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Prospective Study to Evaluate the Role of Thyme-Primula Syrup in the Amelioration of TAA-Induced Nephrotoxicity in Adult Male Albino Rats

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Abstract

NEPHROTOXICITY, which may lead to chronic kidney disease, is considered a serious health problem, particularly in developing nations. Thyme and primula extracts have demonstrated great pharmacological benefits, such as antioxidative, anti-inflammatory and anti-allergenic properties against various diseases. The objective of the current study was to explore the protective effect of using thyme and primula extracts in ameliorating nephrotoxicity in a thioacetamide (TAA) induced rat model. 24 rats were divided into 4 groups (n=6/group) for a 6-week study: Group 1 (Control): received 0.9% saline via intraperitoneal (i.p.) injection, triweekly, Group 2 (TAA): administered TAA (200 mg/kg b.wt.) i.p., triweekly, dissolved in saline, Group 3 (TAA + Thyme-Primula): received TAA as in Group 2, plus 12 mg/kg b.wt. thyme-primula syrup orally once daily, Group 4 (Thyme-Primula): received 12 mg/kg b.wt. thyme-primula syrup orally once daily. The obtained results showed a significant decrease in the levels of urea, creatinine, lipocalin-2 (LCN-2), α2-macroglobulin (α2M), interferon gamma (IFN-γ), interleukin-4 (IL-4), and malondialdehyde (MDA), and a significant increase in the levels of superoxide dismutase (SOD), and glutathione (GSH) in TAA- treated animals. Additionally, western blotting and agarose gel electrophoresis in the treated animals were significantly down-regulated in kidney injury molecule-1 (KIM-1) which was accompanied by mild smearing and significant reduction in DNA fragmentation. All these improvements were confirmed by hematoxylin and eosin (H&E) and vascular endothelial growth factor (VEGF) immunohistochemical examinations. Thus, thyme-primula syrup can provide a new approach in improving nephrotoxicity in general and ameliorating LCN-2, α2M, IFN-γ and IL-4 levels in particular.

Keywords: Thyme-primula syrup, α2-Macroglobulin; Interferon gamma, Kidney injury molecule-1, Nephrotoxicity.

Introduction

Chronic kidney disease (CKD) is a global health concern that has an impact on many individuals across the world [1], particularly in the developing nations [2]. Chronic kidney disease is closely associated with other health problems, such as diabetes mellitus [3] and cardiovascular disorders [4], however, it is developed from nephrotoxicity, which is mainly defined as kidney injury that results from extended and intense exposure to environmental pollutants, toxicants, chemicals, herbal adulterants, and medications [5].

Approximately 673.7 million individuals were affected by CKD worldwide. In Egypt, a CKD prevalence was of 33% of the total population, drawing the attention to the major problem of the disease both globally and nationally [6].

CKD, nephrotoxicity, and tests measuring kidney function are tightly interconnected to each other. Diagnosis of CKD can be carried out through blood analysis and urine tests, however, and on top of that, inflammation acts as a key element during the development of CKD, for that reason, specific inflammatory biomarkers can be estimated to measure the severity of the inflammation and the disease progression, and consequently, their influence on kidney functionality.

TAA is a well-known carcinogen that has been used to induce nephrotoxicity [1,7] in experimental animals such as rats. Specifically, TAA can cause

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massive damage to the kidney structure in the form of alterations in renal corpuscles, proinflammatory markers production [8], cytokine release, tubular epithelial cell death [9], the infiltration of inflammatory cells within renal tissue, cells degeneration [10,11], and cell death in the proximal renal tubules [12]. Notably TAA-induced nephrotoxicity triggers overproduction of reactive oxygen species (ROS) due to oxidative stress and a reduction in free radical scavenging defence mechanisms [13], which gives rise to a deterioration of cellular biomolecules, like DNA, proteins and lipids [8] which collectively, causes an overall impairment in renal function. According to Pandya et al. [14], elevated blood urea and creatinine levels are indications of kidney failure and are often used in metrics to evaluate renal functions. LCN-2 or neutrophil gelatinase-associated lipocalin (NGAL) is a tiny protein purified from neutrophil granules and plays a pivotal role as a renal damage biomarker even in the earliest alterations detected in kidney diseases. As a transporter protein, LCN-2 accounts for various physiological functions like surge of the immune response, inflammation, and metabolic equilibrium, as reported by Kiran Dahiya et al. [15], and it is found that it undergoes overexpression following renal tubular injury [16]. LCN-2 is expressed in macrophages after contacting apoptotic cells. This LCN-2 induction is activated by sphingosine-1-phophate (S1P) which is secreted by apoptotic cells, as well as the downstream stimulation of the signal transducer and activator of transcription 3 (STAT3) signalling pathway [17]. Worth mentioning, low molecular weight of LCN-2 qualifies it to be filtered through the glomerulus along with its subsequent reabsorption in the proximal convoluted tubules, thus, when renal tubular injury occurs, its filtration and absorption rates change, eventually leading to its detection in urine and serum [18].

α2M is the largest plasma protein that is synthesized chiefly in the liver [19]. In humans, elevated levels of serum α2M can be detected in many clinical disorders, including nephrotic syndrome as reported by Rehman et al. [20]. Likewise, its serum levels also increase in rats suffering from gentamicin-induced kidney failure [21], reflecting its use as a diagnostic marker in kidney injury cases.

When free radicals attack polyunsaturated fatty acids (PUFA), MDA, a 3-carbon low- molecular-weight aldehyde, is created [22]. The serum MDA level evaluates oxidative stress, and the resulted free radicals initiate lipid peroxidation leading to tissue damage in chronic renal failure, which propose MDA as a biochemical marker [23].

Natural killer (NK) cells are innate lymphocytes that have an important function in the survival of stressed autologous cells [24]. Damaged cells that show decreased or abnormal major histocompatibility complex class I produce cellular stress ligands which bind to the receptors present on NK cells to activate them. This activation may result in the generation of inflammatory cytokines, proliferation of NK cells, and cytotoxic effects [25]. Worth mentioning, IFN-γ is a proinflammatory cytokine that is generated by NK cells and has been widely linked to renal damage in both human and experimental animals [26].

On the other hand, IL-4 is considered as an essential cytokine that improves renal tubular injury in kidney diseases since it causes macrophage (M2) phenotype polarization which was firmly associated with the recovery of renal tubular injury in acute kidney disease models [27]. Recent research has shown the critical function of IL-4 in inflammation not only by preventing the production of proinflammatory cytokines, but also through the upregulation of some anti-inflammatory mediators [28].

CKD patients suffer from an increase in oxidative stress, inflammation [29], immune system deficiencies via the activation of peripheral blood polymorphonuclear (PMNs) and mononuclear (MNs) leukocytes [30]. According to Mihai et al. [31], leukocyte activation promotes the overproduction of ROS, boosting proinflammatory cytokines production. The overproduction of ROS occurs through the mitochondrial respiratory chain dysregulation, impairment of the antioxidant systems, including SOD and GSH levels [32] that lead to the oxidation of macromolecules that consequently causes tissue damage [33].

KIM-1 is an apical transmembrane protein in the cells of the proximal renal tubules [34]. During the normal body state, KIM-1 can be detected at extremely low levels, however, during inflammation or renal fibrosis, its level escalates considerably [35]. More precisely, after renal damage, matrix-metalloproteinase (MMP) causes the extracellular domain of the KIM-1 protein to be separated from the membrane, which raises the concentration of KIM-1 in the urine. Consequently, urine KIM-1 levels in individuals with acute kidney damage may increase up to 100-fold following the dissociation of this ectodomain [36]. According to Vaidya et al. [37], KIM-1 is a very sensitive biomarker for nephrotoxicity in comparison to the other traditional markers.

On the other hand, VEGF binding to its receptor (VEGFR) can induce angiogenesis [38]. Recently, it has been reported that VEGF has been involved in the advancement of CKD [39].

