Sexual Dimorphism of Protective Effect of Nigella Sativa Oil on Nephrotoxicity Induced by Cisplatin in Adult Albino Rats

Original Article

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ABSTRACT

Background: Sex difference was reported to affect both the process of nephrotoxicity induced by Cisplatin and the different protectant strategies. Nigella sativa seed and oil exhibited antioxidant and anti-inflammatory properties with proven protection against Cisplatin-induced nephrotoxicity in male rats. The current work aimed to clarify the role of sex difference in Cisplatin-induced nephrotoxicity, and the possible sex-related nephroprotective effect of Nigella s. oil.

Materials and Methods: The study included thirty-six adult albino rats (18 males and 18 females). Rats were randomly classified into 6 groups, 3 groups for each sex, group 1&2: control groups; group 3&4: each rat in these groups received a single dose of 6 mg/kg Body weight (BW) Cisplatin injection on the 6th day; and group 5&6: each rat in these groups received 2 ml/kg BW Nigella s. oil orally for 11 consecutive days and a single dose of 6 mg/kg BW Cisplatin injection. The rats were sacrificed on the 12th day. Serum creatinine and urea concentrations were estimated. Histopathological procedures using Hx & E, Masson's Trichrome, and PAS stains and immunohistochemical procedures using NFκB P65 and PPARγ antibodies were done for kidney sections.

Results: Cisplatin treatment induced significant body weight loss, significant increase of normalized kidney weight, significant elevation of serum creatinine and serum urea levels, histopathological changes of kidney tissues, marked increase of NFκB-P65 antibody expression within the renal tubular cells, and marked decrease of PPARγ antibody expression in the nuclei of renal tubular cells for both sexes, but male rats were significantly more affected than female rats. Nigella s. oil has ameliorated all the above changes in both sexes without sexual dimorphism.

Conclusion: Female rats were more resistant to nephrotoxicity induced by Cisplatin, while Nigella s. oil has ameliorated cisplatin-induced nephrotoxicity for both male and female rats without apparent sexual dimorphism.

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Key Words: Gender difference; NFκB; PPARγ; sex difference.

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Background

Cisplatin is a regularly used chemotherapeutic drug. It is very effective against a broad spectrum of malignancies[1,2]. Unfortunately, Cisplatin intake is accompanied with several side effects[3]. Acute kidney injury was reported in 31.5% of adult patients receiving a single dose of Cisplatin of more than 50 mg/m2[4]. The mechanisms for describing the pathogenesis of Cisplatin-induced nephrotoxicity involve severe inflammatory response, oxidative stress, toxic metabolites, as well as apoptosis[1].

Nigella sativa (Nigella s.) seed and oil are good food additives that exhibit strong anti-inflammatory and antioxidant properties and there is a growing body of literatures suggesting that they may protect against Cisplatin-induced nephrotoxicity in male rats[5-9]. Many experimental studies[10-14] reported that sex difference has a great role in the process of Cisplatin-induced nephrotoxicity and in the protective effect of some drugs and supplements.

The aim of this study was to clarify the role of sex difference in Cisplatin nephrotoxicity and the protective effect of Nigella s. oil for both sexes.

MATERIALS AND METHODS

Chemicals

Cisplatin, manufactured by Mylan S.A.S., France, (Cisplatine Mylan) was purchased from a local pharmacy. Nigella S. oil was obtained from Isis Company for food processing, a subsidiary of SEKEM Holding, Cairo, Egypt.

Animals and study groups

Thirty-six adult albino rats (18 males and 18 females), 20 weeks old were obtained from Animal House,

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Faculty of Veterinary Medicine, Zagazig University. For acclimatization, all rats were subjected to passive preliminaries for 2 weeks to adapt themselves to their new environment. The rat groups were housed in separate cages which were well-ventilated. They are kept under standard conditions, with free access to water ad libitum and the standard diet. All the experimental procedures were permitted and performed according to the instructions of The Institutional Animal Care and Use Committee, Zagazig University (ZU-IACUC/3/F/18/2023).

Rats were randomly classified into 6 groups, 3 groups for males and 3 groups for females, each group contain 6 rats. The six groups were 1- control male (C, M) group, and 2- control female (C, F) group in which rats received 0.9% normal saline orally by gavage for 11 consecutive days and an intraperitoneal injection of 2 ml 0.9% normal saline on the 6th day of the experiment, 3- cisplatin male (CP, M) group, and 4- cisplatin female (CP, F) group in which rats received normal saline orally for 11 consecutive days and an intraperitoneal injection of a single dose cisplatin (6 mg/Kg BW) in 0.9% normal saline on the 6th day of the experiment, 5- Nigella s. oil and cisplatin male (NS + CP, M) group, and 6- Nigella s. oil and cisplatin female (NS + CP, F) group in which rats received Nigella s. oil (2 ml/Kg BW) orally by gavage for 11 consecutive days and an intraperitoneal single dose cisplatin injection (6 mg/Kg BW) in 0.9% normal saline on the 6th day of the experiment.

Experimental Methodology

The rats of all groups were weighed, anesthetized by intraperitoneal injection of thiopental (120 mg/kg), and sacrificed on the 12th day. Blood samples were immediately aspirated by cardiac puncture. The blood was centrifuged and serum kept at -20°C until it was assayed. Both kidneys were immediately removed, weighed and fixed in formalin for histopathological procedures.

Body Weight Changes

The body weight of each rat in all groups was measured and recorded at the beginning and at the end of the experiment and the percentage (%) body weight change for each group of rats was obtained.

Normalized Kidney Weight

The kidneys of each rat were weighed and the normalized kidney weight (kidney weight per 100 g of body weight) was calculated and recorded.

Serum Biochemical Assay

Serum creatinine concentrations were estimated by Jaffe colorimetric method using Reactivos GPL kits, Cod. SU015 (Barcelona, Spain), and serum urea concentrations were estimated by o-Phthalaldehyde colorimetric method using Reactivos GPL kits, Cod. SU040 (Barcelona, Spain), according to manufacturer`s instructions.

Histopathological Procedures

The kidneys were fixed in 10% buffered formalin solution and then paraffin blocks were prepared. Sections of 4 µm thickness were obtained and stained with Hematoxylin and Eosin (Hx&E) stain, Masson's Trichrome stain, and Periodic acid–Schiff (PAS) stain as described by Dey[16]. Examination, analysis and morphometric studies of the stained kidney sections were done using light microscopy (Lecia ICC50 W) and Leica Q Win plus Image Analysis System (Leica Micros Imaging Solutions Ltd, Cambridge, UK) in Image Analysis Unit of Human Anatomy and Embryology Department, Zagazig University.

Hematoxylin and Eosin- stained slides were used to estimate the tubular injury score. Tubular injury was defined as sloughing of tubular epithelium, dilatation of tubules, and intratubular cast formation. Scoring of tubular injury was done by grading the percentage of injured tubules in 10 randomly chosen, non-overlapping fields, under a magnification of X400 by the computerized image analysis system as follows: score 0: equals normal; score 1: equal or less than 10%; score 2: from 11 to 25%; score 3: from 26 to 45%; score 4: from 46 to 75%; and score 5: more than 75%[15]. Collagen was evaluated in Masson's Trichrome-stained slides and glycogen components were evaluated in PAS-stained slides using ImageJ software (NIH, USA) by gray threshold method. Ten random field were photographed for the slide. The photographs were converted to 32 bit gray scale pictures. The threshold tool on ImageJ software was used for detection the percent of collagen and glycogen within the image.

