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RESEARCH ARTICLE

Therapeutic Effect of Oroxylin A Against Bisphenol A-induced Kidney Damage in Rats: a Histological and Biochemical Study

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A B S T R A C T

Bisphenol A (BPA) is a synthetic chemical compound used in plastic products that adversely affects health. BPA exposure is associated with multiple organ toxicity, particularly renal toxicity. Oroxylin A (ORA) is a flavonoid that has potential therapeutical properties. The current study explored the effect of different doses of ORA on BPA-prompted inflammatory responses and oxidative stress in kidneys. The experiment was executed on ninety-six rats (200 ± 20 g) that were categorized into 8 groups. The impact of orally treated various doses (15 mg/kg, 30 mg/kg and 60 mg/kg) of ORA was investigated against the toxic effects caused by 10 mg/kg of BPA. After 56 days of the experiment, rats were euthanized to detect the level of renal function markers (urea and creatinine), oxidative stress markers, inflammatory markers, antioxidant enzyme activity and histological architecture. According to the results, BPA administration decreased the glutathione level as well as activities of glutathione peroxidase, glutathione reductase, catalase, glutathione-S-transferase and superoxide dismutase, while increasing the level of the oxidative stress markers (malondialdehyde, reactive oxygen species and hydrogen peroxide). BPA exposure significantly increased the level of urea and creatinine, while decreasing creatinine clearance. Moreover, BPA exposure remarkably increased inflammatory responses by augmenting the activity of cyclooxygenase-2 and levels of nuclear factor kappa-B, tumor necrosis factor-α, interleukin-6 and interleukin-1β. Besides, histopathological damages were also observed in renal tissues followed by BPA exposure. Co-administration of ORA ameliorated the toxic effects of BPA on renal tissue in a dose-dependent manner. Thus, it is deduced that ORA might be used to counter the harmful effects of BPA on renal tissues.

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INTRODUCTION

BPA, a crystalline compound that is extensively used as a raw material in the production of plastic products (Muhammad *et al*., 2016). Owing to its resistance to high temperature, material strength and low moisture absorption, BPA is mainly used to manufacture water bottles, water pipes, toys, food containers, electronic devices, medical equipment and thermal paper (Huang *et al*., 2012). BPA is absorbed from the gastrointestinal tract and it immediately converted into BPA glucuronide in the liver, which is eliminated through the kidneys. It is abundantly reported in urine, placenta and amniotic fluid (Carwile *et al*., 2011). BPA enters the body through the skin, respiratory and alimentary canal due to its small diameter and it causes deleterious effects on animals and humans (Vandenberg *et al*., 2010; Ma *et al*., 2019). It has also been reported that BPA induces damage in the body by altering the antioxidant status (Shirani *et al*., 2019) and may affect the developmental process and reproductive system by inducing cellular injury even at low concentrations (Vandenberg *et al*., 2010). Moreover, neuroendocrine and immune systems are also affected by BPA exposure (Ma *et al*., 2019; Faheem and Bhandari, 2021).

Kidneys are the vital organs that regulate toxic metabolites, electrolyte balance, body fluids, acid-base balance, homeostasis and normal blood pressure in the body (Hertzberg *et al*., 2017). Different endogenous and exogenous environmental toxicants may induce injury in the kidneys which leads to a functional defect in the detoxification and excretion process. (Hertzberg *et al*., 2017). BPA exposure provokes renal mitochondrial swelling as well as reduces mitochondrial membrane potential by prompting the production of ROS (Kobroob *et al*., 2018). Overproduction of ROS causes oxidative stress leading to DNA damage and abnormal expression of proteins (Zeng *et al*., 2019). Moreover, BPA exposure induces glomerulus injury as well as it causes renal epithelial damage (Alekhyasita *et al*., 2019). BPA also promotes damage to the structural integrity of renal tissues which is indicated by disturbed levels of renal markers such as urea and creatinine (Shirani *et al*., 2019). Furthermore, a previous study has also shown that BPA triggers inflammatory responses in the renal tissues (Ma *et al*., 2019).