Historically, natural products have been used extensively to treat diverse disorders. Plant-based treatment has been presented as an old human tradition for many years and still offers an ongoing

practice to date [40]. It is a healthy, pure, and safe remedy as it is collected from natural herbal resources [41]. Recently, massive attention has been directed towards adopting the use of natural antioxidants as alternatives to synthetic ones which may show adverse side effects on health [42]. Thus, a great spectrum of natural products has been developed as existing drug candidates in the market [40]. A one major drawback of these liquid herbal extracts is that these products are sometimes should be dissolved in water and alcohol during their pharmaceutical production which make them unsuitable for some patients such as those who suffer from liver diseases or some patients of definite religious beliefs [43]. Thyme-primula syrup is a well-known herbal formula that has been prescribed broadly in the management of many pulmonary complications [44,45]. Specifically, a fixed combination of thymi herba and primula radix extract reduced the bronchitis severity score in acute bronchitis, both in adults and children [46] and it has been approved that a mixture of thyme and primula extracts had similar effects in a rat model of inflammation given in different doses [47]. This herbal formula consists of an aqueous extract of the aerial part of the thyme plant in addition to the root extract of primula [44]. Multiple studies have reported the antioxidant and anti-inflammatory activities of the thyme extract against renal oxidative stress, nephrotoxicity, and renal inflammation [48]. In addition, Primula extract also displays powerful antioxidant, anti-inflammatory, and anti-apoptotic activities against nephrotoxicity as illustrated by Turan et al. [49]. As a result, the individual effects of thyme or primula extracts on TAA-induced nephrotoxicity have been explored in previous studies, which demonstrate their protective roles through antioxidant, anti-inflammatory and anti-apoptotic mechanisms. Based on this established knowledge, this study aims at investigating the potential synergistic or additive effect of combining both extracts, which have not been previously reported, as far as we know. We believe that this approach will afford new visions into the enhanced therapeutic potential of the combined formulation in improving nephrotoxicity in general and ameliorating LCN-2, α2M, IFN-γ and IL-4 levels in particular.

Material and Methods

Chemicals

TAA was obtained from Alpha Chemika (India). Thyme-primula syrup from SANA pharma (Jordan). The syrup concentration /10 mls (500 mg non-alcoholic thyme fluid extract, 32.5 mg primula dried extract and 16.5 mg potassium sorbate) [44]. Urea and creatinine kits were obtained from Diachem Ltd (Budafoki út, Hungary). LCN-2 ELISA kit was obtained from MyBioSource Co. (California – USA). MDA, total GSH and SOD colorimetric kinetic diagnostic kits were purchased from Cell Biolabs lnc Co. (California – USA). α2M and IL-4 ELISA kits were obtained from Kamiya Biomedical Co. (Washington – USA), while IFN-γ ELISA kit was obtained from Cusabio Co. (Texas – USA). Unstained Page Ruler Unstained Protein Ladder was purchased from ThermoFisher Scientific (USA). The reference protein utilized was the β-actin Loading Control Antibody (cat. no. 4967S) and the main antibody, anti-TIM3 (KIM) antibody, was of (cat. no. FNab10125). Both were from FineTest, Wuhan Fine Biotech Co., Ltd. (China). The source of all primary antibodies was from ThermoFisher Scientific (Massachusetts, USA). Secondary antibody biotin-XX goat anti-rabbit IgG (H+L) (cat. no. 1305936) was from ThermoFisher Scientific (Massachusetts, USA). The VEGF polyclonal antibody, cat. no. PA5-85171 was bought from Invitrogen, ThermoScientific (USA) and tissue sections were covered with 1–3 drops EnVision FLEX link Detection Reagent, cat. no. K8000 (Dako, Denmark).

Animals Grouping

Twenty-four adults' male Wistar albino rats with an average body weight of 180-200 gm were purchased from VACSERA, Giza, Egypt. Animals were allowed a 1-week pre-experimentation acclimation to adjust to lab circumstances (temperature 22±2 ℃, relative humidity of 50-60% and a photoperiod of 12-hour light/dark cycle) to avoid any complications during the experiment. They received a normal diet and water *ad libitum* with fresh supplies presented daily. The present work was permitted by the Scientific Research Ethics Committee - Faculty of Science - Ain Shams University **(Approval Code: ASU-SCI/ZOOL/2023/2/3).** All animal experimentation that was carried out in the current study complied with the ARRIVE guidelines.

Experimental Design

Rats were randomly distributed into 4 groups, each group consists of 6 rats (n=6 based on the ethical considerations to reduce animal use in accordance with the 3Rs principles outlined by National Research Council, 2011). According to the upcoming plan: Group 1 (-ve control group): animals received vehicle-containing 0.9% saline via intraperitoneally (i.p.) injection triweekly for 6 weeks. Group 2 (TAA group): to induce nephrotoxicity, animals received thioacetamide were administered (TAA) 200 mg/kg body weight (b.wt.) via (i.p.) injection, three times per week for six weeks, dissolved in a 0.9% saline solution, following the protocol described by Alomar [1]. Animals in Group 3 (TAA+Thyme-primula syrup) TAA dissolved in 0.9% saline (i.p.) at 200 mg/kg b.wt. three times per week for six weeks, consistent with Group 2. Additionally, this group received oral administration of thyme-primula syrup at a dosage of 12 mg/kg b.wt. once daily for the entire six-week

experimental period, following the method outlined by Issa et al. [44]. Group 4 (Thyme-primula syrup group): animals received thyme-primula syrup via the same route and dose as in the previous group over the 6 weeks of the experiment. The body weights of all animals were recorded weekly during the entire experimental period, and by the end of the experiment, the relative and absolute weights of kidney tissues were recorded as well.

Tissue Preparation and Sample Collection

According to Harikrishnan et al. [50], all rats were anesthetized by inhaling isoflurane at the end of the experiment. Blood samples were drawn using the orbital plexus bleeding technique for biochemical analysis. To extract sera, blood samples were collected in sterile red-top collection tubes. The tubes were then allowed to clot at room temperature and centrifuged for 20 min at 3000 rpm. After that, sera were divided into tiny aliquots and stored for biochemical analysis at -20°C in labeled Eppendorf tubes. However, rats have quickly undergone cervical dislocation, and their kidneys were collected. The collected kidneys were immediately immersed in ice-cold saline. Each rat was given two transverse slices of its right kidney, with the first being homogenized using a homogenizer MPW302 Poland in phosphate buffer saline (PBS) solution at pH 7.4 (10% w/v). After homogenization, the homogenates were centrifuged for 15 min at 4000 rpm and 4°C. The supernatants were then stored at -80°C for the subsequent biochemical analysis. For the proteomics (western blotting analysis for Kim-1 protein expression) and the molecular biology tests (DNA fragmentation by agarose gel), the second part of each right kidney was sustained in PBS solution and frozen at -80°C. The left kidney of each rat was removed, washed in normal 0.9% saline, and fixed in 10% buffered formalin [51] for histological and immunohistochemical investigations.

Biochemical Studies

Blood analysis

Determination of kidney function

The sera samples were used to measure urea levels using the colorimetric technique of Talke and Schubert [52] and creatinine levels using the method of Bartels and Bohmer [53].

Determination of kidney injury marker levels

The collected sera samples were used for the assessment of LCN-2 level by ELISA according to the previously mentioned methods (cat. no. MBS059267). α2M was estimated according to the kit instructions (cat. no. KT-450).

Kidney tissue analysis

Measurement of kidney oxidative stress and antioxidative parameters

Kidney homogenates were prepared and subsequently utilized for the colorimetric kinetic measurement of malondialdehyde (MDA), glutathione (GSH), and superoxide dismutase (SOD), following the protocols specified in the kits (cat. no. STA-330, STA-312 and STA-340, respectively).

Estimation of kidney inflammatory mediators

Kidney homogenates were used for the estimation of IFN-γ and IL-4 by ELISA technique according to the manufacturer's instructions (cat. no. CSB-E04579r and KT-19377, respectively).

To avoid cross reactivity in ELISA technique, some precautions were followed as follow: all standards and samples were run in duplicates, standard curves were complied with each assay; cross-contamination were avoided by using fresh tips and reagents; cross reactivity was avoided by using kit species-specificity; all reagents were at room temperature before use; wells were not allowed to dry during the procedure.

Proteomics and Molecular biology Markers

Western blot analysis

Western blot analysis was used to determine the expression of KIM-1 protein in kidney tissue. Kidney samples were homogenized and processed according to Fischer et al. [54]. The protein contents of the resulting supernatants were stored at -80°C until further analysis. Using 8% SDS-PAGE conventionally, proteins from kidney tissue were separated. Proteins were blotted onto nitrocellulose membranes after electrophoresis. After treatment with a secondary antibody, the blots were immunolabeled with KIM-1 antibodies. Visualized at wavelength 340 nm using a UV Transilluminator gel documentation system (Analytik Jena, USA.). Calculation of the protein concentration was relative to the internal control protein (β-actin). The quantification of the western blots-stained proteins was carried out using the Densitometry Analysis. The calculation was performed using the Analytica Jena software.

