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Preventive Impacts of Black Tea, Green Tea, and *Bidens pilosa* on Renal Stone Formation in Rats: Antioxidant and Anti-Inflammatory Pathways

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Abstract

Previous *in vivo* studies demonstrated that oxalate loading motivate free radical generation and inflammation, thereafter renal tubular cells damage. This study purposed to discover the efficacy of black tea (BT), green tea (GT), and *B. pilosa* tea (PT) aqueous extracts to prevent kidney stone formation. Thirty rats were separated into five groups, (I) fed a balanced diet (control normal, CN). (II) fed potassium oxalate rich diet (KOx) to cause hyperoxaluria, without any treatment. Groups III, IV, and V were fed high oxalate diets concomitantly to tea extracts BT, GT, and PT, in that order. Protein, albumin, creatinine, urea, electrolytes (sodium and potassium) were measured in serum, whereas, oxidative markers and some inflammatory cytokines were measured in kidney homogenates. Total phenolic, total flavonoids and antioxidant capacity tests were assessed in all tea extracts. The PT and GT extract had the highest values of total phenolic and total flavonoids, respectively. Moreover, PT extract showed the highest antioxidant capacity among all tea types. The high oxalate diet (KOx) group altered reduced glutathione (GSH) redox balance, notably lowered serum protein, albumin, sodium, and antioxidant enzymes, but significantly increased malondyaldehyde (MDA) level, creatinine, urea, potassium and nitric oxide (NO) in serum. All inflammation markers were notably ($p<0.05$) elevated in KOx rats. Interestingly, The BT, GT and PT treatment substantially prevented the biochemical changes and restored the observed histological alterations in fed KOx rats. The PT had the strongest influence among other tea extracts. Briefly, these findings suggest that *B. pilosa* tea aqueous extract, among all studied tea extracts, has an ability to reduce inflammation, glutathione redox equilibrium, prevent peroxidative damage, and replenish renal tissue antioxidants. It can be regarded as functional drink agent to protect from the renal oxalate crystallization.

Keywords: Kidney stone, Black tea, Green tea, B. Pilosa Tea, antioxidant, anti-inflammation.

1. Introduction

Nowadays, kidney stones are a serious health concern in several nations [1,2]. The primary causes of kidney stones are genetic, metabolic (excess oxalate production), dietary, and environmental variables; in humans, calcium oxalate is responsible for 60–80% of kidney stones (3-5). In actuality, hyperoxaluria is one of the major risk agents for the development of calcium oxalate stones because of urine calcium oxalate supersaturation (6). The human body experiences an excess in both endogenous oxalate (made through formation of ascorbic acid) and exogenous oxalate (oxalate-rich meals) [7]. Calcium oxalate stones develop directly as a result of dietary oxalate consumption. Researches expected that meals high in oxalate may contribute 50–60% increase in urine oxalate [4]. Elevated urine oxalate levels usually cause acute renal failure, renal tissue deterioration, and urethral oxalate crystal formation [8]. Now widely acknowledge that oxalate crystals cause kidney stones and systemic illnesses such as chronic kidney disease and renal failure, metabolic problems, and cardiovascular illnesses [9]. A variety of techniques, including lithotripsy and surgery, now treat kidney stones, but challenges persist, including rising treatment expenses and undesirable adverse

