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Original Article

Cannabidiol attenuates arsenic-induced nephrotoxicity *via* the NOX4 and NF-kB pathways in mice

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Abstract

Background and purpose: Cannabidiol (CBD) is a phenolic terpene compound with anticancer, antioxidant, anti-inflammatory, antibacterial, neuroprotective, and anticonvulsant properties. Since the effects of CBD on sodium arsenite (As)-induced nephrotoxicity have not been fully determined, this study investigated the effect of CBD on As-induced nephrotoxicity by evaluating the NOX4 and NF-kB pathways in mice.
Experimental approach: 48 male mice were divided into six groups (8 each) including group 1, receiving saline for 14 days; group 2, receiving CBD (10 mg/kg, intraperitoneally) from the 7th to the 14th day; group 3, receiving As (10 mg/kg) for 14 days by gavage; and treatment groups 4-6, receiving CBD (2.5, 5, and 10 mg/kg, i.p.) 1.5 h before As (10 mg/kg by gavage, for 14 days) from the 7th to the 14th day. Mice were anesthetized after overnight fasting on day 15, and the blood sample was collected from their hearts. The level of antioxidants and pro-inflammatory factors, the expression of ROS and TNF-α, NF-kB, NOX4, iNOS, cleaved PARP, and caspase-3 proteins were measured and histological studies were performed.
Findings/Results: Exposure to As significantly increased kidney markers, oxidative stress, apoptosis, and inflammation in mice kidney tissue, and pretreatment with CBD reversed these changes. In addition, CBD significantly decreased the expression of NF-kB and NOX4, and the levels of pro-inflammatory factors and the expression of cleaved PARP and increased the level of antioxidants.
Conclusion and implications: CBD ameliorated As-induced nephrotoxicity related to inhibiting oxidative stress, inflammation, and apoptosis, potentially through the NF-κB/Nox4 pathway.

Keywords: Arsenic; Cannabidiol; Nephrotoxicity;NF-kB; NOX4.

INTRODUCTION

The kidney plays a vital role in the body's homeostasis, which includes the excretion of excess metabolites, regulation of extracellular fluid volume, regulation of electrolytes, acid-base balance, and synthesis and secretion of renin and erythropoietin hormones (1-3). Acute kidney injury is a common disorder characterized by a rapid decrease in glomerular filtration rate, disturbance in the volume and balance of extracellular fluid electrolytes, disturbance in acid-base homeostasis, and retention of blood urea nitrogen (BUN) and creatinine (Cr). Exposure to heavy metals, including sodium arsenite (As), in both acute and chronic forms, can lead to changes in kidney morphology, including tubular dilation and glomerular congestive insufficiency, and ultimately increase kidney cancer risk (4).

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Studies have shown that oxidation, inflammation, and apoptosis are related to As-induced nephrotoxicity, and oxidative stress is one of the effective factors in causing nephrotoxicity (5-7). Studies have shown that As promotes apoptosis through the reduction of antioxidant activity (glutathione, superoxide dismutase (SOD), and catalase (CAT)) (8,9). Also, As significantly increases the expression of nuclear factor kappa B (NF-κB) in the kidney tissue, which ultimately increases the expression of pro-inflammatory mediators including tumor necrosis factor-alpha (TNF-α) and interleukin 1 beta (IL-1β) (10,11). Despite numerous laboratory studies, the mechanism of As nephrotoxicity is unknown, and the treatment of As poisoning is limited to chelator therapy, which has many adverse effects such as hepatotoxicity, neurotoxicity, and nephrotoxicity. Today, attention is paid to natural antioxidants, due to their more effective protective potential than synthetic antioxidants (12) Cannabis (*Cannabis sativa* L.) has been used historically for fibers, oil, and medicinal purposes. The main compounds, delta-9-tetrahydrocannabinol and cannabidiol (CBD), are known for their antioxidant and anti-inflammatory properties, though the mechanisms of their actions are not fully understood (13).

CBD is a non-psychoactive component of marijuana that has anti-inflammatory and antioxidant properties (14). According to the studies, CBD leads to a decrease in the level of pro-inflammatory cytokines and migration and adhesion of immune cells and inhibition and apoptosis of T cells (15). Cannabidiol also reduces the production of reactive oxygen species (ROS) by chelating metal ions (16). Cannabidiol has been reported to have a protective effect on ischemic injury by reducing TNF-α and NADPH oxidase 4 (NOX4) (17). CBD showed a protective role against kidney damage by reducing oxidative factors and NOX enzyme activity and modulating TNFα levels (18). Caspase-3 is a key executioner of apoptosis, responsible for the cleavage of various cellular components, while poly (ADP-ribose) polymerase (PARP) is involved in DNA repair, and its cleavage is a hallmark of apoptosis. Therefore, the assessment of such biomarkers serves as a vital tool to evaluate the extent of apoptotic cell death in kidney tissue (19). This study aimed to evaluate the protective effect of CBD against As-induced nephrotoxicity in mice with emphasis on the role of NOX4 and NF-κB signaling pathways.

