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Research Article

Reno-protective effects of benzbromarone and tart cherry against cyclosporine A-induced nephrotoxicity via SIRT1/PPAR-γ/ABCG2

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ABSTRACT

Cyclosporine A (CsA), a widely used immunosuppressant in organ transplantation, is associated with nephrotoxicity. This research study investigated the potential protective effects of benzbromarone, a uricosuric agent, and tart cherry, a natural antioxidant, against CsA-induced nephrotoxicity in a rat model. Fifty male albino rats were split at random into five groups: control group, CsA group, CsA + benzbromarone group, CsA + tart cherry group, and combination group. Biochemical markers of kidney function, oxidative stress, and inflammation were evaluated after four weeks of treatment. Histopathological analysis of kidney tissue and molecular assessments of SIRT1, PPARγ, URAT1, and ABCG2 expressions were also assessed. Results demonstrated that CsA treatment significantly elevated serum creatinine, and uric acid levels, along with increased oxidative stress markers (MDA) and inflammatory markers (TNF-α, IL-6, NF-κB). Benzbromarone and tart cherry, individually and in combination, significantly reduced these levels. The combination therapy demonstrated superior efficacy, with significant improvements in renal function and greater reductions in oxidative stress and inflammation compared to each drug alone. Histopathological analysis confirmed the protective effects, showing reduced kidney damage in the combination group. Molecular analysis revealed upregulation of SIRT1, PPARγ, and ABCG2, and downregulation of URAT1 in treated groups, with the combination therapy exhibiting the most significant effects. In conclusion, both benzbromarone and tart cherry, especially in combination, protect against CsA-induced nephrotoxicity through antioxidant, anti-inflammatory, and uricosuric activities. These results show that combination therapy might be a promising therapeutic approach for mitigating CsA-associated kidney damage, warranting further clinical investigation.

Keywords: Benzbromarone, Tart cherry, Cyclosporine A, Hyperuricemia, Oxidative stress, Nephrotoxicity.

1. INTRODUCTION

Nephrotoxicity is a problem with a significant incidence, and it is caused by the application of several therapeutic drugs. Nephrotoxicity can profoundly affect kidneys, and in the worst case, lead to renal failure[1]. Several drugs like cancer chemotherapy drugs and drugs used for immunosuppression are well documented for causing nephrotoxicity[2]. Because the kidneys receive a large volume of blood flow and play an important role in excretion of drugs, they are easily damaged

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by drugs. Timely detection and intervention through preventive measures such as hydration and dosing strategies are critical to minimize kidney injury and safeguard patient safety[1,3] Transplant procedures commonly utilize cyclosporine A due to its effectiveness in preventing rejection, an immunological response to foreign body tissues. Through the inhibition of calcineurin by cyclosporine A (CsA), the prevention of rejection is realized through blockade of T cell activity which is an important immune response. More importantly, CsA has a narrow therapeutic index and has severe side effects such as renal toxicity, hypertension, and hyperuricemia[4]. CsA can induce hyperuricemia in as much as 50% of subjects[5,6]. In addition, around 10% of subjects may experience gout. Consistently, due to its nephrotoxicity, blood levels of CsA and kidney function must be monitored in order to reduce the harmful effects of therapy while improving patient outcomes at the same time[7,8] .

At present, researchers still don't fully understand how CsA can damage kidneys[9]. However, according to more recent research, oxidative stress is a major cause and important mechanism[10,11] to cause CsA nephropathy. After being given CsA, the body produces too many reactive oxygen species and lowers the levels of antioxidants like glutathione (GSH) and superoxide dismutase (SOD)[12] . Other previous studies have shown that hyperuricemia exacerbates CsA-induced nephrotoxicity[13]. CsA can hurt renal tubular cells indirectly by increasing the production of osteopontin[14]. Inflammation damages renal cells, characterized by elevated levels of cytokines that promote inflammation, including interleukin 6 (IL-6) and tumor necrosis factor alpha (TNF-α)[15] and one important target molecule of CsA is NF-kB, which helps explain how CsA can damage kidneys[16]. This research intends to address the contribution of these signaling pathways and their 'crosstalk' to the molecular events responsible for CsA nephrotoxin.

The commentary surrounding the traditional treatment of gout with benzbromarone has particularly focused on its possible nephroprotective effects. By promoting the renal excretion of uric acid, benzbromarone lowers serum uric acid levels and reduces the risk of hyperuricemia[17]. A previous study determined that benzbromarone inhibits reabsorption of uric acid via downregulation of URAT1 (Urate transporter 1). Another study showed that benzbromarone exhibits affinity for PPARγ and upregulates the expression of this protein[18]. It has been shown PPARγ modulates the expression of ATP-binding cassette transporter, subfamily G, member 2 (ABCG2)[19]. Benzbromarone exhibits uric acid-lowering effects in addition to anti-inflammatory and free radical scavenging activities, positioning it as a potential therapeutic agent for mitigating CsA-induced toxicity[20].

Tart cherry receives considerable acknowledgement from individuals because of its nutritional value; Its rich phytochemicals such as anthocyanins which are known to have great antioxidants and anti-inflammatory activities. So far tart cherries have been clinically researched for their health benefits[21]. This involves guarding against muscle pain and improving cardiovascular health[21]. These properties make tart cherries useful in enhancing recovery after exercise and decreasing muscle soreness. In addition, tart cherries are a natural source of melatonin which is an antioxidant and a free radical scavenger[22]. It is well established that tart cherry can be protective against chronic CsA nephrotoxicity due to its melatonin antioxidant content and SIRT1/Nrf2 pathway activation[23]. This action leads to the increase in Nrf2 downstream gene products including superoxide dismutase (SOD) and glutathione (GSH)[24]. Some studies have shown the ability to mitigate blood uric acid which helps gout and arthritis[25]. Eating tart cherries may have many health benefits[26].

This study aimed to examine the nephroprotective effects of tart cherries and benzbromarone and their combination against nephrotoxicity induced by CsA in rats. It also explored the molecular mechanisms which explain their nephroprotective effects.