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effects. Specialists should take preventive measures in addition to any needful surgical treatments [10]. A great deal of research has focused on managing the calcium oxalate crystallization in an attempt to find effective therapies and/or preventative drugs versus the production of stones [10]. Numerous studies have demonstrated that the active ingredients in medical herbs have a curative impact on the renal and urinary tract systems [11-13]. Researches have demonstrated the advantageous impacts of antioxidant dietary phytophenols, for instance curcumin, diosmin, rutin, quercetin, epicatechin, epigallocatechin, and hyperoside in preventing urolithiasis, a condition where stones form in the urinary tract [14-17]. Some of the prime ways that these kinds of plants and their plant-based nutrients help treat urolithiasis are by making you pee more, relaxing spasms, and protecting your body from free radicals [16]. They also hinder crystals from shaping, developing, and sticking with each other [18]. The Food and Agriculture Organization (FAO) has recognized *Bidens pilosa* L (Asteraceae family) as a plant that can be eaten since 1975 [19, 20]. World widely, the *Bidens pilosa* L is traditionally used as tea, food and medicinal purposes throughout South America, Africa, and Asia [21]. The ability of *B. pilosa* to scavenge free radicals is well established [22]. People have long used it as a tea to stay cool during the summer. Research suggests that *B. pilosa* possesses active chemicals with a variety of unique bioactivities. Alkaloids, saponins, phenols, glycosides, tannins, quercetin, 3-O-rutinosides, chlorogenic acid, flavonoids, terpenoids, phenylpropanoids, aromatics, and porphyrins are some of the bioactive phytochemicals that are found in *B. pilosa* [22, 23]. Tea, or *Camellia sinensis*, is a popular beverage worldwide. Catechins, flavonols, theaflavins, and arubigins are antioxidant polyphenolic flavonoids found in tea that have a range of pharmacological actions [23]. Various types of tea are derived from the same types of leave, such as green, white, yellow, black, oolong, and Pu-erh. It was proven that the growing circumstances of the plant, the methods used for harvesting, and the processing of the leaves account for the notable variations amongst tea varieties [24, 25]. Prior research has confirmed that green tea extract can effectively prevent calcium oxalate monohydrate from forming *in vitro* [26]. Further, Li et al. [10] have elucidated that the polyphenols in green tea regulate kidney stone crystallization and decrease oxidative stress in rats. While the health benefits of black, green, and B. Pilosa tea have been extensively studied and attributed to their active components, there has been limited research conducted on examining and contrasting the potential connection between consuming these types of tea and protecting against renal tissue damage resulting from high oxalate consumption. Therefore, the current investigation goaled to estimate the biochemical modulatory impacts of Black tea, green tea, and *B. pilosa* tea aqueous extracts on rats that had received experimentally induced hyperoxaluria.

2. Materials and methods

2.1. Materials

2.1.1. Chemicals and raw materials

Potassium oxalate (KOx) was procured from Sigma-Aldrich (St. Louis, MO, USA). Black tea and green tea were obtained from common markets in Cairo, Egypt. The green leaves of *B. pilosa* were provided from a farm in Saudi Arabia, authenticated in Flora and Plant Taxonomy Dept., Agriculture Research Center, Egypt, dried under the sun, and subsequently processed into a fine powder. The tea The reagents and chemicals utilized in this study were all of excellent analytical quality.

2.1.2. Animals

Thirty male adult Wistar albino rats, weighing (150-170 g), were acquired from the Breeding Unit, National Research Centre (NRC) in Cairo, Egypt. Animals were housed in metabolic cages and adapted to lab setting for one week before the onset of the experiments. All animals were humanely treated following the animal care guidelines set by the Medical Research Ethics Committee at NRC, Cairo, Egypt. The research protocol received approval from the Medical Research Ethics Committee (MREC) at NRC. Ethical Approval Certificate No. 18232023

2.1.3. Diets

A maintenance-balanced diet containing corn starch, casein protein, fats, sucrose, and fibers, as well as salt and vitamin mixtures of 62, 14, 5, 10, 5, 3, and 1%, respectively, was formulated following the AIN-93 [27]. A high-oxalate diet containing corn starch, casein protein, fats, sucrose, fibers, and KOx, as well as salt and vitamin mixtures of 57, 14, 5, 10, 5, 5, 3 and 1%, respectively, was prepared in accordance with Gomathi et al. [28].

2.2 Methods

2.2.1. All Tea samples aqueous extraction

The BT, GT, and PT aqueous extracts were all set according to Ramadan et al. [29]. All tea samples extracts made by dissolving quantities equal to 100 mg of powdered leaves for each kg of animal body weight with 0.5 ml boiling water (equal to 3 cups of tea) in glassware, then left covered for 10 min at ambient temperature.