MATERIALS AND METHODS

Chemicals

CBD was obtained from Hansu Biotechnology Co. Ltd. (Yunnan, China). Sodium arsenite was purchased from Sigma Chemical Co. (USA). BUN and Cr assay kits were purchased from Bionic assay kits (Germany). The level of TNF-α was assessed using an enzyme-linked immunosorbent assay (ELISA) kit purchased from PicoKine® (EK0527, Germany). ROS was measured using the OxiSelect™ Intracellular ROS assay kit (green fluorescence, Cat No. STA-342) purchased from Cell Biolabs Inc. (USA). The amounts of SOD measurement kits were purchased from ZellBio (Germany). The expression of NF-κB, NOX4, inducible nitric oxide synthase (iNOS), cleaved PARP, and caspase-3 protein was evaluated using western blotting.

Animals and ethical approval

In this study, 48 NMRI mice weighing (25 ± 2 g) were placed under a 12/12-h light/dark cycle, temperature of 23 ± 2 °C, and relative humidity. All ethical principles of use and care of animals were based on the protocols provided by AJUMS (Ethics No. IR.AJUMS.ABHC.REC.1401.100)

Experimental design

The animals were divided into six groups (8 each): group 1, control (receiving saline for 14 days); group 2, receiving CBD (10 mg/kg, intraperitoneally for 7 days, from the 7th to the 14th day); group 3, receiving As (10 mg/kg by gavage for 14 days); and treatment groups 4-6, receiving CBD (2.5, 5, and 10 mg/kg, respectively, intraperitoneally for 7 days, from the 7th to the 14th day) 1.5 h before As (10 mg/kg by gavage, for 14 days). The CBD and As doses were selected based on previous research (18,20).

The animals were anesthetized with ketamine (90 mg/kg)/xylazine (10 mg/kg) on day 15. The blood samples were collected from the heart. After separating the serum, the sample was stored at -20 °C until the tests were done. The kidneys were extracted and washed with normal saline. The right kidney in 10% formalin solution was transferred to the laboratory for histopathological examination. The left kidney was kept at -70 °C to measure oxidative and inflammatory factors. The amounts of ROS and TNF-α were measured using ELISA. The protein expression of nuclear factor-κB (NF-kB), NOX4, iNOS, PARP, and caspase-3 was measured by western blotting.

Determination of the kidney index

The kidney index was reported as the percentage ratio of kidney weight (g)/body weight (g).

Determination of serum biochemical markers

The levels of BUN and Cr in the serum samples were measured using Bionic assay kits (Germany) with Hitachi 912 auto-analyzer (Japan).

Preparation of kidney tissue homogenate to determine protein content

To determine the protein content, the left kidney tissue was homogenized in a phosphate buffer, using a homogenizer and centrifuged. The supernatant was separated and stored in a freezer at -70 ℃ until the amount of thiobarbituric acid reactive substances (TBARS) and total thiol, the activity of SOD, CAT, ROS, TNF-α, and the expression of NF-kB, NOX4, iNOS, PARP, and caspase-3 proteins were measured. The protein concentration of kidney tissue supernatant was measured by the Bradford method (21).

Determination of total thiol

The total thiol content in kidney tissue was quantified using Ellman's reagent, 5,5'-dithiobis(2-nitrobenzoic acid, DTNB), which produces yellow 5-thio-2-nitrobenzoic acid (TNB). The intensity of the yellow color was measured at 412 nm using a spectrophotometer, and the results were reported as nmol/mg protein (22).

Determination of TBARS

The TBARS level was determined by the method applied by K. Satoh (23). The absorbance of the resultant pink color was measured at 532 nm, with the findings expressed as nmol/mg protein.

Determination of catalase activity

CAT activity was measured using the Shangari method (24). The absorbance was recorded at 410 nm with a microplate reader, and the results were expressed as IU/mg protein (25).

Activity determination of SOD

SOD activity was determined colorimetrically using an enzymatic assay kit from ZellBio (Germany) and reported as IU/mg protein.

Determination of TNF-α

The TNF-α level in kidney tissue was assessed using an ELISA kit. The absorbance was measured at 450 nm, and the results were expressed as pg/mg protein.

Determination of ROS

Kidney tissues were homogenized to prepare a 10% homogenate in cold 40 mM Tris-HCl buffer (pH 7.4). The homogenate supernatant was then mixed with the cell-permeable fluorescent probe 2', 7'-dichlorodihydro-fluorescin diacetate (DCFDA) at a final concentration of 10 µM, dissolved in dimethyl sulfoxide (DMSO). The mixture was incubated at 37 ℃ for 30 min in a dark place. After incubation, the solution was removed, and the cells were washed multiple times with Dulbecco's phosphate-buffered saline (DPBS). Subsequently, the fluorescence intensity of the oxidized form of DCFDA was measured using a fluorescence plate reader at an excitation wavelength of 480 nm and an emission wavelength of 530 nm. The ROS levels were quantified by comparing the fluorescence readings against a standard curve generated with the provided DCF standard.

