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Original Article

Comparative renoprotective effect of *Tamarix dioica* leaf extracts and metformin against acetaminophen-induced renal toxicity in Swiss albino mice: Novel insights on renoprotection and therapeutic potential

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Abstract

Nephrotoxicity is characterized by the adverse effects on kidney function caused by various substances, including hazardous chemicals and drugs. This study aimed to investigate the neuroprotective properties of aqueous, methanolic, and ethanolic extracts of *Tamarix dioica* leaf against acetaminophen-induced kidney damage and compare their efficacy with metformin, a known neuroprotective agent. Thirty-six albino mice were randomly divided into six groups, including a standard control group, an acetaminophen-toxified group, a positive control group treated with metformin (at a dose of 200 mg/kg body weight), and three experimental groups treated with aqueous, methanolic, and ethanolic *T. dioica* extracts (at a dose of 400 mg/kg body weight each). The neuroprotective potential of the *T. dioica* extracts was assessed by evaluating hematological markers, electrolyte levels (Na+, K+, Cl-), antioxidant enzymes (CAT, SOD, MDA), renal function tests (urea and creatinine), and toxicity markers (SGOT and SGPT). Additionally, histopathological analysis was conducted to observe any pathological changes in kidney tissues stained with hematoxylin and eosin. The results demonstrated that the *T. dioica* leaf extracts effectively restored all the indicators and antioxidant enzyme levels to normal, significantly differing from the elevated levels observed in the acetaminophen control group ($p < 0.05$). Furthermore, histopathological examination revealed regeneration of glomeruli and renal tubules in the stained tissues. These findings suggest that *T. dioica* leaf extracts can potentially mitigate acetaminophen-induced nephrotoxicity.

Keywords: *Tamarix dioica*, Neuroprotective effect, Toxicity markers, Renal function tests, Antioxidative enzymes.

1. Introduction

Local folklore is a sort of cultural transmission that has been preserved in families, tribes, and communities around the world with respect to the usage of medicinal herbs [1]. Humans have used medicinal plants or their extracts for a wide variety of medical purposes ever since recorded history. This practice has yielded many useful medications, including analgesics, antitussives, antihyper-

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tensives, cardiotonic, anticancer drugs and antimalarials [2]. To my surprise, I found that plants are a great place to get bioactive compounds. Millions of years of evolution and adaptation have allowed them to develop structurally varied secondary metabolites that can endure bacteria, insects, fungus, and weather. Because of their ethnopharmacological value, they served as a pivotal ingredient in the development of many pharmaceuticals and play their vital role in drug discovery [3].

When the kidneys are exposed to a medicine or toxin, a condition known as nephrotoxicity may develop [4] and this condition leads to malfunctioning of this vital organ for making it unable to excrete harmful substances being eliminated from the body. Nephrotoxicity may temporarily raise BUN and creatinine. These levels may be high owing to dehydration and ultimately renal failure. Early diagnosis and treatment of elevated BUN and creatinine levels may prevent kidney damage [5]. The treatment for kidney illness is lifelong, costly, unusual, and may even be hazardous, making it a huge public health challenge in nations with little resources. Accidents, medications, surgeries, low blood pressure from shock, blockages of the urinary bladder or kidney, and serious infections are also important risk factors for the manifestation of various types of renal failures. Aminoglycoside antibiotics, nonsteroidal anti-inflammatory drugs (acetaminophen), and chemotherapeutic agents are some examples of therapeutic agents that can cause kidney disease [6].

Several degenerative disorders, such as chronic hyperglycemia and chronic renal disease, have been linked to an increase in free radicals, which in turn has led to an increase in reactive oxygen species. Oxidation of cellular components, including proteins, lipids, and DNA, results from the excessive creation of these free radicals and the fall in cellular antioxidants. It causes cellular damage and ultimately apoptosis. Metformin has been shown to reduce oxidative stress and endoplasmic reticulum stress, both of which contribute to the death of cells and antioxidative potential has also been observed to improve by the use of metformin both in experimental and in human studies [7]. It is highly selective in its cytotoxic effects on cancer stem cells, contributing to a potent anti-tumor impact. In addition, it protects the kidneys, which is called renoprotection [8].

Tamarix dioica (Tamaricaceae), is traditionally used as a carminative, diuretic, and for treating splenic and hepatic inflammation [9]. *T. dioica* Roxb belongs to tamaricaceae. Tamarix (tamarisk) consists of evergreen shrubs or trees that grow between 1 and 18 meters tall. It includes approximately 50 to 60 species of flowering plants [9]. Reddish bark covers this 6-meter evergreen shrub or small tree. Three-millimeter flowers. Pink or purple unisexual blooms. Cylindrical spikes are densely packed. Fruit is a cone-shaped capsule up to 5mm long with three valves. Several phytochemical investigations on various Tamarix species have identified a range of phytochemicals, with polyphenolic compounds such as flavonoids, phenolic acids, and tannins being among the most significant [10]. In countries across Asia and Africa, including Pakistan, Algeria, Iran, and India, where tamarisk grows naturally, local populations traditionally use this plant for medicinal purposes [9]. *T. dioica* is used not only as a diuretic but also as a carminative and in the therapy of splenic and hepatic inflammation. The crude extracts of *T. dioica* leaves have been shown to contain cytotoxic, antimicrobial, and antifungal properties [11]. Polyphenolic chemicals such as phenolic acids, flavonoids, and tannins have been shown to play a significant role in the phytochemistry of many Tamarix species. Furthermore, tamarisk is a native plant in several Asian and African countries, including Saudi Arabia, Pakistan, India, and Algeria, where it is used for medical purposes. Chemical components of plant parts have been examined. Tannins, phlobatannins, flavonoids, phenols, steroids, saponins, and terpenoids were present, but glycosides, alkaloids, amino acids, and protein were not. There are several therapeutically beneficial chemical components found in the leaves, including rhamnetin, polyphenols, flavanols, and β -sitosterol [9].

