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Research Article

Impact of aging on kidneys of male Wistar albino rats: the protective antiaging role of resveratrol

Wasan Waadallah Hassawi*
Department of Anatomy, College of Medicine, University of Mosul, Mosul, Iraq
Maha Abdul-Jabbar Al-sammak
Department of Anatomy, College of Medicine, University of Mosul, Mosul, Iraq

*Corresponding author. E-mail: wasanwaadhass@uomosul.edu.iq

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Abstract
Because aging leads to multiple health problems associated with changes in the structure and functions of a different organ system, this study aimed to assess the effect of aging on the histology of renal tissue in rats of various ages and the possible protective role of resveratrol. In the present study, twenty-four male Wistar albino rats were separated into three groups of eight animals each, including rats adult aged (6 months), and old-aged (24 months old). The elderly resveratrol-treated group was administered 25 mg/kg/day of resveratrol diluted in distilled water orally via gastric tube. The treatment lasted three months. At the end of the experiment, blood was drawn for serum creatinine analysis, the animals were sacrificed, and the kidneys were removed and processed for histological investigation. The findings revealed variable changes in kidney tissue, including glomerulosclerosis, compensatory glomerular hypertrophy, tubulointerstitial fibrosis, thickened glomerular basement membrane, arterial sclerosis, tubular dilatation with cast formation, atrophy of the tubules, infiltration of inflammatory cells, a rise in the rate of apoptotic cells, and a decrease in the glomerular number. These histological changes were associated with increased serum creatinine levels and kidney malondialdehyde (MDA), a marker of lipid peroxidation. The use of RES (Resveratrol) improved the creatinine level with a decrease in the MDA and improved the histological changes of aged kidney. As a result, the progression of aging was accompanied by different histological variations that interfered with the physiological functioning of the kidney, predisposing older persons to renal illnesses. Utilizing RES as a prophylactic and/or therapeutic medicine for aging-related renal changes is also possible.

Keywords: Ageing, Albino rates, Apoptosis, Glomerulosclerosis, Kidney, Resveratrol

INTRODUCTION

A biological phenomenon known as aging is the gradual weakening of cellular and organismal functions over time (Kirkwood, 2005). Aging results in a decrease in the capacity to preserve an individual's inner environment when faced with changes in the external environment. Aging also increases sensitivity to trauma, illnesses, and several other types of stress. As a result, the person loses the ability to maintain homeostasis (Viña *et al.*, 2007). A series of researches revealed that the ageing kidney causes an enhanced risk of nephrotoxic damage. Clinical studies on the geriatric population support these experimental design results, which have an elevated rate and increased severity of acute kidney damage (Pascual *et al.*, 1990).

Researchers reviewed plenty of investigations on the mechanisms that are altered with age and may increase the kidney's susceptibility to injury. These mechanisms include hemodynamics and hypovolemia, which lead to hypoxia and kidney injury; oxidative stress; increased apoptotic cell death; decreased autophagy with ageing; and chronic inflammation, the histological changes associated with nephron loss which is a hallmark of the ageing kidney, glomerulosclerosis, and tubulointerstitial fibrosis (Wang *et al.*, 2014). This fact is supported by Denic *et al.*(2016), who said that from 2.7% of those aged 18-29 to 73% of those aged 70-77, the prevalence of nephrosclerosis rises with age. Both glomerular and tubular function deteriorates in the aging kidney. In the Baltimore trial, the rate of Glomerular Filtration Rate (GFR) loss was found to be three times higher in patients above the age of 40 against those under the age of 40 (Tonelli and Riella, 2014). Studies have shown that the GFR declines by 5-10% per decade beginning at age 35 and

that around half of those over the age of 70 and up have an estimated GFR of less than 60 mL/min/1.73 m^2 (Denic *et al.*, 2016, Oscanoa *et al.*, 2018).

Renal function and renal tissue damage deteriorate with age, and this decline is linked to a diminishing reserve of renal function, making older people more vulnerable to acute renal injury, Chronic kidney diseases (CKD), and an elevated possibility of end-stage renal disease, as well as drug-induced nephrotoxicity are observed (James *et al.*, 2010, Nitta *et al.*, 2014).

Due to the increase in the elderly population, healthful ageing has recently been recognized as a significant issue. The possibility of kidney disease has been rising among the elderly. The degeneration of the kidneys is associated with comorbidities. Therefore, a prospective therapeutic regimen is urgently required to normalize age-related conditions like kidney aging. Age is correlated with a deterioration in renal function and elevates the incidence of renal problems. The current drugs for renal illnesses have their limitations due to their adverse effects; hence natural substances with less negative effects are being tested. It is possible that resveratrol can slow the ageing process in the kidney by modulating many pathogenic variables (such as oxidative stress, state of inflammation, tissue fibrosis, impaired mitochondrial function, cellular senescence, and failure of autophagy) that contribute to kidney ageing.