Determination of protein expression of NF-kB, NOX4, iNOS, PARP, and caspase-3

Kidney tissue samples were homogenized on ice with radioimmunoprecipitation assay (RIPA) lysis buffer (Tris-HCl 50 mM pH 7.6, NaCl 150 mM, EDTA 1 mM, Triton® X-100

0.1%, NaF 1 mM) supplemented with protease inhibitor cocktails (Sigma-Aldrich, MO, USA). The homogenates were centrifuged at 12000 rpm for 15 min at 4 °C, and the supernatant protein content was quantified using the Bradford assay. Equal amounts of protein (20 µg/lane) were loaded in Laemmli-buffer (2% sodium dodecyl sulfate (SDS), 5% 2-mercaptoethanol, 10% glycerol, 0.002% bromphenol blue, 0.0625 M Tris-HCl, pH 6.8) and heated to 95 °C for 10 min. Proteins were separated by 10% SDS polyacrylamide gel electrophoresis (SDS-PAGE) and transferred into polyvinylidene difluoride (PVDF) membranes at a constant voltage of 12 V overnight. After blocking, the PVDF membranes were incubated at 4 °C overnight with various specific primary antibodies in Tris-buffered saline containing 0.1% Tween-20 (TBST) against NF-κB (1:1000, Cat No. D14E12, Cell Signaling Technology, USA), NOX4 (1:1000, Cat No. D8L7U, Cell Signaling Technology), iNOS (1:1000, Cat No. D6B6S, Cell Signaling Technology), PARP (1:1000, Cat No. 46D11, Cell Signaling Technology), and caspase-3 (1:1000, Cat No. D3R6Y, Cell Signaling Technology, USA). The membranes were then incubated for 2 h with the corresponding secondary antibodies. Glyceraldehyde 3-phosphate dehydrogenase (GAPDH) enzyme (1:1000, Cat No. D16H11, Cell Signaling Technology) was used as an internal control for equal loading. Finally, the protein band signals were visualized and detected by enhanced chemiluminescence (ECL; Thermo Scientific, USA) and analyzed by densitometry using ImageJ software.

Histopathological examination

After blood collection, the mouse kidneys were removed immediately, and the suitable kidney tissue of the mice was fixed in 10% formalin. Then, it was dehydrated in graded concentrations of alcohol and embedded in paraffin. The sections of 4-6 µm were prepared and stained with hematoxylin and eosin. Six microscopic slides of each animal were examined to assess congestion of red blood cells (RBCs), infiltration of inflammatory cells, and percentage of damaged proximal tubule. The histological features were graded into four categories: normal (0), weak (1), moderate (2), or intense (3), and the averages were considered.

Statistical analysis

Statistical analysis was performed by GraphPad Prism software (version 9.5.0; GraphPad Software, Inc., San Diego, CA, USA) and the normality of the data was confirmed using the Kolmogorov-Smirnov test. The data are presented as mean ± SEM and analyzed using one-way ANOVA followed by Tukey's post hoc test for multiple comparisons. The *P*-values < 0.05 were considered statistically significant.

RESULTS

Effect of CBD on kidney index

The effect of CBD on the kidney index (kidney weight/body weight) showed that this parameter was significantly reduced in the As group compared to the control group. In the group receiving CBD alone at 10 mg/kg the kidney index compared to the control was not significantly affected. However, compared to the As group, co-treatment of As with CBD resulted in a dose-dependent increase in the kidney index. Specifically, CBD at 5 and 10 mg/kg showed significant improvements (Fig. 1).

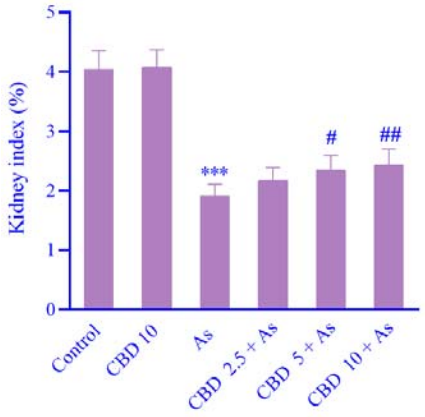


Fig. 1. The effect of As and CBD on kidney index (kidney weight/body weight). The groups received As at 10 mg/kg and/or CBD at 2.5, 5, and 10 mg/kg as shown on the graph. Data are expressed as mean ± SEM. ***P < 0.001 indicates a significant difference compared to the control group; $^{\#}P$ < 0.05 and $^{\#\#}P$ < 0.01 versus the As group. As, Sodium arsenite; CBD, cannabidiol.

Effect of CBD on biomarkers of kidney function

Measurement of the BUN and Cr at the end of the experiment showed that the serum levels of BUN and Cr were significantly elevated in the As group compared to the control group. Treatment with CBD alone at 10 mg/kg did not significantly affect these factors compared to the control group. Co-treatment of As with CBD reduced these biomarkers in a dose-dependent manner in comparison with the As group. CBD at 5 mg/kg significantly improved BUN and Cr levels, while 10 mg/kg of CBD showed a more pronounced improvement (Fig. 2A and B).