In recent years, interest in natural remedies with potential renoprotective effects has grown, particularly as researchers seek alternatives to standard pharmaceutical agents, which can sometimes have adverse effects. Metformin, a widely studied antidiabetic drug, is recognized for its protective effects against renal toxicity due to its antioxidative and anti-inflammatory properties [12]. However, concerns regarding its long-term use and side effects have prompted a search for alternative or supplementary treatments with fewer side effects and natural origin [13]. *T. dioica* has drawn attention due to its high concentration of polyphenolic compounds, such as flavonoids, tannins, and phenolic acids, which are known for their antioxidant capabilities [14]. These compounds are similar to those found in other renoprotective plants like *Camellia sinensis* (green tea) and *Curcuma longa* (turmeric), both of which have demonstrated efficacy in reducing oxidative stress and inflammation in renal tissues [15]. However, unlike these more commonly studied plants, *T. dioica* is also rich in unique bioactive compounds specifically adapted to harsh environmental conditions, which may enhance its stress-resistance properties, making it a promising candidate for nephroprotection [16].

This study aims to investigate the nephroprotective effects of aqueous, methanolic, and ethanolic extracts of *Tamarix dioica* leaves on acetaminophen-induced kidney damage in mice and to compare their efficacy with metformin, a known nephroprotective agent. Specifically, the study hypothesizes that *T. dioica* extracts will restore kidney function markers, balance electrolyte levels, improve antioxidant enzyme activity, and reduce toxicity markers, thus representing renoprotective properties in acetaminophen-induced renal damage. It hypothesizes that these effects may be comparable or superior to metformin, as assessed through hematological, biochemical, and histopathological evaluations.

2. Material and Methodology

2.1. Chemicals

Commercially accessible kits were used to determine renal function. Acetaminophen (Paracetamol/Panadol, GSK) was purchased from Saudi Arabian pharmacies. The remaining analytical-grade chemicals (Merck) were purchased from the Riyadh, Saudi Arabia chemical market.

2.2. Plant collection and storage

T. dioica leaves were purchased at a public market, placed in bags, and assigned date of purchase. The dust particles were removed from the leaves of the *T. dioica* plant by washing them twice with tap water. The washed

leaves were then chopped, and crushed (using pestle mortar and mechanical blender) into a powder are dried in an oven at 45 ℃. The powder was kept in an airtight container until it was needed.

2.3. Preparation of extracts

A portion of this leaf powder (50 g) was subjected to extraction using three different solvents: double-distilled water (DDW), methanol, and ethanol. Each extraction was carried out by soaking 50 g of powder in 500 mL of each solvent separately for 72 hours with intermittent shaking to enhance solubility. After 72 hours, each mixture was filtered through Whatman no. 1 filter paper to ensure a clear filtrate, free from particulate matter. This filtering process was repeated until no further residue appeared on the filter paper, ensuring the purity of the extracts. The resulting aqueous, methanolic, and ethanolic filtrates were then concentrated using a rotary evaporator at a low temperature (below 40°C) to prevent the degradation of heat-sensitive phytochemicals. The concentrated extracts were then carefully transferred to individual airtight containers to prevent oxidation or contamination and were stored at 4°C until further use in biochemical and histopathological analyses. This standardized method allows for precise extraction conditions, aiding reproducibility and consistency across studies.

2.4. Animal handling & housing conditions

Swiss Albino mice of weight 65-75 grams were handled in accordance with the protocol of animal management and welfare of the University of Helsinki, Finland. Animals were kept in a laboratory standard hutch with no limitation access to a rodent's food and drinking water. All methods were carried out in accordance with relevant guidelines and regulations.. The animals were kept in an environment with suitable circumstances, which included a temperature of 25 ± 2 °C, a relative humidity range of 40–50 percent, and a light: dark cycle of 12:12 hours. They were fed on normal standard diet. Practical work was performed in labs of the Faculty of Science, Department of Biology, University of Tabuk, Saudi Arabia.

2.5. Toxicological study

Swiss albino mice good in health were divided at random into four groups, each of which had five mice, were appropriately labelled with the letters A, B, C, and D. They were first put on a fast for three to four hours, with the exception of water, and then given acetaminophen, aqueous, methanolic, and ethanolic extracts along with 0.5% CMC in the following doses: 100 mg/kg to group A, 200 mg/kg to group B, 400 mg/kg, and 2,000 mg/kg of their body weights respectively. OECD guidelines-423, also known as the acute toxic class technique, served as the basis for the study that was carried out [17]. The first twenty-four hours of the experiment were spent carefully observing the animals in order to identify any signs of toxicity, sickness, or death. The initial four hours of observation received special attention and concentration from the researchers. Alterations in the profiles of the behavioural, neurological, and autonomic systems were also evaluated, in addition to those already mentioned. In addition to this, we kept a close eye on them for a total of 14 days and 72 hours to ensure that we had a comprehensive understanding of the toxicity [18].