DNA fragmentation by gel electrophoresis

Proteinase K digestion was used in a normal approach to extract DNA, followed by an extraction method involving a mix of phenol, chloroform and isoamyl alcohol (25:24:1) [55]. DNA pellets, resuspended in loading buffer, were loaded onto a 2% agarose gel containing 1 μg/ml ethidium bromide. The DNA fragments were separated by gel electrophoresis at a constant voltage of 25 V for 12 hours at 4 °C using TAE buffer as the running buffer. Following electrophoresis, the DNA was visualized by ultraviolet (UV) transillumination. (analytic jena UVP transilluminator, Endress+Hauser, Germany) and photographed by a digital camera. DNA damage was assessed to measure the relative intensity of

fragmented DNA bands versus intact genomic DNA ladder by an electronic imaging tool for gel analysis (ImageJ tool) followed by calculating the percentage of DNA damage by number-average length analysis.

Histological Examination

Histological and immuno-histochemical stains for kidney tissue

The collected kidney samples were washed with phosphate-buffered saline (PBS) and then fixed in a 10% buffered formalin solution. Serial sections, 5 μm in thickness, were prepared and subsequently stained with hematoxylin and eosin (H&E) Fischer et al. [56], the kidney sections were assessed for immunohistochemical staining with protein antibody for VEGF, where the sections were stained by rat-specific VEGF polyclonal antibody.

The assessment of immunoreactivity was conducted in a blind manner to all groups; therefore, codes were given to all samples to prevent subjectivity or bias in the assessment of the results [57].

Immunoreactive score calculation

In each region of interest (ROI), immune-positive cells were quantified using a counting grid, and their numbers were compared to the total number of counterstained cells. Digitally discernible stained areas allowed for the determination of the percentage of stained regions using image analysis software. To assess protein expression intensity from the immunohistochemical data, the immune-reactive score (IRS) was calculated. The IRS, ranging from 0 to 12, is derived from the product of the proportion of positive cells (scored 0–4) and the staining intensity (scored 0–3) as shown in table 1.

Statistical Analysis

Analysis of all data of the study was done by the Statistical Package for Social Sciences SPSS window software version 17.0. ANOVA test was used for statistical comparisons between groups followed by LSD test. The data are presented as mean ± standard error of the mean S.E.M. [58]. Significance is indicated in the captions of figures and legends of tables as *: P <0.05: significant, **: P <0.01: highly significant, ***: P <0.001: more highly significant, with 95% of confidence intervals of difference.

Results

Body weights, absolute and relative renal weights

Body weights revealed a significant reduction in group 2 compared with the -ve control group and the body weights returned the closest to normal weight in group 3. The relative kidney weights recorded a significant increase in (TAA group) compared to the -ve control group. After the treatment with thyme-primula syrup, a marked decrease in kidney weights of rats of group 3 was seen, no obvious changes were seen in group 4. Fig. 1 (A-C).

Biochemical studies

Blood analysis

Determination of urea and creatinine in serum

Results illustrated in Fig. 2 (A-B) showed significantly elevated urea and creatinine in TAA group in comparison to -ve control group. However, subsequent treatment with syrup in group 3 showed significantly depleted levels in the same parameters in comparison to TAA group (group 2).

Determination of kidney injury markers (LCN-2 and α2M) in serum

In the current study, LCN-2 values were escalated significantly in group 2 after comparing it to group 1 as (185.50±1.80 and 109.37±0.89, respectively), however, a significant improvement in the same parameter level has been recorded in TAA+thyme-primula syrup group as (150.13±0.77) in comparison to TAA group. Also, animals administered by TAA assigned significantly elevated α2M in comparison to group 1. Quite the opposite, statistically ameliorated results in α2M level were recorded in group 3 when compared to group 2. These were presented in Fig. 3 (A-B).

Kidney tissue analysis

Measurement of oxidative stress (MDA) and antioxidant parameters (GSH and SOD) in kidney tissue

Data presented in fig. 4 (A) exhibited significantly elevated MDA values in group 2 in contrast to group 1. However, significantly reduced values of MDA have been recorded in TAA+thyme-primula syrup group in contrast to group 2. Yet, fig. 4 (B & C respectively) revealed that GSH and SOD values in kidney tissue have been increased in group 3 in comparison to group 2, however, a significant reduction was detected in their values in TAA group in contrast to the group 1.

Estimation of kidney inflammatory mediators (IFN-γ and IL-4)

Results demonstrated in fig. 5 (A-B) showed a significant increase in IFN-γ and IL-4 in kidney tissue after TAA administration in group 2 (TAA) in contrast to group 1. In comparison to TAA group, TAA+thyme-primula syrup group showed enhancement by the action of treatment with thyme-primula syrup.

Molecular markers and proteomics

Effect of thyme-primula syrup on kim-1 protein expression by western blot technique

KIM-1 was analysed by WB to assess nephrotoxicity in rats. Data recorded in fig. (6) and

blots illustrated in fig. (7) showed a significant escalation in KIM-1 protein expression level in renal tissues in animals of group 2 (0.696±0.057) when compared to group 1 with % of change from control by 190.28%. However, after treatment with thyme-primula syrup, animals of group 3 demonstrated a significant diminution in kidney's KIM-1 protein expression level (0.493±0.01) after comparing it to the TAA group. Moreover, group 4 (thyme-primula syrup) showed a significant reduction in kidney tissues' KIM-1 levels (0.296±0.025) when compared to both group 2 and group 3, with % of change from the -ve control by 23.61%.

DNA fragmentation by gel electrophoresis

DNA damage in renal tissues was assessed by smearing as well as the lack of an intact control band as in fig. (8). The electrogram has been developed from the gel electrophoresis represented that in group 2, TAA administration caused expressive oligonucleosome-length degradation of DNA, featured by faint incomplete bands and DNA smearing especially for bands with high molecular size, also a big band appeared on the gel represents a large semi fragmented pieces of DNA indicating incomplete apoptotic fragmentation. Moreover, bands are incomplete and faint in group 2 (lane 2). DNA isolated from normal control (lane 1) showed sharp intact band, and unfragmented DNA detected by intact genomic DNA. However, DNA of rats treated with TAA and thyme-primula syrup (lane 3) shows mild smearing, while no smearing was detected in thyme-primula syrup animals (lane 4) where the bands are complete, homogenous, and brightly stained bands. TAA resulted in a considerable increase in DNA damage percentage by (85%) in group 2 compared to control (0%). Administration of TAA and thyme-primula syrup for group 3 caused a significant minimization in DNA fragmentation percentage to 40%. Also, Thyme-primula syrup for group 4 caused DNA damage by 25%.

Histological and immunohistochemical examination

H&E stain of kidney tissue sections

The kidney sections from rats of group (1) showed a normal kidney histology pattern with a normal architecture of the glomerulus and tubules (Fig. 9 (A) & Fig. 9 (B)). Sections of TAA group showed extensive degeneration of kidney architecture with marked glomerular damage and congestion, mononuclear cell infiltration, tubular necrosis and hyaline casts, desquamation of tubular epithelium and loss of normal architecture (Fig. 9 (C) & Fig. 9 (D)). Kidney tissue sections of group 3 showed less corruption than that in group 2 putting mild mononuclear cell infiltration and mild glomerular congestion into consideration (Fig. 9 (E) & Fig. 9 (F)). The kidney tissue of group 4 showed regular kidney architecture resembles the -ve control group (Fig. 9 (G) & Fig. 9 (H)).

Immunohistochemical with VEGF staining for kidney tissue

Protomorphic images illustrate the expression and localization of VEGF polyclonal antibody in the renal tissue of various experimental groups (Fig. 9 from (I) to Fig. 9 (P)). The images of group 1 showed mild VEGF expression, group 2 showed immunopositivity of VEGF antibody which was shown as membranous, cytoplasmic and perinuclear localization, group 3 revealed moderate VEGF expression compared to group 2, while group 4 showed a trend toward normal kidney morphology with mild VEGF expression. IRS are presented in table (3) and comparative analysis for the % of VEGF reactive cells are shown in table (4) & fig. (10, 11).