The animals were subsequently given fresh extracts.

2.2.2. Estimation of antioxidant potential of all tea aqueous extracts

The total phenolic content (TPC) was measured by the Folin-Ciocalteu method described by Zhang et al. [30]. The total flavonoid content (TFC) was evaluated by Aluminium chloride colorimetric assay based on Horszwald and Andlauer method [31]. The radical scavenging ability of all tea aqueous extracts were tested using different methods. By Using 2,2-diphenylpicrylhydrazyl (DPPH) quenching assay [32], 2,2′-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) (ABTS) scavenging assay [33]. In addition to quantification of tea aqueous extracts for its ferric reducing antioxidant power according to Khatua et al. [33]. All antioxidant potential measurements were performed using microtiter based techniques (microplate reader ThermoScientific Multiskan G0). All samples were run in triplicates.

2.2.3. Animals study design

Five groups of six Wister rats each were classified randomly as follow: Group I (CN) was given a balanced diet, and physiological saline via esophageal gavage, and was served as the control group. In order to induce hyperoxaluria, the experimental groups II to V were fed a high oxalate diet for 21 days [28]. During the experiment time, they were given orally physiological saline for group II (KOx), black tea water extract for group III (BT), green tea water extract for group IV (GT), and *B. pilosa* leaves water extract for group V (PT).

Daily food intake was recorded. The following formula was used to determine any changes in body weight (BW) [34]:

Body weight (BW) gain or loss = BW at the experiment end – BW at the experiment beginning.

2.2.4. Urine, blood and tissue sampling

Before killing (48h), urine was collected for 24 h, then stored at −20 ◦C in an aliquoted state. The animals were put to death by beheading, and the blood was taken from the trunk of the body and placed in tubes free of EDTA. Later, serum was separated using centrifugation (3000 rpm; 10 min). All serum samples kept frozen at -80℃ until later examinations. The kidney was taken out of the body, washed and weighed as soon as the animals were killed. Parts of the kidney were immersed in formalin-saline (10%) to the histopathological examination. Other parts of the kidney were taken from each rat in order to make homogenates (10% w/v) in a cold homogenization buffer (100 mM potassium phosphate buffer, pH 7.4). After centrifuging the homogenates, the supernatants were utilized to carry out the biochemical tests.

2.2.5. Renal function, antioxidant and inflammation indices

As marker of renal function, the values of urea, creatinine, total protein, and albumin were measured colorimetrically in serum samples [35 -38]. In addition to serum electrolytes (sodium and potassium) were determined [39, 40]. Protein in urine samples was determined [41]. Kidney homogenates were analyzed for malondialdehyde (MDA), superoxide dismutase (SOD), nitric oxide (NO) and reduced glutathione (GSH) as antioxidant indices [42-45]. The kidney tumor necrosis factor-alpha (TNF-α), interleukin 6 (IL 6), transforming growth factor 1-beta (TGF 1-β) as inflammation markers were analyzed using Eliza kit (Sunlong Co., Ltd. China) according to the manufacturer's instructions. All biochemical analyses were manually done using commercial kits.

Histopathological examination:

Specimens of kidney were laid in 10% neutrally buffered formalin. Following trimming, the preserved tissues were dehydrated using increasing concentrations of isopropyl alcohol, treated with xylene for cleaning, and washed with cold saline solution. The tissues impregnated with wax of identical grade were encased in paraffin blocks, which were then thinned out to 3-5µ by a rotary microtome. The sections were positioned on glass slides and placed in a tissue floatation bath at 40 °C. After melting the portions in an incubator set at 60°C for five minutes, which were permitted to be cold before being microscopically inspected and treated with hematoxylin and eosin staining [46].

