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Original Article

Sciadopitysin attenuates paraquat induced renal toxicity by modulating Nrf-2/Keap-1 pathway in male albino rats

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Abstract

Paraquat (PQ) is a herbicide that has the potential to instigate nephrotoxicity in animals and human. Sciadopitysin (SPS) is a biflavonoid that is extracted from *Taxus cuspidate* and displays diverse biological activities including anti-oxidant, anti-inflammatory and anti-apoptotic. Therefore, the present investigation was designed to evaluate the mitigative potential of SPS against PQ prompted renal toxicity in albino rats. 48 male albino rats were divided into 4 groups, such as control group, PQ treated group (5 $mgkg^{-1}$), PQ + SPS co-treated group (5 $mgkg^{-1}$ and 2 $mgkg^{-1}$ respectively) and only SPS treated group (2 $mgkg^{-1}$). The exposure of PQ significantly reduced Nrf-2 as well as anti-oxidant enzymes expression, while increasing Keap-1 expression. Moreover, anti-oxidant enzymes such as, glutathione reductase (GSR), superoxide dismutase (SOD), glutathione peroxidase (GPx), catalase (CAT), glutathione-S-transferase (GST), heme oxygenase-1 (HO-1) and glutathione (GSH) activities were decreased. However, in PQ-treated rats malondialdehyde (MDA) and reactive oxygen species (ROS) contents were significantly increased. PQ exposure also increased the serum level of urea, urinary protein, urobilinogen and creatinine while decreased creatinine clearance and albumin protein levels. Moreover, KIM-1 and NGAL levels were also increased in PQ exposed rats. Additionally, inflammatory indices including nuclear factor kappa-B (NF-κB), interleukin-6 (IL-6), tumor necrosis factor-α (TNF-α) interleukin-1β (IL-1β) and cyclooxygenase-2 (COX-2) activity were increased in PQ administrated rats. Besides, it escalated the Bax and Caspase-3 expression. Contrarily, a substantial decrease was observed in anti-apoptotic marker, Bcl-2 expression. The exposure of PQ also induced significant histopathological damages in renal tissues. Nevertheless, SPS supplementation recovered all these damages due to its anti-apoptotic, anti-oxidant and anti-inflammatory nature.

Keywords: Paraquat, Sciadopitysin, Renal damage, Oxidative stress, Inflammation

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Introduction

Herbicides are the chemicals that are used in agriculture sector to control unwanted weeds and have the potential to induce severe damage in the tissues of animals (Mahmood et al., 2021). Paraquat (PQ) is a nonselective and contact herbicide that is used to control invasive grasses (Edori et al., 2013). It is an extremely noxious herbicide to both humans and animals. Humans are exposed to PQ through food, skin contact, or inhalation. According to previous study, PQ exposure leads to death due to the absence of effective treatment (Han et al., 2014; Atashpour et al., 2017; Hu et al., 2017). An extreme exposure of PQ can cause death within 3.5 hours. Organisms exposed to PQ shows mortality rates between 60-80% (Kuan et al., 2016; Nikdad et al., 2020).

PQ causes toxicity by producing reactive oxygen species (ROS) that prompts tissue damage (Zheng et al., 2020). At the physiological level, ROS normally controls growth factors, cell signaling and inflammatory responses, nevertheless excessive production of free radical results in serious damage (Raza et al., 2022; Zeinvand-Lorestani et al., 2015). PQ is reported to cause testicular damage, hepatotoxicity, pulmonary toxicity, neurotoxicity and nephrotoxicity (Kim et al., 2009; Baharuddin et al., 2011; Wu et al., 2012; Blanco-Ayala et al., 2014; Ofoego et al., 2018).

Kidneys are major site of PQ accumulation. One of the major reasons of PQ-induced death is nephrotoxicity (Wei et al., 2014). Renal toxicity caused by PQ is a common adverse effect that is usually the first sign of systemic toxicity. Reduced renal clearance rate and other organ dysfunctions are all symptoms of PQ-induced renal impairment. Previous research on the PQ toxicity indicated a profound increase in blood creatinine level, which suggests a decline in the kidneys' glomerular filtration rate (GFR) (Wunnapuk et al., 2013; Mohamed et al., 2015). Following exposure to PQ, renal tubules lose their characteristic forms and leads to congestion in the blood vessels of the kidneys and glomerular degeneration (Gu et al., 2016).

Antioxidant compounds play an important role in the prevention of multiple diseases (Talas et al., 2014). In the past ten years, numerous researchers have focused on the significance of plant derived anti-oxidants as potential agents for treating various OS-related diseases and toxicities. Flavonoids are an essential class of polyphenols that are reported to show a variety of benefits in the nutraceutical, pharmaceutical and cosmetics industries (Kumar and Pandey, 2013). Biflavonoids exhibit good anti-oxidative capacity and can be employed as antioxidants to shield cells from free radical damage (Ye et al., 2012). Sciadopitysin (SPS) is an important amentoflavone derivative biflavonoid with the 7, 4', 4'-trimethyl ether structure. SPS is isolated from the young branches of *Taxus cuspidate* and displays diverse biological activities i.e., anti-inflammatory, blood glucose regulation, anti-oxidant, cardiovascular protection, and neuroprotective (Dell'Agli et al., 2006; Choi et al., 2006; Gu et al., 2013; Suh et al., 2018; Wenbo et al., 2021). Thus, by considering these therapeutical potentials of SPS into account, the present study was designed to investigate the palliative role of SPS on PQ prompted kidney dysfunction in rats.