Discussion

Kidneys are more prone to the destruction by toxic substances due to the huge volume of blood flowing through them and the high concentrations of toxins that can be concentrated in the renal tubes [1] which may lead to CKD that is considered a significant dangerous life threat [59].

TAA is an industrial toxin which damages the proximal renal tubules [60]. The current study found that rats receiving TAA experienced a notable drop in body weight compared to the control group. Interestingly, these rats undergo a significant increase in both the actual and proportional weight of their kidneys, consistent with the prior findings of Lim et al. [61]. This might stem from the digestive problems, the reduced appetite, or the substantial losses of water, salts, and the proteins due to the kidney injury, ultimately causing dehydration and weight loss [62]. However, the enlarged kidneys in the TAA group point to the toxic effects of TAA, which disrupts trace elements and causes structural kidney damage. Notably, the elevated kidney weights partially returned to normal after treatment with thyme-primula syrup.

The study also found more highly significant elevated levels of creatinine and urea in group 2, which confirms the functional kidney impairment. Zargar et al.,[10] also observed the same finding because these small molecules are normally filtered by the kidney's glomeruli [63]. Creatinine is not reabsorbed and is eliminated from the body, although around 40–50% of urea is reabsorbed in the tubules, as it is related to reabsorption of sodium and water [64].

Also, the current results showed more highly significant increased levels of LCN-2, α2M, MDA in group 2 in comparison to group 1, as well as an important reduction in GSH and SOD compared to their levels in group 1. Also, the concurrent study

showed a more highly significant increase in the inflammatory mediators IFN-γ and IL-4 levels in kidney tissue after TAA administration in group 2 compared to group 1.

LCN-2 shows increased levels in the blood or urine of mice with kidney damage, making it a potential indicator of renal issues [65]. The fate of LCN-2 is influenced by polyamines as the addition of polyamines facilitates its clearance from the bloodstream, whereas their removal stabilizes the protein, possibly causing it to be accumulated in tissues [66]. It was stated that an overproduction of LCN-2 may lead to kidney damage [67,68].

Pejchinovski et al. [69] revealed increased levels of α2M in acute kidney injury (AKI) which was postulated to be due to non-specific leakage and trapping of plasma proteins [70]. Additionally, data recorded in this study revealed a noteworthy increase in MDA levels in TAA animals as Ghanim et al. [71] stated that before. An increase in the free radicals triggers MDA levels [72]. It was found that TAA is metabolized *in vivo* to free radical derivatives, resulting in high levels of MDA [13].

Data presented by Vida et al. [73] revealed reduction in SOD and GSH amounts in TAA rats, markers of oxidative stress [74], resulting in an alteration in the integrity of plasma membranes and mitochondrial membranes initiating the kidney damage [75], impairment of cell proteins, then repression of both cell restoration and division [76].

Preceding studies showed that IFN-γ and IL-4 levels were more significantly increased by TAA administration [11]. TAA-administration triggers an inflammatory response inducing upregulations of many pro-inflammatory cytokines such as IFN-γ, IL-4 inducing renal inflammation [2]. IL-4 upregulation leads to the penetration of neutrophils in the renal tissue and then an induction of renal injury occurs [77].

Despite the scientific data that has been generated in the past decades, a reliable cure for kidney diseases with the low side effects and the low cost remains ambiguous. In the concurrent study, the influence of thyme-primula syrup as a novel combined treatment for nephrotoxicity in rats was investigated.

Thyme extract could help sustain the normal kidney function and structure that were approved by our data. Subsequent treatment with syrup in group 3 (TAA+thyme-primula syrup) revealed a more highly significant decrease in urea and creatinine markers, while more highly significant improvement in LCN-2 level when comparing them to TAA group. In addition, TAA rats treated with thyme-primula (group 3) assigned statistically ameliorated results in MDA, α2M, GSH, SOD levels when compared to TAA group. Also, in comparison to TAA group, group 3 showed wide enhancements by the action of this combined treatment of thyme and primula in the IFN-γ and IL-4 levels.

Research by Soliman et al. [48] indicated thyme extract improvements of urea, creatinine, MDA, GSH, and SOD in mice, confirming its anti-inflammatory effects. Ku and Lin [78] proposed that thymol exerts its anti-inflammatory action by influencing IFN-γ and IL-4. Similarly, Turan et al. [49] observed that treatment with *Primula vulgaris* leaf significantly boosted the antioxidant GSH and lowered the kidney damage markers (blood urea nitrogen and creatinine) along with the indicators of oxidative stress and lipid peroxidation (oxidative stress index and MDA). The high content of phenolic compounds in *Primula vulgaris*, is recognized for its antioxidant [79] and anti-inflammatory [80] abilities, positions it as a potential therapeutic aid in CKD by neutralizing harmful free radicals and regulating inflammatory processes [81]. Furthermore, another study [82] indicated that thymol's antioxidant, anti-inflammatory, and anti-apoptotic characteristics likely contribute to its protective effects on the kidneys, possibly by counteracting gentamicin's tendency to increase serum levels of LCN-2 and KIM-1 in rats. And so, TAA promotes ROS, leading to lipid peroxidation, mitochondrial dysfunction, and cell damage. One of the key inflammatory mediators is IFN-γ, which amplifies immune cell activation and increases pro-inflammatory IL-4. Kidney injury biomarkers such as urea, creatinine and LCN-2 rise significantly during TAA-induced damage, indicating nephrotoxicity, and all these regain to approximately normal values prior Thyme-primula treatment.

On the other hand, KIM-1 has been proven to be a very sensitive biomarker in the early detection of the renal injury and the nephrotoxicity in humans and animal models as well [83]. KIM-1 protein level was assessed by western blot technique which revealed a significant elevation in KIM-1 level in kidneys of group 2 animals by 3 – 4 folds when compared to the -ve control group, thus accounting for nephrotoxicity. This outcome is consistent with Lim et al. [61] and Ghanim et al. [71] which can be explained by the toxic effect of TAA on the proximal and distal tubules [61]; [11]; [7] & [1], or due to KIM-1 correlation to inflammation and fibrosis that accompany the renal disease as Van Timmeren et al. [34] stated, or may be due to the cleavage of KIM-1 ectodomain which consequently leads to its shedding and depositing in urine after proximal tubular injury as declared by Bolignano et al. [84].

Using agarose gel electrophoresis in genomic DNA analysis asserted the apoptosis occurred by TAA and exhibited obvious DNA degradation in kidneys cells. The fragmentation ladder pattern is typically recognized in apoptosis due to endonuclease mediated inter-nucleosomal

fragmentation of DNA. Nevertheless, in necrosis, DNA degradation develops the following cell rupture, when chromatin is digested by cellular proteases and endonucleases enzyme into a smear pattern rather than a ladder pattern, due to proteases action that eliminate the histones and expose the complete DNA length to the nucleases [85]. In the current investigation, agarose gel revealed the mixed smearing and laddering of DNA fragments due to non-specific DNA fragmentation, which might have been caused by both apoptosis and necrosis.

This obtained nephrotoxicity and DNA fragmentation after the administration of TAA may be represented because of intracellular Ca^{2+} escalation. In addition to activating endonuclease (caspase-3/CAD), an increase in cytosolic or intranuclear Ca^{2+} can also activate proteases and/or phospholipases. These enzymes destroy the cells irreversibly by attacking the crucial macromolecular targets such plasma membranes and poly (ADP-ribose) polymerases [86]. Secondly, this intracellular Ca^{2+} escalation may lead to subsequent consequences, like futile redox cycling of Ca^{2+} tilting the normal metabolic balance of the cell toward oxidative stress [87]. Also, DNA laddering throughout this experiment suggested that the intracellular Ca^{2+} dysregulation might be involved [88], or the peroxidative degradation of membrane polyunsaturated fatty acids has been postulated to cause oxidative damage to DNA.

Furthermore, after the treatment with thyme-primula syrup, animals of group 3 showed a significant decrement in the expression level of the renal KIM-1 protein compared to group 2 resulting in a mitigation in the renal tissue damage. This finding was in line with Fouad et al. [82] and Soliman et al. [48]. This observation may be due to the antioxidant activity of thymol that scavenges the released ROS resulting from nephrotoxicity [89]. And since KIM-1 is expressed in kidneys in order to clean debris from the damaged renal cells [90], thyme is proposed to help in restoration of KIM-1 levels in animals treated with thyme-primula syrup.