2. MATERIAL AND METHODS

2.1. Animals

The study sample consisted of fifty male albino rats, each eight weeks old and weighing between 200 and 230 grams. The animals were sourced from the Theodore Bilharz Research Institute (TBRI) in Giza, acquired from and kept under control conditions at the animal house of the Faculty of Pharmacy, Tanta Universitye. The rats were housed in cages made of plastic and bedding of wood shavings was provided. The animals were offered tap water and libitum and routine pelleted food. The rats were given one week of acclimatization before the beginning of the experiments which is a prerequisite to all laboratory work.

The procedures of the study were executed with approval from the Research Ethics Committee at the Faculty of Pharmacy, Tanta University (REC-TP). The study followed institutional and international guidelines set by the Council for International Organization of Medical Sciences (CIOMS). Ethical approval was granted with protocol code TP/RE/10/24M-002."

2.2. Chemicals

Cyclosporine A (CsA) was procured in capsule form under the trademark Neoral® (Novartis) and formulated as a suspension in 1% carboxymethyl cellulose (CMC) for oral administration by gavage. Benzbromarone and tart cherry powder were sourced from Sigma-Aldrich (St. Louis, MO, USA) and were not included in the 1% CMC solution for oral consumption.

2.3. Experimental design

Once the acclimatization period finished, the rats were placed into five groups, each containing ten animals. One of the groups served as a vehicle control which received subcutaneous injections of carboxymethyl cellulose (CMC) once daily for four weeks, at a volume equal to that of the CsA treated group. The CsA group was given CsA subcutaneously at a dose of 25 mg/Kg daily for four weeks[27]. The CsA + benzbromarone group received CsA (subcutaneously) and benzbromarone (orally) for a total of ten days which was the duration of the experiment. They received benzbromarone at 10mg/kg and CsA at 25 mg/kg[28]. Likewise, the CsA + tart cherry group received a combination of tart cherry and CsA for four weeks. CsA was administered at 25mg/kg subcutaneously and tart cherry was given at 500 mg/kg orally[29]. Finally, the combination group received a daily dose of 25mg/kg of CsA subcutaneously, 10mg/kg/kg of benzbromarone and 500mg/kg of tart cherry (orally) for a total of 4 weeks **(Figure 1)**.

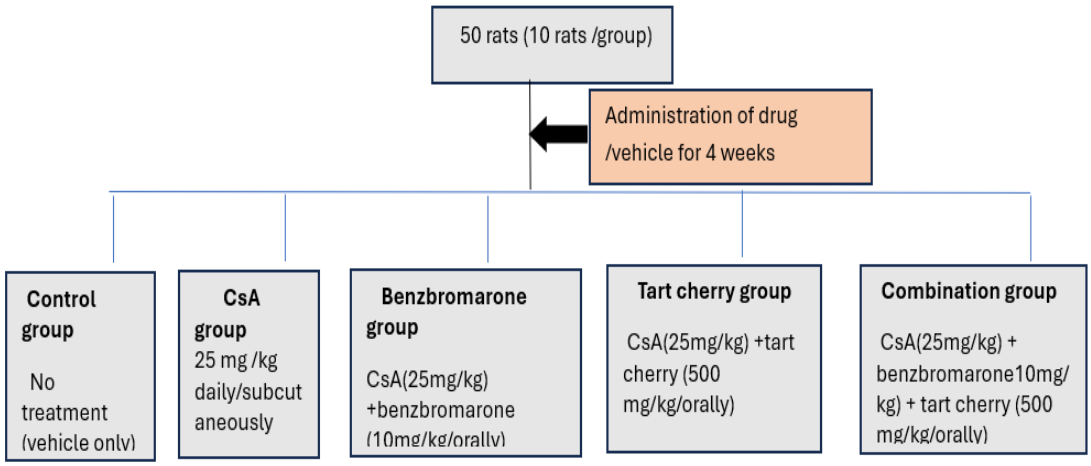

Figure 1. Experimental design

2.4. Sample collection

The animals were euthanized at the end of the study. The whole blood collection was done through cardiac puncture, and serum was extracted and stored at -20 °C for later biochemical assessment. The left kidneys were placed in a 10% neutral buffered formalin for histological examination and the right kidneys were stored at -80°C for molecular and biochemical investigations.

2.5. Determination of kidney biomarkers

Serum creatinine and uric acid levels were measured with a commercial kit from Biodiagnostic Co. [Giza-Egypt]. Measurements were performed with the urease colorimetric method according to (Henry et al., 1957; Fawcett et al.,1960)[30]. This provided accurate assessment of the kidney's functioning in the tested groups.

2.6. Determination of oxidative stress markers in kidney tissue

2.6.1. Superoxide dismutase

The activity of superoxide dismutase (SOD) was assessed using a commercial kit from Biodiagnostic Co (Giza, Egypt) as described previously in the literature [31,32]. The kidney tissues were mixed together in 5–10 mL of cold buffer that had 50 mM potassium phosphate (pH 7.5) and 1 mM EDTA per gram of tissue. The test checks how well the enzyme works in inhibiting the degradation of nitroblue tetrazolium dye by phenazine methosulphate. SOD activities were measured both the control and the sample's absorbance at 560 nm for five minutes at 25°C.

2.6.2. Lipid peroxides (measured as malondialdehyde)

Malondialdehyde (MDA) levels were quantified using the method described in [33] . Mixing MDA and thiobarbituric acid (TBA) in an acidic environment at 95°C for 30 minutes produces a TBA-reactive substance. The absorbance of the pink product was measured at 534 nm utilizing a

Biodiagnostic Co. kit from Giza: Egypt. We quantified the MDA content in the samples colorimetrically.

2.6.3. Glutathione

Glutathione (GSH) levels were also measured with a Biodiagnostic Co. kit, following the protocol in [32]. About 5 to 10 mL of cold buffer with 50 mM potassium phosphate (pH 7.5) and 1 mM EDTA per gram of tissue was mixed with the kidney tissue. A test is done to see how well the enzyme stops phenazine methosulfate from breaking down the nitroblue tetrazolium dye. GSH activities were measured the absorbance at 560 nm for five minutes at 25°C for both the control and treated samples.