Immunohistochemical Procedures

Immunohistochemical staining procedures were done for assessment of nuclear factor-kappa B (NFκB) and peroxisome proliferator-activated receptor gamma (PPARγ) in kidney cortical sections. Sections were dewaxed in xylene and rehydrated in graded alcohol. Antigen retrieval done by immersion in citrate buffer (0.05 M, 6.8 pH) solution. Sections were treated with 0.3 % Hydrogen peroxide and protein block. Then, sections were incubated with the primary antibodies; anti-NF-κB P65 (Santa Cruz, Cat# (F-6): sc-8008, 1:100 dilution) and PPAR gamma/ NR1C3 rabbit polyclonal antibody (Novus Biologicals, Cat# NB120-19481, USA, 1:100 dilution). Thereafter, the slides were washed with phosphate-buffered saline (PBS) and incubated for 30 minutes at room temperature with a goat anti-rabbit secondary antibody (Cat# K4003, EnVision+™ System Horseradish Peroxidase Labelled Polymer; Dako) for polyclonal antibodies. The slides were visualized with Diaminobenzidine kit and then counter stained with Mayer's Hematoxylin stain. Negative control slide was done by replacement of primary antibodies by Phosphate-buffered saline (PBS). The percentage of areas of positive reaction to NFκB and PPARγ antibodies was calculated using ImageJ software (NIH, USA)[17].

Statistical Analysis

Data had been collected, tabulated, and analyzed using statistical methods. It was presented as mean ± standard deviation (SD). The Statistical analysis system SAS (version 9.4, SAS Institute Inc., Cary, NC, USA) was used for its analysis. As appropriate, statistical analysis was done using unpaired Student-t-test, or one-way analysis of variance (ANOVA) followed Tukey's post hoc tests when applicable. A *P-Value* ≤ 0.05 was considered statistically significant.

RESULTS

Body Weight Changes and Normalized Kidney Weight

There were significant body weight loss of rats in both cisplatin male (CP, M) and cisplatin female (CP, F) groups compared to control male (C, M) and control female (C, F) groups with more weight loss in CP, M group. Rats in Nigella s. oil and cisplatin male (NS+CP, M) group, and Nigella s. oil and cisplatin female (NS+CP, F) group showed significantly lesser weight loss compared to CP, M and CP, F groups indicating the ameliorating effect of Nigella s. oil on body weight loss induced by Cisplatin for male and female rats (Table 1).

Normalized kidney weight (the kidney weight per 100 gm BW) was significantly higher in CP, M and CP, F groups compared to control (C, M and C, F) groups and its increase was more significant in CP, M group compared to CP, F group indicating that the kidneys of rats in male group were more affected by Cisplatin treatment than the kidneys of female group. Normalized kidney weight was significantly lower in NS+CP, M and NS+CP, F groups compared to CP, M and CP, F groups indicating the ameliorative effect of Nigella s. oil on the kidneys of both male and female rats (Figure 1A).

Effect on Serum Creatinine

Normal reference value of serum creatinine in albino rats is 1.24 - 1.37 mg/dl[18,19]. There was a significant increase of serum creatinine level in CP, M group (2.57 ± 0.29 mg/dl) compared to C, M group (0.99 ± 0.19 mg/dl) ($P < 0.0001$). Also, there was a significant increase of serum creatinine level in CP, F group (1.64 ± 0.21 mg/dl) compared to C, F group (0.97 ± 0.16 mg/dl) ($P = 0.0002$). The serum level of creatinine was significantly higher in CP, M group compared to CP, F group ($P = 0.0002$) (Figure 1B). This indicated that female rats were more resistant to the effect of Cisplatin on serum creatinine level.

The level of serum creatinine was significantly lower in NS + CP, M group compared to CP, M group (1.45 ± 0.18 Vs 2.57 ± 0.29 mg/dl, respectively. $P = 0.0002$). Also, the level of creatinine was significantly lower in NS + CP, F group compared to CP, F group (1.36 ± 0.12 Vs 1.64 ± 0.21 mg/dl, respectively. $P = 0.0302$). There was no significant difference between NS + CP, M and NS + CP, F groups in creatinine level ($P = 0.3393$), indicating that Nigella s. oil ameliorated the effect of Cisplatin on serum creatinine level in both male and female rats. (Figure 1B).

Effect on Serum urea

Normal reference value of serum urea in albino rats is 33.1- 40.27 mg/dl[18,19]. There was a significant increase of serum urea level in CP, M group (56.4 ± 9.3 mg/dl) compared to C, M group (30.7 ± 3.9 mg/dl) ($P = 0.0006$). Also, there was a significant increase of serum urea level in CP, F group (44.1 ± 4.0 mg/dl) compared to C, F group (29.9 ± 3.5 mg/dl) ($P < 0.0001$). The serum level of urea was significantly higher in CP, M group compared to CP, F group ($P = 0.0198$) (Figure 1C). This indicated that female rats were more resistant to the effect of Cisplatin on serum urea level.

The level of serum urea was significantly lower in NS + CP, M group compared to CP, M group (43.3 ± 4.7 Vs 56.4 ± 9.3mg/dl, respectively. $P = 0.0192$). Also, the level of urea was significantly lower in NS + CP, F group compared to CP, F group (38.9 ± 3.9 Vs 44.1 ± 4.0 mg/dl, respectively. $P = 0.0456$). There was no significant difference between NS + CP, M and NS + CP, F groups in creatinine level ($P = 0.1092$), indicating that Nigella s. oil ameliorated the effect of Cisplatin on serum urea level in both male and female rats. (Figure 1C).

Histopathological Changes and Kidney Tissue Damage

Hematoxylin and Eosin-stained sections: showed normal renal corpuscles and renal tubules in Kidney sections of C, M and C, F groups (Figures 2 A1,B1). Kidney sections of CP, M and CP, F showed marked histological alterations in the form of shrunken glomerular tufts, wide Bowman's space, distorted glomerular basement membrane, tubular degeneration, desquamated epithelium of some tubules, and homogenous acidophilic material in the lumen of some tubules (Figures 2 C1,D1). Those histological alterations were more marked in kidney sections of rats in CP, M group compared to sections of rats in CP, F group which was confirmed by significantly higher tubular injury score (4.67 ± 0.516 Vs 3.5 ± 0.548, P = 0.003) (Figure 5A). Kidney sections of rats in NS+CP, M group, and NS+CP, F group showed lesser degrees of histopathological alterations compared to CP, M and CP, F groups (Figures 2 E1,F1). This was confirmed by a lower tubular injury score compared to Cisplatin (CP, M and CP, F) groups (Figure 5A).