Material and Methods

Chemicals

Analytical grade PQ and SPS were purchased from Sigma-Aldrich (Germany).

Animals

The research was accomplished on 48 adult male albino rats (180-200g weight). The animals were obtained from the Animal House of the University of Agriculture, Faisalabad and were housed in stainless-steel cages at 24-26℃ temperature and in 12-hour light/dark cycle. During the whole experiment, the animals were provided with water & regular feed. The animals were handled as per the protocol approved by the European Union of Animal Care and Experimentation (CEE Council 86/609).

Experimental protocol

48 rats were separated into 4 equal groups (n = 12/group). The following doses were administered to the rats: control group, PQ treated group (5 $mgkg^{-1}$ of PQ), PQ+SPS co-treated group (5 $mgkg^{-1}$ of PQ and 2 $mgkg^{-1}$ of SPS orally), and only SPS supplemented group (2 $mgkg^{-1}$ of SPS). The dose of PQ (5 $mgkg^{-1}$) was selected according to the previous study (Kheiripour et al., 2021). Whereas the dose of SPS (2 $mgkg^{-1}$) was selected in compliance with the earlier study of Cai and Li (2020). The trial was conducted for 30 days. At the end, animals were anaesthetized with ketamine & xylazine, decapitated and blood was

collected in heparinized tubes. Then the blood was centrifuged at 3000 rpm for 15 minutes. After separation, plasma was kept at −20°C until further assessment. The right kidney was kept at −80°C for biological observation. On the other hand, the left kidney was fixed in 10% formalin for histological analysis.

Assessment of biochemical parameters

The method demonstrated by Chance and Maehly (1955) was followed to assess CAT activity. The activity of SOD was appraised by using the approach of Kakkar et al. (1984). GPx content was quantified through the procedure of Lawrence and Burk (1976). The activity of GSR was evaluated by using the methodology of Carlberg and Mannervik (1975). GSH content was calculated by using the spectrophotometric methods stated by Jollow et al. (1974), whereas Younis et al. (2016) methodology was used to estimate GST activity. Additionally, HO-1 activity was appraised by analyzing the formation of bilirubin via the approach of Magee et al. (1999). Whereas, ROS content was evaluated as per the protocol demonstrated by Hayashi et al. (2007) and MDA concentration was measured by using the approach of Ohkawa et al. (1979).

Ribonucleic acid extraction and real-time quantitative reverse transcription polymerase chain reaction

The expression of Nrf-2/Keap-1**,** anti-oxidant enzymes and Caspase-3, Bcl-2 & Bax were evaluated by qRT-PCR. Ribonucleic acid (RNA) was separated by using TRIzol reagent. Total RNA was changed into complementary deoxyribonucleic acid with the help of Fast Quant RT kit (Takara, China). According to Livak and Schmittgen (2001), changes in these expressions were appraised by $2^{-\Delta\Delta CT}$ using β-actin as an interior regulator (Livak and Schmittgen, 2001). Table 1 displays the primer sequences of the target genes as described earlier by Ijaz et al. (2021) and Hamza et al. (2023).

Kidney function markers evaluation

The standard diagnostic kits were employed to assess the urea (Cat No. ab83362), urinary protein (Cat No. 9040), albumin (Cat No. MAK124A), urobilinogen (Cat No. B2012103), creatinine (Cat No. ab65340) and creatinine clearance (Cat No. ab65340) levels. The levels of urine KIM-1 and serum NGAL were evaluated via KIM-1 Quantikine ELISA Kits and NGAL Quantizing ELISA-Kits (R and D Systems Company Ltd. Changning, China) as per the manufacturer's instructions.

Table-1. Primers sequences for the real-time quantitative reverse transcription-polymerase (RT-qPCR).

Gene	Primers 5' -> 3'	Accession number
Nrf-2	F: ACCTTGAACACAGATTTCGGTG	NM_031789.1
	R: TGTGTTCAGTGAAATGCCGGA	
Keap-1	F: ACCGAACCTTCAGTTACACACT	NM_057152.1
	R: ACCACTTTGTGGGCCATGAA	
CAT	F: TGCAGATGTGAAGCGCTTCAA	NM_012520.2
	R: TGGGAGTTGTACTGGTCCAGAA	
SOD	F: AGGAGAAACTGACAGCTGTGTCT	NM_017051.2
	R: AAGATAGTAAGCGTGCTCCCAC	
GPx	F: TGCTCATTGAGAATGTCGCGTC	NM_030826.4
	R: ACCATTCACCTCGCACTTCTCA	
GSR	F: ACCAAGTCCCACATCGAAGTC	NM_053906.2
	R: ATCACTGGTTATCCCCAGGCT	
GST	F: TCGACATGTATGCAGAAGGAGT	NM_031509.2
	R: CTAGGTAAACATCAGCCCTGCT	
HO-1	F: AGGCTTTAAGCTGGTGATGGC	NM_012580.2
	R: ACGCTTTACGTAGTGCTGTGT	
Bax	F: GGCCTTTTTGCTACAGGGTT	NM_017059.2
	R: AGCTCCATGTTGTTGTCCAG	
Bcl-2	F: ACAACATCGCTCTGTGGAT	NM_016993.1
	R: TCAGAGACAGCCAGGAGAA	
Caspase-3	F: ATCCATGGAAGCAAGTCGAT	NM_012922.2
	R: CCTTTTGCTGTGATCTTCCT	
β-actin	F: TACAGCTTCACCACCACAGC	NM_031144
	R: GGAACCGCTCATTGCCGATA	

Inflammatory markers assessment

The inflammatory indices i.e., IL-6 (CSB-E04640r), NF-κB (CSB-E13148r, IL-1β (CSB-E08055r), TNF-α (CSB-E07379r) and COX-2 (CSB-E13399r) activity were assessed via rat ELISA kits. The analysis were performed using an Elisa Plate Reader in accordance with the company's guidelines (Cusabio Technology Llc, Houston, TX, USA).

