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Nephroprotective Effects of Estradiol and Moreinga Oleifera Extract in Ovarictomized Rats.

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Abstract

Estrogen deficiency occurring during menopause seems to increase renal oxidative stress which may predispose to renal malfunctioning. We evaluate the reno-protective effects of estradiol and Moreinga Oleifera supplementation in a rat model of menopause trying to clarify the mechanism(s) by which these therapies may execute its reno- protective effects on oxidative stress and inflammatory changes in the kidney of rat model of menopause. Forty female albino rats were divided into 5 groups, 8 rats in each group, as follows: (Control group) divided into A receive no treatment and B receive vehicle only, (sham group) received sesame oil, ovariectomized non treated group (OV) received sesame oil, Ovariectomized-estradiol treated group (OVEst) and Ovariectomized Moreinga Oleifera treated group (OVMO). Serum urea, creatinine, creatinine clearance and serum tumour necrosis factor α (TNFα) were estimated. Superoxide dismutase (SOD), malondialdehyde (MDA) and Interleukin 10 (IL10) mRNA gene expressions in kidney tissue were measured. Also histopathological, immunohistochemical and morphometrical studies for kidney tissue were performed. OVEST and OVMO showed significant decrease in serum urea, creatinine, TNF-α and MDA level in kidney tissue but significant increase in kidney tissue SOD and IL10 mRNA gene expression in kidney tissue when compared to the OV group. Also, histopathological alterations were ameliorated. The improving effects on the kidney tissues, may be due to the inflammatory gene modifications including reduction of pro-inflammatory cytokines TNF-α and increase in anti-inflammatory IL-10 mRNA expression, reduced oxidative stress and mechanistic downregulation of NF-κB, and iNOS expression.

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INTRODUCTION

Menopause is a turning point in every woman's life, defined as natural permanent cessation of menses for one year without activity of ovarian follicle. It is generally associated with hormonal deficiency, which is a contributory factor for the increased incidence of osteoporosis, cardiovascular diseases, vasomotor disturbances and cognitive deterioration [1]. As the world's population ages the prevalence of menopause is increasing [2]. Its association with risk of chronic kidney disease is increasing globally.

Classically, Estrogens (E2) are the ovarian steroid hormones that have an essential role in female physiology and reproduction. Besides, they affect a variety of physiological and pathological functions of the kidney, including the regulation of hemodynamics, mesangial cells, the mesangial matrix, collagen metabolism, cytokines, the release of inflammatory mediators and glomerular filtration [3]. After menopause, E2 deficiency seemed to play a critical role in the vulnerability and progression of chronic renal diseases and renal oxidative injuries [4]. Postmenopausal sex hormone levels were found to affect renovascular physiology, but the clinical impact of menopause on kidney function is still unclear. There are no guidelines on the use of postmenopausal hormone therapy specific to the population with kidney disease, and studies are limited and conflicting[5]. Oxidative stress usually goes along with menopause related disorders [6]. It resulted from overproduction of reactive oxygen species (ROS), that exceed the body's antioxidant defences capacity [7] and contributes to cellular damage [8].

Also, a low systemic inflammatory status, manifested by increased serum levels of the key proinflammatory cytokines was established to be associated with onset of menopause. The relationship between hormonal deficiency associated with menopause and increased serum levels of proinflammatory cytokines is not yet fully understood. Anti-inflammatory mediators can control proinflammatory cytokine response and activity; their leading representatives are IL-1 antagonist receptor, IL-4,-IL-10, IL-11 and IL-13 [9].

Moringa oleifera Lam. (MO) is an Indian tree referred to as "drumstick tree" that belongs to the Moringaceae family [10] and widely distributed in Asia, Africa and tropical areas of the world [11].

The MO is recognized for its vast therapeutic properties and medicinal uses since ancient times. It has been reported that all its components especially leaves have anti-inflammatory, antioxidant, liver-protective and antihypertensive properties. The leaves are eaten as vegetables of food ingredient because of their high content of vitamins (A, C and E), antioxidants and macronutrients to improve nutritional deficiencies [12]. Also, the strong antioxidant and scavenging ability of M. oleifera has showed a therapeutic role in renal malfunction, reduced the severity of lead-induced nephrotoxicity [13] and a chemoprevention of diseases like cancer.

The objectives of the present study was to evaluate the nephroprotective effects of estradiol and Moringa Oleifera supplementation in menopause with emphasis on oxidative stress and inflammatory alterations trying to elucidate the mechanism(s) by which these therapies may execute its renoprotective effect.

Materials and Methods

Animals and Experimental Design

All procedures conducted in accordance to the Guide of the Care and Use of Laboratory Animals and approved by the Ethical Committee for Scientific Research at Menoufia Faculty of Medicine ; the site of the study. Forty adult female albino rats weighing 150-200 grams, each were used in this investigation. Rats were acclimated for one week and kept on standard laboratory diet and ad libitum water throughout the study period. Constant temperature (22 ± 2 ºC) and normal light–dark cycle.

Rats were divided into five groups (eight animals each):

Group I (Control group): was divided into 2 subgroups included 4 animals for each: Group IA (Negative control): the rats in this group did not receive any treatment. Group IB (Vehicle control group): the rats were injected subcutaneously with 0.2 mL sesame oil, three days / week for 4 weeks.