Masson's Trichrome stained sections

Showed normal thin collagen fibers within the glomerular tuft and between the renal tubules in both C, M and C, F groups (Figures 3 A2,B2). Kidney sections of CP, M group and CP, F group showed interstitial fibrosis, peritubular fibrosis and periglomerular fibrosis (Figures 3 C2,D2). The area percentage of positive Masson's Trichrome stain reaction of CP, M group and CP, F group was significantly higher compared to C, M group and C, F group ($P < 0.0001$& $P < 0.0001$, respectively), and it was significantly higher in C, M group compared to C, F group ($P = 0.0004$) (Figure 5B). Kidney sections of NS+CP, M group and NS+CP, F group showed glomerular and

peritubular fibrosis (Figures 3 E2,F2). The area percentage of positive Masson's Trichrome stain reaction in NS+CP, M group and NS+CP, F group were significantly lower than that in CP, M group and CP, F group ($P < 0.0001$ & $P < 0.0001$, respectively), while there was no significant difference between NS+CP, M group and NS+CP, F group ($P = 0.236$) (Fig.5B). This indicates the ameliorating effect of Nigella s. oil on fibrosis of kidney tissues caused by Cisplatin for both sexes.

PAS-stained sections: showed normal PAS reaction in the glomerular basement membrane, within the glomerular tufts and in the tubular basement membrane with intact brush border of the tubules in C, M and C, F groups (Figures 4 A3,B3). Sections of CP, M and CP, F groups showed deposition of glycated substances within the glomerular tufts, within the tubular lumen and loss of tubular brush borders (Figures 4 C3,D3). The area percentage of positive PAS stain reaction of CP, M group and CP, F group was significantly higher compared to C, M group and C, F group ($P < 0.0001$& $P < 0.0001$, respectively), and it was significantly higher in C, M group compared to C, F group ($P = 0.0014$) (Figure 5C). Sections of NS+CP, M group and NS+CP, F group showed PAS reaction within the glomerular tufts, the glomerular basement membrane, within the lumen of renal tubules with intact brush border of many tubules (Figures 4 E3,F3). The area percentage of positive PAS stain reaction in NS+CP, M group and NS+CP, F group were significantly lower than that in CP, M group and CP, F group ($P < 0.0001$ & $P < 0.0001$, respectively), while there was no significant difference between NS+CP, M group and NS+CP, F group ($P = 0.796$) (Figure 5C). This indicates the ameliorating effect of Nigella s. oil on deposition of glycated substances in kidney tissues caused by Cisplatin for both sexes.

Tubular injury scoring

There were significant tubular injuries in both CP, M and CP, F groups compared to control (C, M and C, F) groups ($P < 0.0001$ & $P < 0.0001$, respectively), but tubular injury score was significantly higher in CP, M group compared to CP, F group ($P = 0.003$) (Figure 5A). This indicates that female rats were more resistant to tubular injuries caused by Cisplatin. Tubular injury scores were significantly lower in NS + CP, M group, and NS + CP, F group compared to CP, M and CP, F groups ($P < 0.0001$ & $P < 0.0001$, respectively), but still tubular injury scores in NS + CP, M group, and NS + CP, F group were significantly higher compared to control (C, M and C, F) groups ($P = 0.0006$ & $P = 0032$, respectively) (Figure 5A). There was no significant difference in tubular injury score between NS + CP, M group and NS + CP, F group ($P = 0.305$). This indicates the ameliorating effect of Nigella s. oil on tubular injury caused by Cisplatin for both sexes.

NFκB Immunohistochemistry

Negative control immunohistochemical slides showed no immunohistochemical reaction (Figure 6).

Cisplatin male and female (CP, M and CP, F) groups showed a marked increase of expression of NFκB-P65 antibody in the cytoplasm and nucleus of renal tubular epithelial cells with a significantly higher area percentage of NFκB-P65 positive immune reaction compared to control male and female (C, M and C, F) groups ($P < 0.0001$ & $P < 0.0001$, respectively) (Figures 5D, 7 C4,D4). The area percentage of NFκB-P65 stain positive immune reaction was significantly higher in Cisplatin male group compared to Cisplatin female group ($P = 0.0002$) (Figure 5D). The expression of NFκB-P65 antibody and the area percentage of NFκB-P65 stain-positive immune reaction were significantly lower in Nigella s. plus Cisplatin (NS + CP, M and NS + CP, F) groups compared to Cisplatin (CP, M and CP, F) groups ($P < 0.0001$ & $P < 0.0001$, respectively). There were no significant differences between rats in NS + CP, M group and NS + CP, F group ($P = 0.134$) (Figures 5D, 7 E4,F4).

PPARγ Immunohistochemistry

The kidney cortical sections of both control male (C, M) and control female (C, F) groups showed marked nuclear expression of PPARγ antibody within the tubular lining epithelium (Figures 8 A5,B5). Cisplatin Male and female (CP, M and CP, F) groups showed a marked decrease of expression of PPARγ antibody (Figures 8 C5,D5) with a significantly lower area percentage of PPARγ stain positive immune reaction compared to control (C, M and C, F) groups ($P < 0.0001$ & $P < 0.0001$, respectively) (Figure 5E).The area percentage of PPARγ stain positive immune reaction was significantly lower in Cisplatin male group compared to Cisplatin female group ($P = 0.0002$) (Figure 5E), indicating that female kidneys were more resistant to injury by Cisplatin. The expression of PPARγ antibody and the area percentage of PPARγ stain positive immune reaction were significantly higher in Nigella s. plus Cisplatin (NS + CP, M and NS + CP, F) groups compared to Cisplatin (CP, M and CP, F) groups ($P < 0.0001$ & $P < 0.0001$, respectively). There were no significant differences between rats in NS + CP, M group and NS + CP, F group ($P = 0.435$) (Figures 5E, 8 E5,F5), indicating the ameliorating effect of Nigella s. oil for kidney injury induced by Cisplatin in both sexes.

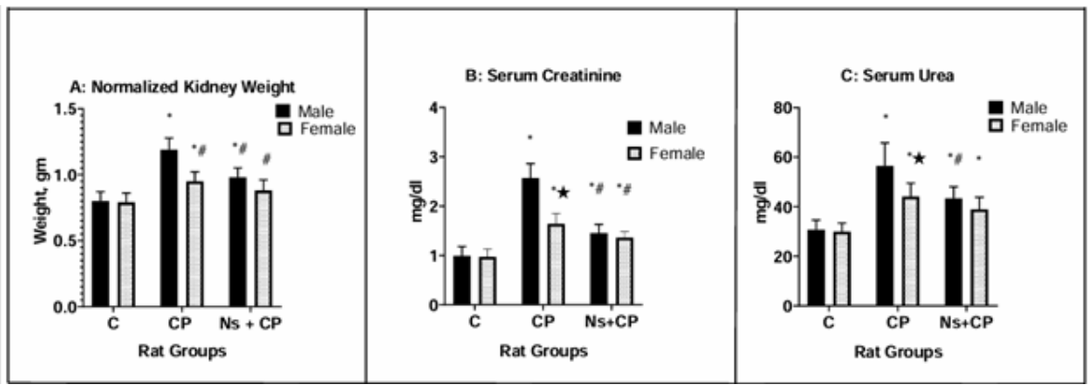

Fig. 1: Histograms showing mean ± SD of (A) normalized kidney weight, (B) serum creatinine, and (C) serum urea for male and female rat groups. C: control groups; CP: Cisplatin groups; Ns + CP: Nigella s. + Cisplatin groups. * Indicates significant difference from control group of the same sex (P less than 0.05), # indicates significant difference from Cisplatin group of the same sex (P less than 0.05), * indicates significant difference from male Cisplatin group (P less than 0.05). Number of rats in each group = 6 rats. Unpaired Student t-test was used for comparison between two groups, and one-way analysis of variance (ANOVA) followed by Tukey's post hoc tests were used for comparison among more than 2 groups.