Histopathological assessment

For histological analysis the left kidney tissues of the rats were used. After cleaning the kidneys with cold saline solution, the tissues were fixed in 10% formalin, then subsequently dehydrated by using increasing grades of ethanol (70, 90 and 100%). After that the tissues were embedded in paraffin wax. Then, 4-5 µm thick slices were cut with the help of 820 Spencer rotatory microtome and stained with Hematoxylin and Eosin (H & E). These slides were examined microscopically, equipped with an automatic camera. ImageJ2X software was used to examine the pictures.

Statistical analysis

The results were expressed as Mean ± SEM. Using Minitab software, One-way analysis of variance (ANOVA) and turkey's test were applied. The significance level was set at $P < 0.05$.

Results

Effects of SPS and PQ on Nrf-2/Keap-1 pathway

PQ exposure led to a significant ($P < 0.05$) decrease in Nrf-2 and anti-oxidant enzymes (SOD, GPx, CAT, GST, HO-1 and GSR) expression, whereas elevated the expression of Keap-1 in PQ treated group as compared to the control group. Nevertheless, SPS treatment increased Nrf-2 and anti-oxidant enzymes expression, while down-regulating the Keap-1 expression. Moreover, only SPS supplemented group presented these expressions that were almost similar to the control group (Figure 1, 2).

Effects of SPS and PQ on antioxidant activities of enzymes

PQ administration significantly ($P < 0.05$) reduced anti-oxidant enzymes, CAT, GPx, SOD, GSH, GSR, HO-1 and GST activities. However, as compared to PQ-intoxicated group, the co-administration of PQ and SPS notably improved anti-oxidant enzymes activities in co-treated group. Moreover, only SPS supplemented group showed similar enzymatic activities as in the control group (Table 2).

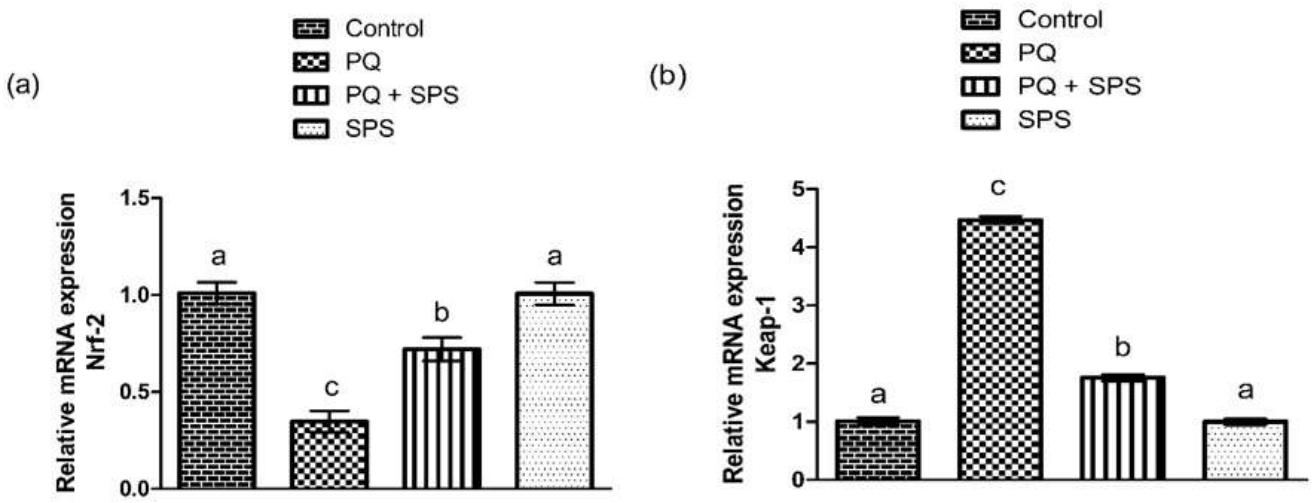

Figure-1. Effect of PQ and SPS on a) Nrf-2, b) Keap-1 expression. Bars are rooted on mean ± SEM values. Dissimilar letters on bars are showing considerable ($P < 0.05$) variation.