2.7. Assessment of inflammatory markers

ELISA kits were used to determine the levels of TNF-α, IL-6, and NF-κB in renal tissues. Kits were purchased from Bioassay (Houston, USA) for TNF-α (Cat. No. 123456), and R&D Systems (Minneapolis, USA) for IL-6 (Cat. No. 654321), and MyBioSource (San Diego, USA) for NF-κB (Cat. No.ER1187). measurements were conducted according to the manufacturers' protocol procedures

2.8. Histopathological examination of kidney tissue

Kidney tissue specimens were preserved in 10% neutral buffered formalin for 72 hours. The tissues were processed through a series of graded ethanol concentrations, cleared with xylene, and embedded in Paraplast tissue embedding medium. The sections were placed on glass slides after being cut with a rotary microtome to a thickness of 4 µm. At least three sections per sample were stained with hematoxylin and eosin (H&E) for general histological assessment. The slides were then evaluated in a blinded manner by an experienced histologist using a full HD microscopic imaging system (Leica Microsystems GmbH, Germany) [34].

2.9. Western blot analysis

To find primary antibodies targeting URAT1 (Abcam, ab123456) and ABCG2 (Santa Cruz, sc-654321) transporters, we made whole-cell lysates from kidney tissues. Total cell extracts with 50 to 100 µg of protein were prepared using sodium dodecyl sulfate sample buffer. We used sodium dodecyl sulfate-polyacrylamide gel electrophoresis (PAGE) on the extracts and then used western blot to look at the results. Protein samples ranging from fifty to one hundred micrograms were mixed with the same amount of sample buffer and subsequently denatured by heating at 95°C for ten minutes.

Semi-dry electroblotting was used to move the proteins to a nitrocellulose or PVDF membrane after gel electrophoresis. For one hour, the membrane was moved around 5% bovine serum albumin (BSA) in tris-buffered saline with 0.05% Tween-20. The membrane was eventually incubated overnight at 4°C with primary antibodies targeting URAT1 and ABCG2. Secondary antibodies conjugated with horseradish peroxidase were subsequently used to incubate the membrane, and sample signals were identified using enhanced chemiluminescence reagents in accordance with the producer's specifications[35].

2.10. Real time polymerase chain reaction for PPAR-γ and SIRT1 gene

Each 100 mg of kidney tissue was treated with one mL of TRIzol™ RNA isolation reagent. We spread out the nucleoprotein complex completely by using a WIteg homogenizer [HG-15D, WIteg Labor Technik GmbH, Korea] for five minutes. We securely sealed the tubes and agitated them to achieve homogeneity after introducing 0.2 mL of chloroform. After an incubation period of 2 to 3 minutes, we centrifuged the tubes at 12,000 g for 15 minutes at 4°C. We spun the mixture at high speed, separating it into three distinct layers: a red phenol-chloroform layer, an interphase, and a colorless water phase. Utilize a pipette to carefully transfer the RNA-laden aqueous phase to a new tube.

Introduce 0.5 mL of isopropanol to the aqueous phase for RNA precipitation. Incubate the tubes at 4°C for ten minutes. A white gel-like RNA pellet was generated following centrifugation of tubes at 12,000 × g for 10 minutes at 4°C. Upon meticulous removal of the supernatant, the RNA pellet persisted. Following a brief vortex, we washed the pellet with one ml of 75% ethanol and centrifuged it for five minutes at 7,500 g at 4°C. Subsequent to ethanol removal, air-dry the RNA pellet for 5 to 10 minutes. The pellet was rehydrated in 20-50 µL of RNase-free water and incubated at 55°C for fifteen minutes to ensure full dissolution.

A reverse transcription kit transformed retrieved RNA into complementary DNA (cDNA). The procedure was used by cDNA for RT-qPCR to quantify the expression levels of the PPAR-E and SIRT1 genes. It was used the Power SYBR® Green PCR Master Mix and the 7500 real-time PCR System in the RT-qPCR reactions. The researchers established the thermal cycling parameters as follows: The procedure commenced with an initial denaturation at 95 °C for four minutes, succeeded by forty cycles consisting of ten seconds at 95°C, thirty seconds at 60°C and ten seconds at 72 °C.

Subsequently, the $2^{-\Delta\Delta Ct}$ method was implemented [36], and the data was presented as fold changes in comparison to the control gene expressions. The primer sequences for B-actin, PPAR-γ, and SIRT1 genes are shown in **Table 1**.

Table 1: Primers' sequences employed in RT-qPCR analysis

Gene	Sequence
PPAR-ϒ [37]	Forward: 5'-GAGTTCCTCAAGCCCTTTGGT-3'
	Reverse: 5'-GGCATCTCTGTGTCAACCATG-3'
SIRT1 [38]	Forward: 5'-GTGTCAGATAAGGAAGGGTTGTTG-3'
	Reverse: 5'-TGGCAAGTGGAGATTGTTGG-3'
B-actin [39]	Forward: 5'-GTGTCAGATAAGGAAGGGTTGTTG-3'
	Reverse: 5'-TGGCAAGTGGAGATTGTTGG-3'

SIRT1: silent information regulator T1, PPAR-γ: peroxisome proliferator-activated receptor gamma

2.11. Statistical analysis

Data were expressed as mean ± standard deviation (SD). Statistical comparisons were conducted using one-way analysis of variance (ANOVA), followed by Tukey's test for multiple comparisons. A p-value of less than 0.05 was considered statistically significant. All analyses were conducted using GraphPad Prism version 5.0 (GraphPad Software, San Diego, CA, USA).

3. RESULTS

3.1. The Combination of Benzbromarone and Tart cherry improves renal function

This study showed that compared to the normal control group, the CsA group had higher levels of serum creatinine (542.67%), and serum uric acid (41.13%), $P < 0.05$. In contrast, the benzbromarone group showed a significant decrease in these markers by 43.67%, and 24.09%, respectively, $P < 0.05$, while the tart cherry group displayed significant reductions of 40.98%, and 20.16%, respectively, compared to the CsA group. In comparison to the group that received CsA, the group that received combination therapy demonstrated significantly greater reductions in serum creatinine and serum uric acid results by 70.12%, and 24.69%, respectively, $P < 0.05$ **(Figure 2)**.

