



Research Article



Ameliorative Effect of Methanolic Extract of Broccoli on Diclofenac Sodium-induced Oxidative Damage in Rat Kidney

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ABSTRACT

Introduction: This study aimed to investigate the potential protective effect of the methanolic extract of broccoli against oxidative stress induced by diclofenac in rats. Non-steroidal anti-inflammatory drugs are known to cause nephrotoxicity, hence the need to explore the therapeutic potential of medicinal plants.

Materials and methods: A total of 48 adult male Wister rats with a maximum age of 2-3 months with an average weight of 220 g were randomly divided into four equal groups (12 in each group). The first group was a control (C) and fed physiological saline without treatment. The second group (BC) was treated with broccoli methanolic extract at a dose of 500 mg/kg/Intraperitoneal injection. The third group (DC) was treated with diclofenac sodium (100 mg/kg, Intra-muscular injection), and the fourth group was treated with diclofenac sodium (100 mg/kg, Intra-muscular injection) and broccoli (500 mg/kg/ Intraperitoneal injection). After blood collection, serum was isolated, and urea, creatinine, interleukin-1, and TNF- α were measured in blood serum. In kidney tissue, malondialdehyde, superoxide dismutase, catalase, and glutathione peroxidase were measured. At the end of the study, the samples were taken for histopathological investigation.

Results: The results of the present study indicated that diclofenac sodium causes severe kidney damage. The creatinine and urea levels significantly increased in the DC group, compared to the control and other treatment groups. The proinflammatory biomarkers in blood serum increased in the DC group and significantly decreased in the BC+DC group compared with control and other treatment groups. These changes were in line with the significant decrease of catalase, and glutathione peroxidase enzyme levels in the DC group and its increase in the BC group. Malondialdehyde increased in the DC group and reached its lowest level in the BC group. Hyperemic changes, accumulation of inflammatory cells, and bleeding were indicators of diclofenac tissue poisoning reported in the kidney.

Conclusion: The results of biochemical and histopathological showed that broccoli extract at the dosage of 500 mg/kg with strong antioxidant potential can play a protective role against diclofenac damage in the kidney.

1. Introduction

Non-steroidal anti-inflammatory drugs (NSAIDs) are commonly used to treat acute muscle pain conditions due to their analgesic and anti-inflammatory properties¹. However, their use at normal doses can cause gastrointestinal issues, while consuming high doses can lead to toxicity, causing acute or long-term chronic disorders such as renal diseases and cardiovascular disorders². Additionally, NSAIDs can also affect the body's oxidative balance³. Diclofenac sodium is a

commonly used NSAID to alleviate arthritis-associated pain and inflammation⁴. It is easily absorbed and accumulates at the site of inflammation, but its use can induce hepatorenal toxicity and cause changes in organs like the kidney, heart, and stomach^{5,6}.

Inflammation and oxidative stress are interconnected, as they increase the activation of transcription factors and gene expression of proinflammatory cytokines, leading to

a vicious cycle of inflammation^{7,8}. Oxidative damage can also decrease the phagocytic activity of leukocytes, further amplifying the inflammatory response^{9,10}. Therefore, it is essential to include natural and herbal compounds with antioxidant potentials, including vitamins, minerals, and phytonutrients, to maintain the body's health¹¹. To combat inflammation and oxidative stress, many people turn to natural remedies like vitamins, minerals, and phytonutrients found in fruits and vegetables^{12,13}. Broccoli is a particularly potent source of these beneficial compounds¹⁴. It contains high levels of Vitamin C, Vitamin E, and carotenoids, which are all powerful antioxidants that can help reduce inflammation and oxidative stress^{15,16}. The flavonoids found in broccoli, such as sulforaphane, kaempferol, and quercetin, have also been shown to have potent anti-inflammatory and antioxidant effects^{17,18}.