Flavonoids are secondary plant metabolites and different classes are found in vegetables and fruits. Flavonoids are characterized by the presence of the 3-carbon along with two benzene rings attached on either side. Flavonoid exhibits antioxidant, anti-inflammatory and antimutagenic properties and are significantly used in the field of pharmacology (Li *et al*., 2016; Dias *et al*., 2021). ORA is an important flavonoid extracted from *Scutellariae radix* (*Scutellaria baicalensis Georgi* dry roots). ORA contains a 2,3-double bond as well as OH groups on 5- and 7- positions and it possesses neuroprotective, anti-cancer, anti-coagulatory and anti-inflammatory activities (Kim and Lee, 2021). Thus, by considering these therapeutical potentials, the present research was formulated to investigate the curative potential of ORA on BPA-instigated renal damage in rats.

MATERIALS AND METHODS

Chemicals: Both ORA and BPA were bought from Sigma-Aldrich (Germany).

Animals: The current research was executed on 96 adult Sprague-Dawley rats weighing 200 ± 20 g. The animal care center of the University of Agriculture, Faisalabad (UAF) was used to perform the experiment. Rats were housed in steel cages at a controlled temperature ranging from 21-25°C. 12 hours light and dark cycle with adequate moisture was also maintained. Before starting the experiment, the rats were acclimatized to the environmental conditions of the lab for seven days. Food pellets and tap water were supplied to the rats after the regular interval as per requirement. Animals handling and treatment were approved by the UAF animal protection and handling committee.

Experimental: The experiment was performed on ninety-six rats and animals were divided randomly into 8 groups (n=12). Following doses of BPA and ORA were provided to the following groups.

Group-I Control: received 0.3 ml vehicle only (0.1% DMSO) by oral gavage.

Group-II BPA: 10 mg/kg of BPA was dissolved in 0.1% DMSO and provided orally. The 10 mg/kg dose of BPA was used following a previous investigation by Olukole *et al*. (2018).

Group III, IV, V Co-treated groups (BPA + ORA1, BPA + ORA2 and BPA + ORA3): Different doses of ORA were given with BPA to investigate the dose-dependent effects of ORA on BPA prompting renal damage. 10 mg/kg of BPA (dissolved in 0.1 % DMSO) were co-administrated with low (15 mg/kg of ORA dissolved in 0.1% DMSO), moderate (30 mg/kg of ORA dissolved in 0.1% DMSO) and high (60 mg/kg of ORA dissolved in 0.1% DMSO) doses of ORA to evaluate desired results and these doses were provided orally. These doses of ORA were used in accordance with a previous investigation of Li *et al*. (2016).

Group VI, VI, VIII ORA-treated groups (ORA1, ORA2 and ORA3): Low (15 mg/kg of ORA dissolved in 0.1% DMSO), moderate (30 mg/kg of ORA dissolved in 0.1% DMSO) and high (60 mg/kg of ORA dissolved in 0.1% DMSO) doses of ORA were orally provided to ORA-treated rats. The experiment was continued for 56 days.

At the end of experimental trial, rats were anesthetized with diethyl ether and decapitated. To separate plasma, trunk blood was drawn into heparinized syringes. Kidneys were isolated and the left kidney was stored in zipper bags and retained at -80°C for the assessment of various biochemical parameters. For histopathological analysis, the right kidney was kept in 10% formalin.