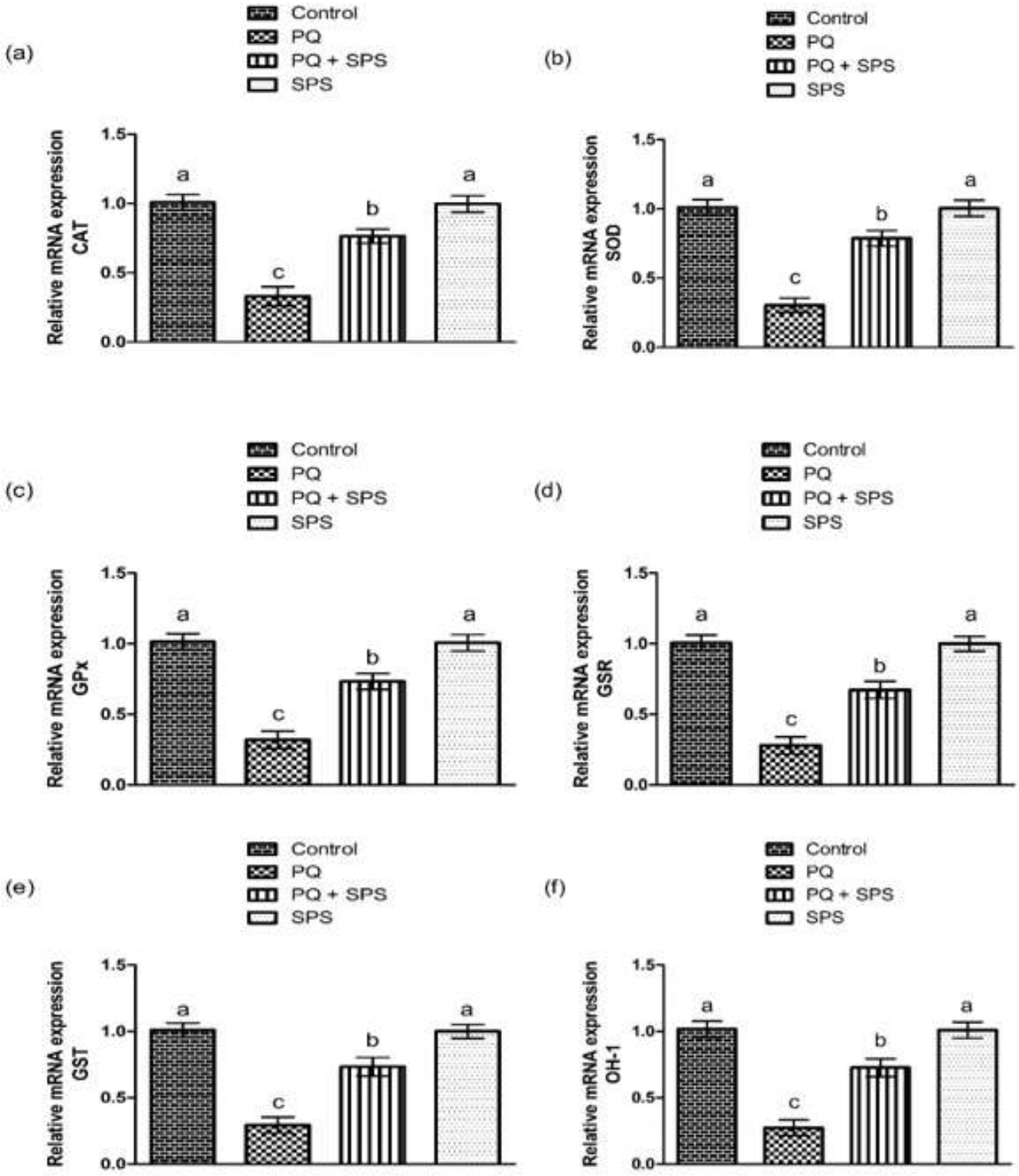

Figure-2. Effect of PQ and SPS on the expression of a) CAT, b) SOD, c) GPx, d) GSR, e) GST and f) HO-1. Bars are rooted on mean ± SEM values. Dissimilar letters on bars are showing considerable ($P < 0.05$) variation.

Table-2. The effect of PQ and SPS on biochemical markers

PARAMETERS	GROUPS			
	Control	PQ	PQ + SPS	SPS
CAT (Umg^{-1} protein)	9.52±0.17^{a}	4.30±0.09^{c}	8.11±0.14^{b}	9.54±0.16^{a}
SOD (Umg^{-1} protein)	7.62±0.18^{a}	3.09±0.08^{c}	6.36±0.14^{b}	7.66±0.19^{a}
GPx (Umg^{-1} protein)	24.11±0.32^{a}	6.33±0.17^{c}	18.63±0.53^{b}	24.27±0.34^{a}
GSR (nM NADPH oxidized/min/mg tissue)	6.81±0.08^{a}	1.63±0.15^{c}	5.57±0.15^{b}	6.84±0.10^{a}
GST (nM/min/mg protein)	29.76±1.05^{a}	11.72±0.44^{c}	24.96±1.01^{b}	29.84±0.91^{a}
GSH (µM/g tissue)	19.54±0.65^{a}	5.45±0.27^{c}	14.53±0.41^{b}	19.75±0.74^{a}
HO-1 (pmoles bilirubin/mg protein/h)	241.36±6.55^{a}	70.15±5.43^{c}	147.44±7.77^{b}	247.11±9.39^{a}
ROS (µmol/g)	1.09±0.11^{b}	10.23±0.53^{a}	2.15±0.16^{b}	1.08±0.11^{b}
MDA (nmol/g)	0.74±0.04^{c}	7.33±0.11^{a}	1.85±0.06^{b}	0.73±0.04^{c}

The values with dissimilar superscripts are notably ($P < 0.05$) dissimilar from other groups.