2.6. Experimental model

There was a total of 36 albino mice, and they were split up into 6 groups each containing six mice. The group I have named the normal controls drank just distilled water (5 ml/kg of body weight, orally) for a week and they were just kept to find the normal conditions of the mice. Group II was named as uremic group because acetaminophen-induced toxicity was observed and in this group by administering: acetaminophen at 2g/Kg of body weight on day 5. Group III was labeled as positive control as they received only metformin @ 200 mg/Kg of body weight (P.O) for 7 days except the 5th day when acetaminophen @ 2g/Kg of body weight was given. Group IV was labelled as test group I as they received only aqueous *T. dioica* extract @ 400 mg/Kg of body weight (P.O) for 7 days except the 5th day when acetaminophen @ 2g/Kg of body weight was given. Group V was labelled as test group II as they received only methanolic *T. dioica* extract @ 400 mg/Kg of body weight (P.O) for 7 days except the 5th day when acetaminophen @ 2g/Kg of body weight was given. Group VI was assigned the tag of test group III received only ethanolic *T. dioica* extract @ 400 mg/Kg of body weight (P.O) for 7 days except the 5th day when acetaminophen @ 2g/Kg of body weight was given. The doses of *T. dioica* extract (400 mg/kg) and metformin (200 mg/kg) used in our study likely align with those from previous studies evaluating the plant's nephroprotective effects. For instance, studies involving other plants, such as *Aerva javanica* and *Acalypha wilkesiana*, have demonstrated nephroprotective effects with doses ranging from 200–400 mg/kg in animal models [19].

The control groups in this study were strategically chosen to ensure a comprehensive evaluation of the effects of Tamarix dioica leaf extracts on acetaminophen-induced nephrotoxicity. Group I served as the normal control, receiving only distilled water to establish baseline conditions. Group II, the uremic control, was exposed to acetaminophen to induce kidney damage, providing a reference point for the toxic effects of acetaminophen alone. Group III, the positive control, received metformin, a well-known renoprotective agent, to compare its protective effects against the T. dioica extracts, validating the experimental setup and ensuring that the observed effects could be attributed to the treatments being tested. Groups IV, V, and VI were the test groups, each receiving different solvent-based T. dioica extracts (aqueous, methanolic, and ethanolic) to assess their individual renoprotective potential. The inclusion of metformin as a positive control is crucial, as it serves as a benchmark for evaluating the efficacy of the plant extracts, ensuring that any beneficial effects observed are significant and comparable to a proven therapeutic agent.

2.7. Body weight variation along the group

Animals were weighed daily leading up to the experiment, once daily throughout the trial, and once more just before they were sacrificed.

2.8. Estimation of Hematological parameters

By day 7 of the experiment, the rats had been killed. The heart was punctured to get blood samples. One portion of the blood was drawn into EDTA-treated sample bottles for hematological analysis by means of an automated hematology machine (Cell-Dyn, Abbott, USA).

This involved measuring parameters like the number of white blood cells, their subtypes (neutrophils, eosinophils, basophils, lymphocytes, and monocytes), the number of red blood cells, and the concentration of hemoglobin and platelets.

2.9. Estimation of Electrolytes

Na^+, K^+, Cl^- electrolyte, urea, creatinine and CRP levels were measured as biochemical indicators of kidney function, using commercially available diagnostic kits**.**

2.10. Estimation of Antioxidative Enzymes Profile

The biochemical activity of catalase was evaluated. Blood was centrifuged and plasma was separated and kidney tissues were also homogenized at the same time separately in 0.05 M Tris Hydrochloric acid (HCl) buffer solution (pH-7.0) keeping the tissue concentration of 50 mg/ml. The homogenate was centrifuged at 10,000 rpm, for 10 min at 4°C. The absorbance at 240 nm was measured after mixing 0.5 ml of hydrogen peroxide (H_2O_2) with 2.5 ml of distilled water in a spectrophotometric cuvette. Separate additions of 40 µl of tissue supernatant and plasma were made, and the measurements were taken at intervals of 30 seconds.

Similarly, to CAT, SOD activity was also evaluated from blood plasma and tissue homogenate. Kidney was homogenized in 100 mM tris cocodylate buffer (ice cold) and homogenized mixture was kept at the concentration of 50 mg/ml while blood was centrifuged at 10,000 rpm for 20 min at 4 °C. The SOD activity of these fluids depends to the extent by which these supernatants prevent pyragallol auto-oxidation. Absorbance at 420 nm was measured for 3 minutes in a spectrophotometer after 2 ml of buffer, 100 µl of 2 mM pyragallol, and 10 µl of supernatant were put into a spectrophotometric cuvette. The enzyme activity that prevented 50% of the autooxidation of pyragallol was considered one unit of SOD [20].