Group II: Sham operated group (Sham group): rats were subjected to the same surgical procedure and anesthesia without removal of the ovaries. Rats were left 1 week for wound healing, then they were injected subcutaneously with 0.2 mL sesame oil, three days / week for 4 weeks.

Group III: Ovariectomized non treated group (OV): ovariectomized rats were left 1 week for wound healing, then they were injected subcutaneously with 0.2 mL sesame oil, three days / week for 4 weeks.

Group IV: Ovariectomized-estradiol treated group (OVEst): ovariectomized rats were left 1 week for wound healing, then they were injected subcutaneously with 17β-estradiol valerate (30 mg/kg body weight) dissolved in 0.2 mL sesame oil, three days / week for 4 weeks *(Sigma-Aldrich Corp., St. Louis, MO, USA)* [14]**.**

Group V: Ovariectomized Moring Oleifera treated group (OVMO): rats were left to have 1 week rest for wound healing, then supplemented with Moringa Oleifera

extract (300 mg/kg BW) dissolved in distilled water orally [15] for 4 weeks.

Surgical procedure of Ovariectomy

After a 7-days of acclimatization, rats of the OVX group underwent a bilateral ovariectomy. Briefly, rats were anesthetized (phenobarbital sodium (60 mg/kg, intraperitoneally (Sigma–Aldrich Chemie GmbH, Steinheim, Germany) and given a preoperative dose of antibiotics and analgesics. In brief under general anesthesia, a 2 cm midline abdominal skin incision was performed. Using artery forceps, the ovarian fat pad was gently grasped, and the ovaries were exposed. After removing the bilateral ovaries, the fat pad was repositioned into the abdomen. Wound closure was performed in two layers, adapting the muscle layer with single stitch sutures (Vicryl 5/0, Johnsons and Johnson, Hong Kong) and the skin layer with stainless steel wound clips. The same procedures were used for the sham control group, except that the ovaries were kept intact. Topical sterilization and antibiotic application were given immediately and daily for 1 week after operation to help wound healing. All rats were given meloxicam (5 mg in 250 mL drinking water, Metacam, Boehringer Ingelheim, Germany) for 5 days postoperatively for pain relief [16]**.**

Plant material (Moringa oleifera):

A total of 2 kg of fresh green leaves of Moringa oleifera (MO) were collected from a local MO

farm located in Sadat City, Menoufia, Egypt (latitude 30.3597; longitude 30.4952). MO leaves were identified and authenticated in Biochemistry department, Faculty of Veterinary Medicine, University of Sadat City. Ethanolic extract of MO leaves (MOLE) was prepared according to **Sinha et al.** [17]**.**

Preparation of Moringa oleifera Extract: fresh leaves of MO were rinsed thoroughly with distilled water to remove dust and debris then shade dried at room temperature after that it was ground into powder. The powder was immersed in 70% ethanol for 48 h at room temperature (22 ℃) with gentle shaking. The contents were filtered through filter paper (Whatmann size No. 1), then the filtrate was dried at room temperature by air current to obtain a semisolid crude extract weighing (250 g) with 12.5% as a crude percent. The extract was stored in airtight container at 4 ℃ until used [15].

Samples collection and analysis

Urine and blood samples collection

By the end of the study, each rat was placed in an individual metabolic cage for urine collection which was placed over a funnel; the top of the funnel was covered by wire mesh to avoid fecal contamination of urine. During urine collection the animal received free access to water, but no food was given to avoid contamination [18]**.** The total voided urine during the next 24-h was measured to calculate urine volume (ml/min). Urine samples were centrifuged at 3000 rotation per minute for 15 minutes to remove any particulates and stored at -20℃ until use for biochemical analysis.

Also , fasting blood samples were collected from the retro-orbital venous plexus, using a fine heparinized capillary tube introduced into the medial epicanthus of the rat's eye. Two millilitres of blood were collected in a clean graduated centrifuge tube, left for clotting at room temperature in a water bath for 10 minutes, and then centrifuged at 3000 r.p.m (rotation per minute) for 20 minutes. The supernatant serum was collected in a dry clean tube and kept at -20°C for further analysis. The collected samples were used for measurement of serum urea, creatinine, and tumour necrosis factor α (TNFα).

Assessment of urine parameters

Glomerular filtration was assessed by creatinine clearance based on serum and urine creatinine levels, with values expressed in mL/min, computed with the formula:

Clcr = urine creatinine (mg/dL) x urine flow (mL/min) /Serum creatinine (mg/dL).

Urine flow was calculated dividing 24 hours of urine volume by 1,440, which corresponds to the number of minutes in 24 hours (60 min x 24h = 1,440): urine flow (mL/min) = value of urine volume (24h)/1,440.[18].

Biochemical analysis

Serum & urinary creatinine (mg/dl) levels were determined by enzymatic colorimetric method using test reagent kits (Bio-diagnostic Company, Egypt) according to the manufacturer's instructions.

Determination of serum TNFα

Serum TNFα (pg/ml) was determined by enzyme linked immunosorbent assay (ELIZA) kits (sunred, Shanghai Biological Technology Co., Ltd, China) according to the manufacturer's instructions.