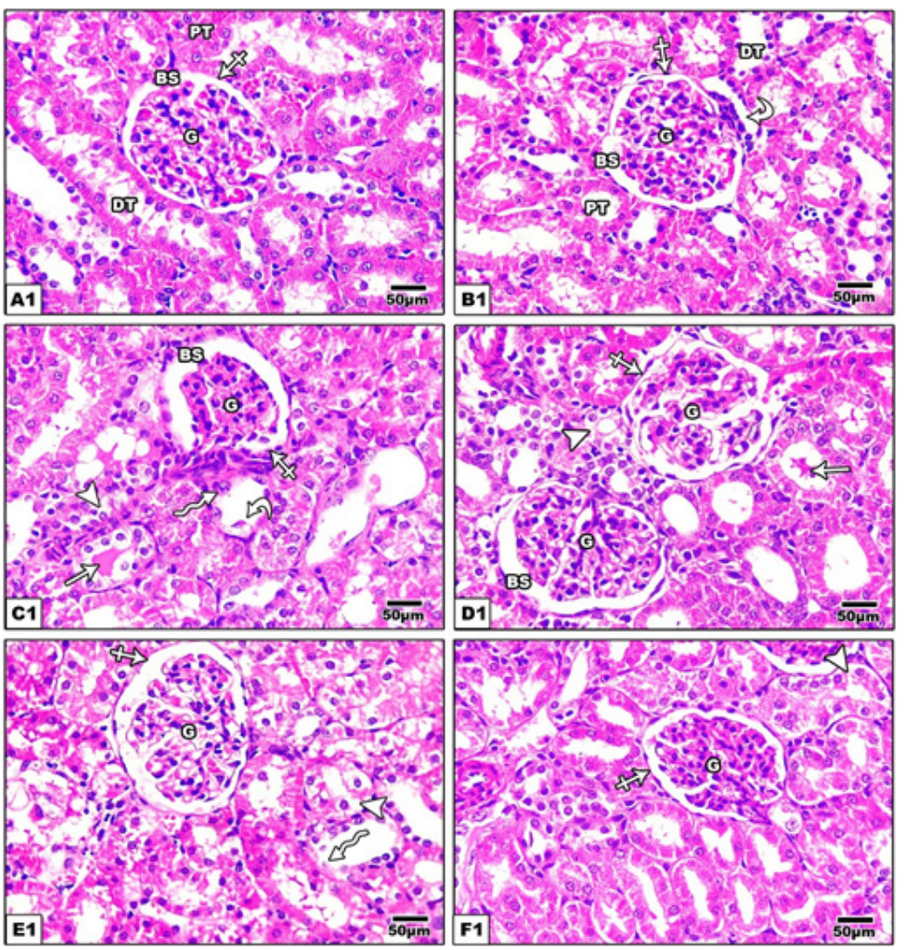

Fig. 2: Illustrative photomicrographs of H&E-stained kidney cortical sections from the different experimental groups. A1 (control male group) and B1 (control female group) showing normal renal corpuscles and renal tubules. G, glomerulus; BS, bowman's space; PT, proximal tubule; DT, distal tubule; crossed arrow, glomerular basement membrane; curved arrow, juxtaglomerular apparatus. C1 (Cisplatin male group) showing shrunken glomerular tufts (G) with wide Bowman's space (BS), distorted glomerular basement membrane (crossed arrow), vacuolar tubular degeneration (white arrowhead), desquamated epithelium of some tubules (curved arrow), nucleomegaly of epithelial lining of some renal tubules (zigzag arrow) and homogenous acidophilic material in the lumen of some tubules (white arrow). D1 (Cisplatin female group) showing shrunken glomeruli (G), wide Bowman's space (BS), distorted glomerular basement membrane (crossed arrow), vacuolar tubular degeneration (white arrowhead) and homogenous acidophilic material in the lumen of some tubules (white arrow). E1 (Nigella s. oil and cisplatin male group) showing distorted glomerular basement membrane (crossed arrow), tubular vacuolar degeneration (white arrowhead), with some ghost nuclei in the epithelium of the tubules (zigzag arrow). F1 (Nigella s. oil and cisplatin female group) showing distorted glomerular basement membrane (crossed arrow) and vacuolar tubular degeneration (white arrowhead). (H&E, X400, bar= 50 µm).

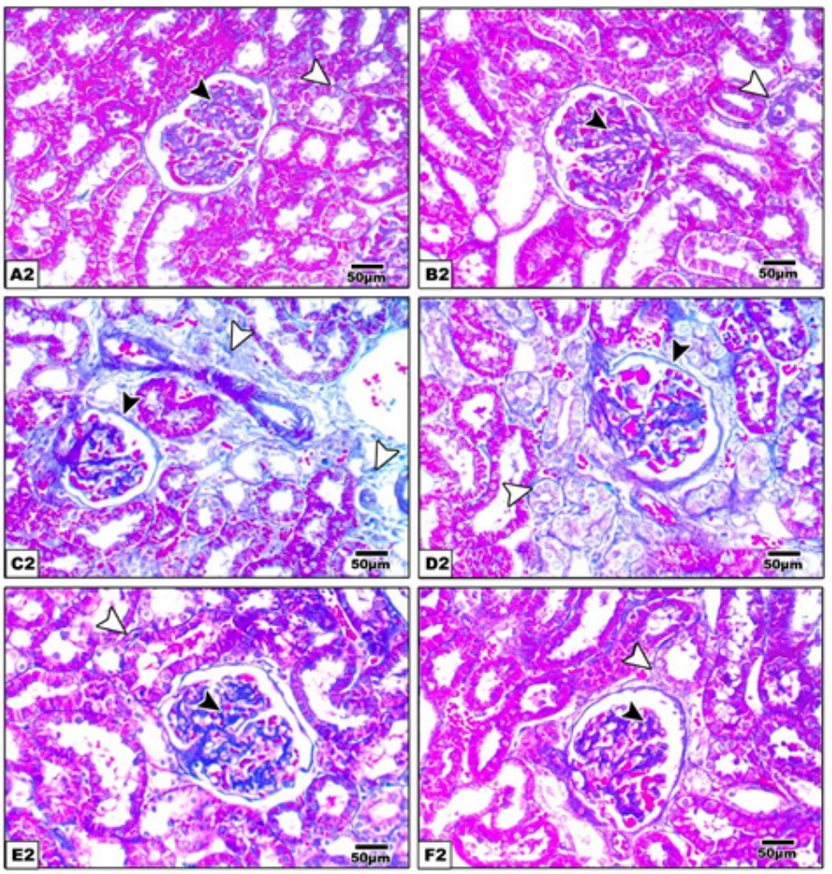

Fig. 3: Illustrative photomicrographs of Masson's Trichrome-stained kidney cortical sections from the different experimental groups. A2 (control male group) and B2 (control female group) showing normal thin collagen fibers within the glomerular tuft (black arrowhead) and between the renal tubules (white arrowhead). C2 (Cisplatin male group) showing interstitial fibrosis (white arrowheads) and periglomerular fibrosis (black arrowhead). D2 (Cisplatin female group) showing periglomerular fibrosis (black arrowhead), peritubular fibrosis (white arrowhead). E2 (Nigella s. oil and cisplatin male group) showing glomerular fibrosis (black arrowhead) and peritubular fibrosis (white arrowhead). F2 (Nigella s. oil and cisplatin female group) showing glomerular and peritubular fibrosis (black and white arrowheads respectively). (Masson's Trichrome stain, X400, bar= 50 µm).