Kidney protection mediated by resveratrol predominantly targets ageing indicators, including SIRT1 (Silent Information Regulator 1), AMPK (Adenosine Monophosphate activated protein kinase), and (Nuclear factor-Kb) NF-kB, and their related signaling pathways (Uddin *et al.*, 2021), resveratrol is a natural Polyphenols compound and a class of bioactive molecules found in plants, have been shown to improve health in a variety of ways. It can be present in various foods, including grapes, peanuts, and blueberries. Antioxidant, anticancer, anti-inflammatory, immunomodulatory, hypotensive, and hypolipidemic qualities are only some of the many biological effects demonstrated by resveratrol. Obesity, cardiovascular disease, cancer, and neurological diseases are just some of the conditions it has been proven to help with (Harikumar and Aggarwal, 2008, Meng *et al.*, 2020). As a result of its ability to reduce oxidative stress, reduce inflammation, boost mitochondrial function, and control apoptosis, it has been the subject of numerous studies demonstrating its value in treating ageing (Wang *et al.*, 2019, Ginés *et al.*, 2017).

Many studies have demonstrated resveratrol's kidney protective role. One such study used resveratrol (5 mg/kg/day) for treating male Wistar rats for 45 days, leading to reduced renal hypertrophy, or mesangial expansion, fibrosis, oxidative damage, DNA damage, caspase-3 protein (Al-Hussaini and Kilarkaje, 2018), and in another study the use of Resveratrol (RES) for16 weeks of treatment with 5 mg/kg/day lead to serum superoxide dismutase (SOD) activity was considerably elevated in diabetic rats, but levels of thiobarbituric acid reactive substances (TBARS) and (oxidized glutathione GSSG/reduced glutathione GSH) were reduced. Anti-inflammatory effects were seen with RES therapy, as plasma levels of the cytokines that trigger inflammation TNF-α and IL-6 decreased (Szkudelska *et al.*, 2020). The present study aimed to focus on the role of RES in the prevention and/or treatment of aging kidneys by RES.

MATERIALS AND METHODS

Animals and housing

Twenty-four male Wister albino rats, with different weights (200-450g) because of different age groups, provided by The animal house of the College of Veterinary Medicine/University of Mosul. The rats were housed in separate groups in polypropylene cages (55 x 37 x 15 cm) with a stainless steel top grill. Ethical approval was obtained from the Research Ethical Committee of College of Medicine/University of Mosul. All animals were examined carefully for general health status, had free access to tap water, and were nourished with commercially prepared *ad libitum* food. They were loaded with the best meals and put under observation for 3 months experimental period.

Experimental design

The rats were distributed into three separate groups (each one of 8 animals) as follows:

Group A (Adult-aged group): The rats in this group were aged 6 months.

Group B (Old age group): The rats in this group were aged 24 months.

Group C (RES-treated old age group): This group of 24 -month-old rats received 25 mg/kg/day of RES orally for three months.

Resveratrol: The medication was bought from an Iraqi pharmacy. Trans-RES is an antiaging medication produced by the NOW FOODS corporation in the United States. As (50mg) per capsule. After being dissolved in distilled water, it was supplied orally to animals at a dose of 25 mg/kg b. w. each day by gastric gavage (Nankivell, 2001).

Relative weight of kidney: At the end of the experiment, the weight of the body and kidneys were taken using an electronic scale, and the kidney weight was calculated using the kidney weight to final body weight ratio, kidney weight /body weight ×100.

Collection of the blood: The blood samples were obtained from the rats' retro-orbital vein to measure the serum creatinine level.

Necropsy: After 3 months, diethyl ether inhalation in a glass desiccator was used to euthanise the animals; a scissor was used to make a longitudinal midline in the

abdominal region and remove both kidneys.

Handling and fixation of tissue: the kidney was washed with normal saline to remove the blood, and then dried on filter papers. The kidneys were cut longitudinally in half, and then kept in neutral buffered formalin at 10%. One piece of the kidney should weigh at least 1 gram, as measured by utilizing an accurate balance (an electronic compact scale) kept in aluminum foil in the refrigerator until utilized for the MDA level estimation.