Tissue sample collection:

At the end of the experiment, rats of all groups were sacrificed by decapitation and both kidneys were excised. Right kidneys were kept at -80°C for estimation of tissue MDA, SOD, and Interleukin 10 (IL10) mRNA expressions. The left kidneys were prepared for histological, immunohistochemical and morphometrical studies.

1-Histological study: the kidney specimens were fixed in 10% buffered formalin then embedded in paraffin. Serial sections of 5-7 μm thickness were cut and subjected to Hematoxylin and eosin (H. and E.) to illustrate the histological structure [19] and Masson trichrome stain for identification of collagen fibers [20]

2-Immunohistochemical study:

Four-micron sections were prepared from the paraffin blocks of all groups. Deparaffinization followed by rehydration of all specimens was performed. Endogenous peroxidase activity was blocked with 3% hydrogen peroxide for 10 min and then incubated with a primary antibody in a humidified chamber at 4 °C overnight. Primary antibody was monoclonal mouse anti-iNOS antibody & anti-NF-κB antibody (Dako, USA) at 1:200 dilution [21]. Human tonsil & human colon cancer was used as a positive control for iNOS &NF-kB respectively. Negative control sections of both markers were prepared by the same procedure but with omitting primary antibody. Finally, Mayer's hematoxylin was used to counterstain the sections.

Morphometrical study:

For quantitative evaluation, five different sections from five different animals in each group were measured employing a Leica DML B2/11888111 microscope supplied with a Leica DFC450 camera. The measured variance was estimated using the software version K1.45 of Image J. The measured data were undertaken using H&E, Masson Trichrome and immunohistochemical sections. For quantitative evaluation, the following parameters were calculated: Mean area percentage of collagen fibers with Masson trichrome stained sections (x 200). Mean area percentage of iNOS & NF-kB immuno-positive intensity in iNOS & NF-kB immuno-stained sections respectively (x 200). This was done in the Anatomy Department, Faculty of Medicine, Menoufia University, Egypt.

Determination of kidney tissue MDA and SOD levels

One small section (one gram) of right kidney was harvested from the rats and precisely weighed. Subsequently, saline was added according to the tissue weight: Saline volume=1:9 (w/v). Following homogenization at 4°C by a DY89-I electric homogenate (Ningbo Scientz Biotechnology Co., Ltd., Ningbo, China), the homogenates were centrifuged at 1,100xg for 15 min at room temperature. MDA and SOD content levels were measured by colorimetric method using test reagent kits (Bio-diagnostic Company, Egypt) according to the manufacturer's protocol.

Quantitative assay of Interleukin 10 mRNA expression

Kidney tissue samples were prepared for total RNA isolation using Qiagen RN easy plus Universal kit from, USA according to manufacturer instructions.

Statistical analysis:

The data were tabulated and analyzed by SPSS (statistical package for the social science software) using statistical package version 20 on IBM compatible computer. Quantitative data were

expressed as mean ± standard error of mean (X± S.E.M). Data from control and test groups were compared using one way ANOVA, followed by Turkey post Hoc test, Probability value of less than 0.05 was considered as statistically significant (P<0.05)

Results:

Results of control groups IA and IB showed no differences. Also, there were no significant differences of sham group compared to control group.

Serum urea was significantly decreased in OVEst and OVMO groups when compared to OV group (41.55 ± 5.89 and 38.8 ± 2.85 Vs 64.5 ± 3.65 mg/dl (P < 0.05). There was no significant difference in serum urea in OVEst and OVMO groups when compared to control group. No significant difference between the two treated groups **(Table 1)** .

Serum creatinine was significantly decreased in OVEst and OVMO groups when compared to OV group (0.36 ± 0.04 and 0.34 ± 0.04 Vs 1.38 ± 0.24 (P < 0.05). There was no significant difference in serum creatinine in OVEst and OVMO groups when compared to control group. No significant difference between the two treated groups **(Table 1).**

Urinary creatinine level was significantly increased in OVEst and OVMO groups when compared to OV group (67.91 ± 1.54 and 68.6 ± 2.14 Vs 60.17 ± 7.64 (P < 0.05). There was no significant difference in urinary creatinine in OVEst and OVMO groups when compared to control group. No significant difference between the two treated groups **(Table 1).**

Creatinine clearance was significantly increased in OVEst and OVMO groups when compared to OV group (2.35 ± 0.52 and 2.33 ± 0.56 Vs 0.69 ± 0.11 P < 0.05). There was no significant difference in creatinine clearance in OVEst and OVMO groups when compared to control group. No significant difference between the two treated groups **(Table 1)**.