Regarding the primula role, Alinezhad et al. [91] illustrated that primula displays a moderate protective effect against the oxidative stress in rat erythrocytes suffering from hemolysis. Also, Alinezhad et al. [92] confirmed the richness of primula with antioxidant compounds that dropped the oxidative stress in rats' brains induced by Fe^{2+} lipid peroxidation. Thus, it can be thought that the recent reduction in the renal KIM-1 protein expression level is probably due to the dual antioxidant activity of thyme-primula syrup which reduced the oxidative stress in nephrotoxic rats, that ultimately led to a decline in KIM-1 levels.

It is worth mentioning there is no available literature regarding the ameliorative role of combining thyme and primula on KIM-1 expression and α2M in rats, thus, our current study supports the use of thyme-primula syrup in kidney diseases.

Here in, the administration of thyme-primula syrup in TAA rats caused a reduction in the fragmented DNA %, highlighting the ameliorative effect of thyme-primula syrup that caused a drop in the percentage of DNA damage from 85% to 40%. This was in line with Panahi Kokhdan et al. [93] and Abdelgawad et al. [94]. This may be attributed to its high antioxidant properties that improved the renal cells' damage. Also, this reduction in the DNA damage is perhaps owing to the anti-inflammatory, and anti-apoptotic activities of thymol as declared by Mahran et al. [95].

This study showed that the co-administration of primula to thymol has doubled the magnitude of improvements that took place in the DNA integrity of kidney cells in the rats exposed to TAA. This is compatible with Turan et al. [49] and ALİYAZICIOĞLU et al. [96]. This might be because of the richness of primula in saponins, tannins, alkaloids, terpenes, and phenolic compounds that showed antigenotoxic and anti-inflammatory properties [97]. It is noteworthy that phenolic compounds in primula exert massive antioxidant activity because of their chain breaking, hydrogen donating, metal chelating, and the free radical scavenging capability [98]. Based on the above, the western blot analysis revealing elevated KIM-1 expression in TAA-treated rats, alongside increased DNA fragmentation that was observed in gel electrophoresis, collectively indicates significant renal injury at both the protein and genomics. Also, treatment with thyme-primula syrup markedly reduced both KIM-1 expression and DNA damage, indicating the ameliorative effect of the syrup.

Through the present research, H&E images of kidney sections in rats of -ve control group showed a normal kidney histology pattern with a normal architecture of the glomerulus and tubules. Otherwise, in group 2, TAA caused kidney injury which marked by glomerular damage and congestion, mononuclear cell infiltration, tubular necrosis and hyaline casts, desquamation of tubular epithelium and the loss of normal architecture. Our observations are compatible with those obtained by Alshahrani et al. [99].

As TAA is extensively used in experimental animals of renal toxicity [100], it was stated that it is quickly processed to TAA-S-oxide and ROS [101]. These reactive metabolites are concerned with the pathogenesis of renal impairment as the kidneys are more susceptible to damage than other organs [102].

In the kidney, VEGF and its receptors are widely expressed in different types of cells, it is expressed most prominently in the glomerular podocytes, the distal tubules, collecting ducts, and to a fewer extent in some proximal tubules [103], whereas VEGF receptors are basically expressed by the endothelial cells of the glomerular and the peritubular capillaries [104]. Results presented in this study showed a strong positive VEGF expression in TAA group. This result is consistent with Kim et al. [105]. Liu et al. [106] also showed that VEGF expression in the glomerular epithelial cells essentially maintain the normal glomerular functions and provide a filtration barrier, whereas the aberrant increased expression of VEGF may potentially be detrimental in terms of the renal functions.

Besides, the current investigation showed that thyme abated the histopathological damage induced by TAA in group 3. These consequences were approved by Abd El-Kader et al. [107]. These results obviously demonstrate the antioxidant and protective effect of thyme extract. In addition, the VEGF immunostaining was returned to normal after treatment with thyme-primula syrup and this was in line with Saravanan & Pari [108]. On the other hand, Prasad & Prasad [109] revealed that primula components aid in the treatment of tubular congestion and glomerular degeneration. Yamabe et al. [110] reported that protocatechuic acid in primula, improved the histological appearance of the rat kidneys and reduced the tubular cell damage, these results are compatible with our own study data.

Conclusion

Generally, through this present novel study, it could be observed by biochemical, molecular, proteomics, and histopathological examination that the combined treatment of thyme and primula together in a syrup enhanced the treatment of nephrotoxicity provoked by TAA in rats by letting down the oxidative stress, inflammatory mediators, kidney function profile, kidney injury markers, and DNA fragmentation, as well as, increasing antioxidant enzymes that all together, ameliorated the kidney damage and restored its histological architecture. The present work has a limitation as by addressing it in future studies, it will improve the assay accuracy and strengthen the translational potential of the findings. This limitation includes the adjoining of another experimental group that examines a reference drug prescribed to patients of CKD or nephrotoxicity to compare it to the present syrup formulation.

Abbreviations

ANOVA: Analysis of variance; b.wt.: Body Weight; CKD: Chronic kidney disease; GSH: Glutathione; H&E: Hematoxylin and Eosin; i.p.: Intraperitoneal; IL: interleukin; IFN-γ: Interferon gamma; IRS: Immune-reactive score; KIM-1: kidney injury molecule-1; LCN-2: Lipocaline-2; MDA: Malondialdehyde; MMP: Matrix-metalloproteinase; MNs: Mononuclear; NGAL: Neutrophil gelatinase-associated lipocalin; NK: Natural killer; PBS: Phosphate buffer saline; PGE2: Prostaglandin E2; PMNs: Polymorphonuclear; PUFA: Polyunsaturated fatty acids; PVE: Primula vulgaris leaf; ROI: Region of interest; ROS: Reactive oxygen species; SIP: Sphingosine-1-phophate; SOD: Superoxide dismutase; TAA: Thioacetamide; TNF-α: Tumor necrosis factor-α; VEGF: Vascular endothelial growth factor; α2M: α2-macroglobulin.

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Declaration of Conflict of Interest

The authors declare that there is no conflict of interest.

Ethical of approval

This study follows the ethics guidelines of the Scientific Research Ethics Committee, Faculty of Science, Ain Shams University, Egypt (Approval Code: ASU-SCI/ZOOL/2023/2/3).

TABLE 1. The immune-reactive score (IRS) for immunohistochemical data interpretation

A (percentage of positive cells)	B (intensity of staining)	IRS score (multiplication of A and B)
0 = no positive cells	0 = no colour reaction	0-1 = negative
1 = <10% of positive cells	1 = mild reaction	2-3 = mild
2 = 11-50% positive cells	2 = moderate reaction	4-8 = moderate
3 = 51-80% positive cells	3 = intense reaction	9-12 = strongly positive
4 = >80% positive cells	**Final IRS score (A × B): 0-12**	

TABLE 2. Interpretation of DNA fragmentation

	Lane 1	Lane 2	Lane 3	Lane 4
DNA fragmentation	Negative	Positive	Positive	Positive
Band shape	Regular, sharp	Incomplete, irregular outlines	Intact, irregular outlines	Intact, irregular outlines
DNA smearing	negative	+3 "marked"	+1 "mild"	negative
% of DNA damage	0	85%	40%	25%
DNA integrity	Excellent	Extremely poor	fair	good

DNA fragmentation score test results: 15% or less fragmentation – excellent DNA integrity, 15-to 25% DNA fragmentation – good to fair, 25 – 50% DNA fragmentation – fair to poor, 50% or greater DNA fragmentation – extremely poor DNA integrity.