Sulforaphane is one of the most studied flavonoids in broccoli, and it has been found to have numerous health benefits¹⁹. Studies have shown that sulforaphane can protect against neurodegenerative diseases such as Alzheimer's and Parkinson's disease by reducing inflammation, oxidative stress, and neuronal loss^{20,21}. Sulforaphane has also been beneficial in treating other conditions, such as cancer, diabetes, and cardiovascular disease²²⁻²⁴.

To investigate the effect of broccoli extract on diclofenac poisoning, a study was conducted on rats to observe any changes in the biochemical and histopathological parameters of the kidney. This research aims to find a way to control and prevent renal toxicity caused by NSAIDs, emphasizing the importance of natural and herbal remedies in treating or preventing such conditions.

2. Materials and Methods

2.1. Ethical approval

All procedures were approved by the Animal Care Committee of Veterinary Medicine, Islamic Azad University of Sanandaj, Sanandaj, Iran. The principles of laboratory animal care were followed, and specific international laws were observed.

2.2. Animals

A group of 48 adult male Wister rats, with an average weight of 220 g and a maximum age of 2-3 months, were obtained from the laboratory animal unit of the Islamic Azad University of Sanandaj. The rats were kept under laboratory conditions and had free water and commercial food access. They were housed in cages with appropriate ventilation, a minimum of 50 percent humidity, 24°C temperature, and a 12-hour light/dark cycle. The rats were divided into four groups, with 12 rats in each group. The first group served as the control and received only physiological saline without any treatment. The second group was treated with broccoli methanolic extract at a

dose of 500 mg/kg via intraperitoneal injection and was labeled as the BC group. The third group was treated with diclofenac sodium (DC) at a dose of 100 mg/kg via intramuscular injection and was labeled as the DC group. The fourth group was treated with both broccoli (500 mg/kg via intraperitoneal injection) and diclofenac sodium (100 mg/kg via intramuscular injection) and was labeled as the BC plus DC group.

2.3. Serum biochemical analysis

Throughout 28 days, blood samples were collected from the hearts of the rats under anesthesia on the final day of the experiment. The blood was then processed using a centrifuge at 3000 rpm for 10 minutes to isolate the serum. The levels of urea, creatinine, interleukin-1, and TNF- α in the serum were measured using a Pars test kit and an autoanalyzer²⁵.

2.4. Measurement of renal oxidative stress parameters

At the end of the experimental period, the left and right kidneys of the rats were surgically removed. The kidneys were washed in a saline and ice bath and homogenized in a 1:10 (w:v) ratio with ice-cold 150 mM KC1. The amount of protein and malondialdehyde (MDA) in the homogenized tissue was measured promptly. The homogenized tissue was then stored at -70°C to measure the catalase (CAT) and superoxide dismutase (SOD) enzymes at a later time.

2.4.1. Catalase and superoxide dismutase measurements

The Claiborne method, as described by Haque et al. (2003), was used to measure catalase activity. To do this, the sample was combined with 0.09 M H₂O₂, 0.1 M phosphate buffer, and PMS (10%) up to a volume of 3 ml. The mixture was left for 30 seconds before the absorption was read at a wavelength of 240 nm using a spectrophotometer. The superoxide dismutase enzyme's activity was determined by the level of inhibition of the reaction between xanthine and xanthine oxidase, which produced superoxide radicals and led to the formation of a red color when reacting with 2-(4-iodophenyl)-3-(4-nitrophenyl)-5-phenyltetrazolium chloride.

2.4.2. Malondialdehyde and glutathione peroxidase measurements

Wright et al. used their method to measure the level of malondialdehyde. This involved adding TAC 10% in a volume that was twice that of the homogenized kidney tissue, followed by intense vortexing and centrifugation at 2500 rpm for 10 minutes. Next, TBA 0.67% was added in the same volume as the supernatant solution. The mixture was then placed in a water bath at 100 degrees for an hour, and the absorption was read at 532 nm to determine the level of malondialdehyde.