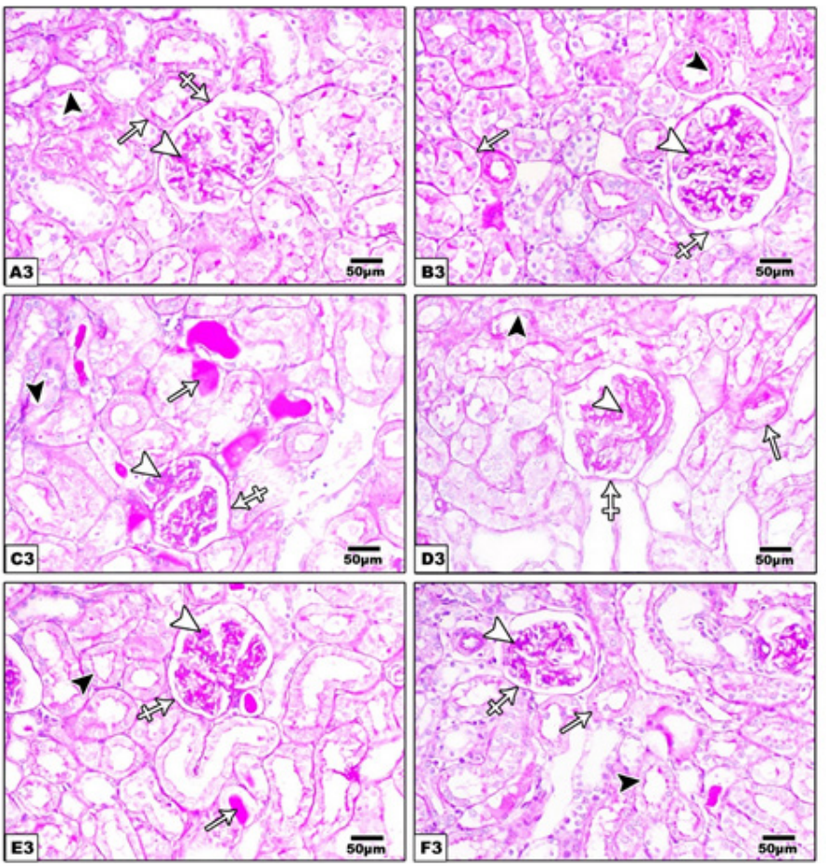

Fig. 4: Illustrative photomicrographs of PAS-stained kidney cortical sections from the different experimental groups. A3 (control male group) and B3 (control female group): showing normal PAS reaction in the glomerular basement membrane (crossed arrow), within the glomerular tufts (white arrowhead), in the tubular basement membrane (white arrow) and intact brush border of the tubules (black arrowhead). C3 (Cisplatin male group) showing deposition of glycated substances within the glomerular tufts (white arrowhead), in the glomerular basement membrane (crossed arrow), within the lumen of the renal tubules (white arrow) and loss of the brush border of the tubules (black arrowhead). D3 (Cisplatin female group) showing deposition of PAS-positive substances within the glomerular tufts (white arrowhead), the glomerular basement membrane (crossed arrow), the tubular basement membranes (white arrow) and loss of the brush border of the tubules (black arrowhead). E3 (Nigella s. oil and cisplatin male group) showing PAS reaction within the glomerular tufts (white arrowhead), the glomerular basement membrane (crossed arrow), within the lumen of renal tubules (white arrow) and intact brush border of some of the tubules (black arrowhead). F3 (Nigella s. oil and cisplatin female group) showing PAS-positive reaction within the glomerular basement membrane (crossed arrow), within the glomerular tufts (white arrowhead), in the tubular basement membranes (white arrow) and intact brush border of some of the tubules (black arrowhead). (PAS Stain, X400, bar= 50 µm).

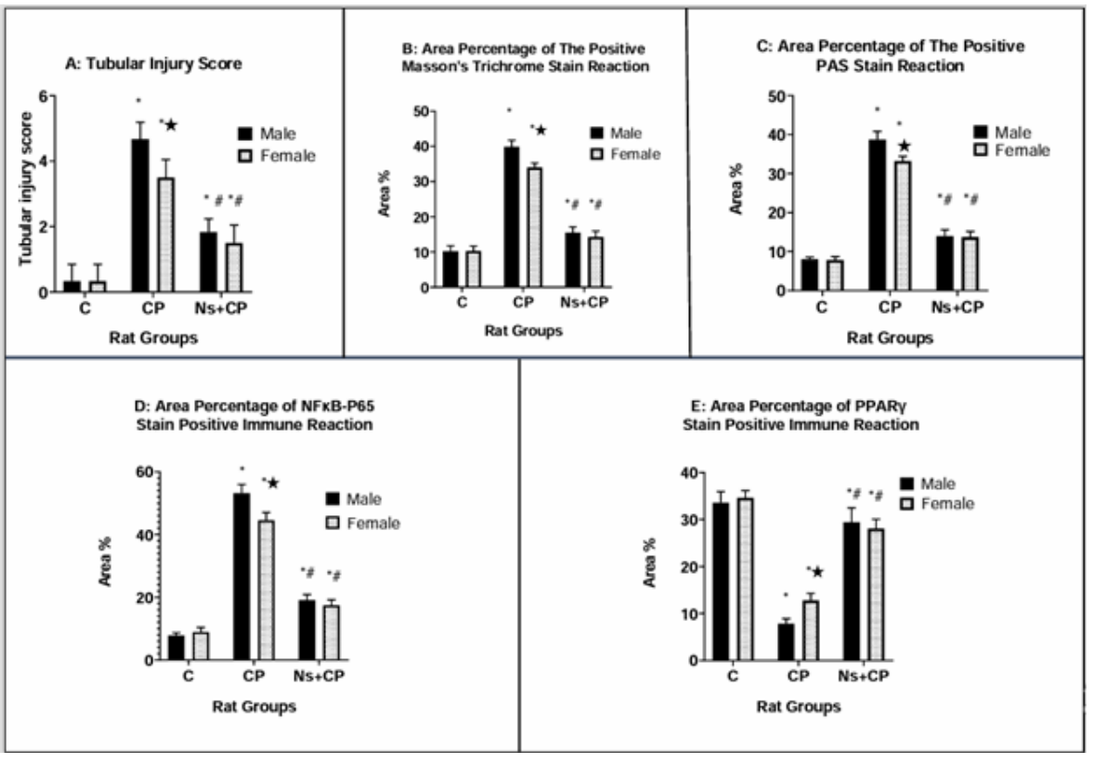

Fig. 5: Histograms showing mean ± SD of (A) tubular injury score, (B) area percentage of the positive Masson's Trichrome stain reaction, (C) area percentage of the positive PAS stain reaction, (D) area percentage of NFkB-P65 stain positive immune reaction, and (E), area percentage of PPARγ stain positive immune reaction for male and female rat groups. C: control groups; CP: Cisplatin groups; Ns + CP: Nigella s. + Cisplatin groups. * Indicates significant difference from control group of the same sex (P less than 0.05), # indicates significant difference from Cisplatin group of the same sex (P less than 0.05), * indicates significant difference from male Cisplatin group (P less than 0.05). Number of rats in each group = 6 rats. Unpaired Student t-test was used for comparison between two groups and one-way analysis of variance (ANOVA) followed by Tukey's post hoc tests were used for comparison among more than 2 groups.