Statistical analysis:

All analyses were done using the SPSS for Windows, version 20.0 (SPSS Inc., Chicago, IL, USA). All results are expressed as mean ± Standard deviation (SD) and evaluated using the one-way analysis of variance (ANOVA) test. We set statistical significance at $p \leq 0.05$ for all comparisons

3. Results

3.1. Estimation of antioxidant potential of all tea water extracts

The TPC values for the BT, GT, and PT extracts were 11.21, 14.33, and 15.25 mg GAE/ml, respectively, while their TFC values were 9.56±0.61, 15.21±0.25, and 2.38±0.14 mg QE/ml, in that order (Table 1). The PT had the highest TPC, while the GT had the

highest TFC among all studied tea type extracts. The antioxidant capacity of all tea type aqueous extracts was measured using the DPPH and ABTS methods, with the PT extract showing the highest ability to scavenge DPPH and ABTS with values 86.89±3.36% and 70.18±2.16%, respectively (Table 1). The GT extract had lower antioxidant capacity values of 48.9±0.0 for DPPH and 30.9±1.22 for ABTS. Obtained results of reducing power assay showed that the PT extract had the highest value of 864.05±6.55 µg Vit C E/ml, while GT was notably the lowest 197.06±4.34 µg Vit C E/ml) among all the tea extracts (Table 1).

Table 1: Total Phenolic, flavonoid constituents and antioxidant potential of all tea aqueous extracts

	BT	GT	PT
TPC (mg GAE/ml)	11.21 ±0.16	14.33 ±0.21	15.25 ±0.26
TFC (mg QE/ml)	2.38 ±0.14	15.21 ±0.25	9.56 ±0.61
DPPH radical scavenging (%)	82.97 ±0.24	48.90 ±0.20	86.89 ±3.36
ABTS radical scavenging (%)	48.83 ±1.66	30.90 ±1.22	70.18 ±2.16
Reducing power activity (µg VitC E/ml)	443.96 ±5.65	197.06 ±4.34	864.05 ±6.55

The values represent the means ±SD of three findings
BT: Black tea, GT: Green tea, PT B. pilosa tea, TPC: Total phenolic content, TFC: Total flavonoid content

3.2. Efficacy of all tea aqueous extracts on body weight, food intake and urinary output

This study assessed the capability of aqueous extracts of BT, GT, and PT to prevent renal damage resulted from high fed oxalate diet. The findings (Table 2) showed that the untreated rats fed on the oxalate diet experienced a substantial ($p < 0.5$) decrease by 21.2% in their ultimate BW and body weight gain (BWG) when contrasted to the normal group. Conversely, rats given extracts of BT, GT, and PT indicated a slight rise in their final BW. Similar trends were observed for total feed intake and feed efficiency ratio parameters. The kidney weight in the KOx group had a substantial ($p \leq 0.5$) decrease by 32.7% when contrasted to the normal group, whereas all tea extract groups showed slight rise in their kidney weight. Rats fed with KOx exhibited significantly higher urine output within a 24-hour period. By day 21, the urine volume recorded was 11.08±0.21 ml/day, reflecting a 310% increase comparable to the urine output of normal control rats. Utilizing of BT, GT, and PT aqueous extracts in conjunction with an oxalate diet significantly lower urine volume compared to the group not receiving treatment with an oxalate diet.

Table 2: Efficacy of All tea types aqueous extracts on the growth performance and urine volume

	Groups				
	CN	**KOx**	**BT**	**GT**	**PT**
IBW (g)	159.17 a ±2.27	159.50^{a} ±2.85	159.67 a ±3.36	159.50^{a} ±2.62	159.67^{a} ±2.12
FBW (g)	196.67^{b} ±3.36	155.00^{a} ±3.17	161.83^{a} ±3.58	157.17^{a} ±3.30	160.50^{a} ±2.16
BWG (g)	37.50^{b} ±3.92	-4.50^{a} ±4.79	2.17^{a} ±6.39	-2.33^{a} ±5.27	0.83^{a} ±2.91
Total feed intake (g)	364.17^{b} ±4.24	334.67^{a} ±2.95	374.50^{bc} ±3.68	377.33^{c} ±3.55	371.17^{bc} ±3.34
Feed efficiency ratio	0.10^{b} ±0.01	-0.01^{a} ±0.01	0.01^{a} ±0.01	-0.01^{a} ±0.01	0.01^{a} ±0.01
Kidney weight (g)	1.13^{b} ±0.05	0.76^{a} ±0.06	0.87^{a} ±0.07	0.89^{a} ±0.04	0.90^{a} ±0.05
Relative kidney weight	0.58^{a} ±0.03	0.49^{a} ±0.04	0.54^{a} ±0.04	0.57^{a} ±0.03	0.56^{a} ±0.03
urine volume (ml/24 h)	3.57^{a} ±0.24	11.08^{e} ±0.21	6.50^{d} ±0.59	5.25^{bc} ±0.73	4.42^{b} ±0.30