Table-3. The effect of PQ and SPS on renal function markers

PARAMETERS	GROUPS			
	Control	PQ	PQ + SPS	SPS
Urea (mg/dl)	14.65±0.36^{c}	53.48±0.92^{b}	22.70±0.42^{a}	14.52±0.34^{c}
Creatinine (mg/dl)	1.41±0.08^{c}	6.60±0.15^{a}	2.52±0.09^{b}	1.38 0±0.08^{c}
Creatinine Clearance (mL/min)	1.83±0.06^{a}	0.52±0.07^{c}	1.17±0.05^{b}	1.86±0.07^{a}
Albumin (mg/dl)	9.36±0.65^{a}	3.07±0.08^{b}	8.04±0.32^{a}	9.45±0.69^{a}
Urobilinogen (mg/dl)	3.99±0.18^{c}	16.38±0.63^{a}	6.14±0.42^{b}	3.95±0.18^{c}
Urinary proteins (mg/dl)	2.07±0.19^{c}	29.37±0.72^{a}	8.35±0.69^{b}	2.05±0.21^{c}
Urinary KIM-1 (ng/ml)	0.23±0.10^{c}	2.63±0.04^{a}	1.34±0.11^{b}	0.21±0.09^{c}
NGAL (ng/ml)	0.52±0.08^{c}	3.29±0.13^{a}	1.32 ± 0.06^{b}	0.51±0.08^{c}

The values with dissimilar superscripts are notably ($P < 0.05$) dissimilar from other groups.

Effects of SPS and PQ on oxidative stress markers

PQ exposure substantially ($P < 0.05$) increased ROS and MDA contents in PQ treated group as compared to the control group. Nonetheless, ROS and MDA levels were markedly reduced in the co-treated animals as compared to PQ treated group. Moreover, only SPS supplemented group showed ROS and MDA contents almost similar to the control group (Table 2).

Effects of SPS and PQ on renal markers

PQ exposure significantly ($P < 0.05$) increased creatinine, urinary proteins, urea, urobilinogen, urinary KIM-1 as well as NGAL levels, while a significant reduction was observed in albumin and creatinine clearance levels. However, the co-treatment of PQ+SPS showed a remarkable reduction in urobilinogen, urinary protein, creatinine, urea, KIM-1 and NGAL levels, on the other hand a profound increase in albumin and creatinine clearance was observed. Moreover, only SPS supplemented group showed similar level of these markers as in the control group (Table 3).

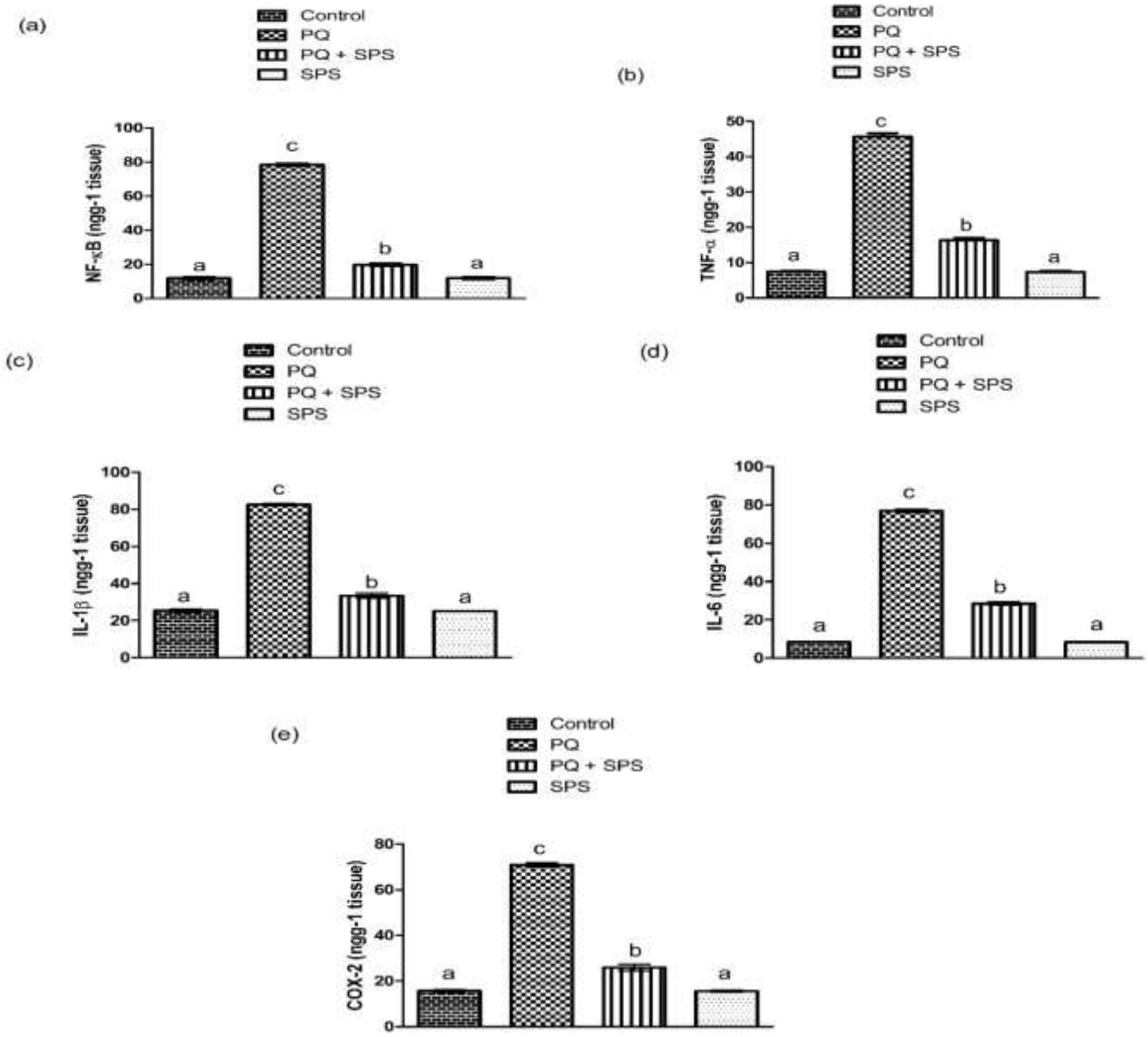

Figure-3. Effect of PQ and SPS on a) NF-κB, b) TNF-α, c) IL-1β, d), f) IL-6 level and e) COX-2 activity. Bars are rooted on mean ± SEM values. Dissimilar letters on bars are showing considerable