Additionally, the combination group exhibited significant decreases in serum creatinine, and serum uric acid compared to benzbromarone group (45.76%, 0.79%, respectively) and tart cherry group (49.38%, 5.67%, respectively, $P < 0.05$ **(Figure 2)**.

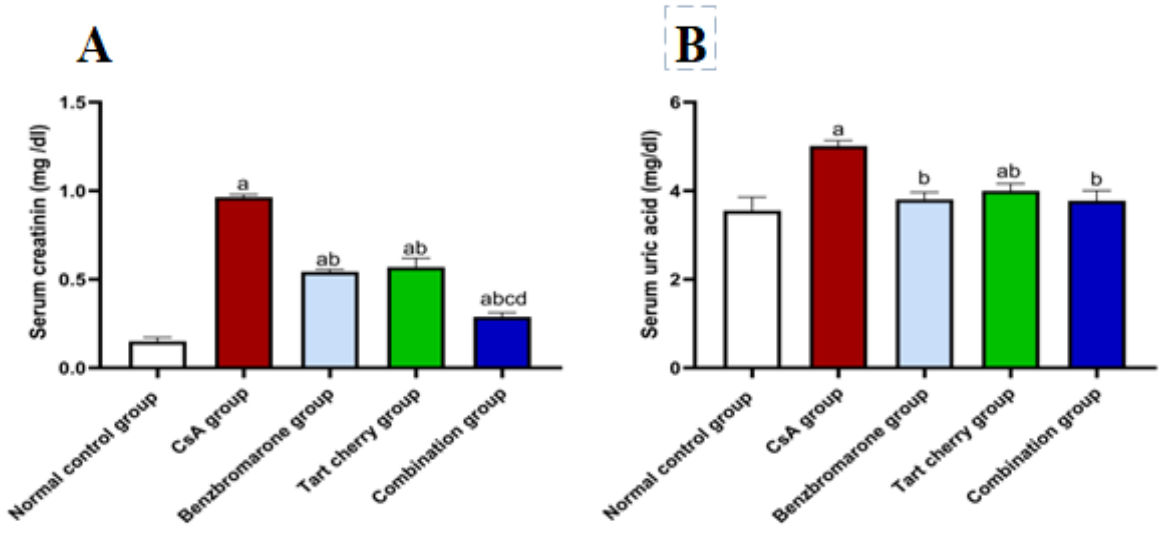

Figure 2. Effects of benzbromarone, tart cherry, and their combination on renal function markers. (A) Serum creatinine levels and (B) serum uric acid levels. Data are expressed as mean ± SD (n=10). The following letters indicate statistically significant differences at $p < 0.05$ compared to their respective groups: a: normal control group, b: CsA group, c: benzbromarone group, and d: tart cherry group.

3.2.The combination of benzbromarone and tart cherry attenuates tissue oxidative stress parameters

CsA group demonstrated a statistically significant increase in MDA content, rising by 245.25% compared to the normal control group. However, the benzbromarone group (40.56%), tart cherry group (39.1%), and combination group (56.10%) significantly reduced MDA content compared to the CsA group. Additionally, the combination group demonstrated a significant reduction in MDA levels by 45.76% compared to the benzbromarone group and 27.87% compared with tart cherry group, $P < 0.05$. **(Figure 3)**.

In addition, the results showed a significant reduction in SOD (64.17%) and GSH activities (56.59%) in the CsA group relative to the normal control group. Compared to the CsA group, the benzbromarone group significantly increased SOD and GSH activities (75.9% and 85.17%, respectively), also, the tart cherry group caused significant increment in SOD and GSH activities (76.45% and 65.17%, respectively), and the combination group had higher levels of SOD and GSH activities (131.30% and 100.63%, respectively). The activities of SOD and GSH were significantly elevated in the combination group relative to the benzbromarone group (31.56% and 8.35%, respectively) and the tart cherry group (31.09% and 21.47%, respectively) $P < 0.05$. **(Figure3).**

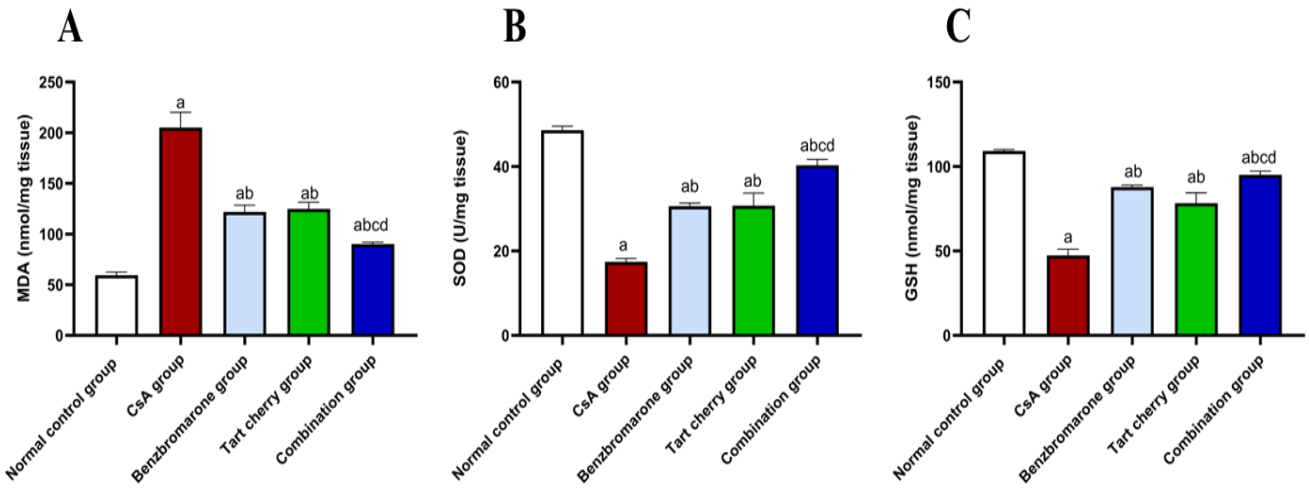

Figure 3. Impact of benzbromarone, tart cherry, and their combination on oxidative stress markers in renal tissues. (A) Malondialdehyde (MDA) levels, (B) superoxide dismutase (SOD) activity, and (C) glutathione (GSH) activity were analyzed. Data are presented as mean ± SD. The following letters indicate statistically significant differences at $p < 0.05$ compared to their respective groups: a: normal control group, b: CsA group, c: benzbromarone group, and d: tart cherry group.