Biochemical assay: For biochemical assays, the renal tissues (left) were homogenized in 3mL phosphate buffer saline (PBS) and centrifuged at 12000rpm for 15 minutes. The supernatant was used to assess oxidative stress markers and antioxidants. Chance and Maehly's (1955) protocol was used to estimate the catalase (CAT) activity. Superoxide dismutase (SOD) activity was computed by the methodology stated by Nishikimi *et al*. (1972). The glutathione peroxidase (GPx) activity was estimated according to the process of Rotruck *et al*. (1973). However, the concentration of glutathione reductase (GSR) was evaluated by the method explained by Carlberg and Mannervik (1975). Jollow *et al*. (1974) protocol was followed to assess the activity of glutathione (GSH), while the activity of glutathione-S-transferase (GST) was estimated by the protocol of Habig *et al*. (1974). The level of malondialdehyde (MDA) was computed through the technique stated by Zhang *et al*. (2014). However, reactive oxygen species (ROS) levels and hydrogen peroxide (H_2O_2) concentrations were detected by the method explained by Hayashi *et al*. (2007) and Pick and Keisari (1981).

Renal biomarkers assessment: The quantity of creatinine, urea and creatine was evaluated according to the laboratory protocol described by the methodology stated in the guide provided with standard Randox lab kits Crumlin, Co. Antrim, UK.

Inflammatory markers assessment: The renal inflammatory markers were assessed from renal homogenate by using commercially available kits. The activity of cyclooxygenase-2 (COX-2) as well as the level of nuclear factor kappa-B (NF-κB), tumor necrosis factor-α (TNF-α),

Table 1: Effects of ORA and BPA on antioxidant enzyme activities and oxidative stress markers in different groups

PARAMETERS	GROUPS							
	Control	BPA	BPA + ORA1	BPA + ORA2	BPA + ORA3	ORA1	ORA2	ORA3
CAT (Umg^{-1} protein)	10.65±1.85^{a}	4.19±1.30^{d}	6.20±1.36^{c}	6.88±1.74^{bc}	7.21±2.75^{b}	10.79±2.90^{a}	11.09±4.21^{a}	11.92±3.37^{a}
SOD (Umg^{-1} protein)	7.71±1.91^{a}	3.41±1.41^{c}	4.86±2.61^{bc}	5.09±1.81^{bc}	6.20±2.01^{ab}	7.85±3.61^{a}	7.89±3.32^{a}	7.92±4.15^{a}
GPx (Umg^{-1} protein)	21.21±4.62^{a}	9.11±2.20^{d}	13.29±4.93^{c}	16.01±5.04^{bc}	17.74±6.36^{b}	21.41±4.09^{a}	21.91±3.83^{a}	22.19±5.70^{a}
GSR (nM NADPH oxidized/min/mg tissue)	7.76±1.66^{a}	2.01±0.88^{d}	4.33±0.94^{c}	6.00±0.94^{b}	6.18±1.36^{b}	7.89±1.37^{a}	8.07±2.15^{a}	8.26±1.30^{a}
GST (nM/min/mg protein)	27.33±3.01^{a}	11.63±2.23e	15.69±2.50^{d}	19.56±4.14^{c}	22.85±4.56^{b}	27.41±3.01^{a}	27.89±3.15^{a}	29.00±3.76^{a}
GSH (nM/min/mg protein)	14.55±1.80^{b}	4.23±2.00^{e}	7.78±1.58^{d}	10.78±1.67^{c}	10.83±1.96^{c}	14.91±1.78^{ab}	15.33±2.04^{ab}	16.18±1.76^{a}
H_2O_2 (µM/min/mg protein)	1.73±0.45^{d}	9.37±1.49^{a}	4.45±0.57^{b}	4.16±0.79^{b}	2.46±0.92^{c}	1.71±0.46^{d}	1.69±0.66^{d}	1.62±0.66^{d}
ROS (Umg^{-1} tissue)	1.36±0.26^{d}	7.70±1.83^{a}	3.94±0.64^{b}	3.25±0.78^{c}	3.11±0.55^{c}	1.35±0.27^{d}	1.29±0.30^{d}	1.26±0.30^{d}
MDA (nmol/mg protein)	0.57±0.18^{e}	3.09±0.59^{a}	2.58±0.45^{b}	1.73±0.44^{c}	1.48±0.19^{d}	0.56±0.20^{e}	0.52±0.18^{e}	0.51±0.18^{e}

Means ± SEM in the same row that show different superscripts represents statistical difference.