To measure glutathione peroxidase, the method described by Paglia and Valentine was used. This involves

measuring the oxidation reaction of glutathione (GSH) via cumene hydroperoxide using light absorption at a wavelength of 340 nm²⁶.

2.5. Preparation of methanolic extract from broccoli

In April 2021, broccoli (scientific name: *Brassica oleracea*) was gathered from a farm in Hamadan and verified by the Herbarium Center of Kurdistan University (No: 30073). The broccoli heads were cut into small florets after removing the leaves and hard stems. Two grams of these dried florets were mixed with 200 mL of 80% methanol and left at 25°C overnight. The solution was then filtered using (German Whatman) paper, and the methanol was removed using a vacuum rotary machine (IKA-RV 8). The resulting substance was dried in an oven and lyophilized to form a powder. This powder was then dissolved in sterile distilled water at a concentration of 0.1 g/mL and injected into rats intraperitoneally in suitable doses. The tolerable dose of 500 was determined with the aid of previous research and a pilot project.

2.6. Histopathological investigation

The kidneys were immersed in a 10% formaldehyde buffer to preserve them and were then examined pathologically. To do this, the kidneys were stained with HandE stain. The evaluation involved looking for indicators of inflammatory infiltration cells, hyperemia, and hemorrhage in the kidney tissue.

2.7. Data analysis

The data were checked for normality using the Kolmogorov-Smirnov test. Descriptive statistics were then presented as the mean value \pm standard deviation. Next, the results were analyzed using a statistical method called one-way analysis of variance (ANOVA), which was followed by the Tukey post hoc test. The significance level for the results was set at less than 0.05.

3. Results

3.1. Biochemical findings

The concentration of creatinine in cases of kidney damage caused by DC peaked at 1.52 ± 0.17 mg/dl, which differed significantly from the other groups ($p < 0.05$). The DC group showed the highest amount of urea, while the C group showed the lowest. The difference in serum urea concentration between the DC and other groups was also significant ($p < 0.05$, Figure 1).

The DC group exhibited an increase in proinflammatory biomarkers (TNF- α and IL-1) in their blood serum, while a significant decrease was observed in the DC plus BC and BC groups ($p < 0.05$). These changes were consistent with a significant decrease in the levels of GPx and CAT enzymes in the DC group and an increase in the BC group ($p < 0.05$). The MDA levels increased in the DC group but decreased to their lowest level in the BC group (Table 1).

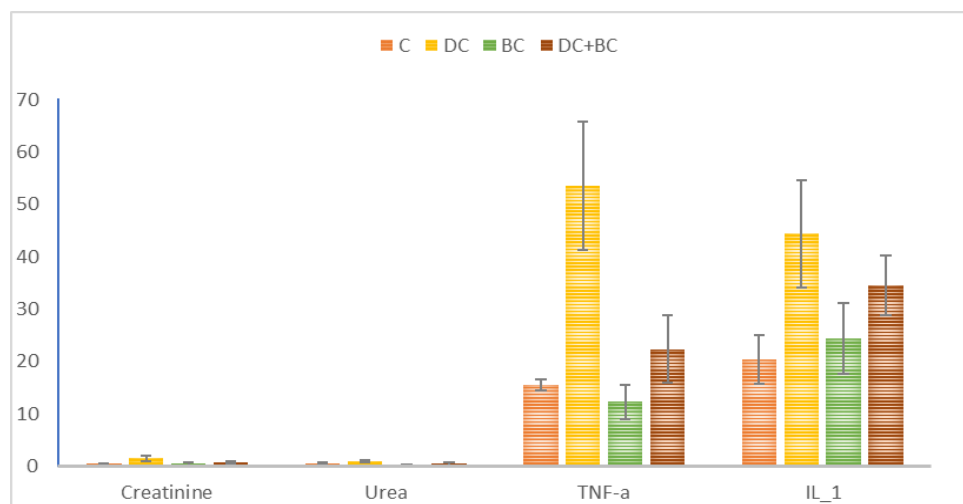


Figure 1. Serum biochemical findings of experimental groups

C: Control, DC: Diclofenac sodium at a dose of 100 mg/kg, BC: Broccoli methanolic extract at a dose of 500 mg/kg