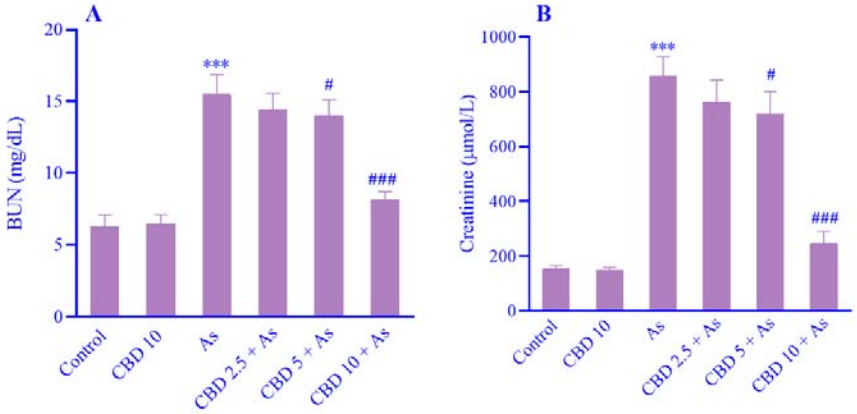


Fig. 2. The effect of As and CBD on the levels of (A) BUN and (B) Cr in the serum. The groups received As at 10 mg/kg and/or CBD at 2.5, 5, and 10 mg/kg as shown on the graphs. Data are expressed as mean ± SEM. ***$P < 0.001$ indicates significant differences compared to the control group; $^{\#}P < 0.05$ and $^{\#\#\#}P < 0.001$ versus the As group. As, Sodium arsenite; CBD, cannabidiol; BUN, blood urea nitrogen; Cr, creatinine.

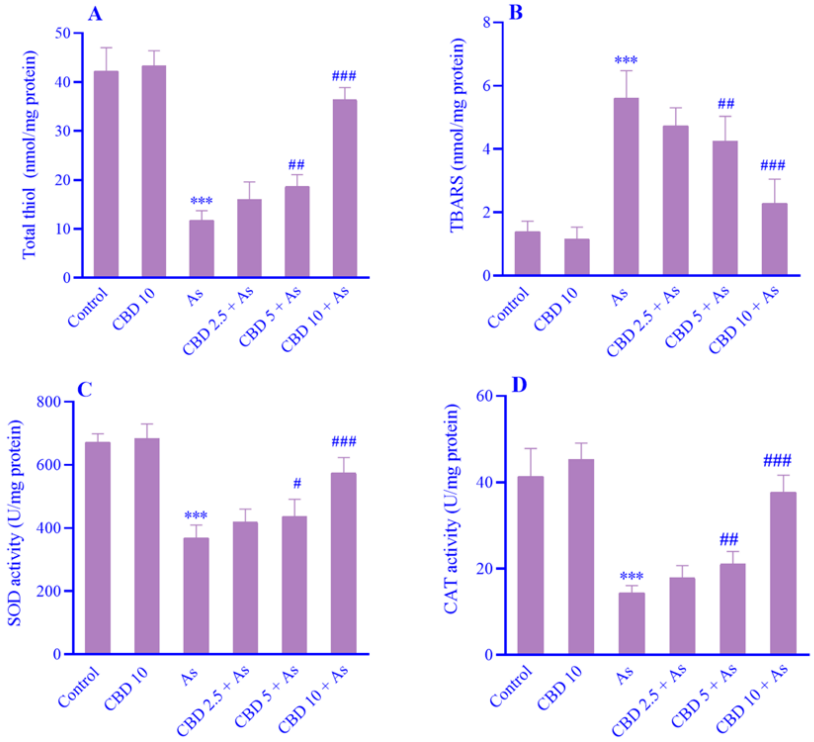


Fig. 3. The effect of As and CBD on (A) TT levels, (B) TBARS levels, (C) SOD activity, and (D) CAT activity. The groups received As at 10 mg/kg and/or CBD at 2.5, 5, and 10 mg/kg as shown on the graphs. Data are expressed as mean ± SEM. ***$P < 0.001$ indicates significant differences compared to the control group; $^{\#}P < 0.05$, $^{\#\#}P < 0.01$, and $^{\#\#\#}P < 0.001$ versus the As group. As, Sodium arsenite; CBD, cannabidiol; TT; total thiol; TBARS, thiobarbituric acid reactive substances; SOD, superoxide dismutase; CAT, catalase.

Effect of CBD on oxidative markers of kidney tissue

In the As group, the activity of SOD and CAT enzymes, and the amount of total thiol significantly decreased, while TBARS levels strikingly increased compared to the control group. In the group receiving CBD alone at 10 mg/kg, no significant alterations in these markers were observed compared to the control. However, co-treatment of As with CBD led to a significant increase in the activity of SOD and CAT and total thiol levels, and a decrease in TBARS levels in the treatment groups. These changes were significant in the groups receiving CBD at 5 and 10 mg/kg, indicating a dose-dependent improvement in oxidative stress markers (Fig. 3 A-D).

Effect of CBD on TNF-α and ROS levels

The results showed that ROS and TNF-α levels in the As-treated group significantly elevated compared to the control group. Receiving CBD alone at 10 mg/kg did not significantly affect the amount of ROS and TNF-α compared to the control. However, co-treatment of As with CBD at 2.5, 5, and 10 mg/kg resulted in a dose-dependent decrease in both TNF-α and ROS levels compared to the As group. In particular, the doses 5 and 10 mg/kg of CBD showed significant improvements in both TNF-α and ROS levels (Fig. 4 A and B).