Effects of SPS and PQ on renal inflammatory mediators

PQ exposure noticeably ($P < 0.05$) increased inflammatory indices (NF- κB, IL-1β, TNF-α, IL-6 and COX-2 activity) as compared to the control group. Nevertheless, PQ+SPS co-treatment significantly decreased inflammatory indices in comparison to the PQ treated group. Moreover, in only SPS supplemented animals these markers were close to the control group (Figure 3).

Effects of SPS and PQ on renal apoptotic marker

PQ exposure significantly ($p < 0.05$) increased Caspase-3 and Bax expression in PQ treated group in comparison to the control group. Whereas, Bcl-2 expression was decreased in PQ treated group. However, the co-administration of PQ+SPS considerably ($P < 0.05$) decreased Caspase-3 and Bax expression, while elevated the Bcl-2 expression as compared to PQ treated group. Additionally, in only SPS supplemented group these expressions were almost similar to the control group (Figure 4).

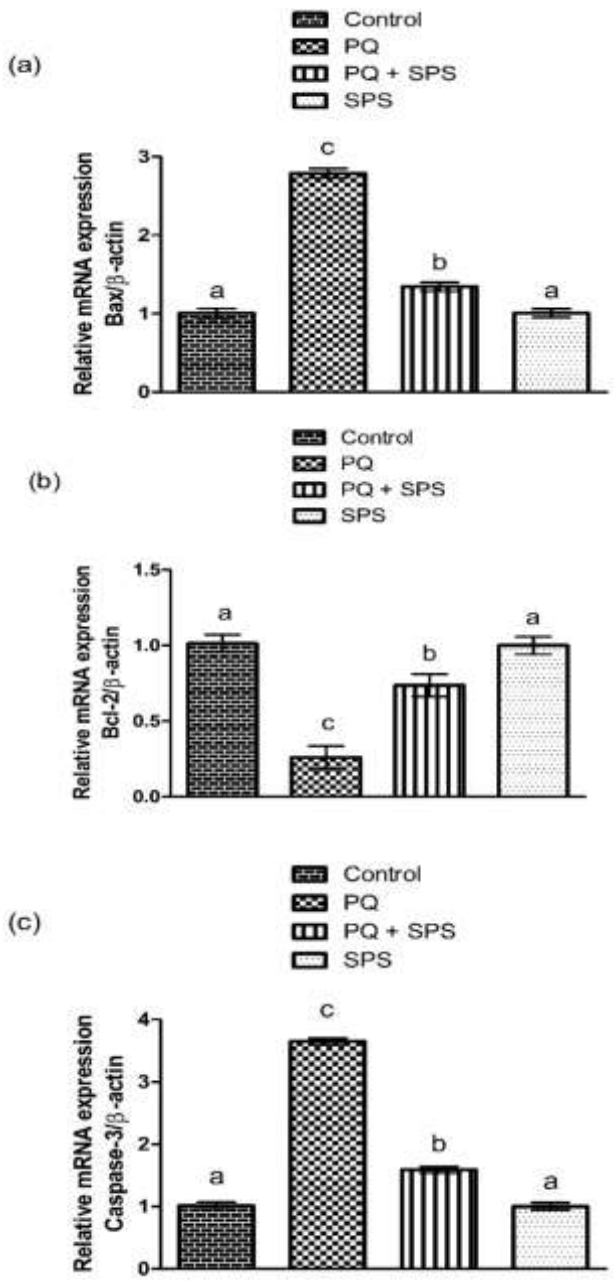

Figure-4. Effect of PQ and SPS on a) Bax, b) Bcl-2 and c) Caspase-3 expression. Bars are rooted on mean ± SEM values. Dissimilar letters on bars are showing considerable ($P < 0.05$) variation.

Effects of PQ and SPS on renal histology

PQ exposure induced severe histopathological abnormalities such as tubular, interstitial and glomerular disruption, damage to Bowman capsule, loss of cellular differentiation, inflammatory response and tubular necrosis in comparison to the control animals. However, PQ+SPS co-administration significantly improved the renal histological profile in the co-treated group. Moreover, in only SPS supplemented group the histological profile of renal tissues was almost similar to the control group (Figure 5).