Table 2: Effects of ORA and BPA on inflammatory markers in different groups

PARAMETERS	GROUPS							
	Control	BPA	BPA + ORA1	BPA + ORA2	BPA + ORA3	ORA1	ORA2	ORA3
NF-κB (ng/g tissue)	23.46±1.56^{e}	80.89±3.94^{a}	41.87±2.94^{b}	39.42±4.90^{c}	31.97±5.23^{d}	23.27±1.51^{e}	22.64±1.28^{e}	22.05±1.34^{e}
TNF-α (ng/g tissue)	10.95±2.09^{e}	24.49±2.33^{a}	18.10±1.44^{b}	15.38±1.08^{c}	13.49±1.34^{d}	10.74±2.14^{e}	10.65±1.95^{e}	10.47±2.27^{e}
IL-1β (ng/g tissue)	26.70±1.75^{d}	84.32±3.49^{a}	40.54±3.48^{b}	35.83±3.19^{c}	34.45±3.86^{c}	26.52±2.39^{d}	25.53±2.05^{d}	24.96±1.97^{d}
IL-6 (ng/g tissue)	8.91±1.98^{d}	25.74±1.57^{a}	16.94±1.90^{b}	15.85±2.35^{b}	13.81±1.67^{c}	8.61±1.77^{d}	8.54±1.65^{d}	8.11±1.82^{d}
COX-2 (ng/g tissue)	16.22±2.15^{e}	73.01±3.69^{a}	41.40±2.95^{b}	30.62±3.26^{c}	24.33±4.03^{d}	16.15±2.11^{e}	15.58±1.89^{e}	15.01±1.89^{e}

Means ± SEM in the same row that shows different superscripts represents the statistical difference.

Table 3: Effects of ORA and BPA on renal markers in different groups

PARAMETERS	GROUPS							
	Control	BPA	BPA + ORA1	BPA + ORA2	BPA + ORA3	ORA1	ORA2	ORA3
Urea (mg/dL)	18.97±1.56^{e}	42.03±3.42^{a}	28.11±1.71^{b}	26.26±1.51^{c}	24.49±2.12^{d}	18.81±1.35^{e}	18.20±1.87^{ef}	17.02±2.36^{f}
Creatinine (mg/dL)	1.50±0.17^{d}	5.95±0.48^{a}	3.00±0.13^{b}	2.72±0.21^{c}	2.65±0.19^{c}	1.46±0.17^{d}	1.44±0.16^{d}	1.38±0.14^{d}
Creatinine Clearance (mL/min)	1.81±0.17^{a}	0.42±0.16^{e}	1.15±0.07^{d}	1.28±0.06^{c}	1.47±0.10^{b}	1.81±0.18^{a}	1.85±0.14^{a}	1.88±0.21^{a}

Means ± SEM in the same row that shows different superscripts represents the statistical difference.

interleukin-6 (IL-6), interleukin-1β (IL-1β) was estimated with the help of a rat ELISA kit (Shanghai-YL-Biotech. Co. Ltd., China). Analysis was carried out according to the manufacturer's guidelines using ELISA Plate Reader (BioTek, Winooski-VT, USA).

Histopathological examination: For histopathological examination, the samples of the kidney were fixed in 10 percent formalin buffer solution and dehydrated in the ascending grades of ethyl alcohol (80, 90 and 100 percent). After dehydration, tissue specimens were embedded in paraffin wax and 4-5µm thin slices were cut down with microtome and stained by hematoxylin-eosin (H&E) stain and analyzed with the compound microscope (Nikon, 187842, Japan). Leica-LB and image-J2x software was used to take photographs as well as the examination of specimens.

Statistical Analysis: All the data were exhibited as Mean ± SEM. ANOVA (One-way analysis of variance) followed by the Tukey test was used to check pairwise comparison among different groups. The $P < 0.05$ was considered as the level of significance.