Tissue and blood supernatants were obtained and used for MDA estimation. Kidney tissues were homogenized at 50 mg/ml concentration in 0.1 M phosphate buffer of pH 7 (ice cold). The blood and homogenate were centrifuged at 10,000 rpm for 5 min at 4°C and supernatants were collected separately. 0.5 ml of each supernatant was mixed separately with 0.5 ml of normal saline and 2 ml of TBA-TCA mixture and finally boiled at 100 °C for 10 min and then cooled to room temperature and centrifuged again at 4000 rpm for 10 min. The supernatant and the plasma both were placed in a cuvette of a spectrophotometer and analyzed at 535 nm [21].

2.11. Estimation of Toxicity markers

1 ml of the active GOT and GPT reagents were placed in a spectrophotometric cuvette separately, and then 0.05 ml of plasma was added to each cuvette. The mixture was stirred and incubated at room temperature for 1 min. The absorbance was taken after the interval of every 30 seconds at 340 nm.

2.12. Histopathological studies

For histopathological examination, the kidneys from all experimental groups were removed and fixed in Bouin's solution, followed by paraffin embedding. Sections 5 µm thick were prepared using a microtome and stained with hematoxylin and eosin to highlight tissue morphology. These stained slides were analyzed under a compound microscope at 40x magnification to assess potential pathological changes in the glomeruli and renal tubules. The primary focus was on identifying morphological anomalies, such as signs of inflammation, necrosis, or regeneration. However, the degree of regeneration in glomeruli and renal tubules was not quantified using a specific scoring system or numerical measures in this study.

2.13. Statistical Analysis

A sample size of 36 albino mice was calculated to provide 80% power with a significance level of 0.05, with six mice per group. Data were analyzed using one-way ANOVA followed by Tukey's HSD post-hoc test to assess significant differences between groups. A p-value of < 0.05 was considered statistically significant.

3. Results

3.1. Body weight variation along the group

It was reported that the original body weight of the experimental animals had been updated to the final body weight. Seven days later, the ultimate body weight of animals in groups I and V was considerably (p 0.05) higher than that of animals in groups II, III, IV, and VI. When blended plant extract was administered to group V animals, their body weight tended to be more comparable to that of group I animals. Group II animals, who were subjected to acetaminophen-induced uremia and oxidative stress, had their ultimate body weight significantly reduced in comparison to groups I and V. We found no statistically significant difference in body weight between animals labelled as group III, group IV, or group VI. This weight variations are described in figure 1.

3.2. Estimation of hematological parameters

The results indicated significant changes in several hematological parameters, particularly in the white blood cell (WBC) and red blood cell (RBC) counts, which were lower in Group III (metformin-treated) and the experimental groups IV, V, and VI (treated with aqueous, methanolic, and ethanolic T. dioica extracts), compared to the acetaminophen-treated control (Group II) ($P < 0.05$). Hemoglobin levels were significantly reduced in Group II but were elevated in Groups III, IV, V, and VI, suggesting a restorative effect of metformin and T. dioica extracts. However, no

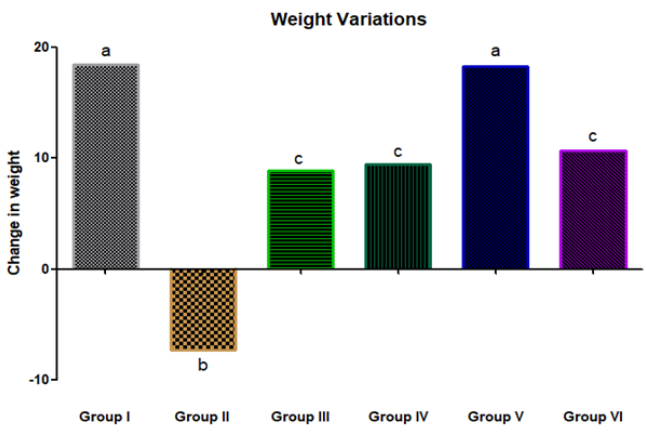

Fig. 1. Therapeutic effectiveness of *T. dioica* extract in rats with acetaminophen-induced renal failure. (Values with superscripts a, b, and c indicate statistically significant ($P < 0.05$) variation).

Table 1. Effects of different extracts on hematological parameters.

Parameter	Group I	Group II	Group III	Group IV	Group V	Group VI
WBC (×10^9/ L)	9.067 ± 0.752	7.46 ± 1.392	4.9 ± 0.656*	5.46 ± 0.867*	5.13 ± 0.551*	5.87 ± 0.81*
RBC (×10^{12}/ L)	9.13 ± 0.23	9.56 ± 0.76	7.13 ± 0.57*	6.99 ± 0.48*	7.19 ± 0.61*	7.80 ± 0.21
Hemoglobin (g/dL)	15.46 ± 0.516	5.54 ± 0.85	12.31 ± 0.77*	15.85 ± 0.73	15.95 ± 0.43	14.95 ± 0.39
Hematocrit (g/dL)	57.20 ± 1.99	57.58 ± 3.65	35.62 ± 3.11*	54.43 ± 1.81	55.39 ± 1.76	59.33 ± 1.06
Mean Corpuscular Volume (fL)	67.44 ± 0.91	65.40 ± 1.13	56.66 ± 0.33*	69.16 ± 1.03	65.31 ± 1.42	66.19 ± 1.14
Mean Corpuscular Hemoglobin (pg)	19.71 ± 0.18	17.85 ± 1.05	18.87 ± 0.44	17.80 ± 0.21	18.54 ± 0.29	17.96 ± 1.08
Mean Corpuscular Hemoglobin Concentration (g/dL)	28.17 ± 0.15	27.35 ± 1.02	30.51 ± 0.65*	28.66 ± 0.67*	29.69 ± 0.59*	28.12 ± 0.59*
Platelet (×10^9/ L)	621.79 ± 51.83	568.99 ± 95.24	251.84 ± 50.13*	671.64 ± 55.78	358.97 ± 49.07*	388.77 ± 51.03*
Lymphocyte (%)	84.86 ± 4.08	82.13 ± 4.19	81.38 ± 5.98	86.14 ± 3.12	83.29 ± 3.91	82.97 ± 1.25
Neutrophils (×10^9/ L)	11.08 ± 3.58	11.93 ± 3.48	14.50 ± 5.19	13.28 ± 3.08	13.99 ± 2.98	14.23 ± 3.16
Eosinophils (×10^9/ L)	1.59 ± 0.43	1.4 ± 0.86	1.95 ± 0.21	1.4 ± 0.34	1.61 ± 0.21	1.68 ± 0.76
Basophils (×10^9/ L)	1.11 ± 0.25	2.46 ± 0.49	2.51 ± 1.56	3.44 ± 2.13	2.96 ± 1.81	2.77 ± 1.91