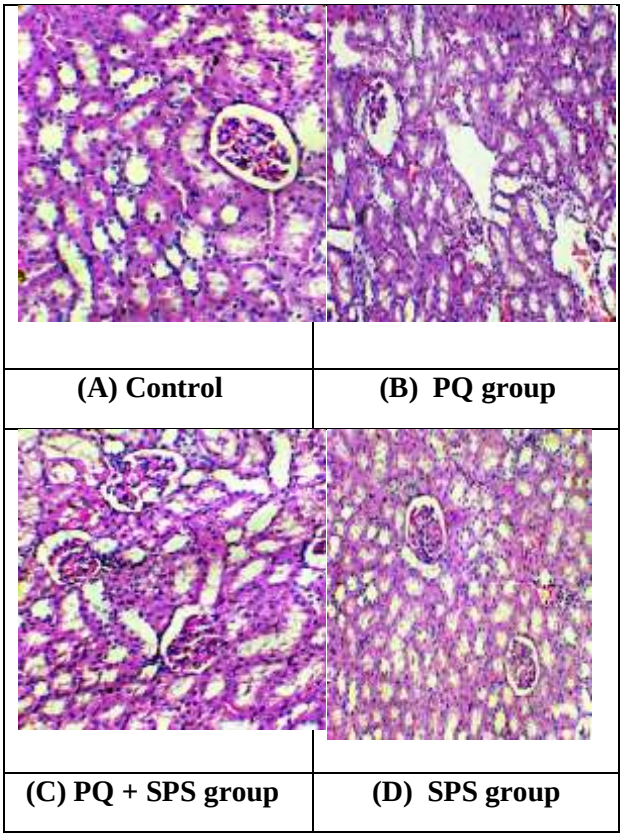

Figure-5. Photomicrographs of rat renal tissues. A) Control group presenting normal histology. B) PQ exposure prompted adverse alterations in renal tissues i.e., tubular structural damage, widespread necrosis, tubular cell desquamation, dilatation of proximal tubules and vacuolization. C) PQ + SPS group displayed restored histology of renal tissues. D) SPS group showing normal histology almost as in the control rats.

Discussion

The present research was formulated to estimate the ameliorative role of SPS against PQ-instigated renal damage in rats. It was reported that herbicides have the potential to induce severe damage in the tissues of animals (Mahmood et al., 2021). Paraquat (PQ) is an organic herbicide that is often employed in agriculture to remove unwanted weeds. PQ is highly soluble in water and it poses a substantial risk to human health, whether consumed on purpose or by

accident. PQ poisoning results in high death rate from 60% to 70% yearly due to the lack of efficient treatment (Amirshahrokhi and Bohlooli, 2013; Nunes et al., 2017). PQ damages various organs in humans especially it leads to kidney failure. PQ exposure causes OS, inflammation, apoptosis, and nephrotoxicity (Wei et al., 2014). Nowadays, multiple ailments, including renal dysfunction are treated with plant based medicinal herbs (Valipour et al., 2016). Sciadopitysin (SPS) is a biflavonoid reported in *Taxus cuspidate* and it displays versatile pharmacological properties, such as anti-oxidants, anti-inflammatory, and neuroprotective (Choi et al., 2006; Gu et al., 2013; Suh et al., 2018; Suh et al., 2022).

PQ exposure decreased Nrf-2 along with anti-oxidant genes expression, besides escalated the expression of Keap-1. Nrf-2 is a crucial transcription factor that has a major role in controlling the oxidative and electrophilic stress (Vomund et al., 2017). Generally, Keap-1 interacts with Nrf-2, regulates its stability and acts as its inhibitor (Pintard et al., 2004). During ROS production, Nrf-2 detaches from Keap-1 via some structural modifications and moves into the nucleus. In the nucleus it interacts with small MAF proteins. Then, the heterodimers bind to the anti-oxidant responsive elements & instigate the expression of cytoprotective genes (Telkoparan-Akillilar et al., 2019). Nrf-2 performs a vital role in controlling the induction of anti-oxidant enzymes (CAT, SOD, GPx, GSR and HO-1) (Li and Kong, 2009; Digaleh et al., 2013; Hawkes et al., 2014). However, under excessive ROS production the expression of Keap-1 increased, while decreasing the expression of Nrf-2 and anti-oxidant genes (Yang et al., 2022). Therefore, lowered Nrf-2 expression consequently reduces the expression of antioxidant genes. However, the supplementation of SPS escalated the expression of Nrf-2 that was further confirmed by the augmented expression of anti-oxidant genes (CAT, SOD, GPx, GSR and HO-1), on the other hand, it reduced the expression of Keap-1. So, it can be stated that SPS may be used as a pharmacological candidate to regulate Nrf-2/Keap-1 pathway under excessive OS.

PQ administration lowered anti-oxidant enzymes (CAT, GSR, SOD, GST, GPx, HO-1 and GSH) activities, while escalating ROS and MDA levels, which led to OS and ultimately renal damage. The antioxidant enzymes are the first wall of protection that lower the production of ROS and safeguard the macromolecules (DNA, lipids and proteins) (Ighodaro and Akinloye, 2018). Excess free radicals can cause tissue damage and have a significant role in the development and progression of many diseases (Talas et al., 2009). CAT is one of the crucial anti-oxidant enzymes that is involved in the catabolism of H_2O_2 (Selamoglu, 2014). SOD transforms superoxide free radicals into H_2O_2 and O_2 (Ighodaro and Akinloye, 2018). GPx reduces OS by scavenging the hydrogen peroxide (Safhi, 2018). GST is mostly involved in detoxification processes (Allocati et al., 2018). GSR maintains the level of GSH that enable GPx to retain its continuous action (Ali et al., 2020). GSH protects cells from OS by lowering the levels of H_2O_2 and other peroxides (Deponte, 2013). HO-1 is a cytoprotective enzyme that shows the ability to break down the heme and plays a notable role in the regulation of cellular homeostasis (Bai et al., 2017). MDA is a detrimental byproduct of lipid peroxidation (LP) and its level might reflect the harm caused by ROS and LP (Yu et al., 2018). Anti-oxidants are responsible for ROS regulation, however excessive ROS production in the body overwhelms the anti-oxidant enzymes, which then results in OS (Lushchak, 2014). Our study revealed that SPS + PQ treatment improved enzymatic activity; however, the contents of ROS and MDA were significantly decreased due to its anti-oxidant potential.