RESULTS

Protecting impact of ORA on biochemical assays: Table 1 illustrate the relative changes in antioxidant enzyme activity. BPA exposure significantly ($P < 0.05$) decreased the level of GSH and activities of GPx, GSR, CAT, GST and SOD, while increased the level of MDA, ROS and H_2O_2. On the other hand, ORA co-treatment along with BPA elevated the level of GSH and activities of GPx, GSR, CAT, GST. However, the increase in the activity of SOD in BPA + ORA1 and BPA + ORA2 co-treated groups was non-significant. A significant ($P < 0.05$) decreased in the level of MDA, ROS and H_2O_2 was detected after ORA treatment. Nonetheless, in all co-treated groups the highest activities of antioxidant enzymes were observed in BPA + ORA3 treated group. Moreover, insignificant differences were observed between control group and only ORA treated group.

Protecting impact of ORA on inflammatory markers: Table 2 displays the relative alterations in the values of inflammatory parameters. BPA exposure significantly ($P < 0.05$) increased the COX-2 activities and NF-kB, TNF-α, IL-6, IL-1β levels as compared to control group, whereas ORA co-treatment along with BPA lowered the COX-2 activities and NF-kB, TNF-α, IL-6, IL-1β levels as compared to BPA treated rats. However, the highest decrease was observed in BPA+ORA3 treated rats. Moreover, the values of inflammatory biomarkers in control group as well as in ORA treated group were near to each other.

Protecting impact of ORA on renal function markers: Table 3 demonstrated the changes in urea and creatinine level. BPA treatment significantly ($P < 0.05$) decreased creatinine clearance and increased urea and creatinine level. Whereas, ORA showed a reduction in urea and creatinine level and an increased in creatinine clearance as compared to rats administered only BPA. However, group VIII administrated with BPA + 60 mg/kg of ORA showed highest decline in urea, and creatinine and an increase in creatinine clearance. Furthermore, the only ORA-treated rats did not present significant differences with the control group.