3.2. The combination of benzbromarone and tart cherry enhance SIRTI and PPAR-γ expression

Using PCR to measure SIRT1 and PPAR-γ content showed that both markers were significantly lower in the CsA group relative to the normal control group, by 66.37% and 72.61%, respectively. There was a significant increase in benzbromarone group (142.01% for SIRT1 and 159.71% for PPAR-γ), the tart cherry group (155.74% for SIRT1 and 169.42% for PPAR-γ), and the combination group (5180.47% for SIRT1 and 217.63% for PPAR-γ) compared to CsA. Also, the combination group had much higher amounts of SIRT1 and PPAR-γ than the benzbromarone group (15.89% and 22.30%, respectively) and the tart cherry group (9.67% and 17.89%, respectively) $P < 0.05$ **(Figure 4)**.

3.3. The combination of benzbromarone and tart cherry reduces inflammatory cytokines

ELISA assays indicated that the concentrations of TNF-α, IL-6, and NF-κB were markedly elevated in the CsA group relative to the normal control group. TNF-α levels rose by 493.31%, IL-6 levels rose by 247.69%, and NF-κB levels rose by107.06% compared to normal group .But when Comparing to the CsA group, their levels were much significant lower in the benzbromarone group (55.61% for TNF-α, 43.69% for IL-6, and 33.70% for NF-κB), the tart cherry group (56.34% for TNF-α, 40.86% for IL-6, and 35.33% for NF-κB), and the combination group (72.46% for TNF-α, 63.88% for IL-6, and 46.73% for NF-κB). TNF-α, IL-6, and NF-κB levels were also significantly lower in the combination group compared to the benzbromarone group (37.97%, 35.85%, and 19.66%, respectively) and the tart cherry group (36.93%, 38.93%, and 17.63% respectively) $P < 0.05$ **(Figure 5)**

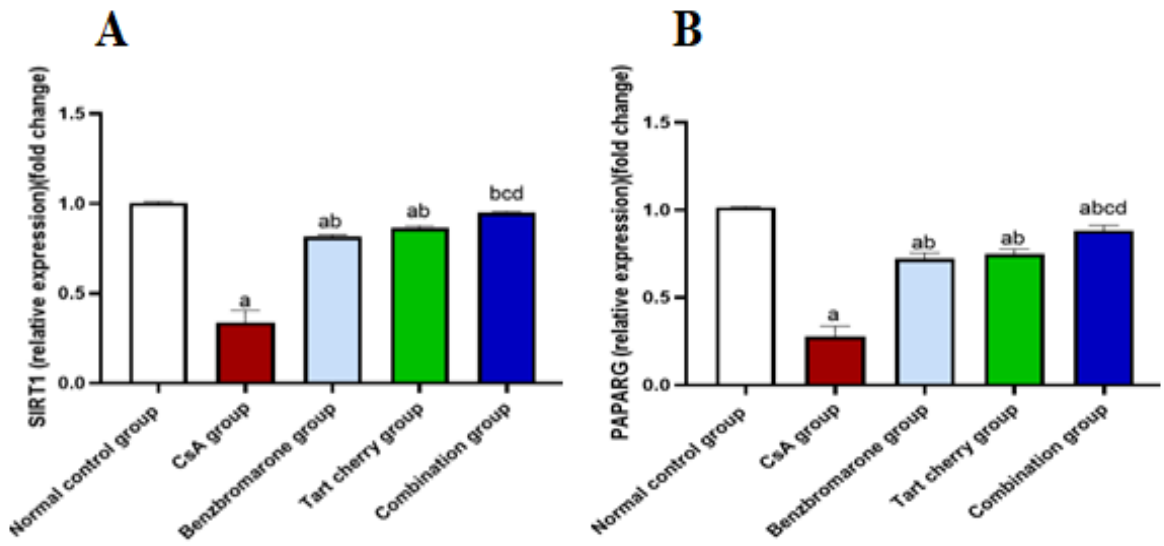

Figure 4. Gene expression analysis of SIRT1 and PPAR-γ in renal tissues following treatment with benzbromarone, tart cherry, and their combination. (A) SIRT1 expression and (B) PPAR-γ expression levels SIRT1: silent information regulator T1; and PPAR-γ: peroxisome proliferator-activated receptor gamma. Results are shown as mean ± SD. The following letters indicate statistically significant differences at $p < 0.05$ compared to their respective groups: a: normal control group, b: CsA group, c: benzbromarone group, and d: tart cherry group.

3.4. The combination of benzbromarone and tart cherry enhances renal histopathology

As shown in **(Figure 6)**, the kidney section of the normal control group displayed normal, organized histological features, including renal parenchyma with apparent intact glomeruli, renal tubular segments with nearly intact tubular epithelium, and intact vasculatures.

The CsA group exhibited moderate to severe tubular degenerative changes, characterized by cortical and corticomedullary junctions, marked interstitial inflammatory cell infiltrates, moderately congested interstitial blood vessels, or hemorrhagic spots.

The benzbromarone group displayed minimally degenerated tubular segments and an abundance of apparent intact tubular segments; however, it also displayed persistent interstitial congested vasculatures or hemorrhagic spots, along with mild inflammatory cell infiltrates. The tart cherry group exhibited nearly identical shapes to those of the benzbromarone group. Furthermore, the kidney section of the Combining group revealed more ordered histological characteristics of renal parenchyma compared with other groups. Minimal sporadic records of tubular degenerative changes were observed, with numerous apparent intact tubular segments, a mild persistent infiltration of interstitial inflammatory cells, and congested vasculatures.