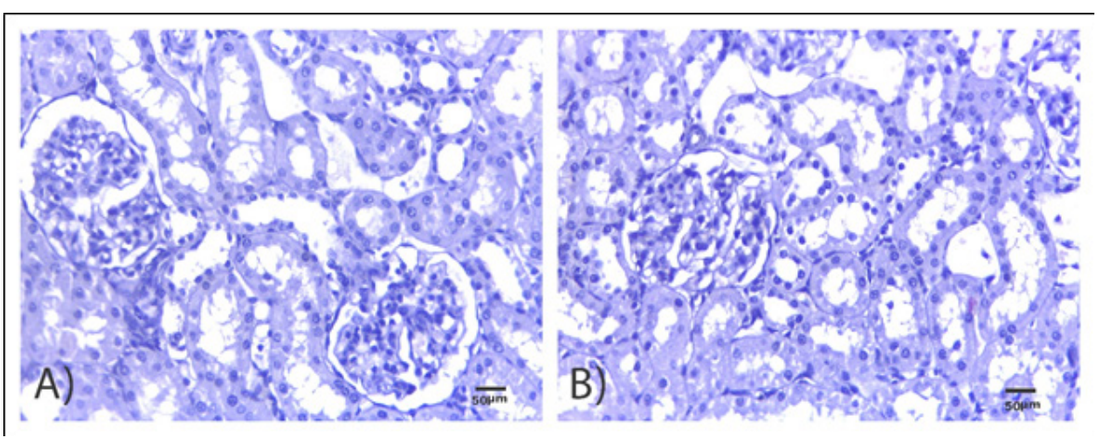

Fig. 6: Illustrative photomicrographs of negative control immunohistochemical slides in kidney cortical sections. No immunohistochemical reaction can be seen. A) control male group. B) control female group. (X400, bar= 50 µm)

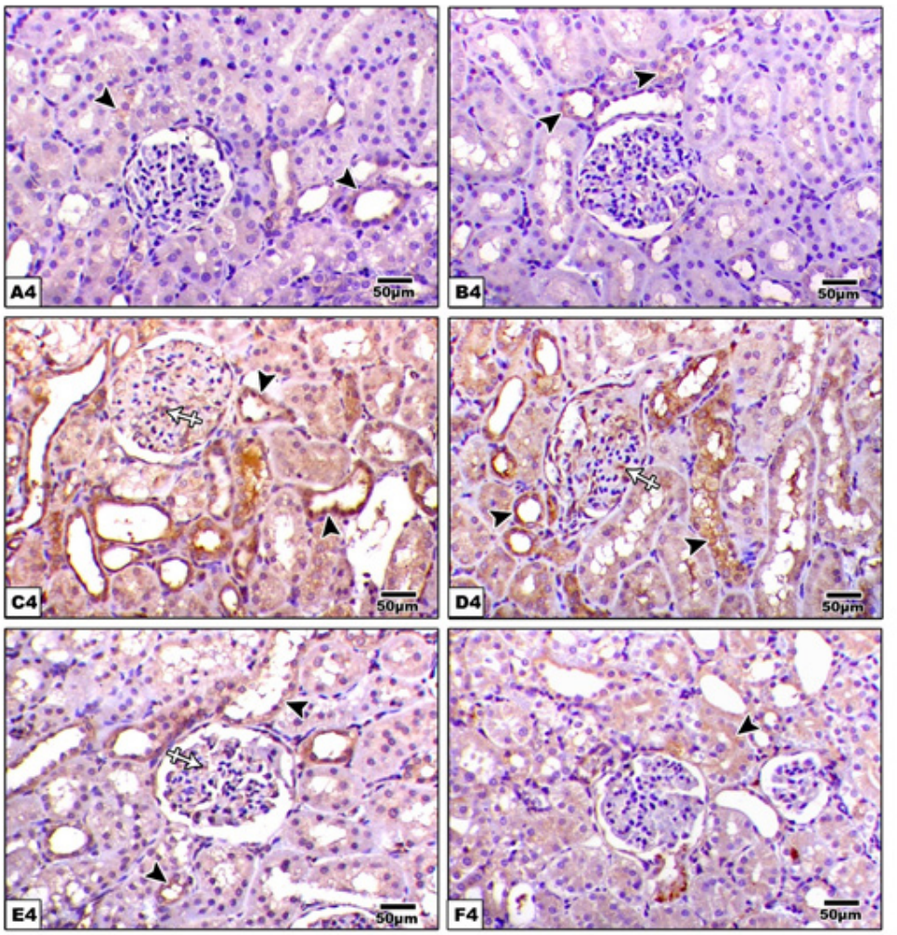

Fig. 7: Illustrative photomicrographs showing immune expression of NFκB in kidney cortical sections from the different experimental groups. A4 (control male group) and B4 (control female group) showing normal cytoplasmic expression of NFκB-P65 antibody within the tubular lining epithelium (black arrowheads). C4 (cisplatin male group) and D4 (cisplatin female group) showing the cytoplasmic expression of NFκB-P65 antibody within the tubular lining epithelium (black arrowheads), and within the renal glomeruli (crossed arrow), E4 (Nigella s. oil and cisplatin male group) and F4 (Nigella s. oil and cisplatin female group) showing the cytoplasmic expression of NFκB-P65 antibody within tubular lining epithelium (black arrowheads) and within the glomerulus (crossed arrow). (NFκB-P65 IHC, X400, bar = 50 µm).