The values in every column with distinct letters differ dramatically ($p<0.05$).
CN: control normal, KOx: high oxalate, BT: black tea , GT: green tea, PT: *pilosa* tea, IBW: Initial boy weight, FBW: Final body weight, BWG: body weight gain

3.3. Effect of all tea aqueous extracts on renal function and electrolytes.

In the high oxalate diet (KOx) group show a notable rise in serum ($p<0.05$) in serum creatinine, urea, and potassium (Figure 1A, 1B, and 1F), While show a notable decline in serum total protein, albumin and sodium (Figure 1C, 1D, and 1E). Whereas, all tea types extract groups showed an adverse significant ($p<0.05$) impacts on all mentioned parameters in serum (Figure 1). led to a notable rise in serum protein, albumin, and sodium, as well as a notable decline in serum creatinine, urea, and potassium ($P < 0.05$). Among all tea extract groups, the PT displayed a more effective impact along with an oxalate diet dose than to use green tea or black tea extracts in water (Figure 1A-F). The high oxalate diet shown a considerable ($p<0.05$) increment in urine protein

compared to the normal control group (Figure 1G). But, when BT, GT, and PT aqueous extracts were applied with an oxalate diet, there was a substantial ($p<0.05$) drop in urine protein contrasted with the group that was only given oxalate diet (KOx). The impact of *B. pilosa* was more potent than other types of tea aqueous extracts (Figure 1G).

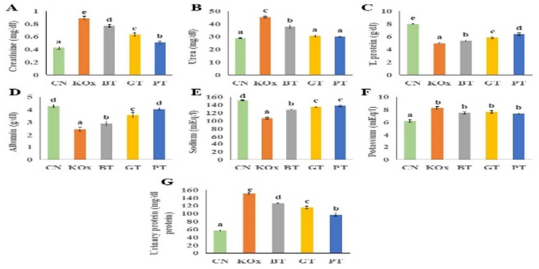

Figure 1: Kidney functions of control normal (CN), oxalate (KOx), black tea (BT), green tea (GT) and *B. pilosa* (PT) groups. Creatinine (A), urea (B), T.protein (C), albumin (D), sodium (E), and potassium (F) in serum sample. Finally, (G) urinary protein

3.4. Effect of all tea extracts on renal antioxidant activities and inflammation

Due to the intake of an oxalate rich diet, the MDA concentration in kidney homogenates considerably increased by 2.5 times in rats fed with KOx diet contrasted with the normal control. Nevertheless, the MDA values were notably lower in rats treated with BT, GT and PT in comparison to those fed with KOx (Fig 2 A). The SOD enzyme activity and non-enzymatic GSH antioxidant were significantly ($p\leq0.05$) reduced, in addition to significant depletion in reduced NO in rats fed with KOx diet. The adverse effect of high oxalate in SOD, GSH and NO were considerably restored ($p \leq 0.05$) in BT, GT and PT treated rats (Fig 2. B, C, D). The results concluded that all tea types aqueous extracts had a renoprotective impact against oxalate formation in different degrees.

The BT, GT, and PT aqueous extracts showed an important anti renal inflammation. The inflammation parameters TNF-α, IL 6, and TGF 1-β showed notable rising ($P \leq 0.05$) in normal group contrasted with oxalate diet untreated group (Fig. 2 E, 2F and 2G). However, the concomitant use of BT, GT, and PT aqueous extracts with oxalate diet resulted in a notable decline in the mentioned parameters compared to oxalate diet untreated group. Among all types of tea aqueous extracts, it was observed that PT had the most beneficial impact on parameters indicating inflammation.