Kidney tissue MDA level was significantly decreased in OVEst and OVMO groups when compared to OV group (0.21 ± 0.13 and 0.19 ± 0.13 Vs 2.14 ± 0.32 P < 0.05). There was no significant difference in kidney tissue MDA in OVEst and OVMO groups when compared to control group. No significant difference between the two treated groups.**(Figure 1).**

Kidney tissue SOD level was significantly increased in OVEst and OVMO groups when compared to OV group (78.79 ± 4.32 and 81.96 ± 4.6 Vs 60.34 ± 5.21 P < 0.05). There was no significant difference in kidney tissue SOD in OVEst and OVMO groups when compared to control group. No significant difference between the two treated groups **(Figure 1).**

Serum TNF-α level was significantly decreased in OVEst and OVMO groups when compared to OV group (89.15 ± 2.71 and 87.33 ± 3.95 Vs 153.9 ± 4.83P < 0.05). There was no significant difference in serum TNF-α in OVEst and OVMO groups when compared to control group. No significant difference between the two treated groups **(Figure 2).**

IL10 mRNA gene expression in kidney tissue was significantly increased in OVEst and OVMO groups when compared to OV group (1.15 ± 0.07 and 1.18 ± 0.02 Vs 0.72 ± 0.12. P < 0.05). There was no significant difference in kidney tissue IL10 mRNA gene expression in OVEst and OVMO groups when compared to control group. No significant difference between the two treated groups **(Figure 2).**

Histological results:

Regarding group II (Sham group), it showed similar histological, and immunohistochemical picture as group I (control group).

Hematoxylin and eosin results

H. & E.-stained sections of the renal cortex of group I (control group) revealed renal corpuscles, formed of glomerular capillaries surrounded by a parietal and visceral layer of Bowman′s capsule with the urinary space in-between. The lining cells of proximal convoluted tubules were low columnar, and the cytoplasm was strongly acidophilic with spherical basal nuclei. The lining cells of distal convoluted tubules were low cuboidal with less acidophilic cytoplasm and rounded central nuclei (Fig. 3 A).

H. & E.-stained sections of the renal cortex of group III (OV group) showed renal corpuscles with shrunken glomeruli and Bowman`s space widening others with ruptured capsule. The lining epithelial cells of PCT appeared vacuolated with shrunken dark nuclei. Tubular degeneration leaving hyaline material was noticed. Intense interstitial cellular infiltration as well as congested blood capillaries were detected (Fig. 3 B & C).

Group IV (OVEST) revealed improvement in the form of presence of increased numbers of normal renal corpuscles, but few affected still present. Tubular changes: vacuoles in the cytoplasm and pyknotic nuclei were still present. Minimal cellular infiltration and congested blood capillaries were noticed (Fig. 3 D).

Group V (OVMO) showed marked amelioration in the renal cortical histological picture back to normal. Normal renal corpuscles, PCT, DCT were noticed. Only congested blood capillaries were still present (Fig. 3 E).

Masson`s trichrome stain results:

Group I showed minimal collagen fibers deposition in the interstitium and around renal corpuscles. Group III showed apparent increase in collagen deposition, while group IV revealed moderate amount of collagen fibers in the interstitium. Minimal amount of collagen fibers was noticed in group V (Fig. 4).

Immunostain results:

Weak immunoreaction for iNOS and NF-KB was detected in group I, while strong cytoplasmic immunoreaction for iNOS and NF-kB was detected in the cells of renal corpuscles and tubules of the cortex of group III. Minimal to moderate reaction for iNOS and NF-kB was noticed in renal cortex sections of group IV. Marked reduction in the immunoreaction in group V-stained sections was detected (Fig. 5 & Fig.6).

Table 1: Serum Urea, Serum creatinine and Creatinine clearance in Control, Sham, Ovariectomized (OV) , Ovarictomized estradiol treated (OVEst) and Ovariectomized moreinga treated (OVMO) groups. Data were expressed as mean ±S.E. (n=10). One way ANOVA

Variable	Control	Sham	OV	OVEst	OVMO
Serum Urea(mg/dl)	37.1 ± 1.04	36.51 ± 1.02	64.5 ± 3.65	41.55 ± 5.89 *	38.8 ± 2.85 *
Serum Creatinine(mg/dl)	0.32 ± 0.04	0.34 ± 0.05	1.38 ± 0.24	0.36 ± 0.04 *	0.34 ± 0.04 *
Urine creatinine(mg/dl)	70.29 ± 3.26	68.14 ± 2.48	60.17 ± 7.64	67.91 ± 1.54 *	68.6 ± 2.14 *
Creatinine clearance	2.44 ± 0.57	2.4 ± 0.54	0.69 ± 0.11	2.35 ± 0.52 *	2.33 ± 0.56 *

* $P < 0.05$ compared to ovariectomized non treated group

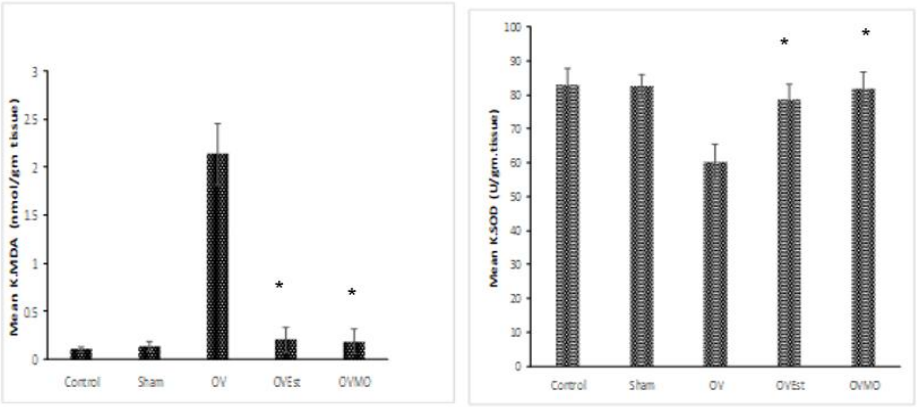

Figure 1: Kidney MDA and SOD in Control, Sham, Ovarictomized (OV) , Ovarictomized estradiol treated (OVEst) and Ovarictomized moreinga treated (OVMO) groups. Data were expressed as mean ±S.E. (n=10). One way ANOVA: *p<0.05, vs. ovariectomized non treated.