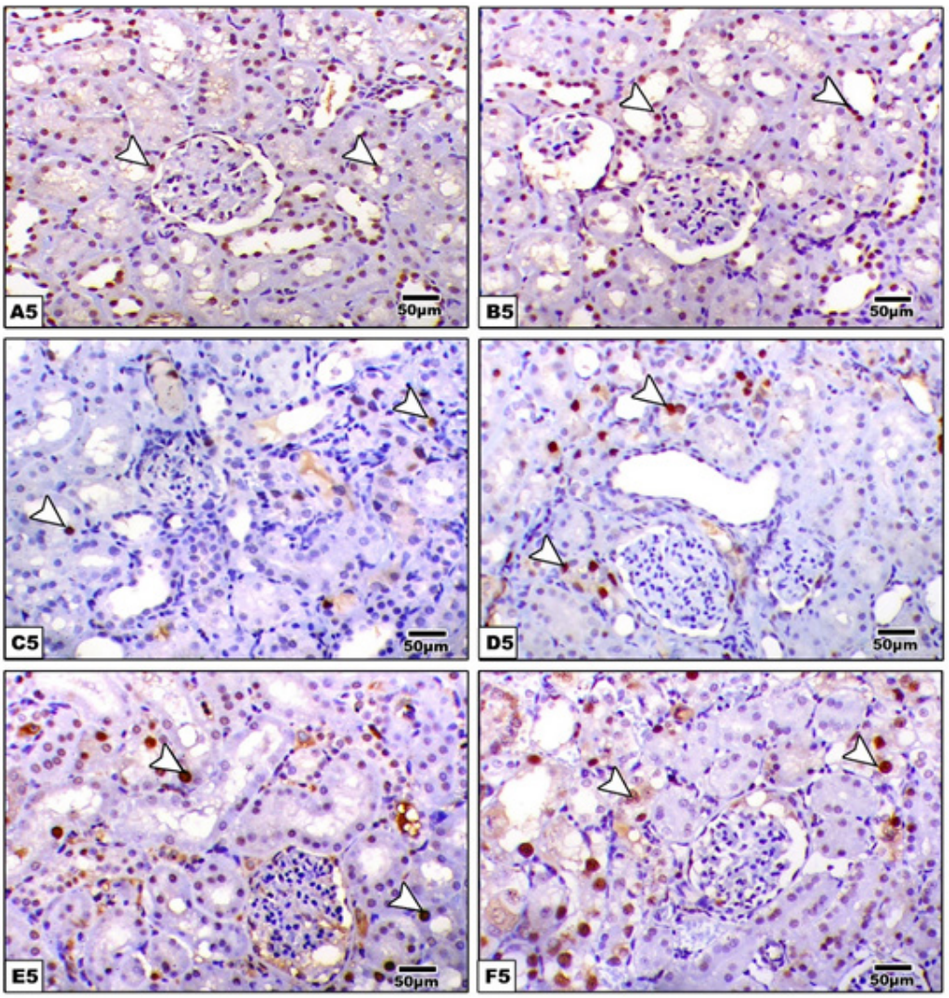

Fig. 8: Illustrative photomicrographs showing immune expression of PPARγ in kidney cortical sections from the different experimental groups. A5 (control male group) and B5 (control female group) showing normal nuclear expression of PPARγ antibody within the tubular lining epithelium (white arrowheads); C5 (cisplatin male group) and D5 (cisplatin female group) showing the nuclear expression of PPARγ antibody within the tubular lining epithelium (white arrowheads), E5 (Nigella s. oil and cisplatin male group) and F5 (Nigella s. oil and cisplatin female group) showing the nuclear expression of PPARγ antibody within the tubular lining epithelium (white arrowheads). (PPARγ IHC, X400, bar = 50 µm).

Table 1: Body Weight (gm) Before and After Treatment of Rat Groups

Groups (6 rats in each group)	Before Treatment (Mean ± SD)	After Treatment (Mean ± SD)	% Change	*P-Value*
Male Groups				
C, M	215.2 ± 11.41	234.2 ± 12.42	+ 8.83%	0.0188
CP, M	218.2 ± 12.06	184.5 ± 13.25[a]	- 15.44%	0.0008
NS+CP, M	213.3 ±12.12	198.3 ± 15.82[a]	- 7.03%	0.104
P-Value	0.8118	0.0008		
Female Groups				
C, F	170.0 ± 7.75	180.0 ± 9.57	+ 5.88%	0.0918
CP, F	166.7 ± 8.16	151.7 ± 8.98[b]	- 9.0%	0.0258
NS+CP, F	169.7 ± 8.41	160.5 ± 7.34[b]	٪٥,٤٢	0.1008
P-Value	0.7399	0.0038		

Abbreviations: C,M = Control male; C,F = Control female; CP,M = Cisplatin male; CP,F = Cisplatin female; NS+CP,M = Nigella s. oil and Cisplatin male; NS+CP,F = Nigella s. oil and Cisplatin female. N = number, [a]P indicates significant difference from control male group, [b]P indicates significant difference from control female group.

DISCUSSION

The pathogenesis of nephrotoxicity induced by Cisplatin involves mainly severe inflammatory response and oxidative stress[20,21]. Nephrotoxicity induced by Cisplatin is greatly influenced by sex disparities[10]. Nigella s. exhibits strong antioxidant and anti-inflammatory properties with evident ameliorating effects on nephrotoxicity induced by Cisplatin in male rats[5-9]. To our knowledge, there were no published experimental studies reporting the efficacy of Nigella s. in the protection against nephrotoxicity induced by Cisplatin according to sex difference.

Consistent with many other studies[12,22-24], the current study showed significant weight loss of rats in both Cisplatin male (CP, M) group and cisplatin female (CP, F) group indicating that Cisplatin induced significant loss of weight in both male and female rats and weight loss was significantly more in Cisplatin male group.

The main causes of body weight loss induced by Cisplatin, as reported by Garcia *et al*.[25], and Lin *et al*.[26] were anorexia and gastrointestinal disturbances, which might indicate lesser degrees of anorexia and gastrointestinal disturbances occurred in female rats.

Also, the current study showed that weight loss was significantly lesser for rats in NS + CP, M group, and NS + CP, F group compared to CP, M and CP, F groups indicating the ameliorating effect of Nigella s. oil on body weight loss induced by Cisplatin for male and female rats, while Jilanchi, *et al*. (a)[27], Motamedi *et al*.[28], Haghighi *et al*.[29] and Jilanchi *et al*. (b)[30] showed that pomegranate flower extract, losartan, and vitamin E protected male rats from weight loss induced by Cisplatin and exaggerated it in female rats. El-Arabey[31] considers phytoestrogen component of pomegranate flower extract is the cause of the different effect in female rats. Also, he considers estrogen may eliminate the renoprotective role of losartan and vitamin E.

Normalized kidney weight can be considered as one of the parameters for evaluating the degree of nephrotoxicity and kidney tissue damage for experimental studies[30,31,32]. The current study, consistent with many studies,[12,29,30,33] showed a significant increase in the normalized kidney weight of rats in male Cisplatin (CP, M) group compared to female Cisplatin (CP, F) group as a result of cisplatin injection indicating more kidney damage in male rats. In the current study, normalized kidney weight was significantly lower in male and female Nigella s. and Cisplatin groups (NS + CP, M and NS + CP, F) compared to Cisplatin groups (CP, M and CP, F) indicating Nigella s. oil ameliorated the effect of Cisplatin on normalized kidney weight for both sex while enalapril[12], losartan[29], and vitamin E[30] failed to do this effect in female rats.

Consistent with the results of many studies[11,12,29,33,34], the current study showed deterioration of kidney function with significant elevations of serum creatinine and serum urea in male and female rats treated with Cisplatin in CP, M and CP, F groups. The elevation in serum creatinine and urea was significantly more in CP, M group compared to CP, F group. In the current study, Nigella s. oil ameliorated the deterioration of kidney function for both male and female rats in Ns + CP, M and Ns + CP, F groups, while bosentan[34], gamma-aminobutyric acid[14], and enalapril[12] failed to ameliorate the elevation of serum levels of creatinine and urea induced by Cisplatin in male rats and exaggerated it in female rats. This can be attributed to sex-dependent renin angiotensin system (RAS) response[12,14,34].