3.5. Histological examination.

The histological examination of the kidney revealed no evidence of CaOx crystal deposition or other morphological alterations in the kidney of control rats (Fig. 3 A). In contrast, the kidney of rats fed with KOx displayed crystal deposits in the tubules, dilation of the collecting tubules, necrosis of tubular epithelium, inflammation, edema, and congestion in the interstitium (Fig. 3 B). It's interesting to note that the high calcium oxalat-treated group's histological alterations were significantly avoided by BT, GT, and *B. pilosa* tea aqueous extract treatment (Fig. 3 C, D, and E).

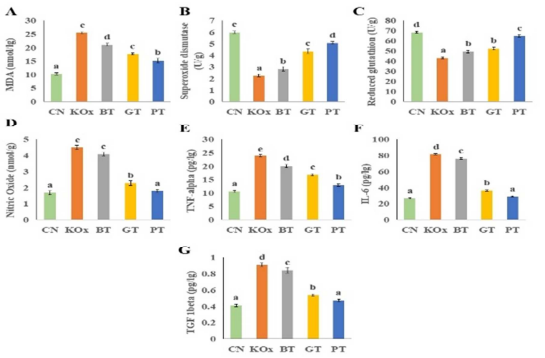

Figure 2: Oxidative markers and inflammatory cytokines in kidney homogenates of control normal (CN), oxalate (KOx), black tea (BT), green tea (GT) and *B. pilosa* (PT) groups. MDA (A), SOD (B), GSH (C), NO (D), TNF-α (E), IL-6 (F) and TGF-1β (G)

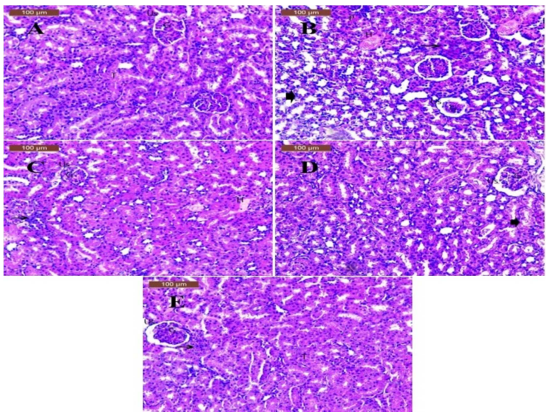

Figure 3: Photomicrographs of histopathological alterations and CaOx crystal deposition in renal sections. (A) control normal (CN), (B) high oxalate (KOx), (C) black tea (BT), (D) green tea (GT) and (E) *B. pilosa* tea (PT) groups. The CaOx deposition indicated by black arrows. All groups tissue were stained with hematoxylin and eosin (H & E). the scale were all 100 µm

4. Discussion

Hypercaluria, which causes injury in renal tissue and stimulates the development of calcium oxalate crystals, is caused by increased endogenous oxalate synthesis and enhanced intestinal oxalate absorption (secondary hyperoxaluria) [7, 47]. However, due to the existence of bioactive substances including

phenolic acids, alkaloids, terpenoids, and flavonoids that have lately been displayed to have significant anti-litholytic impacts, plants are one of the anti-litholytic remedies [10-13]. In this work, renal damage in rats treated with oxalate was assessed in response to aqueous extracts of BT, GT, and PT.