Effect of CBD on the expression of kidney proteins

The protein expression of NF-κB, NOX4, iNOS, cleaved PARP, and caspase-3 in the As group significantly increased compared to the control group. The expression of the mentioned protein did not change following the administration of CBD alone at 10 mg/kg versus the control group. However, the groups treated with As and CBD (5 and 10 mg/kg) indicated a significant reduction in the expression of all the examined proteins compared to the As group which was dose-dependent (Fig. 5 A-E). Furthermore, the co-treatment of As with CBD at 2.5 mg/kg could significantly decrease caspase-3 expression versus As group (Fig. 5E).

The effect of CBD on histopathological markers

Hematoxylin and eosin staining of the kidney showed that the tissue structure of the kidney was normal in the control and the group treated with CBD at 10 mg/kg. In the As group, the percentage of damaged kidney tissue tubes increased significantly compared to the control group. The groups receiving CBD indicated a reduction of tissue damage in a dose-dependent manner. Specifically, administration of CBD at 5 mg/kg brought about a significant improvement in histopathological markers while 10 mg/kg of CBD demonstrated a more effective attenuation of tissue damage (Fig. 6 and Table 1). The effect of CBD on congestion of RBCs, infiltration of inflammatory cells, and damaged proximal tubule (%) of the kidney in the As toxicity is shown in Table 1.

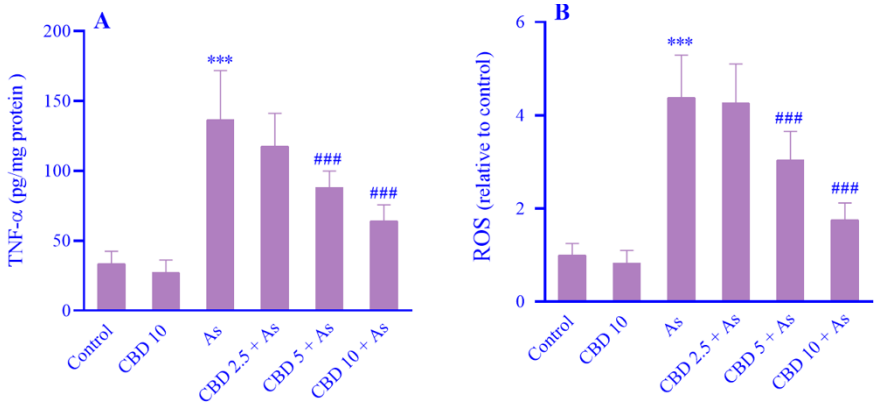
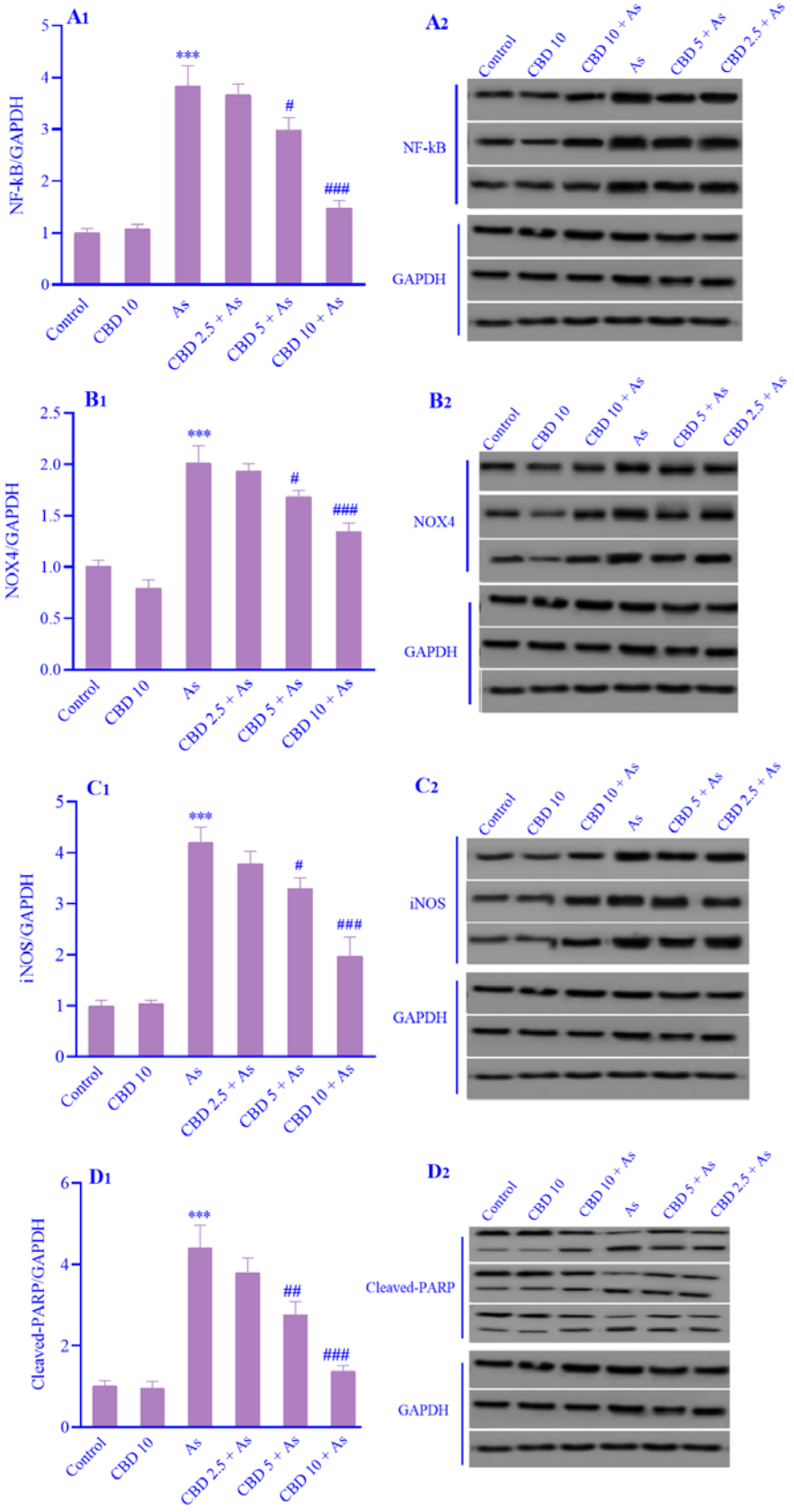