Table 1. Renal oxidative stress parameters of experimental groups

Parameters	Groups			
	C	DC	BC	DC plus BC
GPx (U/g)	150.47 \pm 13.45	45.30 \pm 16.03 ^a	170.12 \pm 12.45 ^b	93.27 \pm 9.28 ^{a, b}
SOD (U/g)	30.24 \pm 5.17	10.12 \pm 3.22 ^a	29.26 \pm 4.16 ^b	22.15 \pm 10 ^b
CAT (U/g)	27.39 \pm 3.48	5.39 \pm 1.25 ^a	28.23 \pm 7.60 ^b	15.58 \pm 6.19 ^{b, c}
MDA (nmol/g)	1.55 \pm 0.45	10.12 \pm 3.44 ^a	1.40 \pm 0.12 ^b	6.12 \pm 2.23 ^{a, b, c}

C: Control, DC: Diclofenac sodium at a dose of 100 mg/kg, BC: Broccoli methanolic extract at a dose of 500 mg/kg, GPx: Glutathione peroxidase, SOD: Superoxide dismutase, CAT: Catalase, MDA: Malondialdehyde

^{a, b, c}, Different superscripts letters in the same group mean significant differences ($p < 0.05$)

Table 2. Inflammatory infiltration cells, hyperemia, and hemorrhage indicators of kidney tissue

Parameters	C	DC	BC	DC plus BC
Inflammatory infiltration cell	0.03 ± 0.01	5.15 ± 1.10 ^a	0.04 ± 0.12	0.78 ± 0.5
Hyperemia	0.04 ± 0.01	3.56 ± 0.47	0.02 ± 0.01	1.03 ± 0.26
hemorrhage	0.02 ± 0.02	2.35 ± 0.5	0.03 ± 0.02	1.12 ± 0.67

C: Control, DC: Diclofenac sodium at a dose of 100 mg/kg, BC: Broccoli methanolic extract at a dose of 500 mg/kg,

^{a,b,c}, Different superscripts letters in the same group mean significant differences ($p < 0.05$)

3.2. Histopathological findings

Regarding the histological structure of kidney tissue, the control and broccoli groups demonstrated healthy tissue without any complications. However, hyperemic changes, inflammatory cell accumulation, and bleeding were observed in the kidney tissue, indicating tissue poisoning caused by diclofenac (Table 2).

4. Discussion

Non-steroidal anti-inflammatory drugs (NSAIDs) are widely used to treat pain and inflammation²⁷. However, the administration of high doses of NSAIDs, such as diclofenac sodium, can cause several adverse reactions²⁸. Diclofenac sodium is known to cause renal toxicity, which can lead to acute kidney injury or chronic kidney disease²⁹. Renal toxicity induced by diclofenac is thought to be due to oxidative stress caused by the generation of reactive oxygen species (ROS) or reactive nitrogen species (RNS)³⁰.

ROS are generated in the body as a byproduct of normal metabolic processes. They can cause oxidative stress when their production exceeds the body's ability to detoxify them³¹. Oxidative stress can lead to cellular damage and dysfunction, including in the kidneys³². The body has several enzymatic and non-enzymatic antioxidant defenses to deactivate ROS and prevent oxidative stress³³.

In this study, it was observed that diclofenac administration increased blood urea, creatinine, and oxidative stress levels. Furthermore, diclofenac-induced renal tissue damage was associated with increased oxidative stress and morphological changes consistent with renal damage. However, the administration of broccoli at a dose of 500 mg/kg mitigated the negative effects of diclofenac, suggesting that it has antioxidant properties.