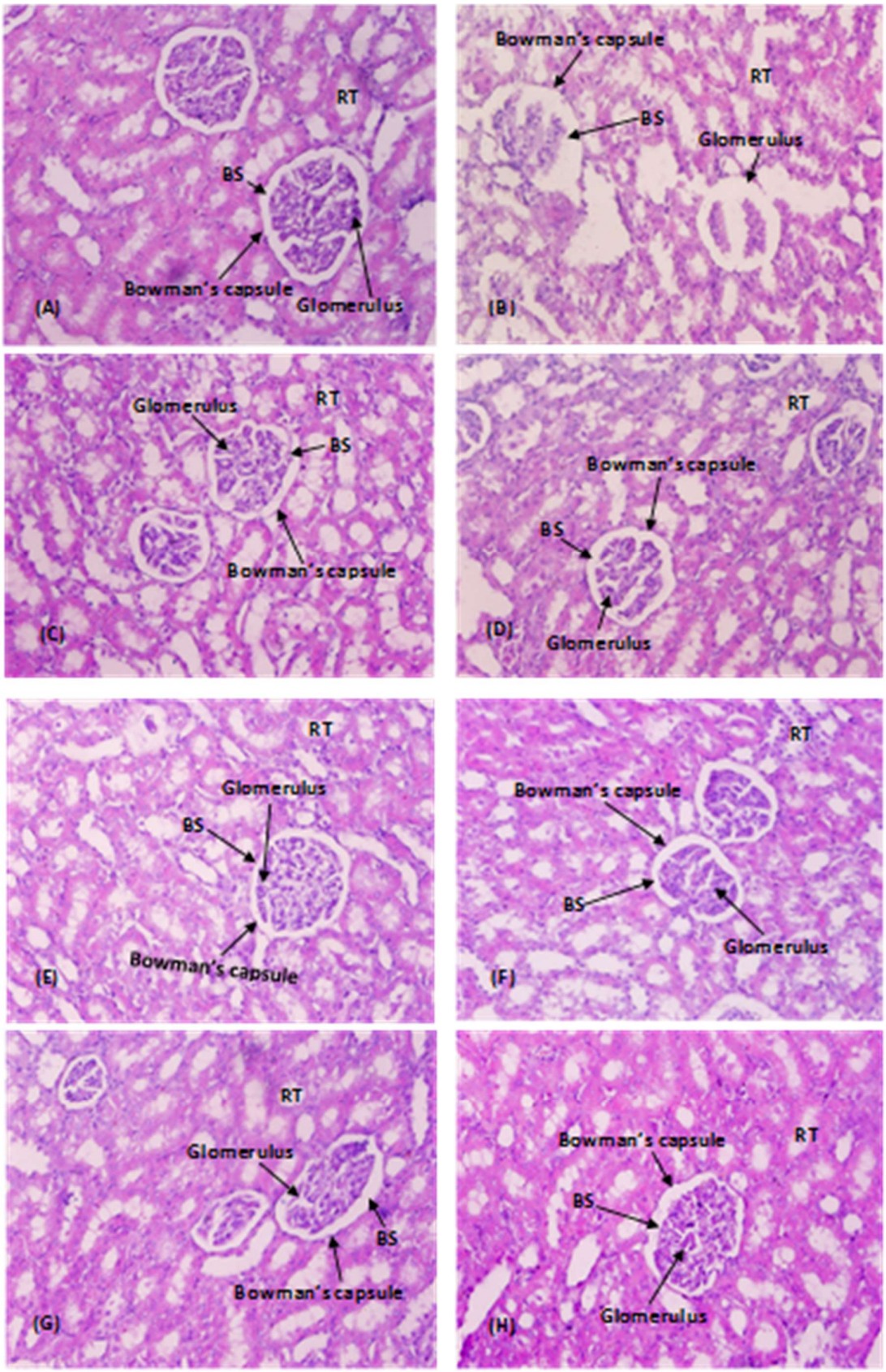

Fig. 1: Protective effects of ORA on BPA-prompted histopathological damages in renal tissues (H & E/ 400X). (A) Control group; (B) BPA intoxicated rats (10 mg/kg); (C) BPA (10 mg/kg) + ORA1 (15 mg/kg) treated group; (D) BPA (10 mg/kg) + ORA2 (30 mg/kg) treated group; (E) BPA (10 mg/kg) + ORA3 (60 mg/kg) treated group; (F) ORA1 treated rats (15 mg/kg); (G) ORA2 treated rats (30 mg/kg); (H) ORA3 treated rats (60 mg/kg). BS: Bowman's space, RT: Renal tubules.

Protecting impact of ORA on histology of renal tissues: Fig. 1 represents the changes in histopathology of renal tissues. Exposure of BPA considerably increased renal damage. BPA intoxication induced damage in the Bowman capsule, glomerular shrinkage, cellular swelling, and an increase in the space between the glomerulus and the capsule wall (Fig. 1B). However, co-treatment of ORA and BPA resulted in a dose-dependent reduction the renal damage; Restored cellular swelling as well as reduced damage in the bowman capsule (Fig. 1C,1D,1E). However, the renal tissues of rats treated with only ORA showed normal histology comparable to control (Fig. 1F, 1G, 1H).

DISCUSSION

In the present study, oral administration of BPA potentially dysregulated the antioxidant system by bringing down the activity of antioxidant enzymes such as SOD, CAT, GSR, GPx, GST and GSH. On the other hand, BPA intoxicated rats showed a higher level of ROS and H_2O_2 concentration which induced renal oxidative stress and ultimately instigated renal injury. Furthermore, escalation in MDA level was also observed. CAT, a hemeprotein, is involved in the transformation of H_2O_2 into H_2O and O_2 and protects the cells from oxidative impairment. Superoxide dismutase defends against free radicals by transforming them into slightly less harmful radicals (Nieskens *et al*., 2018). GSR transforms the glutathione disulfide into GSH and it shields the mammalian cells from oxidative damage by decreasing hydrogen peroxide and other peroxides levels (Deponte, 2013). Thus, it is indispensable to regulate the normal activity of these enzymes for the reduction of renal deterioration and oxidative stress. However, ORA treatment significantly increased the activities of antioxidant enzymes which lowered the level of MDA, ROS and H_2O_2 concentration. Our results were in line with Zhu *et al*. (2022) who demonstrated the antioxidant property of ORA involved in the reduction of oxidative damage caused by UV radiations. The current findings showed that ORA exhibit anti-oxidant activity due to its ability to scavenge free radicals.