There is a wide range of severity of histological changes induced by cisplatin. It may be localized to the S3 segment of the proximal tubules, where Cisplatin is mainly accumulated or extended to distal tubules and glomeruli[1,35].

In the current work, Cisplatin induced histopathological changes in kidney tissues for both sexes in CP, M and CP, F groups. These changes were more marked in rats of CP, M group. The differences in the degree of histopathological changes of kidney tissues between male and female rats were confirmed by significantly higher tubular injury score, area percentage of Masson's Trichrome stain reaction, and area percentage of PAS stain reaction of male rats' kidney sections compared to female rats' kidney sections. Also, sexual dimorphism of Cisplatin-induced nephrotoxicity was reported by Eshraghi-Jazi *et al*.[22], Nematbakhsh *et al*.[23], Zamani *et al*.[12], Shi *et al*.[11] and Hwang *et al*.[24].

The causes of Sexual dimorphism of Cisplatin-nephrotoxicity are not well determined[35]. El-Arabey[31] considered the endogenous sex hormones to have a role by their different effect on the organic cation transporter (OCT2), a mediator for the uptake of Cisplatin into tubular cells. Urakami *et al*.[37] study showed that testosterone intake increased the level of organic cation transporter (OCT2), but estradiol intake moderately decreased the level of OCT2 in both sexes. Marcu[36] considered that sex differences in renal circulation play a role in sexual dimorphism. Kander *et al*.[38] and Eshraghi-Jazi and Nematbakhsh[10] considered that females are more resistant to Cisplatin toxic effect on the kidney because they have lower oxidative stress as well as reactive oxygen species (ROS) formation than males.

Tumor necrosis factor α (TNF-α) activates chemokines and proinflammatory cytokines triggering oxidative stress leading to exacerbation of kidney injury. Hydroxyl free radicals which are produced by Cisplatin, ultimately regulate TNF-α production that activates NF-κB which is an essential pathway for the production of inflammatory mediators[39]. NF-κB is located mainly in the tubular cells and to a lesser degree in the glomeruli[40]. PPARγ is a potent anti-inflammatory agent, acting by inhibiting the NF-κB pathway[39,41]. Also, PPARγ is one of the first responders during oxidative stress by inducing many antioxidant molecules, protecting the cells from apoptosis[42].

The current study showed significant increase in NF-κB expression and significant decrease in PPARγ expression

in kidney sections of male and female rats treated with Cisplatin (CP, M and CP, F groups) in comparison to control (C, M and C, F) groups. There were significant differences between male and female Cisplatin-treated rats indicating that female rats had a lower inflammatory response and a lower oxidative stress response to Cisplatin treatment.

The current study showed significant decrease of NF-κB expression and significant increase of PPARγ expression in male and female rats in Nigella s. and Cisplatin (Ns + CP, M and Ns + CP, F) groups compared to cisplatin (CP, M and CP, F) groups without significant differences between both sexes, but not reaching the level of control (C, M and C, F) groups. These data indicated the ameliorating effect of Nigella s. oil on inflammatory response and oxidative stress induced by Cisplatin in kidney tissues for both male and female rats.

Oxidative stress is attenuated by Nigella s. by a mechanism involving the regulation of antioxidant enzymes and molecules as well as the reduction of ROS. The mechanism of the anti-inflammatory effect of Nigella s. is "probably" due to inhibiting pro-inflammatory cytokines, inflammatory enzymes, leukotriene C4 synthase and 5-lipoxygenase[9].

CONCLUSIONS AND RECOMMENDATION

The results of this study proved the sexual dimorphism of Cisplatin-induced nephrotoxicity. Nigella s. oil has an ameliorating effect on kidney tissue damage and the deterioration of kidney function induced by Cisplatin treatment for both male and female rats, and this is most probably due to its potent anti-inflammatory and antioxidant properties.

The results of the current study suggest using Nigella s. oil as a food additive for male and female patients treated with Cisplatin, but this needs clinical trials to confirm its beneficial effect on human beings without decreasing the tumoricidal effect of Cisplatin.

CONFLICT OF INTERESTS

There are no conflicts of interest

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الملخص العربى

تأثير نوع الجنس في الدور الوقائي لزيت حبة البركة من التسمم الكلوي بعقار سيسبلاتين في الجرذان البيضاء البالغة

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الخلفية: ذكرت العديد من الدراسات التجريبية أن إختلاف الجنس له دور كبير في عملية التسمم الكلوي التي يسببها عقار سيسبلاتين مع نتائج متنوعة لإستراتيجيات الحماية. تظهر حبة البركة خصائص قوية مضادة للأكسدة ومضادة للالتهابات مع تأثيرات وقائية من السمية الكلوية لعقارسيسبلاتين في ذكور الجرذان. تم تنفيذ الدراسة الحالية لتوضيح دور إختلاف الجنس في السمية الكلوية لعقارسيسبلاتين، والتأثيرالوقائى المحتمل لزيت حبة البركة طبقا لنوع الجنس.
المواد وطرق البحث: تضمنت هذة الدراسة ثمانية عشر ذكرًا و ثمانية عشر أنثى من الجرذان البيضاء البالغة. تم تصنيف ذكور وإناث الجرذان بشكل عشوائي إلى ٣ مجموعات لكل جنس، وهي مجموعة ضابطة، ومجموعة تلقت جرعة واحدة من حقن سيسبلاتين ٦ مجم/كجم من وزن الجسم، ومجموعة تلقت ٢ مل/كجم من زيت حبة البركة عن طريق الفم لمدة ١١ يوما متتاليا وجرعة واحدة من حقن سيسبلاتين ٦ مجم/كجم من وزن الجسم. تم وزن و تخدير وذبح الجرذان في اليوم الثانى عشر من التجربة. تم سحب عينات الدم لتقدير تركيزات الكرياتينين واليوريا في الدم. تم إستئصال كلتا الكليتين على الفور، ووزنهما، وتثبيتهما في الفورمالين من أجل الدراسات النسيجية والكيميائية المناعية.
النتائج: تسبب العلاج بعقار سيسبلاتين في فقدان وزن الجسم بشكل كبير، وزيادة كبيرة في الوزن النسبى للكلى، وارتفاع كبير في مستويات الكرياتينين واليوريا في الدم، وتغيرات نسيجية مرضية لأنسجة الكلى، وزيادة ملحوظة في ظهور الأجسام المضادة (NFκB-P٦٥)داخل الخلايا الأنبوبية الكلوية، وانخفاض ملحوظ في ظهور الأجسام المضادة (PPARγ) داخل الخلايا الأنبوبية الكلوية لكل من ذكور وإناث الجرذان، ولكن ذكورالجرذان كانت الأكثر تأثراً بشكل ملحوظ مقارنةً بإناث الجرذان. وثبت أن استخدام زيت حبة البركة قد قلل من جميع التغيرات المذكورة أعلاه في كل من ذكور وإناث الجرذان.
الخلاصة: أثبتت الدراسة أن إناث الجرذان أكثر مقاومة للسمية الكلوية التي يسببها عقارسيسبلاتين من ذكور الجرذان وأن زيت حبة البركة قد قلل من هذه السمية الكلوية لكل من ذكور وإناث الفئران دون تأثير لنوع جنس الجرذان.