TABLE 3. Immune-reactive Score (IRS) of VEGF in renal tissue of the different experimental groups

Sample no	Group	Positive cells		Fluorescence intensity		IRS (0-12)
		Percentage (%)	Score	Intensity	Score	
G1 (1)	Negative control	40	2	mild	1	2
G1 (2)	Negative control	42	2	mild	1	2
G1 (3)	Negative control	38	2	mild	1	2
G2 (1)	TAA group	80	3	intense	3	9
G2 (2)	TAA group	72	3	intense	3	9
G2 (3)	TAA group	85	4	intense	3	12
G3 (1)	TAA+Thyme-primula syrup	70	3	moderate	2	6
G3 (2)	TAA+Thyme-primula syrup	65	3	moderate	2	6
G3 (3)	TAA+Thyme-primula syrup	58	3	moderate	2	6
G4 (1)	Thyme-primula syrup	50	2	mild	1	2
G4 (2)	Thyme-primula syrup	48	2	mild	1	2
G4 (3)	Thyme-primula syrup	46	2	mild	1	2

TABLE 4. Comparative analysis for the % of VEGF reactive cells using ANOVA test

ANOVA test for % of VEGF positive cells	
F	73.80
P value	0.0128
P value summary	*
Statistically significant ($P < 0.05$)?	Yes
R squared	0.9736

TABLE 5. Comparative analysis for VEGF protein expression (IRS) using ANOVA test.

ANOVA test for VEGF protein expression (IRS)	
F	27.9
P value	0.001
P value summary	***
Statistically significant ($P < 0.05$)?	Yes
R squared	0.91

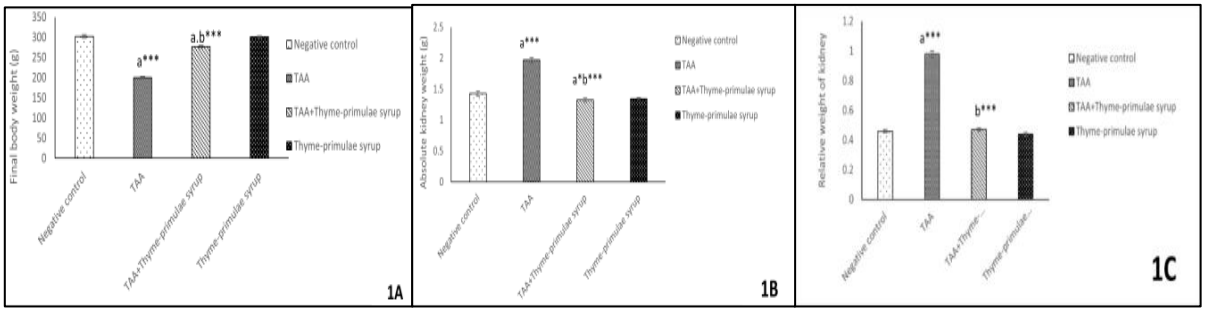

Fig. 1. The graphs present the mean ± standard error of the mean (S.E.M.) for (A) body weight, (B) absolute kidney weight, and (C) relative kidney weight across the different experimental groups. Error bars indicate the S.E.M., calculated from 6 rats per group. Statistical analysis was performed using ANOVA. The symbol 'a' denotes a significant difference compared to the negative control group, while 'b' indicates a significant difference compared to the TAA group. Significance levels are indicated as follows: * = *P* < 0.05, * = *P* < 0.001. TAA represents thioacetamide.**

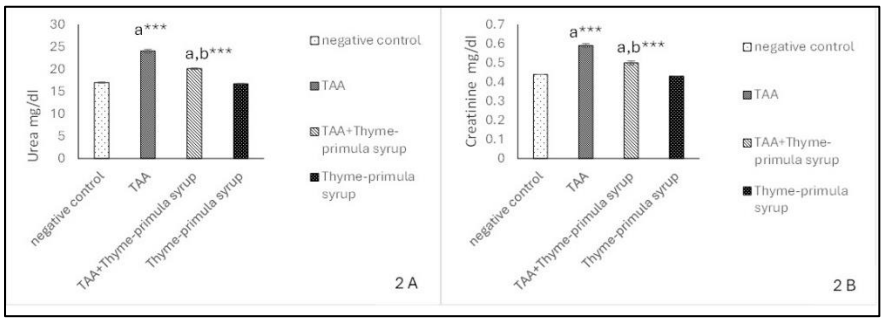

Fig. 2. The graphs illustrate the mean ± S.E.M. for (A) serum urea levels (mg/dl) and (B) serum creatinine levels (mg/dl) in the various experimental groups. Error bars represent the S.E.M., derived from 6 rats per group. Statistical analysis was conducted using ANOVA. The symbol 'a' signifies a significant difference compared to the negative control group, and 'b' denotes a significant difference compared to the TAA group. Significance is indicated by * = *P* < 0.001. TAA stands for thioacetamide.**

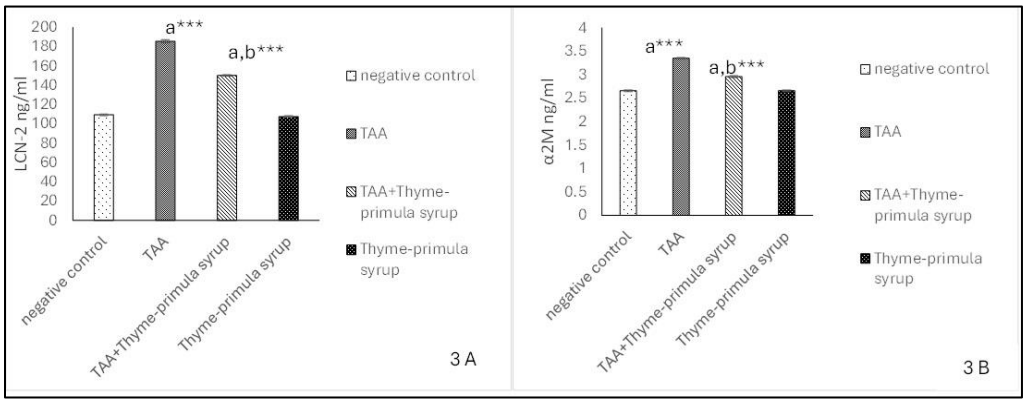

Fig. 3. The graphs depict the mean ± S.E.M. for (A) serum lipocalin-2 (LCN-2) levels (ng/ml) and (B) serum α2-macroglobulin (α2M) levels (ng/ml) in the different experimental groups. Error bars represent the S.E.M., calculated from 6 rats per group. Statistical analysis was performed using ANOVA. The symbol 'a' indicates a significant difference compared to the negative control group, and 'b' signifies a significant difference compared to the TAA group. Significance is denoted by * = *P* < 0.001. TAA represents thioacetamide; LCN-2 stands for lipocalin-2; α2M denotes α2-macroglobulin.**

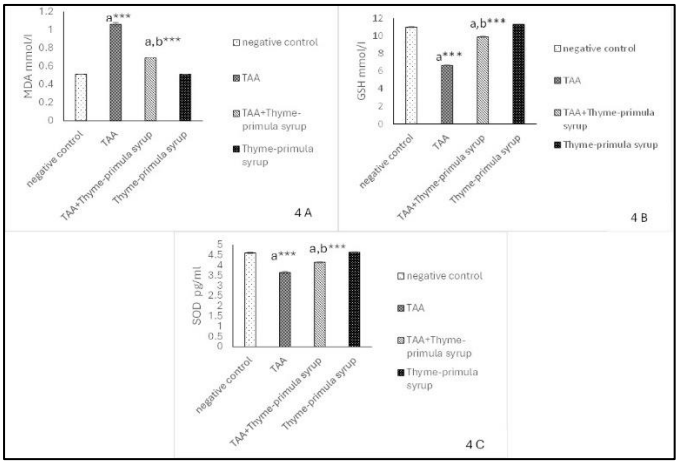

Fig. 4. The graphs show the mean ± S.E.M. for (A) kidney malondialdehyde (MDA) levels (mmol/l), (B) kidney glutathione (GSH) levels (mmol/l), and (C) kidney superoxide dismutase (SOD) levels (pg/ml) across the different experimental groups. Error bars represent the S.E.M., derived from 6 rats per group. Statistical analysis was conducted using ANOVA. The symbol 'a' indicates a significant difference compared to the negative control group, and 'b' signifies a significant difference compared to the TAA group. Significance is indicated by * = *P* < 0.001. TAA represents thioacetamide; MDA stands for malondialdehyde; GSH denotes glutathione; SOD represents superoxide dismutase.**