PQ exposure prompted a notable increase in urea, urinary protein, creatinine & urobilinogen levels, while lowering the levels of albumin protein and creatinine clearance. Raised serum levels of creatinine are used as biochemical indicator of renal injury (Sahu et al., 2020). During muscle metabolism, creatine and phosphocreatine are converted into creatinine, a non-protein nitrogenous molecule that is immediately removed by glomerular filtration (Sepulveda, 2019). The secretion of creatinine also depends on Glomerular filtration rate (GFR), therefore any change that slows glomerular filtration causes escalation in serum creatinine level (Padma and Sundaram, 2020). Although urobilinogen is not a component of urine, but PQ toxicity increases the appearance of urobilinogen in urine (Younis et al., 2018), Our studies strongly endorsed the findings of Sharifi-Rigi and Heidarian (2019), who reported that GFR was decreased drastically following PQ exposure (Gu et al., 2016; Shahzad et al., 2017; Sharifi-Rigi and Heidarian, 2019). Intensive renal failure is characterized by elevated urinary creatinine, as well as urinary proteins and decreased albumin protein and creatinine clearance levels (Khan et al.,

2010). However, the supplementation of SPS reduced creatinine, urobilinogen and urea levels while, increasing the levels of albumin protein and creatinine clearance that may be due to the improved GFR.

PQ treatment markedly increased urinary KIM-1 and NGAL levels. It is reported that KIM-1 and NGAL are the two most well-known kidney function markers (Lei et al., 2018). In healthy kidney tissue, KIM-1 is almost completely absent, nonetheless it is observed in the early stages of renal damage (Luo et al., 2016). NGAL is the cytosolic protein that is released into the urine as well as proximal-distal tubule (Mori et al., 2005). Following renal damage, it is released in high concentration into the blood and eliminated through the urine (Yim, 2015). However, the supplementation of SPS in SPS+PQ treated rats lowered the levels of urinary KIM-1 & NGAL due to its nephroprotective potential.

According to our findings, PQ treatment increased IL-1β, NF-κB, IL-6, TNF-α levels and COX-2 activity. Pro-inflammatory cytokines i.e., IL-6, TNF-α, IL-1β and COX-2 expression is significantly increased by NF-κB activation and that leads to acute inflammation and ROS-related damages (Kandemir et al., 2018). Additionally, COX-2 is also a significant inflammatory mediator that contributes to inflammation (Gandhi et al., 2017). Our outcomes revealed that COX-2 activity was elevated in the PQ administered group, reflecting the inflammatory state. However, the co-administration of SPS+PQ resulted in a considerable decrease in inflammatory indices due to its anti-inflammatory nature. Nile et al. (2018) have reported that flavonoids may possess some structural characteristics that affect inflammatory responses by lowering the levels of inflammatory markers (Nile et al., 2018).

PQ exposure elevated Bax and Caspase-3 expression, whereas suppressing the expression of Bcl-2. Proteins of Bcl-2 and Caspase families play a key role in apoptotic cell death. Bcl-2 is a protein (anti-apoptotic) that prevents apoptosis. Contrarily, apoptotic protein Bax functions as an antagonist and has been shown to accelerate cell death (Hou et al., 2021). An imbalance in Bax and Bcl-2 expression leads to an augmented cytochrome C liberation into the cytoplasm by changing the permeability of the mitochondrial membrane and activates Caspase 3 (Siddiqui et al., 2015). Caspase-3 is associated to cysteine protease family, which is involved in the breaking of cellular proteins and leads to apoptotic cell death (Lei et al., 2018). However, SPS therapy prevented these kidney damages by up surging Bcl-2 expression, while lowering Bax & Caspase-3, due to its anti-apoptotic nature.

The findings of this research indicated that control and SPS supplemented group displayed normal histological profile of kidney. Whereas, PQ instigated severe histopathological changes in kidney of PQ exposed rats. PQ-treated group showed considerable damages in the bowman capsule, glomeruli, distal and proximal convoluted tubules and interstitial disturbance as well as a significant loss of cellular differentiation, a prodigious inflammatory response and tubular necrosis. These outcomes are in line with earlier research showing that PQ exposure causes kidney malfunction and damage (Damain et al., 1992; Akinloye et al., 2013; Gao et al., 2015). However, SPS+PQ co-administration significantly improved these histopathological abnormalities due to its renoprotective, anti-oxidant, anti-inflammatory and anti-apoptotic properties.

Conclusion

The outcomes of our investigation apprise that PQ causes nephrotoxicity in rats by decreasing anti-oxidant enzymatic activity and increasing the levels of OS markers, inflammatory, apoptotic markers and histopathological anomalies. However, SPS exhibited excellent attenuative effects against all the PQ induced renal damages and improved the histological architecture of kidney. Thus, it is assumed that SPS may have some protective potential to cope with renal impairment in human as well as in animals.

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Contribution of Authors

Ijaz MU & Kalsoom A: Planned the study, performed the experiments, wrote the first draft, read and approved the final manuscript

Hamza A: Performed the experiment, reviewed the literature, read and approved the final manuscript

Ehsan N: Helped in statistical analysis, read and approved the final manuscript