Fig. 4. The effect of As and CBD on the levels of (A) TNF-α and (B) ROS. The groups received As at 10 mg/kg and/or CBD at 2.5, 5, and 10 mg/kg as shown on the graphs. Data are expressed as mean ± SEM. ***$P < 0.001$ indicates significant differences compared to the control group; $^{###}P < 0.001$ versus the As group. As, Sodium arsenite; CBD, cannabidiol; TNF-α, tumor necrosis factor alpha; ROS, reactive oxygen species.


A1
NF-kB/GAPDH
***
#
###
Control
CBD 10
As
CBD 2.5 + As
CBD 5 + As
CBD 10 + As
A2
NF-kB
GAPDH
B1
NOX4/GAPDH
B2
NOX4
C1
iNOS/GAPDH
C2
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D2
Cleaved-PARP

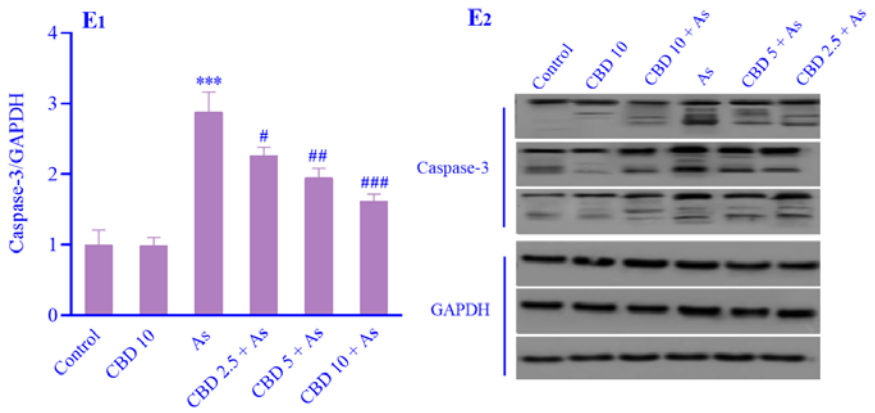

Fig. 5. The effect of As and CBD on the kidney protein expression: (A_1) NF-κB, (B_1) NOX4, (C_1) iNOS, (D_1) cleaved PARP, and (E_1) caspase-3 protein. (A_2-E_2) represent western blotting images for the corresponding proteins and GAPDH in groups treated with CBD (2.5, 5, 10 mg/kg) and/or As (10 mg/kg). Data are expressed as mean ± SEM. $^{***}P < 0.001$ indicates significant differences compared to the control group. $^{\#}P < 0.05$, $^{\#\#}P < 0.01$, and $^{\#\#\#}P < 0.001$ versus the As group. As, Sodium arsenite; CBD, cannabidiol; NF-κB, nuclear factor kappa-light-chain-enhancer of activated B cells; NOX4, NADPH oxidase 4; PARP, poly (ADP-ribose) polymerase.