The highest TPC value of PT is attributed to 33 different phenolic compounds was belong to *B. pilosa* leaves constituents such as caffeic acid, p-coumaric acid and pyro-catechin [20, 21, 48]. While green tea showed the highest value of TFC, which attributed to the presence of flavan-3-ols monomers, catechins and its derivatives that make up around 18–30% of the dry weight content of fresh leaves [23, 24, 49]. Moreover, among all tea extracts the *B. pilosa* exhibited the greatest capacity to scavenging free radical by DPPH and ABTS method, as well as a significant antioxidant capability in reducing ferric ion. The hot water extracts of PT aerial parts displayed significant antioxidant activity for either in vitro or in vivo assays, caused by its high content of phenolic acids in leaves [21, 48]. In this study TFC were 14.33 and 11.21 mg GAE/ml extract for GT and BT, respectively. These data harmonious with former study on green tea from Argentina displayed TFC ranged from 14.32 to 21.02% of GAE [50]. Furthermore, Almajano and colleagues demonstrated that the TPC in green tea surpassed that of black tea [51]. The TPC value of our study GT was lower than green tea leaves extract from West Java in Indonesia with value 36.64 mg GAE/mg extract [52]. The Phenol content might be varied among different tea types dependent on several factors including geographical locations, environmental reasons, processing, degree of ripeness, storage condition, and analytical equipment. [13, 23, 25].

In a rat model, our findings showed that feeding animals a potassium oxalate-rich diet for 21 days led to formation of kidney stone, as well as activation of oxidation and inflammation pathways. This experimental period was indeed enough to detect the deteriorations that induced by high KOx diet, as seen in previous study by Crestani and colleagues [53], who used high sodium oxalate diet for three weeks on Wistar rats. Previously, many studies had looked at the impact of high KOx diet on rats at various experimental days 0, 7,14,21,28 [28, 54]. The authors detected considerable increases in urinary oxalate and calcium excretion from day 7 to day 21, followed by a decrease at day 28. These reductions indicated that the deposition of CaOx crystals in renal tubules, leading to renal dysfunctions was obvious after three weeks feeding on high oxalate diet. In this study, the KOx group reported a considerable reduction in their final body weight compared to normal rats group (CN). These findings were consistence with previous studies [47, 55], but not the same as Gomathi et al. study [28], who saw a minor rise in oxalate-treated rats relative to control rats. Groups that administered with BT or PT extract, conversely, showed a little rise in their ultimate body weight. Due to the possibility of enhanced calorie expenditure from green tea, the GT group also had a decrease in their final body weight. The results were in line with other studies [28, 47, 53]. Our findings showed that the urine volume of the KOx group was considerably greater than the volume in rats fed normal diet (NC) group. In contrast to KOx group, the BT, GT, and PT groups progressively displayed significant decreases in urine volume values.

The biochemical results showed that the KOx group had much higher amounts of creatinine, urea, potassium, and 24-hour urinary protein than the normal rats (CN) group. In contrast, there was a substantial drop in albumin, total protein, and salt levels in the KOx group when contrasted to the CN group. Oxalate toxicity is primarily caused by oxidative stress, which leads to tissue destruction, damage to the renal epithelium, and high levels of urea, phosphate, and creatinine in the kidneys. Increased phosphate excretion may make calcium oxalate stone formation more susceptible to heterogenous nucleation [8, 28, 53]. Conversely, exogenous antioxidant supplements have the ability to minimize the harmful impacts of oxalate and prevent stone development. In this study, rats that fed a lot of oxalate and were then given extracts from either BT, GT, or PT had lower levels of creatinine, urea, potassium, and the 24-hour urinary protein. On the other hand, their levels of albumin, total protein, and sodium went up compared to the KOx group. The PT aqueous extract was the most promising extract for restoring kidney function. This finding was consistence with recent study explained that PT was effective protector against the toxic effect of CCl4 on kidney function in rats [56]. The fact that antioxidants in tested tea types were shown in this study may have helped explain why they protect the kidneys from oxalate toxicity [14-17].