Analysis of variance (ANOVA) using Dunnett's post hoc test for multiple comparisons *significantly different from group II. * Values showing significant importance as values have P <0.05.

Table 2. Effects of different extracts on serum electrolytes.

Parameter	Group I	Group II	Group III	Group IV	Group V	Group VI
Potassium (mmol/L)	6.35 ± 0.22	7.28 ± 0.66	5.58 ± 0.35	5.78 ± 0.17	5.96 ± 0.31	5.49 ± 0.28
Sodium (mmol/L)	135.59 ± 1.95	142.35 ± 2.19	149.05 ± 1.95*	151.57 ± 1.98*	155.93 ± 1.07*	152.09 ± 1.87*
Chloride (mmol/L)	113.08 ± 5.71	98.85 ± 6.31	106.22 ± 1.88	108.51 ± 1.77	108.98 ± 1.67	110.57 ± 1.86

Analysis of variance (ANOVA) using Dunnett's post hoc test for multiple comparisons *significantly different from group II.

significant differences were observed in the counts of lymphocytes, neutrophils, eosinophils, or basophils. Notably, platelet counts showed significant changes ($P < 0.05$) in Groups III, V, and VI compared to Group II, indicating the potential effect of treatments on platelet regulation. Additionally, mean corpuscular hemoglobin concentration (MCHC) was significantly altered ($P < 0.05$) in all treated groups (III, IV, V, and VI), further supporting the potential hematological benefits of these treatments. These findings are summarized in Table 1.

3.3. Estimation of electrolytes

The electrolyte analysis revealed significant differences in sodium (Na+) levels between the groups. In particular, sodium levels were significantly elevated in Groups III, IV, V, and VI (treated with metformin and Tamarix dioica extracts) compared to Group II (acetaminophen-treated group) ($P < 0.05$), as shown in Table 2. This suggests that the treatments may have contributed to the restoration of electrolyte balance, particularly in terms of sodium regulation. However, potassium (K+) and chloride (Cl-) levels did not show significant differences across the groups, indicating that these electrolytes may not be as affected by the treatments or the induced nephrotoxicity. These findings highlight the potential role of T. dioica extracts and metformin in improving electrolyte homeostasis, specifically sodium levels, under conditions of nephrotoxicity.

3.4. Assessment of renal function

In this investigation, the plasma urea (Figure 2), creatinine (Figure 3), and serum CRP concentrations (Figure 4) in Group II animals were significantly higher than those in Group I, Group III, Group IV, Group V, and Group VI owing to acetaminophen exposure. *T. dioica* aqueous,

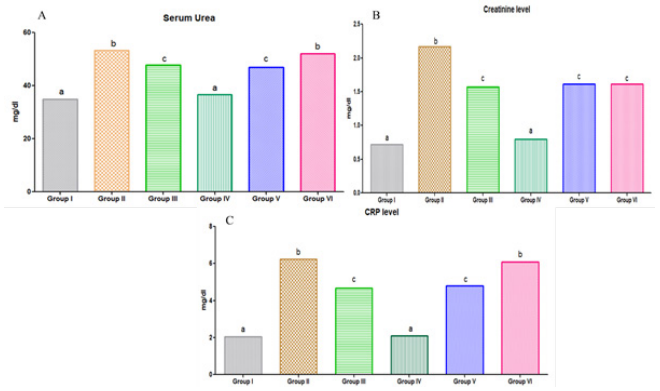

Fig. 2. Effect of different extracts of *T. dioica* on (A) plasma urea, (B) plasma creatinine level and (C) CRP level.

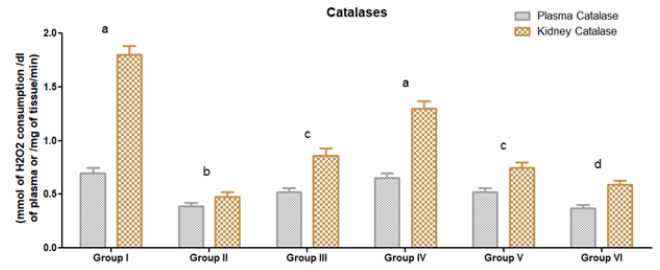

Fig. 3. Effect of different extracts of *T. dioica* on plasma and kidney catalase level (ANOVA followed t-test. Bars for specific data differ from each other significantly ($p < 0.05$)).