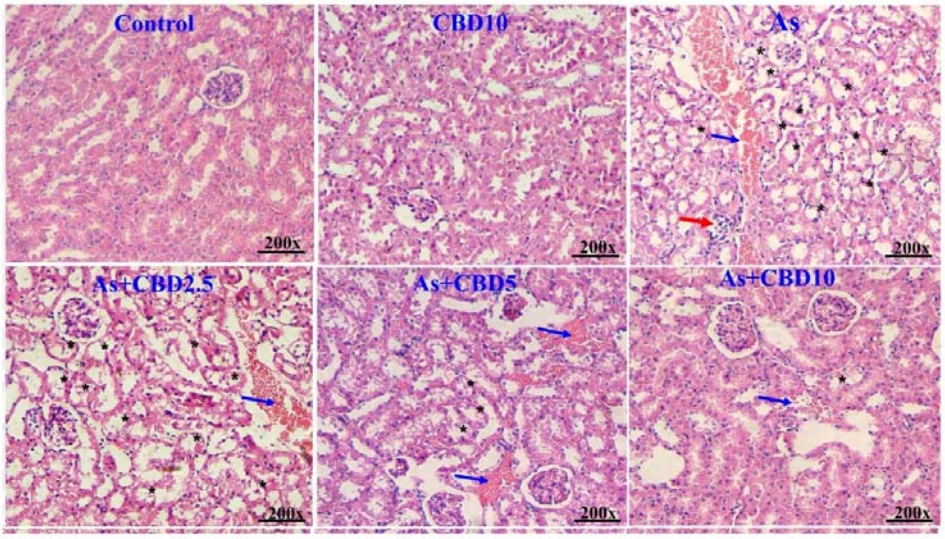

Fig. 6. Light microscopy examination of kidney tissue sections stained by hematoxylin and eosin in the groups received As at 10 mg/kg and/or CBD at 2.5, 5, and 10 mg/kg as shown on the graphs. The red arrow indicates inflammation, the blue arrow for congestion of red blood cells, and the asterisks for damaged proximal tubules. Magnification: × 200. As, Sodium arsenite; CBD, cannabidiol.

Table. 1. The effect of CBD on congestion of RBCs, infiltration of inflammatory cells, and damaged proximal tubule (%) of kidney in the As toxicity. $^{***}P < 0.001$ indicates significant differences compared to the control group; $^{\#}P < 0.05$ and $^{\#\#\#}P < 0.001$ versus the As group.

Groups	Congestion of RBCs	Infiltration of inflammatory cells	Damaged proximal tubule (%)
Control	0.11 ± 0.04	0.06 ± 0.02	0.00 ± 0.00
As (10 mg/kg)	1.44 ± 0.14***	0.89 ± 0.12***	9.61 ± 1.54***
CBD (10 mg/kg)	0.095 ± 0.02	0.04 ± 0.00	0.00 ± 0.00
CBD (2.5 mg/kg)+ As (10 mg/kg)	1.28 ± 0.22***	0.67 ± 0.11***	9.33 ± 1.86***
CBD (5 mg/kg) + As (10 mg/kg)	0.53 ± 0.16#	0.56 ± 0.17#	5.17 ± 0.28#
CBD (10 mg/kg) + As (10 mg/kg)	0.18 ± 0.05###	0.17 ± 0.04###	2.80 ± 0.19###

As, Sodium arsenite; CBD, cannabidiol; RBC, red blood cells.

DISCUSSION

This study confirmed that CBD attenuates As-induced nephrotoxicity in mice. Environmental pollution such as heavy metals, air pollutants, chemicals, drinking water, and contaminated food is the leading cause of disease, disability, and death worldwide (26). Arsenic is a heavy metal found in groundwater and food and can lead to kidney cancer (27). BUN and Cr are considered the markers of kidney damage and increasing such markers leads to their release from the kidney into the blood and causes oxidative stress and nephrotoxicity. In this study, exposure to a dose of 10 mg/kg of As, led to an elevation of nitrogenous waste products such as BUN and Cr which is consistent with previous studies (28).

CBD treatment remarkably inhibited elevations of BUN and Cr in a dose-dependent manner, which demonstrated that CBD could alleviate As-induced nephrotoxicity (Fig. 2 A and B).

Arsenic promotes apoptosis by reducing the activity of antioxidants total thiol, SOD, and CAT (29). In this study, the activity of antioxidant enzymes of SOD, and CAT and the amount of total thiol decreased in the As group. In contrast, these markers increased in the groups receiving CBD (Fig. 7). According to the findings of the previously conducted studies, CBD reduces the production of ROS by increasing the antioxidant enzymes (30). In our study, exposure to As increased the level of ROS while CBD led to the removal of ROS and the reduction of TBARS levels production. In this study, As significantly increased the expression of NF-kB in the kidney tissue, which ultimately increased the expression of pro-inflammatory mediator of TNF-α which complied with the results of the studies conducted by Hofmann *et al.* and Hsu *et al.* (31,32).