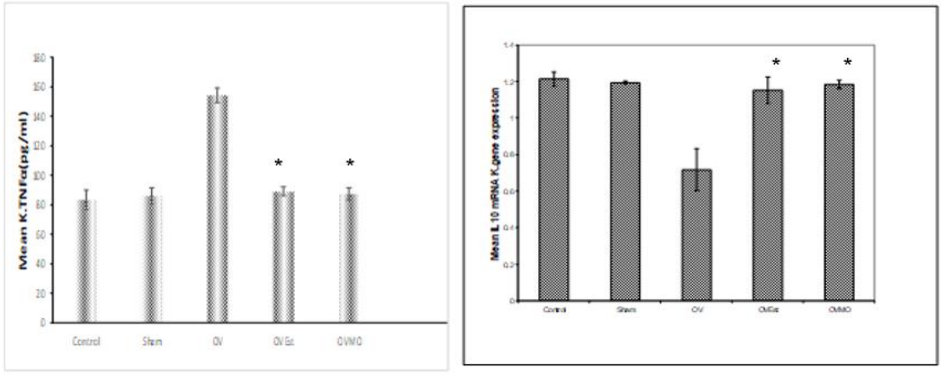

Figure 2: Kidney TNFα and IL10 mRNA Kidney gene expression in Control, Sham, Ovarictomized (OV) , Ovarictomized estradiol treated (OVEst) and Ovarictomized moreinga treated (OVMO) groups. Data were expressed as mean ±S.E. (n=10). One way ANOVA: *p<0.05, vs. ovariectomized non treated.

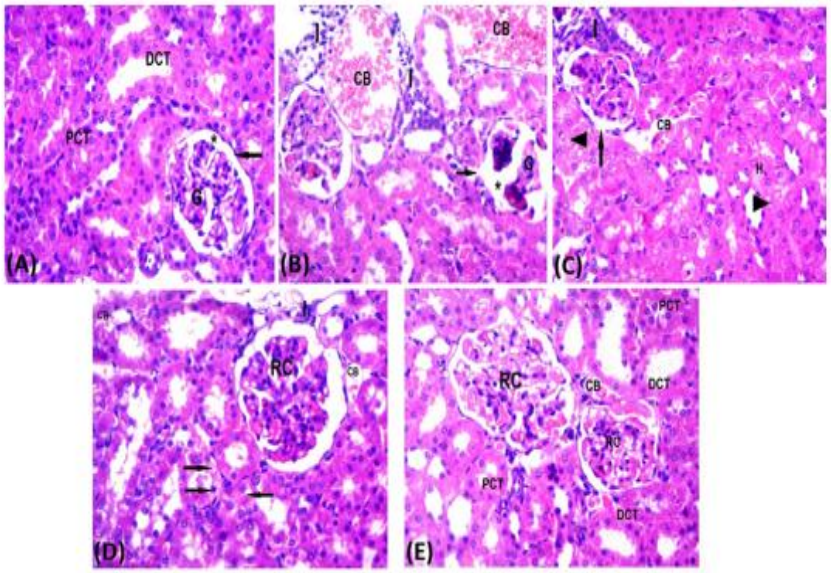

Fig. 3: A photomicrograph of a section of the renal cortex showing; **A:** group I (control) renal corpuscles, formed of a glomerulus (G) surrounded by a parietal layer of Bowman′s capsule (→) with the urinary space (*) in-between. The proximal convoluted tubules (PCT) are lined with low columnar with spherical basal nuclei and strong acidophilic cytoplasm. The distal convoluted tubules (DCT) are lined with low cuboidal with central rounded nuclei and faint acidophilic cytoplasm. **B & C:** (group III) renal corpuscles with shrunken glomeruli (G) and Bowman`s space widening (*) others with ruptured capsule (→). The lining epithelial cells of PCT appeared vacuolated with shrunken dark nuclei (►). Tubular degeneration leaving hyaline material (H) was noticed. Intense interstitial cellular infiltration (I) as well as congested blood capillaries (CB) was detected. **D:** (group IV) increased numbers of normal renal corpuscles (RC) but few affected still present. Tubular changes: vacuoles in the cytoplasm and small, dark nuclei (→) are noticed. Minimal cellular infiltration (I) and congested blood capillaries (CB) are present. **E:** (group V) normal renal corpuscles (RC), PCT & DCT cells were noticed. Only congested blood capillaries were still present. **(H. & E. x 400).**