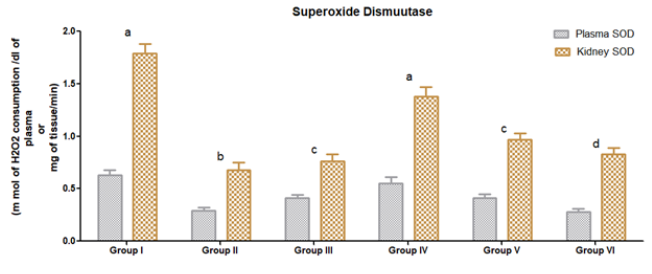

Fig. 4. Effect of different extracts of *T. dioica* on plasma and kidney SOD level (ANOVA followed t-test. Bars for specific data differ from each other significantly ($p < 0.05$)).

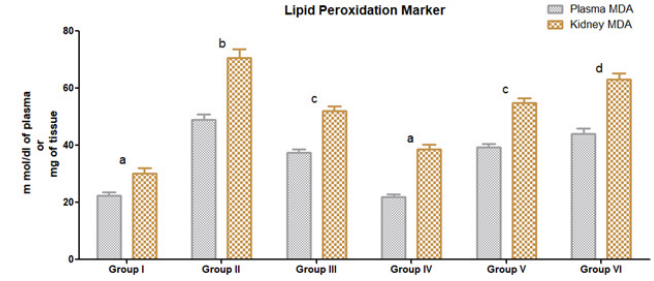

Fig. 5. Effect of different extracts of *T. dioica* on plasma and kidney MDA level (ANOVA followed t-test. Bars for specific data differ from each other significantly ($p < 0.05$)).

methanolic, and ethanolic extracts were orally administered to Group IV, V, and VI animals respectively, resulting in a substantial drop in plasma urea, creatinine, and serum CRP. Group IV animals had plasma urea, creatinine, and CRP levels that were almost identical to group I animals.

Data with various superscripts (a, b, c) vary substantially from one another ($P< 0.05$), as determined by an ANOVA.

3.5. Estimation of antioxidative enzymes profile

Figures 5 and 6 depict the results of antioxidant enzyme activity in various test groups. Groups IV, V, and VI animals treated with *T. dioica* extracts had higher plasma SOD and catalase levels following acetaminophen administration group II animals. Similar kind of results were also observed in group III animals.

Similar amounts of plasma SOD and catalase were observed in the group IV and group I animals. Hence, in comparison to the GII group (the uremic group), the plasma levels of SOD and catalase were higher in the group I, III, IV, V and VI animals ($P < 0.05$).

3.6. Estimation of toxicity markers

The levels of malondialdehyde (MDA) in the plasma and renal tissue of several animal groups are shown in Fig. 7. Surprisingly, animals in groups IV and V who were given *T. dioica* extracts in aqueous and methanolic forms respectively had MDA levels in their plasma and kidney tissue that were on par with those of animals in groups I and III. Nevertheless, compared to the MDA levels in plasma and kidney tissue of group II mice were considerably ($P < 0.05$) higher than in the control and *T. dioica* extract-treated groups.

Toxicity due to uremia and oxidative stress caused by acetaminophen was investigated by measuring blood levels of glutamate oxaloacetate transaminase (SGOT) and glutamate pyruvate transaminase (SGPT). Serum GOT and GPT activity were elevated in Group II compared to the control group by statistically significant amounts ($P < 0.05$). This significantly altered levels of SGOT and SGPT in Group II, confirming the toxicity of the high-dose acetaminophen. The toxicity caused by acetaminophen was considerably decreased ($P < 0.05$) when *T. dioica* extracts were given in groups IV, V, and VI, as well as in group III, which received metformin as well. The results regarding SGPT and SGOT are summarized in Table 3.

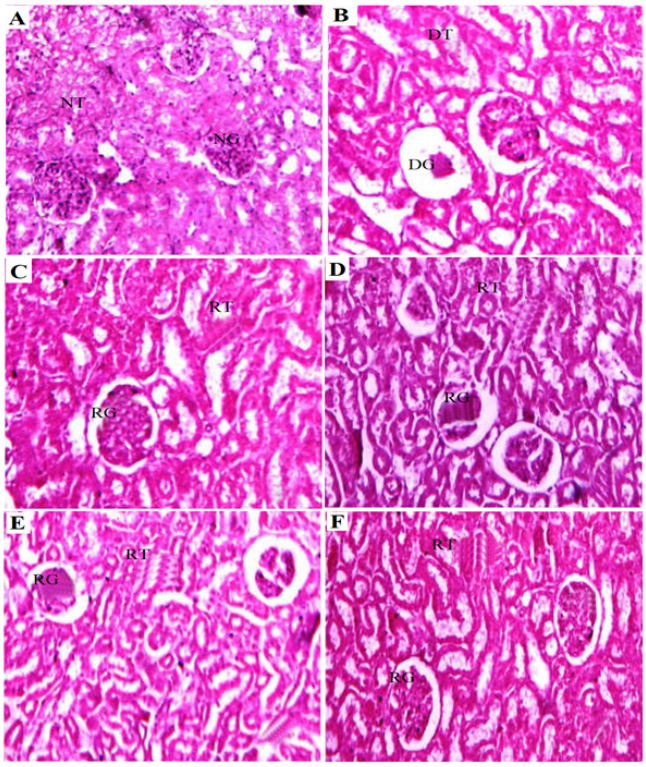

Fig. 6. Histopathological analysis of Kidney tissues. A; Group I, B; Group II, C; Group III, D; Group IV, E; Group V, F; Group VI. NG; Normal Glomerulus, NT; Normal Tubule RG; Repaired Glomerulus, RT; Repaired Tubule.