Overproduction of free oxygen radicals triggers distinct transcription factors, causing some genes implicated in inflammatory pathways to express differently. Two of the most prevalent upstream pro-inflammatory cytokines are TNF-α and IL 6. Acute kidney damage triggers the activation of TGF-β1, which can regulate cellular responses to the toxin in a positive or negative way. The TGF-β1, which is elevated by any extended kidney damage, promotes renal fibrosis [57]. Consequently, this study determined TGF-β1, TNF-α, and IL 6. Our data displayed that the KOx group's rats had much higher quantity of MDA, NO, IL 6, TNF-α, and TGF-1β

than the normal rats. In contrast, the KOx group's SOD and GSH values dramatically dropped when compared to the CN group. The rats in the BT, GT, and PT groups had much lower amounts of MDA, NO, IL 6, TNF-α, and TGF-β1 than the rats in the KOx group. Alternatively, their levels of SOD and GSH went up. The PT extract was the most promising extract for reducing oxidative indicators and inflammatory cytokines, as well as reviving antioxidant enzyme activity. The phytochemicals in BT, GT, or PT may be responsible for these benefits, which include renoprotection against oxalate toxicity. Recent research explained that polyphenols decrease inflammation and oxidative stress via activating antioxidant enzymes [58]. Phenolic acids and flavonoid derivatives like epigallocatechin-3-gallate (EGCG), rutin, apigenin, and naringenin have been shown in many studies to reduce inflammation [59]. In rats, quercetin had a unique impact comparable to those fed KOx, as it led to a decrease in lipid peroxidation and antioxidant depletion, reduced CaOx aggregation in both urine and kidneys, and changed the expression of genes involved in regulating CaOx levels and antioxidant like GPx and SOD [28]. Recent study stated that quercetin and rutin are abundant polyphenols in BT, GT, or PT, exerted a reno-protective effect against oxalate [60]. Rutin inhibited the increase in calcium and oxalate and returned them to nearly normal levels. Additionally, it lowered CaOx levels, caused less kidney damage and histological alterations in the kidneys compared to those of animals given calculi, and decreased lipid peroxidation. The EGCG may be accountable for green tea's renoprotective effects, as well as squalene and ethyl caffeate in *B. pilosa* [21, 23, 48, 61]. The EGCG and squalene may directly stop reactive oxygen species (ROS) production and disrupt the Nrf2-Keap1-Cul-3 complex, allowing Nrf2 to enter the nucleus and bind to specific region in the genes that produce antioxidant enzymes. In addition, EGCG and ethyl caffeate own potential to suppress ROS-mediated inflammation by blocking the NF-κB signaling pathway, particularly by blocking the degradation of IκB induced by phosphorylation, ultimately preventing NF-κB from binding to DNA.

The histopathological examination of renal tissue displayed massive structural differences between CN and KOx groups tissues, indicating the severely impairment in renal function might be occurred in rats fed KOx diet. These results were concordant with previous studies [10, 28, 62] which has indicated a clear connection between oxidative stress, renal injury and presence of CaOx crystals in hyperoxaluric rats. This interplay is a key factor in the retention of crystals and the eventual formation of stones. All Tea extracts treated groups, particularly GT and PT displayed a normal morphology of renal tissue and reduced crystal deposition when compared to KOx fed rats. The obtained histology results were concordant with former studies [23, 26, 28, 56]. The gotten results, might propose that drinking tea, in particular *B. pilosa* has played a significant role in modulating the renal oxalate crystallization *in vivo*.

Conclusion

The present study indicated that the concomitant administration of black tea, green tea and *B. pilosa* leaves aqueous extract with high oxalate diet could be protective from kidney stone formation. All tea extracts showed abilities to quenching the harmful effect of free radical that produced from loaded oxalate diet through increasing the SOD and GSH activities and NO, as well as decline lipid peroxidation, and inflammation parameters in renal homogenates. Furthermore, all tea extracts displayed positive effect through reduction of renal function indices and electrolytes in serum comparable to loaded oxalate diet rat. All Tea extracts treatment rats displayed normal morphology and decreased crystal deposition in renal tissue. In rat stone model, this study proved the relationship between drinking tea, in particular *B. pilosa* to prevent and cure the oxalate crystallization in kidney. To our knowledge, the comparing between different tea types intake on formation of kidney stone was not adjusted enough in previous studies. So, we wish that our work will provide some useful data and information on stone disease therapy.

Conflict of interest

The authors declare that they have no competing interest.

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