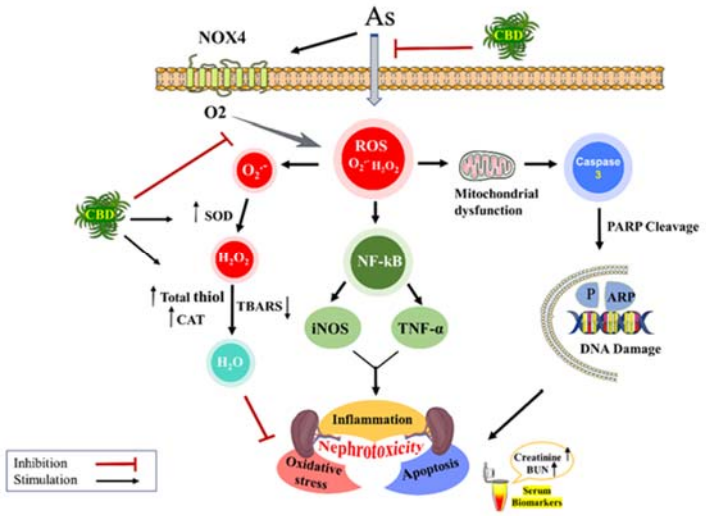

Fig. 7. The effect of As toxicity and co-treatment with CBD on the nephrotoxicity in mice. Aarsenic-induced nephrotoxicity is characterized by increased ROS production through the activation of NOX4 and NF-kB, resulting in elevated levels of inflammatory mediators such as TNF-α and iNOS. Arsenic also induces apoptosis *via* caspase-3 activation, leading to PARP cleavage, and contributes to increased lipid oxidation (TBARS) by reducing antioxidant factors (SOD, CAT, and total thiol). CBD treatment mitigates these pathological changes by decreasing NOX4 expression, ROS production, and NF-kB activation. It improves kidney function markers (Cr BUN) and reduces the expression of inflammatory mediators (TNF-α and iNOS) as well as apoptosis factors (Caspase-3). Furthermore, CBD treatment restores antioxidant levels. The protective effects of CBD were dose-dependent, with significant benefits observed at doses of 5 and 10 mg/kg. ROS, Reactive oxygen species; NOX4, NADPH oxidase 4; NF-kB, nuclear factor kappa-light-chain-enhancer of activated B cells; TNF-α, tumor necrosis factor-alpha; SOD, superoxide dismutase; CAT, catalase; TBARS, thiobarbituric acid reactive substances; Cr, creatinine; BUN, blood urea nitrogen; iNOS, inducible nitric oxide synthase; PARP, poly (ADP-ribose) polymerase; CBD, cannabidiol; As sodium arsenite.

NOX oxidases are membrane-bound enzyme complexes contributing to producing oxygen free radicals, oxidative stress, and renal fibrosis (33-37). In the present study, co-treatment of As with CBD at 5 and 10 mg/kg, decreased the expression of NF-kB, NOX4, iNOS, cleaved PARP, and caspase-3 proteins compared to the As-treated group. The expression of NF-kB, NOX4, iNOS, cleaved PARP, and caspase-3 proteins increased in the As group compared to the control group. Based on the western blotting results, the band intensity of NF-kB, NOX4, iNOS, cleaved PARP, and caspase-3 proteins was significantly higher in animals treated with CBD 10 mg/kg compared to As group, indicating more protein expression. Arsenic is excreted through the kidney and causes kidney damage through the necrosis of tubular epithelial cells, mainly the proximal part (4). In this study, histopathological investigations showed inflammation and dilated sinusoids in As group. In the groups treated with As and CBD (5 and 10 mg/kg), the widening of the sinusoids and the infiltration of inflammatory cells were reduced to a large extent.

Therefore, this study showed that CBD mitigates arsenic-induced kidney toxicity in mice by enhancing antioxidant defense, reducing lipid peroxidation and inflammation, and decreasing proteins related to oxidative stress and cell death. The 10 mg/kg dose was the most effective. These results suggest CBD's potential for treating arsenic-induced nephrotoxicity, but further investigation is required to elucidate the precise molecular pathways underlying CBD's protective action, examine its pharmacokinetics and pharmacodynamics in diverse animal models, and evaluate its long-term effects, dose-response relationships, and potential interactions with other compounds in various experimental conditions. Additionally, studies exploring CBD's impact on different routes of arsenic exposure and its efficacy across various stages of kidney injury progression in animal models would be valuable.

CONCLUSION

This study confirmed that CBD attenuates As-induced nephrotoxicity in mice. CBD led to the strengthening of antioxidant defense, reduction of lipid peroxidation, inflammation and expression of proteins of NF-kB, NOX4, iNOS, cleaved PARP, and caspase-3. The dose of 10 mg/kg of CBD showed better results than 5, and 2.5 mg/kg. Finally, the findings of the present study provide evidence that CBD may serve as a potential therapeutic agent for the prevention and treatment of arsenic-induced nephrotoxicity. However, further research is needed to understand its molecular mechanisms and pharmacological properties. Studies should also assess its long-term effects, dose-response relationships, and efficacy in different stages of kidney injury.

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Conflict of interest statement

The authors declared that no conflict of interest in this study.

Authors' contributions

The study's conceptualization and design were jointly developed by M. Mahdavinia, M. Salehcheh, and A. Vadizadeh. Methodological frameworks were established by M. Mahdavinia, M. Salehcheh, M. Rashno, and A. Vadizadeh. Formal analysis and investigative procedures were carried out by M. Mahdavinia, M. Salehcheh, L. Khorsandi, M. Rashno, H. Kalantar, and A. Vadizadeh. The initial manuscript draft was prepared by M. Mahdavinia, H. Kalantar, and A. Vadizadeh, while subsequent review and editing were performed by M. Mahdavinia, M. Salehcheh, A. Vadizadeh, and L. Khorsandi. Funding acquisition was managed by A. Vadizadeh. Resources were provided by M. Mahdavinia, M. Salehcheh, and A. Vadizadeh. The study was supervised by M. Mahdavinia, M. Salehcheh, H. Kalantar, L. Khorsandi, and M. Rashno. The finalized article was read and approved by all authors.

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