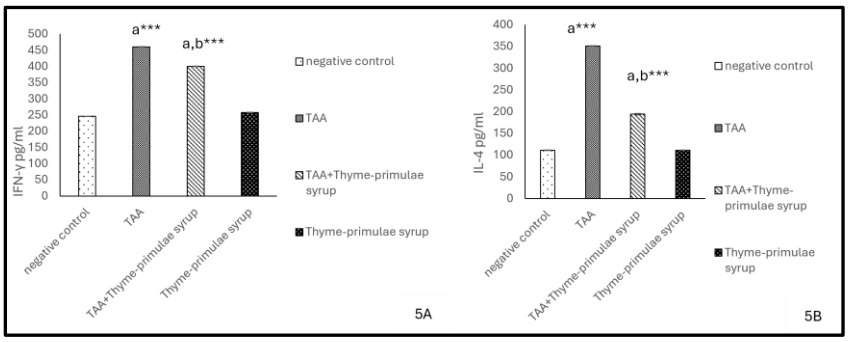

Fig. 5. The graphs present the mean ± standard error of the mean (S.E.M.) for (A) kidney interferon-gamma (IFN-γ) levels (pg/ml) and (B) kidney interleukin-4 (IL-4) levels (pg/ml) across the different experimental groups. Error bars indicate the S.E.M., calculated from 6 rats per group. Statistical analysis was performed using ANOVA. The symbol 'a' denotes a significant difference compared to the negative control group, while 'b' indicates a significant difference compared to the TAA group. Significance levels are indicated as follows: * = *P* < 0.001. TAA represents thioacetamide; IFN-γ stands for interferon-gamma; IL-4 denotes interleukin-4.**

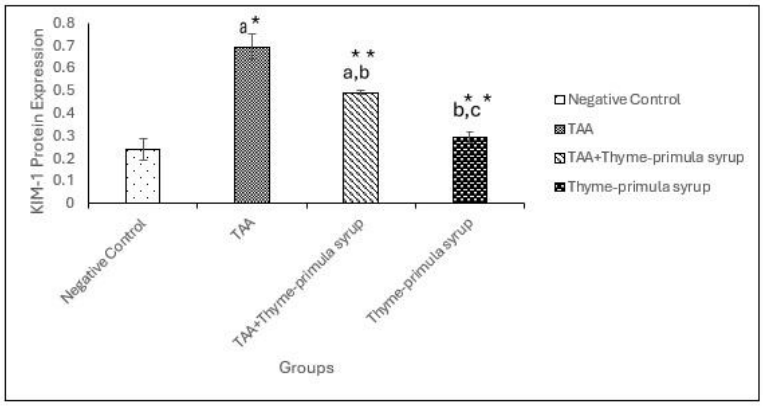

Fig. 6. The graph illustrates the mean ± S.E.M. of the relative quantity of kidney injury molecule-1 (KIM-1) protein expression in the renal tissue of different groups, as determined by Western blot (WB) analysis and normalized to β-actin intensity. Statistical analysis was conducted using ANOVA. The symbol 'a' signifies a significant difference compared to the negative control group, 'b' denotes a significant difference compared to the TAA group, and 'c' indicates a significant difference compared to the TAA+thyme-primula syrup group. Significance is indicated by * = *P* < 0.05. TAA stands for thioacetamide.

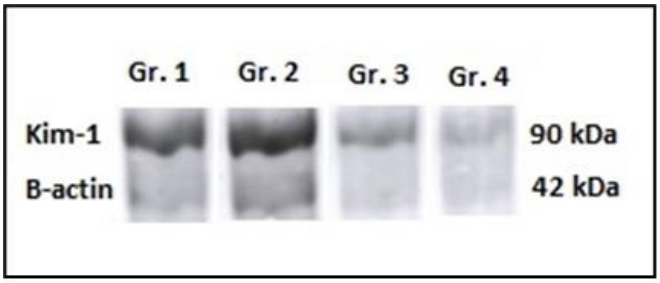

Fig. 7. Representative western blots analysis depicting KIM-1 protein levels performed on protein extract from kidney tissue as intensity relative to β-actin as a housekeeping protein. Gr. 1: -ve control; Gr. 2: TAA; Gr. 3: TAA+thyme-primula syrup; Gr. 4: thyme-primula syrup. Each lane represents 1 rat from each group. Original blots are included within the supplementary western blot data "Full-length blots are presented in Supplementary Fig. (1)"

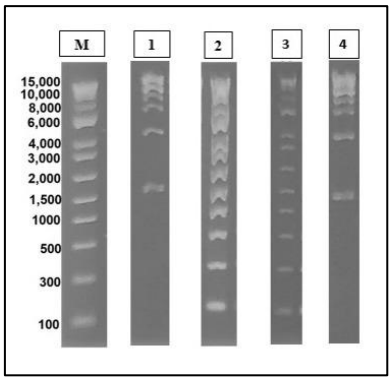

Fig. 8. 2% Agarose gel electrophoresis of DNA attained from rat renal tissue, where 1 = negative control group, 2 = TAA group, 3 = TAA+thyme-primula syrup group, 4= Thyme-primula syrup group. From the gel image it is evident that there is intact DNA in the normal control lane (1). TAA treatment introduced DNA damage, marked by the smearing of DNA lane (2). Whereas there is a decrease in DNA smearing lane (3) and no smearing is present in lane (4). (M) represents DNA ladder "Full-length gel is presented in Supplementary Figure (2)"

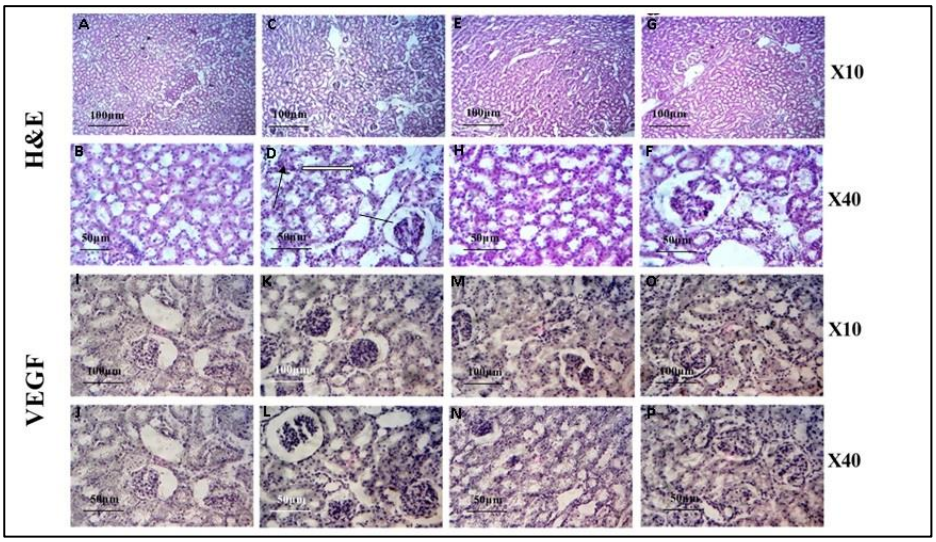

Fig. 9. Photomicrographs of kidney tissues from adult albino rats across different experimental groups

(A) and (B) (Group 1: Negative Control): Representative images showing normal kidney architecture (H&E staining; 100X and 400X magnification, respectively).

(C) and (D) (Group 2: TAA Group): Images revealing extensive degeneration of kidney architecture, including marked glomerular damage and congestion (indicated by a line), mononuclear cell infiltration, tubular necrosis (indicated by a hollow arrow), hyaline casts (indicated by a solid arrow), and desquamation of the tubular epithelium (H&E staining; 100X and 400X magnification, respectively).

(E) and (F) (Group 3: TAA + Thyme-Primula Syrup): Images showing less severe damage compared to Group 2, characterized by mild mononuclear cell infiltration and mild glomerular congestion (H&E staining; 100X and 400X magnification, respectively).

(G) and (H) (Group 4: Thyme-Primula Syrup): Images showing approaching normal kidney architecture with no apparent alterations (H&E staining; 100X and 400X magnification, respectively).

The same figure also illustrates the expression and localization of vascular endothelial growth factor (VEGF) polyclonal antibody in the renal tissue of various experimental groups:

(I) and (J) (Group 1: Negative Control): Images showing mild VEGF expression (VEGF immunostaining; 100X and 400X magnification, respectively).

(K) and (L) (Group 2: TAA Group): Images revealing strong positive VEGF expression (VEGF immunostaining; 100X and 400X magnification, respectively).