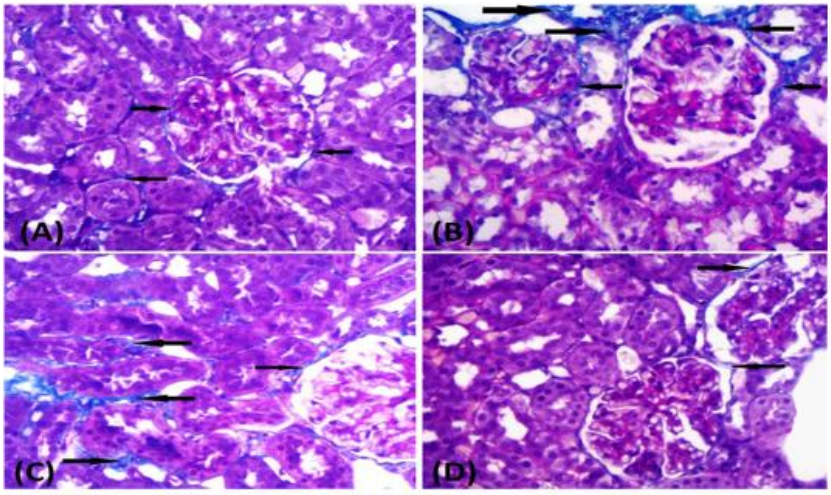

Fig. 4: A photomicrograph of Masson`s trichrome stained kidney sections showing;(A) Control: minimal collagen fibers deposition in the interstitium and around renal corpuscles (→). (B) Group III: apparent increase in collagen deposition around corpuscle and tubules (→). (C) Group IV: moderate amount of collagen fibers in the interstitium and around corpuscles (→). (D) Group V: Minimal amount of collagen fibers surrounding corpuscle (→). **(Masson`s trichrome x 200).**

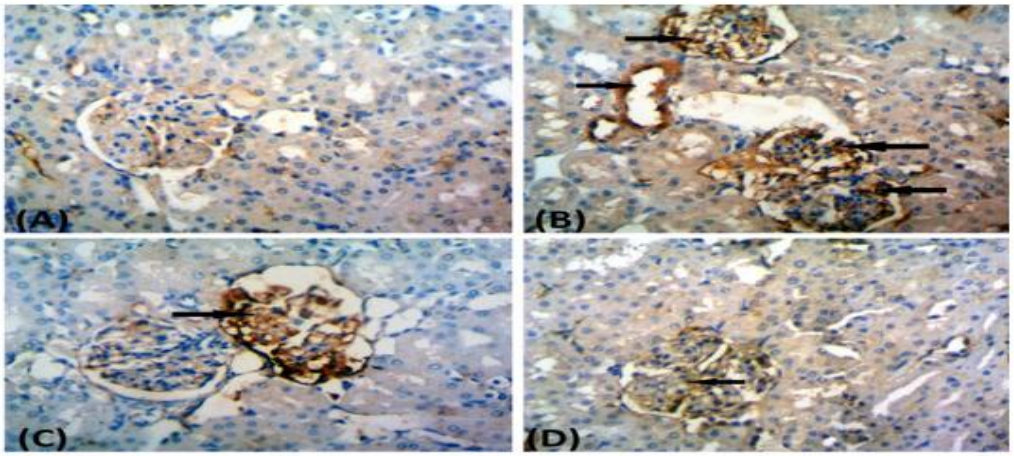

Fig. 5: A photomicrograph of iNOS immunostained kidney sections showing ;(A) Control: weak immunoreaction for iNOS in the renal corpuscle and renal tubules cells. (B) Group III: strong cytoplasmic immunoreaction in both corpuscular and tubular cells (→). (C) Group IV: Minimal to moderate cytoplasmic brown reaction for iNOS (→). (D) Group V: weak reaction for iNOS in the cells of renal cortex in this group (→). **(iNOS x 200)**

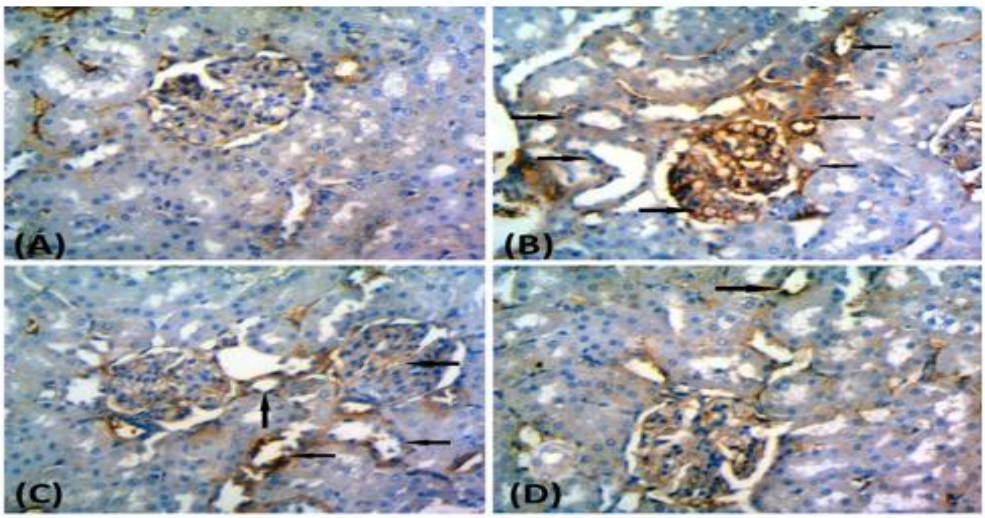

Fig. 6: A photomicrograph of NF-KB immunostained kidney sections showing ;(A) Control: weak immunoreaction for NF-KB in the renal corpuscle and renal tubules cells. (B) Group III: strong immunoreaction in both corpuscular and tubular cells in the form of brown coloration of the cytoplasm (→). (C) Group IV: Minimal to moderate cytoplasmic brown reaction for NF-KB (→). (D) Group V: minimal reaction for NF- kB in the cells of renal corpuscles and tubules in this group (→). **(NF-Kb x 200)**