3.7. Histopathological studies

Figure 8 (A-F) displays the results of a histological examination of the kidney tissue of untreated and treated mice. Section A shows the typical organization of renal tissue. Both the glomerulus and the renal tubules seemed normal in control group I of mice. Kidney tissue damage caused by acetaminophen in a uremic animal model was

Table 3. SGPT and SGOT the toxicity markers in different groups.

Toxicity Markers		Groups					
		Group I	Group II	Group III	Group IV	Group V	Group VI
SGOT	(Unit/L of	17.7 ± 0.02^a	35.6 ± 0.04^b	21.8 ± 0.06^c	18.7 ± 0.02^a	18.1 ± 0.04^a	20.6 ± 0.04^c
SGPT	Serum)	26.4 ± 0.022^a	42.4 ± 0.026^b	32.1 ± 0.023^c	27.2 ± 0.025^a	28.6 ± 0.03^a	33.1 ± 0.028^c

shown in section B (Group II). Extensive tubular degeneration, necrosis, cell swelling, and a damaged glomerulus were all on display in this particular segment. The histological structure of kidney tissues was partially restored in the other sections (C, D, E, and F). It was observed that these parts were in better condition than section B.

4. Discussion

Medieval medicines came from medicinal herbs. Plant treatments formerly addressed many diseases. Nonetheless, safety is essential before usage [22]. Unless its toxicity is known, the extract will not be used. Several plants have medical properties; thus, their toxicity must be assessed. Medicinal plant overdoses and poisonings have occurred. Traditional medications are usually used without determining a safe dosage. This has had numerous negative effects. When it comes to treating a wide range of serious illnesses, traditional medicine has long relied on *T. dioica* as the most effective and widely accepted medicinal plant. The plant's phytoconstituents, among which phenolics are the edible polyphenols, are responsible for a wide variety of health benefits when introduced to an experimental animal model and in the human body. Extracts of the plant *T. dioica* have potent anti-oxidant properties and aid in lowering systemic toxicity by boosting levels of antioxidant enzymes (GSH, SOD, and CAT) [23].

Metformin, a well-known anti-diabetic and renoprotective agent, was used as a positive control in this study to compare its efficacy against the protective effects of Tamarix dioica leaf extracts on acetaminophen-induced nephrotoxicity. Acetaminophen, or paracetamol, a common analgesic and antipyretic, can cause severe renal and hepatic toxicity due to its metabolite, NAPQI, which depletes glutathione levels and leads to oxidative damage in the kidneys [24]. Acetaminophen is a multipurpose medication used to treat pain, fever, and inflammation and is classified as NSAID. The liver metabolizes it through cytochrome p450 enzyme and the intermediate product, N acetyl-p-benzoquinone imine (NAPQI), which is hazardous to the liver is produced [25]. In this study, metformin treatment (200 mg/kg body weight) significantly improved the hematological and biochemical parameters in the acetaminophen-induced toxicity group, indicating its renoprotective effects. Interestingly, the *T. dioica* extracts also showed promising results, as they significantly restored various markers, such as RBC count, hemoglobin levels, urea, creatinine, and electrolyte balance, demonstrating similar protective effects.

Hematological characteristics may be used to determine toxicity in living systems. The hematopoietic system is rare as an organ of interest due to the several critical roles played by blood cells. There is a turnover rate of roughly 1–3 million red blood cells, white blood cells, and platelets in a healthy adult human body; however, this number may be changed in aberrant physiological or pathological situations such as hemolytic anemia or suppressive inflammation. Many alkylating cytotoxic medicines altered hematological parameters, including the pace at which new blood cells formed and their typical range. Acetaminophen generates oxidative stress, which may be linked to red blood cell (RBC) destruction, leading to a drop in both RBC count and hemoglobin levels when used on a regular basis [26]. Current results demonstrated that several *T. dioica* extracts reduced red blood cells, hemoglobin, hematocrit, mean corpuscular volume, mean corpuscular hemoglobin, and mean corpuscular hemoglobin concentration, reducing blood oxygen-carrying capacity and causing anemia. It has been shown that *Acalypha wilkesiana* extracts cause a reduction in red blood cell count, packed cell volume, hemoglobin, and lymphocytes in rats [27].

This finding is consistent with earlier studies that reported the nephroprotective effects of Tamarix spp. extracts [28]. The plant's ability to restore electrolyte balance, particularly sodium (Na^+), and its modulation of oxidative stress markers, suggest that it holds significant promise for future therapeutic applications in preventing or treating nephrotoxicity induced by various drugs, including acetaminophen.

