^3/ml	0.47±0.04	0.42±0.04	0.3528
Monocytes, 10^3/ml	0.60±0.04	0.50±0.06	0.1842
Monocytes, %	6.12±0.44	5.29±0.23	0.1068
Mean corpuscular volume, fl	8.39±0.25	7.45±0.30*	0.0275
Haemoglobin, g/l	13.98±0.15	13.06±0.26*	0.0063

*: Significant difference between the groups

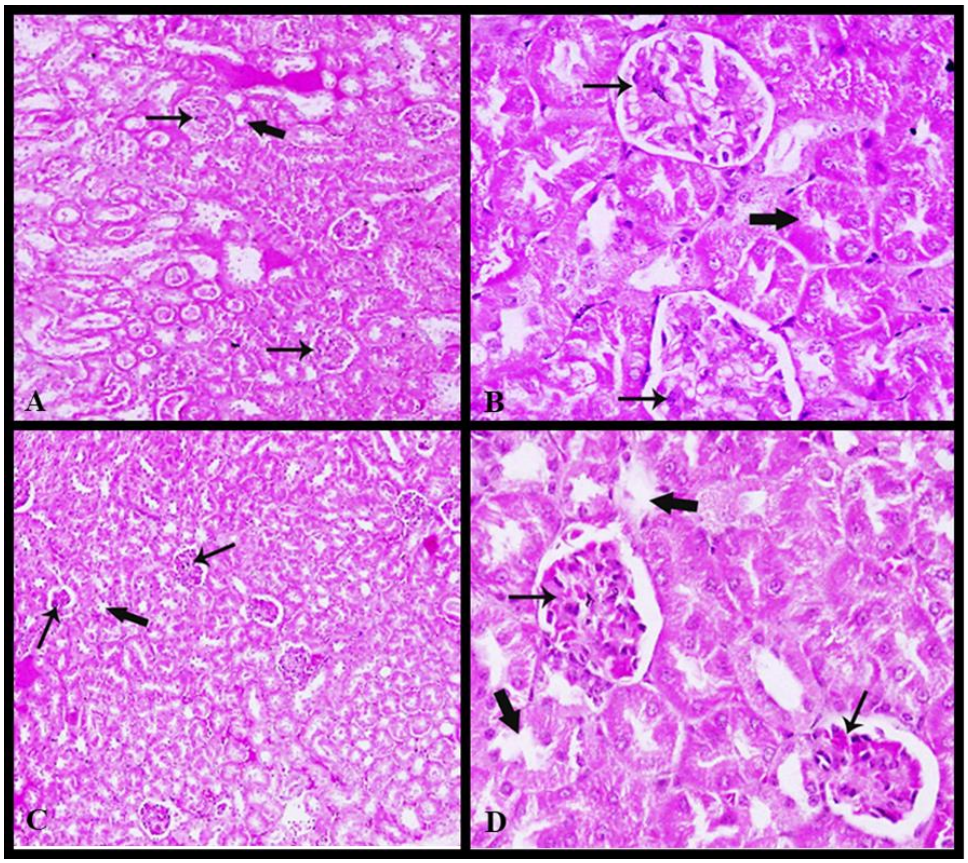

Fig. 3: Kidney section displaying Glomerulus (thin arrow), Renal tubules (thick arrow). (A, B) photo of control group. (C, D) showing tubular degeneration, after 5% of TT for 15 days

4. Discussion

As TT is a common weed but has significant value in the ancient medicine system such as Ayurveda, Chinese, Siddha, etc. which is traditionally used for its pharmacological activities in various health ailments (Adaikan et al., 2001) and significantly improving sexual activity (Singh et al., 2012). Recently investigation on TT revealed that it has secondary metabolites such as antiaging (Iacono et al., 2010) and anti-inflammatory activities (Oh et al., 2012). However, till today there are fewer studies carried out to find the toxicity of the plant. The critical assessment of the data obtained from our study gives the notion that utilization of 5% spines extract of the TT has detrimental effects on the BALB/c mice. The present study treated BALB/c mice for 15 days with 5% spines extract of TT on parameters such as physiological, biochemical (function tests for liver and kidney), haematological and histopathological. Results revealed that food intake was reduced among TT group mice due to which animal body weight was reduced after 15 days from baseline. Additionally, increased Urinary Na+ may be due to kidney problems or hypernatremia measured high levels of Na+ in blood, symptoms also include an increase in thirst found in TT group mice (Arambewela et al., 2016). Moreover, increased urinary K+ shows the symptoms of Hyperkalaemia which is caused due to kidney failure, (Kamboj et al.,

2011) less Urinary Calcium (hypoparathyroidism) was recorded with increased Ca2+ serum electrolytes shows the hypernatremia condition is present and calcitonin condition acts on bone cells to increase calcium levels in blood among TT group mice (Yu and Sharma, 2021).

With increased Urinary Nitrogen the blood had low levels of it, usually also suggest due to the low protein diet or due to parenchymal liver disease (Seki et al., 2019). As low Urinary creatinine plasma creatinine was increased which implies due to poor clearance of creatinine due to kidney malfunction (Shahbaz and Gupta, 2021). An elevation in blood pressure leads to an increase in GFR. Moreover, the observed increase in random blood glucosem levels indicates a prediabetic condition. The administration of 5% TT spine extracts affected the CBC in the TT group mice, resulting in a slight increase in WBC and Granulocytes, indicative of a response to infection(s).

Furthermore, the animals in the TT group (mice) exhibited reduced Hemoglobin levels and diminished MCV, suggesting the presence of iron deficiency and anemia. Conversely, the elevated levels of ALP, ALT, AST, BIL, CHO, HDL, and TRI may be attributed to hepatorenal syndrome or acute interstitial nephritis/tubular necrosis among the TT group mice (Talasaz et al., 2010). Due to the consumption of the 5% spines extract of TT several biochemical changes occurred, hence resulting in morphological changes occurred in the histopathological profile of the renal tubule as well as the degeneration of cortical tubule epithelial cells was observed for TT group mice (Talasaz et al., 2010).

5. Conclusions

Thus, the utilization of TT in this study yielded adverse outcomes, prompting caution regarding the consumption of TT extracts. It is worth noting that in this study, we used the raw material of TT spines. However, conducting extraction and active ingredient preparation could potentially reveal more detailed effects. Our intention was to highlight the possibility of certain individuals experiencing tissue toxicity when using raw natural products, particularly if prolonged use is involved. To gain a comprehensive understanding of the underlying mechanisms of the adverse effects observed with the 5% spines extract of TT, further investigations at the molecular level are warranted. Additionally, the isolation of specific metabolites for subsequent toxicological investigations is imperative prior to considering the usage of TT as herbal medicine. Based on our findings, it is prudent to refrain from using TT as a dietary supplement for the treatment of various diseases.

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Acknowledgment

We acknowledge Taif University Researchers Supporting Project number (TURSP-2020/269), Taif University, Kingdom of Saudi Arabia.

Compliance with ethical standards

Institutional review board statement

All procedures carried out in the study containing animals were in accordance with the ethical standards of the Ethical Committee of Deanship of Taif University. All mice were sacrificed under anaesthesia; efforts were made to reduce pain and suffering to the mice, and with the Helsinki Declaration of 1964 and its later amendments or comparable ethical standards.

Conflict of interest

The author(s) declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

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