Broccoli is a rich source of various nutrients, including vitamin C, vitamin A, and glutathione, which are potent antioxidants³⁴. These antioxidants can neutralize ROS and prevent oxidative stress³⁵. Therefore, the protective effects of broccoli observed in this study could be attributed to its antioxidant content.

The present study confirms previous findings that the administration of diclofenac sodium leads to an increase in serum levels of urea, uric acid, and creatinine. Urea is a byproduct of protein metabolism and its accumulation in the blood can be attributed to a decrease in glomerular filtration rate (GFR) caused by diclofenac sodium³⁶. This drug inhibits the production of prostaglandins, which play a vital role in maintaining GFR. Uric acid is a metabolic product of purine metabolism, and its accumulation can result from either increased purine intake or impaired

renal function³⁷. Similarly, increased serum creatinine levels can be an indicator of reduced GFR caused by diclofenac sodium's effect on renal prostaglandins³⁸.

The study also indicated an increase in the activity of antioxidant enzymes, such as glutathione peroxidase and superoxide dismutase, as well as a decrease in the level of malondialdehyde in the BC group. These findings suggest that the antioxidant defense system is effective in reducing oxidative stress in this group. Broccoli extract contains vitamins C and E, polyphenols, and flavonoids are known to have antioxidant properties. These compounds may have contributed to the observed reduction in oxidative stress.

In contrast, the DC group showed an increase in oxidative stress as evidenced by decreased antioxidant enzyme activity and increased malondialdehyde levels. This suggests that diclofenac may disrupt the oxidant-antioxidant balance in the body. Moreover, the histological findings and presence of inflammatory cells in the kidney tissue of the DC group indicate the presence of an inflammation process, which is associated with an increase in proinflammatory cytokines. Despite the presence of anti-inflammatory compounds and phytoestrogens in broccoli, their effect on reducing inflammation in the DC+BC group was not significant.

The recent study's findings are consistent with Raeeszadeh et al.'s studies in 2021 and 2022 regarding broccoli's ability to improve kidney damage caused by lead and arsenic^{39,40}. The study also found that broccoli's ability to increase the level of SOD enzyme antioxidants is in line with another study by Raeeszadeh et al. in 2022, which demonstrated broccoli's superior effectiveness in reducing oxidative stress parameters compared to vitamins C and E in sperm freezing⁴¹.

Broccoli is a rich source of polyphenols, vitamins A, C, and E, flavonoids, glycosides, beta-carotene, and xanthine compounds, all of which have potent antioxidant effects. Broccoli also contains 1% sulforaphane, which reduces the damage caused by oxidative stress to DNA and lipids by inhibiting 8-hydroxy-2-deoxyguanosine. Dharmender and Gurinder found that exposure of female albino rats to triazophos could be reversed by the antioxidant properties of broccoli extract, resulting in improved stress biomarkers and reduced lipid peroxidation levels⁴².

Additionally, broccoli's antioxidant properties can be enhanced by various processing methods, making it a unique and potent herbal compound. Basha et al. demonstrated that calcium could reverse lead-induced alterations in the cholinergic and aminergic systems of rats' hippocampus and cerebellum, respectively. Broccoli, which is high in calcium, may be effective in reducing lead-induced toxicity⁴³.

5. Conclusion

The study's biochemical and histopathological results indicated that administering broccoli extract at a dosage of 500 mg/kg, which has potent antioxidant potential, can provide protective effects against diclofenac-induced damage to the kidneys.

Declarations

Competing interests

The authors have declared no conflicts of interest.

Authors' contributions

Mahdeih Raeeszadeh designed the study and performed the sampling, statistical analysis, and practical procedures. Pouria Ahmadi Simab wrote the draft of the manuscript. All authors check the final proof of the article and the statistical results.

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Ethical considerations

The authors declare that this manuscript is original and has not been submitted elsewhere for possible publication. The authors also declare that the data used/presented in this manuscript has not been fabricated.

Availability of data and materials

The data presented in this study are available on request from the corresponding author.

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