Discussion

Estradiol (E2) is an essential hormone for normal cellular growth and differentiation, it is also important for maintenance of normal kidney functions [22] and regulation of the renal homeostatic activities [4]. The nephroprotective effects of estrogens is one of its important actions, estrogens decreasing glomerulosclerosis and tubulo-interstitial fibrosis. Thus, the abrupt reduction in E2 levels by removal of the ovaries accelerates the progression of the renal functional, structural, and pathological changes with deterioration of excretory and endocrine functions of the kidney [23]. The incidence of kidney diseases is known to be gender-related [24]. The resistance of kidneys in female gender to the progression of renal disease is most frequently attributed to ovarian sex hormones which suggest a protective role of estradiol against the progression of chronic renal diseases [4]. While decreased synthesis of E2 makes the kidney more vulnerable to renal oxidative injuries [23]. Research has revealed that the effect of E2 deficiency on kidney tissue is widespread and includes infiltration of tubulointerstitial inflammatory cells, tubular atrophy and dilation, glomerulosclerosis, adhesion of Bowman's capsule, and glomerular hypertrophy with tubulointerstitial sclerosis [22].

The main objective of this study was to determine the protective role of Moreinga oleifera leaves extract and estrogen replacement against post ovariectomy renal dysfunction. Signifying the role of estrogen and M.O in decreasing the progression of renal diseases after menopause.

Urine volume, urine creatinine, serum creatinine, and serum urea were measured as an index of renal function. A significant increase in their levels was in Ovariectomized (OV) group when compared with the control group. The increased level of SCr in OV group can be explained either by increased SCr production by muscles because of absence of estrogen anabolic effect [25] or by the decrease SCr clearance by the kidneys due to decreased renal perfusion and GFR induced by oxidative stress mediated by ovariectomy [26]. In ovariectomized estradiol treated (OVEst) group, there was significant decrease in serum urea and serum creatinine indicate the improvement of renal function, provide evidence for the protection provided by ovarian sex hormones against oxidative damage of the kidney. In ovariectomized M. oleifera treated (OVMO) group there was significant decrease in serum creatinine, and serum urea. These findings were supported by previous studies which showed that feeding of diets containing different concentrations of MO to rats revealed a significant decrease of the plasma levels of creatinine and urea [27,28,29].

Oxidative stress caused by E2 deficiency can reduce the activity of superoxide dismutase (SOD), catalase (CAT), and glutathione (GSH) enzymes, which clean free oxygen radicals in cells [30]. Reduction of these radical cleaning enzymes causes the accumulation of free radicals in cells (ROS). The imbalance between the increased production of ROS and the decreased antioxidant capacity results in a persistent formation of toxic lipid peroxidation products, contributing to the development of serious complications affecting the enzymatic antioxidant defense system in many tissues participating in pathogenesis of some conditions such as glomerulosclerosis and renal disorders [23]. The current study showed that excess ROS apparently contributes to the etiology

of renal complications in OV group because there was significant increase in the kidney MDA levels in this group when compared by control group. This result was supported by data from Gross et al., providing evidence that all female kidney tissues increased ROS after ovariectomy [31].

Sex steroid hormones, especially estriol and estradiol, are natural antioxidants [30]. Estrogens exerts protective effects through ROS-scavenging chain-breaking antioxidant activity as hydrogen donors from their phenol-hydroxyl ring. It can induce antioxidant enzyme expression by stimulating the antioxidant defense system and inhibiting the formation of lipid peroxides in plasma and liver tissues *in vitro* [23]. Some studies had indicated that the estrogen supplementation can improve the levels of MDA as a marker of oxidative stress in menopausal female thus reducing the renal diseases [32]. These data support our results in this study which showed significant decrease in MDA in estrogen treated group OVEst compared to OV group.

Oxidative stress is an important event that has been related to pathogenesis of some conditions affecting the enzymatic antioxidant defense system in renal tissue. The reduced activities of the antioxidant system could be due to the increased production of free radicals [33]. One of the most important intracellular antioxidant systems is the glutathione redox cycle. Glutathione is one of the essential compounds for maintaining cell integrity because of its reducing properties and participation in the cell metabolism [32]. In this study, it was indicated that SOD level in kidney tissues was reduced in the OV rats these results agreed with data from Ulas & Cay [30]. In OVEst group there was significant increase in SOD level when compared with OV group, estrogen can induce antioxidant enzyme expression by stimulating the antioxidant defense system, by activating the expression of anti-inflammatory cytokines and interleukin [34]. The protective effects of E2 also are explained by activation of nitric oxide synthases system and proinflammatory properties response [32]. This result support the hypothesis of protection against oxidative damage provided by ovarian sex hormones.