The results of the present study are comparable to what was discovered in prior research in which male albino rats were given acetaminophen. Concentrations of plasma urea and creatinine were altered after administration of *T. dioica* extract in all acetaminophen-treated rats. C-reactive protein (CRP) is a biomarker of tissue inflammation that is produced during the acute phase of the immune response. It was discovered that the liver is the primary source of this protein [29]. Inflamed illnesses are associated with a sharp increase in serum CRP levels. As inflammation occurs, oxidative stress, lipid peroxidation, and tissue damage all rise, and CRP levels follow suit. It's a simple way to monitor how the condition is progressing. Angiotensin-converting enzyme (ACE) inhibitors, found in many plant foods including phenolic and flavonoid chemicals, reduce the body's inflammatory response to Ang II and, in turn, lower blood CRP levels [30].

Creatinine levels rise when the kidneys are unable to excrete the waste product normally, as occurs with various disorders or after the use of incompatible medications that disrupt normal renal function. [31] predicted that increased serum creatinine levels may be an indicator of kidney injury, particularly to the nephrons responsible for filtering blood. Serum creatinine levels were a good marker for the identification of chronic kidney disease, and when they were over the reference range, renal failure was a predicted prognosis. Serum urea concentration is often seen as a more accurate indicator of renal function than serum creatinine concentration [32]. Histopathological examination revealed that after 7 days of *T. dioica* extract treatment, kidney structures in all groups had recovered to the same extent as the control following acetaminophen toxicity. This is consistent with the findings of earlier studies on the nephroprotective action of *T. dioica* extracts.

T. dioica provides renoprotection through its rich content of antioxidant compounds like flavonoids, tannins, and polyphenols, which help neutralize reactive oxygen species and reduce oxidative stress. Additionally, the plant's active compounds modulate inflammatory pathways and enhance cellular regeneration, aiding kidney tissue repair. Its ability to boost antioxidant enzyme levels, such as SOD, CAT, and GSH, further contributes to its nephroprotective effects [33]. When administered at therapeutic levels, NAPQI binds to cellular GSH and is then urined out. Acetaminophen overdose, however, prevents NAPQI from binding to cellular GSH and further depletes GSH levels in the body. Because of its ability to attach to cellular proteins, NAPQI in excess causes lipid peroxidation, which leads to the death of kidney cells. Hence, the excess of acetaminophen produces alterations in metabolic

symptoms including plasma urea, creatinine, MDA, Hb%, total RBC count, GSH, SOD, catalase levels, and KIM-1 level, and significantly occurs the disorganisations of renal and liver tissue [34].

Large amounts of serum glutamic oxaloacetic transaminase (SGOT) are found in the heart, followed by the liver, the kidneys, and the skeletal muscles. Myocardial infarction, liver disorders such as cirrhosis, viral hepatitis, liver necrosis, kidney diseases like uremia, chronic renal disease, and skeletal muscle diseases all result in an increased SGOT level in the body [35]. The liver and kidney have higher levels of SGPT. Oxidative stress damages these organs, raising serum enzyme levels. Infective hepatitis, liver cirrhosis, uremia, acute renal disease, chronic kidney disease, and skeletal muscle injury enhance the SGPT level. These findings suggest that while metformin remains a gold standard, *T. dioica* offers a natural and potentially safer alternative with renoprotective properties that deserve further exploration.

5. Conclusion

It was observed that extracts of *T. dioica* leaves administered at a dose of 400 mg/kg had the strongest neuroprotective properties and prevented acetaminophen-induced toxicity on blood biochemical parameters to the greatest degree. The methanolic extract of *T. dioica* demonstrated the greatest and most comparable neuroprotective efficacy against the neurotoxicity caused by acetaminophen.

Future prospective & recommendations

A limitation of this study is the lack of identification and quantification of specific active compounds in Tamarix dioica that contribute to its renoprotective effects. Future studies should focus on isolating these compounds and evaluating their individual contributions to kidney protection through in vitro and in vivo models. Advanced techniques such as GC-MS, LC-MS, and NMR spectroscopy can be employed to determine the molecular structure of these active compounds. Further testing in various animal models and clinical trials will be essential to fully understand their therapeutic potential and refine their use in clinical practice.

Author contribution

Conceptualization: AA, CM, FKRA, ZMM &WSA. Methodology: RMA, ZA, FKRA, ZMM & HMA. Software and Visualization: RHA, IA, CM & NAS. Formal analysis: AA, NSA, NAS, &MHS. Investigation: AA, ZMM, ZA, RHA, &NSA . Writing original draft preparation: AA, HMA, WSA, & ZMM. Editing: MHS, NSA, &NSA,FKRA & IA. Supervision: IA, NAS, CM HMA, AA. Project administration: HMA. Funding acquisition, Hailah M. Almohaimeed. Submission, Waheeb S. Aggad & MHS. All authors have read and agreed to the published version of the manuscript.

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Ethical Approvals

Institutional Review Board Statement Ethical approval was taken by the Institutional Review Board of HEALTH SCIENCE RESEARCH CENTER , Princess Nourah bint Abdulrahman University , Riyadh, Saudi Arabia. with Registration number of HA-01-R-104 and IRB log number, 23-0040E.

Conflicts of interest

Authors declare no conflict of interest.

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