(M) and (N) (Group 3: TAA + Thyme-Primula Syrup): Images showing moderate VEGF expression compared to Group 2 (VEGF immunostaining; 100X and 400X magnification, respectively).

(O) and (P) (Group 4: Thyme-Primula Syrup): Images appeared more morphologically similar to normal kidney architecture with mild VEGF expression (VEGF immunostaining; 100X and 400X magnification, respectively).

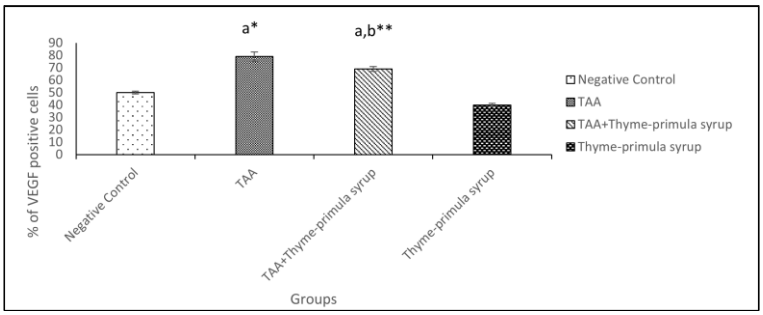

Fig. 10. The graph illustrates the mean ± S.E.M. of the % of VEGF immunoreactive cells in the renal tissue of different groups. Statistical analysis was conducted using ANOVA. The symbol 'a' signifies a significant difference compared to the negative control group, 'b' denotes a significant difference compared to the TAA group. Significance is indicated by * = *P* < 0.05, ** = *P* < 0.01. TAA stands for thioacetamide.

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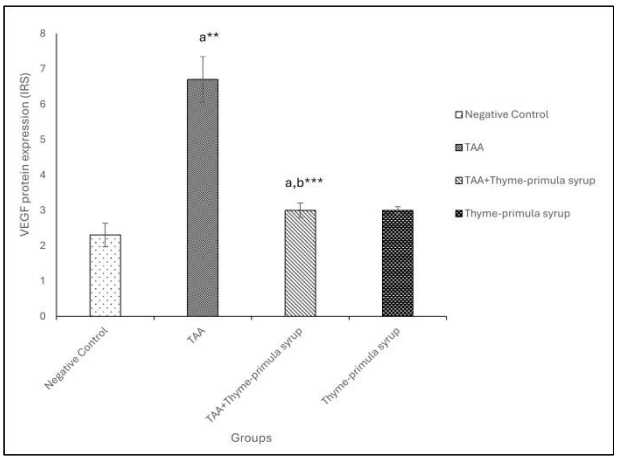
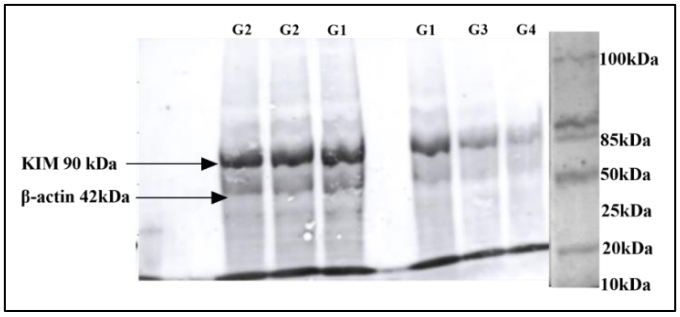
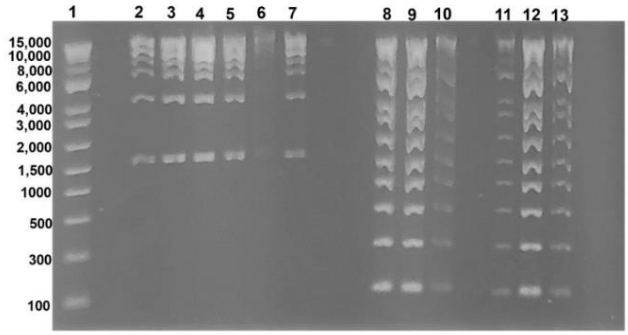


Fig. 11. The graph illustrates the mean ± S.E.M. of the VEGF protein expression (IRS) in kidney tissue of different groups. Statistical analysis was conducted using ANOVA. The symbol 'a' signifies a significant difference compared to the negative control group, 'b' denotes a significant difference compared to the TAA group. Significance is indicated by ** = *P* < 0.01, * = *P* < 0.001. TAA stands for thioacetamide, IRS stands for immunoreactive score.**



Supplementary Fig. 1. Supplementary western blot data



Supplementary Fig. 2. Supplementary Gel electrophoresis data

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دراسة مستقبلية لتقييم دور شراب الزعتر-زهرة الربيع في تحسين السمية الكلوية المحدثة بالثيوأسيتاميد TAA في ذكور الجرذان البيضاء البالغة

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الملخص

يُعتبر تسمم الكلى (اعتلال الكلى) والذي قد يؤدي إلى مرض الكلى المزمن، مشكلة صحية خطيرة، خاصة في الدول النامية. وقد أظهرت مستخلصات الزعتر وزهرة الربيع فوائد دوائية كبيرة، مثل خصائصها المضادة للأكسدة والالتهابات والحساسية ضد الأمراض المختلفة. كان الهدف من الدراسة الحالية هو استكشاف التأثير الوقائي لاستخدام مستخلصات الزعتر وزهرة الربيع في تحسين تسمم الكلى في نموذج الفئران المستحث بالثيواسيتاميد (TAA). تم تقسيم ٢٤ فأرًا إلى ٤ مجموعات (ن=٦/مجموعة) لدراسة استمرت ٦ أسابيع: المجموعة ١ (الضابطة): تلقت محلول ملحي ٠,٩% عن طريق الحقن داخل الصفاق (i.p.)، ثلاث مرات أسبوعيًا. المجموعة ٢ (TAA): أعطيت TAA (٢٠٠ ملجم/كجم من وزن الجسم) عن طريق الحقن داخل الصفاق، ثلاث مرات أسبوعيًا، مذابًا في محلول ملحي. المجموعة ٣ (TAA + زعتر-زهرة الربيع): تلقت TAA كما في المجموعة ٢، بالإضافة إلى ١٢ ملجم/كجم من وزن الجسم من شراب الزعتر-زهرة الربيع عن طريق الفم مرة واحدة يوميًا. المجموعة ٤ (زعتر-زهرة الربيع): تلقت ١٢ ملجم/كجم من وزن الجسم من شراب الزعتر-زهرة الربيع عن طريق الفم مرة واحدة يوميًا. أظهرت النتائج التي تم الحصول عليها انخفاضًا كبيرًا في مستويات اليوريا والكرياتينين و(LCN-2) Lipoocalin-2 وα٢-ماكروجلوبيولين (α2M) والإنترفيرون جاما (IFN-γ) والإنترلوكين-٤ (IL-4) والمالونديالدهيد (MDA)، وزيادة كبيرة في مستويات إنزيم فوق أكسيد الديسموتاز (SOD) والجلوتاثيون (GSH) في الحيوانات المعالجة بالـ TAA. بالإضافة إلى ذلك، أظهرت تقنية وسترن بلوتنج والتفريد الكهربائي بجل الأجاروز في الحيوانات المعالجة تنظيمًا سلبيًا كبيرًا في جزيء إصابة الكلى-١ (KIM-1) والذي كان مصحوبًا بمسح خفيف وانخفاض كبير في تجزؤ الحمض النووي (DNA). تم تأكيد كل هذه التحسينات عن طريق فحص النسيجي بواسطة صبغة الهيماتوكسيلين والإيوزين (H&E) والفحوصات الكيميائية المناعية لعامل نمو بطانة الأوعية الدموية (VEGF). وبالتالي، يمكن لشراب الزعتر-زهرة الربيع أن يوفر نهجًا جديدًا في تحسين تسمم الكلى بشكل عام وتحسين مستويات LCN-2 و α2M و IFN-γ و IL-4 بشكل خاص.

الكلمات الدالة: ثيوأسيتاميد ، ألفا ٢ (α2M) ، الإنترفيرون جاما (IFN-γ) ، التعبير الجيني لجزيء إصابة الكلى -١ (KIM-1) ، سمية الكلى.