3.5. The combination of benzbromarone and tart cherry inhibit URAT1 transporter protein expression

As shown in **(Figure7)**, Western blot analysis of URAT1 content demonstrated a significant rise in the CsA group, with levels up by 402.95% relative to the normal control group. In contrast, the benzbromarone group (53.42%), tart cherry group (52.45%), and combination group (69.67%) significantly reduced URAT1 levels compared to the CsA group. Additionally, the combination group had a significant further reduction in URAT1 levels, with decreases of 34.87% relative to the benzbromarone group and 36.21% compared to the tart cherry group $P < 0.05$.

3.6. The combination of benzbromarone and tart cherry enhances ABCG2 transporter protein expression

As shown in **(Figure 7),** measuring ABCG2 content by western blot indicated a notable reduction in the CsA group, with levels dropping by 642.57% compared to the normal control group. However, the benzbromarone group (53.33%), tart cherry group (58.33%), and the combination group (68.27%) significantly increased ABCG2 levels compared to the CsA group. Furthermore, the combination group exhibited a substantial decrease in ABCG2 levels in comparison to the benzbromarone group (32.00%) and the tart cherry group (23.84%) $P < 0.05$.

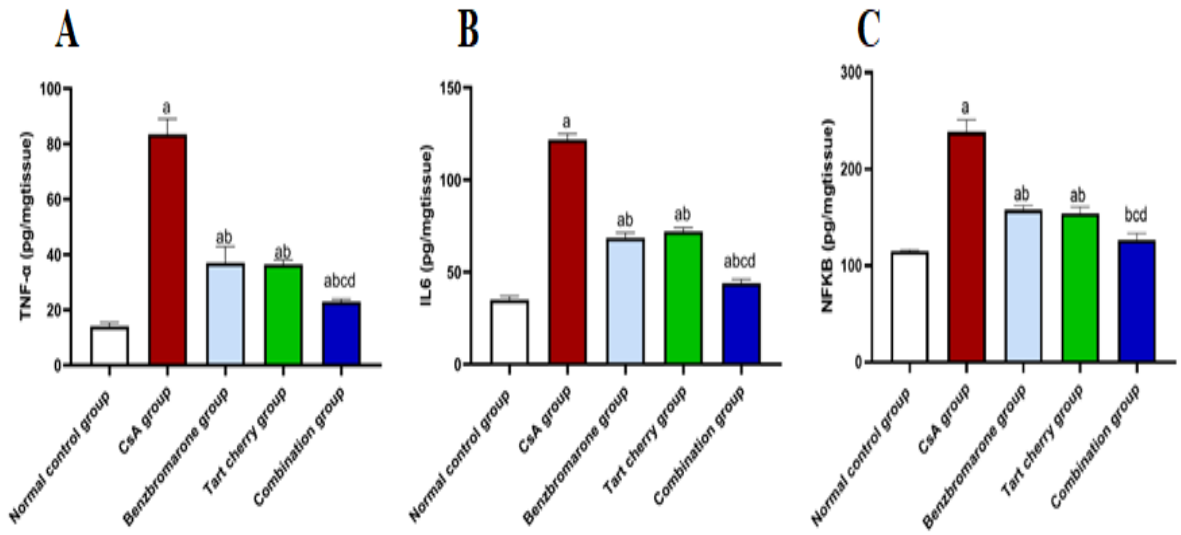

Figure 5. Reduction of inflammatory cytokines in renal tissues by benzbromarone, tart cherry, and their combination. (A) TNF-α, (B) IL-6, and (C) NF-κB levels. TNF-α: Tumor necrosis factor α, IL-6: Interleukin 6, and NF-κB: Nuclear factor kappa B. Data are expressed as mean ± SD. The following letters indicate statistically significant differences at $p < 0.05$ compared to their respective groups: a: normal control group, b: CsA group, c: benzbromarone group, and d: tart cherry group.