According to our results, a substantial elevation in urea and creatinine levels was detected after BPA administration, while creatinine clearance was significantly reduced. Urea and creatinine level is used to assess the normal functions of kidneys (Sahu *et al*., 2020). Urea is the byproduct of protein metabolism while, creatinine is a nitrogenous substance that is generated by phosphocreatine and creatine and predominantly eliminated via glomerular filtration (Sepulveda, 2019). The signs of adverse oxidative damage to the renal cells are due to elevated urea, creatinine, and reduced creatinine clearance (Shirani *et al*., 2019) which reflects a low glomerular filtration rate (Kobroob *et al*., 2018). However, co-administration with ORA+BPA reduced the levels of urea and creatinine possibly by improving the glomerulus filtration rate that is indicated by increased creatinine clearance. Thus, ORA might be used to normalized the level of renal functions markers.

BPA exposure significantly augmented the level of the inflammatory markers including IL-6, IL-1β, COX-2, TNF-α, NF-κB. NF-κB stimulation leads to the increased the level of pro-inflammatory cytokines including TNF-α, IL-6, NF-κB, COX-2 and IL-1β (Kandemir *et al*., 2018). The level of these markers causes overproduction of ROS which leads to acute inflammatory responses (Kandemir *et al*., 2018). Cyclooxygenase-2 is also an important marker that induces inflammation in the renal tissues (Gandhi *et al*., 2017). Furthermore, our results were in line with Ma *et al*. (2019), who reported that BPA treatment damaged renal tissues. Our results revealed that ORA supplementation suppress the activation of NF-κB, responsible for inflammation. Therefore, it is deduced that ORA might be used to counter the inflammatory responses of BPA on renal tissues. Nile *et al*. (2018) also reported that flavonoids may hold some structural features which influence the inflammatory responses by depleting the level of the inflammatory markers.

Microscopic observation of the control group displayed the normal structure of renal tissues, rounded epithelial cells and normal glomerular interstitium. BPA intoxicated rats showed cellular swelling, glomerular shrinkage, damage in the Bowman capsule and an increase in the space between the glomerulus and the capsule wall. These alternations in renal tissues are associated with oxidative stress and inflammatory responses induced by BPA intoxication. Alekhyasita *et al*. (2019) also reported glomerular shrinkage, renal cylindrical epithelial damage and interstitial inflammatory invasion in the kidney followed by BPA exposure. However, 60 mg/kg of ORA showed the highest ameliorative effects on the structural changes persuaded by BPA, while the other doses (15 mg/kg and 30 mg/kg) of ORA partially improved the morphology of the kidney in a dose-dependent manner. These protective benefits of ORA may be ascribed to free radical scavenging potential as well as its antioxidant and anti-inflammatory potential.

Conclusions: In conclusion, ORA has a remarkable capability to prevent BPA-prompted oxidative damage. ORA treatment improved renal markers, oxidative stress markers, inflammatory markers, antioxidant enzyme activity and histological architecture. The renoprotective characteristics of ORA may be ascribed to its anti-inflammatory and antioxidant properties. Taken together, it can be deduced that ORA may offer some clinical potential to restore renal dysfunction followed by BPA exposure.

Authors contribution: AI and GZ designed the study. AI, MUI and NE Performed the experiments. MI, KA and HN performed statistical analysis. GZ supervised the study. AI and GZ wrote the manuscript. All the authors read and approved the final version of the manuscript.

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