Moringa oleifera is one of the potential sources of compounds with strong antioxidant effect [35]. In our study in OVMO treated group there was significant decrease in MDA and significant increase in SOD level when compared with OV group, The bioactive compounds founded in MO helps to increase antioxidant level in body and decreases malondialdehyde. This result was supported by data from Kushwaha et.al., [36] who revealed the reducing power of MO leaves powder and its ability to improve the antioxidant levels and decrease the marker of oxidative stress.

Evidence from human and animal studies showed that a decrease of estrogen levels is accompanied by increased production of pro-inflammatory cytokines such as IL-6 and TNF-α [37]. The increased pro-inflammatory TNF alpha is balanced by increase of anti-inflammatory cytokines IL-10, IL-12, and IL-4 as a compensatory mechanism, by which these cytokines reduce the oxidative stress effects [38]. While treatment with estrogen in ovariectomized rats were reported to reduce levels of TNF-α and IL-6 [39]. The changes of IL-10 and IL-12 levels in menopausal state are still not fully clarified, with some studies reporting an increase and others a decrease during menopause [40]. These results support our study which showed significant

decrease in IL-10 and significant increase in TNF-α in OV group compared with control group, while there was significant decrease in TNF-α and significant increase in IL-10 in OVEst group compared to OV group.

In OVMO treated group there was significant decrease in TNF-α and significant increase in IL-10 compared to OV group. MO extract can be a potent inhibitor of inflammation, this result was supported by data from Tan et al., [41] who reported that MO extract had significant effect on inhibiting the production of proinflammatory cytokines (TNF-α, IL-1β, IL-6, and PGE2) whereas it increased expression of anti-inflammatory cytokines, IL-10.

These findings of oxidative stress are accompanied by the morphological alteration in OV group, that showed renal corpuscles with shrunken glomeruli and Bowman`s space widening others with ruptured capsule. The lining epithelial cells of PCT appeared vacuolated with shrunken dark nuclei. Tubular degeneration leaving hyaline material was noticed. Intense interstitial cellular infiltration as well as congested blood capillaries were detected.

The protective effect of E2 was approved histopathologically in OVEst group by improvement in the form of presence of increased numbers of normal renal corpuscles, but few affected still present. Tubular changes, vacuoles in the cytoplasm and pyknotic nuclei were still present. Minimal cellular infiltration and congested blood capillaries were noticed. Several previous studies have attributed the protective effects of E2 to antioxidant [30], and proinflammatory properties response [40]. OVMO group showed marked amelioration in the renal cortical histological picture back to normal. Normal renal corpuscles, PCT, DCT were noticed. Only congested blood capillaries were still present.

The current study showed the presence of glomerulosclerosis and interstitial fibrosis in the OV group, this was evidenced by the significant increase in deposition of collagen fiber in the glomerulus and in the interstitium. This may be due to chronic inflammation confirmed by the significant increase in lipid peroxidation end product MDA and TNF-α pro-inflammatory cytokine in this study. Also, the present study showed significant decrease in collagen fiber deposition in the interstitium and within the glomeruli of the OVEst and OVMO groups. This might suggest the anti-fibrotic action of estrogen and moringa. This could be explained by regressive effects of estrogen and moringa on inflammation (reduction of TNF-α) with subsequent regressed fibrosis. This was in accordance with **Vrachnis** et al. [39] and Tan et al., [41].

Expression of NF-κB and iNOS showed a significant increase in the OV group in comparison to the control group, this increase was in parallel with TNF-α levels. NF-κB, play a key role in the pathophysiology of clinically important diseases, stimulates the synthesis of inflammatory mediators including TNF-α [42]. The upregulated iNOS markedly increased NO level that may react with ROS or superoxide anion to produce peroxynitrite to cause more lipid peroxidation and renal injury [43]. Targeting NF-κB could therefore be essential in therapeutic strategies aimed at suppressing inflammation [44]. In this study, estrogen and moringa treatments induced down-regulation of NF-κB and iNOS expression

showing evidence for their anti-inflammatory action. Consequently, the renal levels of TNF-α were considerably regained their levels. These results are consistent with the literature that inhibition of NF-κB and iNOS signaling pathways could block excess IL-1β, IL-6, TNF-α, and NO levels [41].

Conclusion

In conclusion, we found that moreinga oleifera and estradiol attenuated renal injury in ovariectomized rats. they exert their improving effects on the kidney tissues, in part, due to their antioxidant activity and inflammatory gene modifications including reduction of pro-inflammatory cytokines TNF-α and increase in anti-inflammatory IL-10 mRNA expression.

Moreinga oleifera is expected to be preferable than estradiol supplementation due to fewer side effects being a natural food stuff. That may be clarified in future research.

Compliance with Ethical Standards

Funding: Nil

Disclosure of potential conflicts: The authors declare no competing of interest.

Ethical approval: Rats were reared and treated in accordance with the experimental protocol that approved by the local ethical committee of the faculty of Medicine, Menoufia University with approval code 10/2022PHYS24 (Ulas Met al., 2011) [45].

Informed consent: not applicable as our study did not involve humans.

Authors contribution: The authors declare that all data were generated in-house and that no paper mill was used.

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