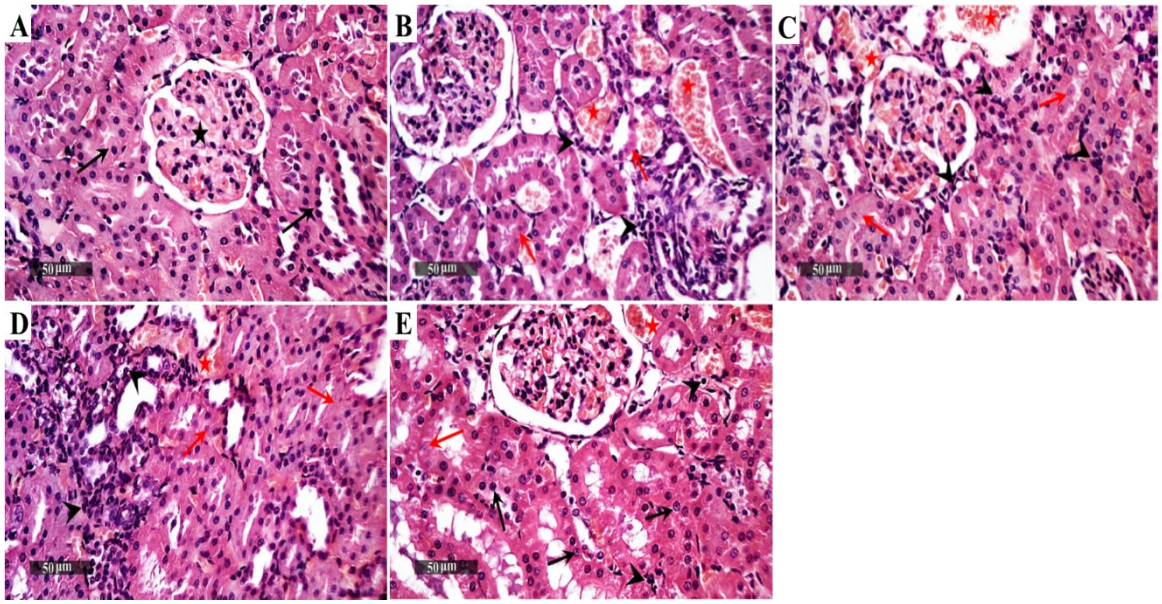

Figure 6. The combination of benzbromarone and tart cherry improves renal histopathology. A: The normal control group's kidney section shows organized histological features with nearly intact tubular epithelium (arrow) and preserved vasculature. B: Cyclosporine group showing moderate to severe records of tubular degenerative changes (red arrow) with marked interstitial inflammatory cells infiltrates (arrow head) and moderate congested interstitial BVs or hemorrhagic spots (red star), C: Benzbromarone group showing moderate to severe records of tubular degenerative changes (red arrow) with marked interstitial inflammatory cells infiltrates (arrow head) and moderate congested interstitial BVs (red star), D: Tart cherry group showing almost the same records as benzbromarone samples, E: The combination group exhibited better-ordered histological features associated with renal parenchyma compared to samples from other groups, minimal sporadic tubular degenerative alterations (red arrow), several apparently intact tubular segments (black arrow), mild persistent interstitial inflammatory cell infiltrate (arrowhead), and congested vasculatures (red star). BVS: Blood vessels. X200, bar = 50 µm.

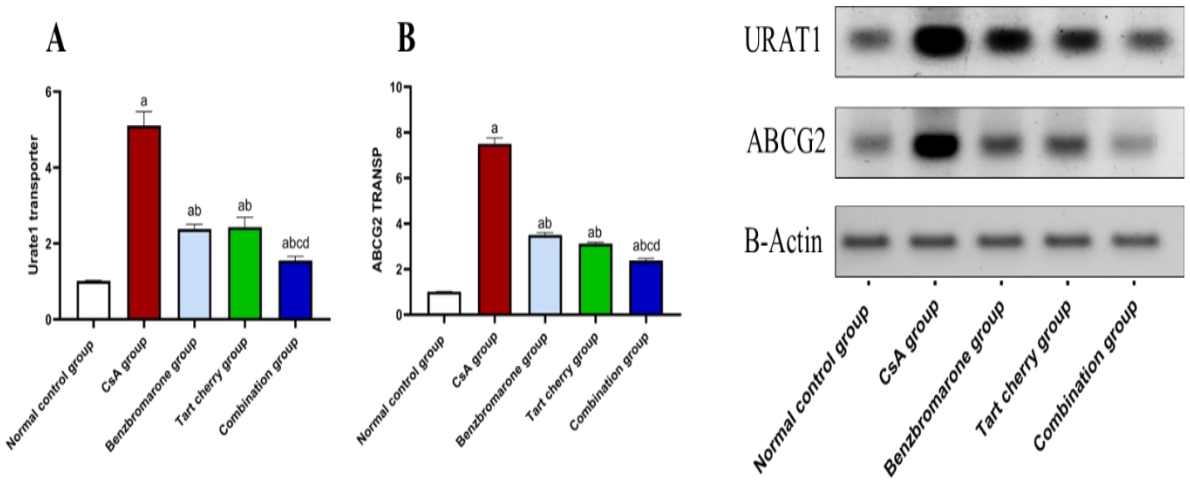

Figure 7. Effect of benzbromarone, tart cherry, and their combination on URAT1 and ABCG2 protein expression. (A) URAT1 expression and (B) ABCG2 expression were analyzed by western blotting. URAT1 refers to the urate anion exchanger, while ABCG2 is the ATP-binding cassette transporter G2. Results are presented as mean ± SD. The following letters indicate statistically significant differences at $p < 0.05$ compared to their respective groups: a: normal control group, b: CsA group, c: benzbromarone group, and d: tart cherry group.

4. DISCUSSION

The current study found that benzbromarone and tart cherry had strong antioxidants, anti-inflammatory, and nephroprotective effects. These effects effectively lowered the damage induced by CsA. Prolonged administration of CsA is understood to bring about oxidative stress, hyperuricemia and consequent cellular injury which ultimately results in chronic nephrotoxicity marked by progressive and irreversible tubule atrophy, tubular epithelial cell apoptosis, and interstitial fibrosis which may ultimately necessitate renal transplantation[40,41]. This work sought to investigate the impact of benzbromarone and tart cherry on hyperuricemia and kidney damage in a chronic CsA-induced renal disease model. Results suggest that both of these substances, and especially their combination, are very effective in reducing these negative outcomes.

The nephrotoxic effects of CsA made the kidneys work less well, as shown by significantly higher levels of serum creatinine and serum uric acid compared to the normal control group. This research confirms the results reported by (El-Kashef, et al 2017)[42]. It has been determined that hyperuricemia enhances the nephrotoxic impacts of CsA [43]. CsA usage is also known to cause hyperuricemia due to the decreased uric acid excretion[44,45]. In the model of CsA nephropathy, serum uric acid concentration increased but the fractional urate excretion was reduced[43]. The treatment with tart cherry and/or benzbromarone snacks elevated cupric salt excretion at the same time preventing further generation therefore protecting against hyperuricemia and nephrotoxicity induced by CsA.

It has been asserted that oxidative stress is the primary mechanism underlying the CsA-induced nephrotoxicity [46]. From the present study, remarkable oxidative stress developed in the kidneys over four weeks of CsA administration along with notable impairment in renal functions. This provoked an increase in ROS and MDA concentration, while SOD and GSH activity was lowered. These findings corroborate previously published data [47,48]. The cited studies found that treatment of rats with benzbromarone or tart cherry, or both, together reduced ROS, increased body antioxidant defenses, and improved kidney function after CsA treatment.

The histological evidence supports the above statement by showing that there is a notable reduction in histopathological changes in the benzbromarone, tart cherry, and combination groups as opposed to the CsA group. From this, it is clear that the therapies used have not only mitigated oxidative stress levels but also considerably protected the cells from direct CsA induced tissue injuries.

SIRT1 has been shown to mitigate oxidative stress, inflammatory stimuli, cellular senescence and apoptosis [49]. The current study shows that the CsA group reduced SIRT1 expression significantly, however the benzbromarones, tart cherry, and combination groups showed significant increases. SIRT1 is a constituent protein of virtually all cells and is important in the pathophysiology, progression, and treatment efforts of many conditions. The deletion of SIRT1 was found

to drastically increase ROS along with a more elevated inflammatory response from the kidney, pancreas, and brain. The literature provides evidence for SIRT1's modulating role in oxidative stress and inflammation, reinforcing its critical role in disease mitigation [50].

The SIRT1 protein is implicated in the regulation of various histone and non-histone substrates, encompassing PPARγ [51]. Among the PPAR family, PPARγ is well known for expressing anti-inflammatory effects in many tissues[52,53]. This transcription factor whose action is controlled by ligands is critical in several pathophysiological processes, most importantly, the regulation of inflammation. The findings of this research displayed through PCR analysis demonstrate that the use of benzbromarone alone or in combination with tart cherry greatly upregulated the PPARγ expression in comparison to the CsA group. Both metabolism and inflammation can be regulated by the interaction which takes place between PPARγ and SIRT1 [54,55].

SIRT1 is capable of forming a direct complex with NF-κB and removing acetyl groups from the p65 subunit, thus inhibiting transcriptional activity of NF-κB[56]. Also, the interventional capacity of SIRT1 to block the activity of NF-κB is mediated by activating PPAR-α and PGC-1[57]. The results from the current study showed that CsA greatly enhances inflammation markers including NF-κB, IL-6, TNF-α, and others. On the contrary, the application of benzbromarone, tart cherry, or both proved effective in inflammatory signaling inhibition. Some previous studies have indicated that PPAR-α agonists like benzbromarone are able to inhibit NF-κB and inflammation[58]. Our data corroborates these findings. Additionally, benzbromarone induced an anti-inflammatory effect through inhibition of the p38 MAPK/NF-κB p65 signaling pathway as well as its downstream effector molecules like TNF-α and IL-6 which in turn rescued renal tissue from apoptotic cell death[58]. Therefore, benzbromarone could be an effective treatment option for CsA induced nephrotoxicity.

The elimination of urate by the kidneys is crucial, and this is accomplished through processes such as glomerular filtration, tubular reabsorption, and secretion. Urate transporters are pivotal in this, and especially significant for renal reabsorption of uric acid is urate transporter 1 (URAT1)[59]. The current observations indicate that benzbromarone, tart cherry, or both, separately from CsA were able to markedly upregulate the expression of URAT1 transporter. Benzbromarone facilitates uric acid excretion by inhibiting proximal tubular URAT1[18,60]. Moreover, Benzbromarone also mitigates the oxidative stress caused by uric acid through inhibition of its transport into vascular endothelial and smooth muscle cells[61].

The urate transporter ABCG2 is located in the ileum of hyperuricemic mice. It is one of the major contributors in the metabolism of uric acid. Scientists have proved that SIRT1 increases ABCG2 expression in a human colon cancer cell line. The results evident portrayed higher expression of ABCG2 in the benzbromarone, tart cherry, and combination treatment groups when juxtaposed with the CsA group. Urate transporters like ABCG2 are essential to the excretion of uric acid by human kidneys. ABCG2 works as a transporter which assists in the renal excretion of uric acid[62] **.**Various Research indicates ABCG2 facilitates uric acid excretion through the intestines, functioning as means of acid removal in both humans and rats, which doesn't involve the kidneys. The level of ABCG2 expression is associated with increased urate secretion[63,64]. This seems to mean that SIRT1 has control over uric acid levels by regulating ABCG2 expression in the ileum. This will make the kidneys and intestines more efficient in clearing out the urate.

5. CONCLUSION

In summary, although CsA is an indispensable medication in the context of organ transplantation, its broader application is limited due to its nephrotoxic effects. The research presented here shows how benzbromarone, an anti-hyperuricemic drug, and tart cherry, a natural product, individually and synergistically protect the kidneys against CsA induced nephrotoxicity. Combined therapy was more efficacious than either drug alone because it decreased oxidative stress while enhancing the SIRT1/PPAR-γ/ABCG2 axis activity. These data indicate that benzbromarone and tart cherry together may provide a new way to mitigate the nephrotoxic effects resulting from cyclosporine.

Recommendations

As the results show, we suggest benzbromarone and tart cherry as possible clinical therapeutics to counter the adverse nephrotoxic effects of cyclosporine. These drugs exhibit potent anti-inflammatory and oxidative suppressive properties which aid in hyperuricemia. Their distinctive actions, such as lower URAT1 and increase ABCG2, SIRT1, and PPAR-γ expression, along with other factors are astonishing and suggest these different mechanisms can aid in more renal protective approaches. Nonetheless, more clinical studies are needed to confirm these results in people and refine dosage habits for maximum therapeutic potency.

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Data availability

Data that supports the study's findings are presented in the publication or supplemental data.

Authorship contribution statement

1) Conceptualization: Nageh Ahmed El-Mahdy, Sally El-Sayed Abu-Risha, Laila Ahmed Ramadan. 2)Data curation, Methodology, Writing-original draft: Sally El-Sayed Abu-Risha, Yasmin Tarek Saad Ali 3) Formal analysis, writing-review & editing: Nageh Ahmed El-Mawy, Sally El-Sayed Abu-Risha, Yasmin Tarek Saad Ali 4) Supervision: Nageh Ahmed El-Mawy, Sally El-Sayed Abu-Risha, Laila Ahmed Ramadan.

Declarations

Conflict of interest No conflicts of interest exist, according to the authors with the publishing of this article

Ethical approval

The experimental procedures were conducted under the approval of the Research Ethics Committee at the Faculty of Pharmacy, Tanta University (REC-TP). The study adhered to both institutional guidelines and the standards of the Council for International Organizations of Medical Sciences (CIOMS) (Committee reference number: TP/RE/10/24M-002).

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