Tissue processing

Dehydration with increasing alcohol concentrations: 70%, 90%, and absolute alcohol clearing with Three changes of xylene impregnation and immersion of tissues into three changes of paraffin wax create a solid block containing the tissues that are ready for Sectioning of 4-5 mm thick sections using a Reichart rotary microtome, followed by flotation of the sections in a bath of warm water at 37 °C for stretching. Tissue Sections were then put on cleaned glass slides. Deparaffinization with two xylene changes was accomplished using ascending grades of alcohol and tap water: absolute alcohol, 95% alcohol, and 70% alcohol. Staining the slides with Hematoxylin and Eosin (H&E) stain (Abdulqader *et al.*, 2022b, Abdulqader *et al.*, 2022a), Masson's Trichome (MT) stain, and Periodic Acid-Schiff stain (PAS)(Hegazy and Hegazy, 2015).

TUNEL Apoptosis assay

This test was done with the tunnel apoptosis assay kit (HRP-DAB). A simple, quick, and highly sensitive approach for detecting cell apoptosis is the Elabscience® TUNEL Apoptosis Assay Kit. Under standard optical microscopy, cell apoptosis can be identified after biotin-dUTP labelling and DAB staining.

MDA (Malondialdehyde)

MDA was detected in kidney tissue by using an MDA (malonaldehyde) ELISA Kit from Elabscience Biotechnology (E-EL-0060). after doing tissue homogenization. then the homogenate fluid put in a centrifuge 5–10 min. at 5000 rounds to get the supernatant fluid to carry out the ELIZA assay. The value of the control group was considered standard and used to compare with the results of the treated group.

Micro-morphometric measurement

Many parameters were estimated in the current study including the number of glomeruli, the diameter of glomeruli GD (µm), the diameter of proximal and distal convoluted tubule PCT and DCT(µm), in a microscope with a magnification of 40x. For morphometric estimation, special digital camera (OMAX 18 MP, China) with USB 3.0 was used.

Statistical analysis

IBM SPSS statistical analysis version 21 was utilized to analyze the data. To compare the mean differences among the experimental groups, used analysis of one-way variance (ANOVA), then a post-hoc Duncan test. The statistical results were stated as a mean ± SE. The means Differences were regarded as significant at $P<0.05$.

RESULTS

Vital parameters

In the present study, kidney weight in gram/ 100grams of body weight was significantly ($p\leq 0.05$) decreased in old age rats (group B) (0.278± 0.011) compared to control rats (group A) (0.341±0.009), while in the treated rats with RES (group C) showed significant ($p\leq0.05$) improvement in kidney weight (0.310± 0.004) compared to old age group . The level of serum creatinine was raised significantly ($p\leq 0.05$) in the old age rats (0.84±0.07), in comparison to the control group (0.55±0.03). However, the treated group with RES showed a significant reduction of serum creatinine (0.54±0.02) compared to the old age group .The mean concentration of oxidative stress marker MDA in the renal tissue elevated significantly ($p\leq 0.05$) in the old age group (69.14± 3.88) compared to the control group (31.71±0 .28). However, there was a statistical reduction in MDA of RES treated groups (48.32± 1.83), compared to old age group (Fig.1).

Histological findings

The findings of Hematoxylin and Eosin (H&E) stain showed that in Group A (control group), the light microscopic showed normal histological findings, in which the renal cortex and medulla comprise the kidney parts. The renal corpuscle, proximal and distal convoluted tubules (PCT), and (DCT) are all found in the cortex. The Bowman's capsule is a double-walled capsule around the renal corpuscle, which is made of several loops of endothelial-lined blood capillaries and rests on the basement membrane. A large, highly acidophilic cuboidal epithelium with a thick brush border and round, basally positioned nuclei lines the PCT. It also had a narrow lumen. The DCT had a broad lumen, a tiny cuboidal epithelium that was faintly stained, and spherical, apically positioned nuclei . In Group B (Old age group), the kidney sections showed the presence of asymmetrical sizes of the glomeruli; some decreased in size with atrophied glomeruli and widening of the Bowmen's space, while at the same time, other glomeruli enlarged and hypertrophied as a means of compensation; the absence of the Bowmen's space and congested glomeruli could also be seen. Some glomeruli showed inflammatory cell infiltration and

hemorrhage in the glomerular tuft. Other glomeruli showed segmental glomerulosclerosis with destroyed glomerular tufts. The kidney sections of this group showed a dilated and cystic appearance of the tubules with cast formation inside the tubule and hybridization of the kidney tubules, and the epithelium of the tubule was flattened and had a different appearance of hydropic changes and vacuolization in the cytoplasm of the epithelial cell. Peritubular fibrosis and tubular atrophy were also present. The interstitial tissue changes showed interstitial fibrosis , the presence of inflammatory cell infiltration, and the thickening of the blood vessel wall within the interstitial tissue . In Group C (Resveratrol treated group, the kidney sections showed improvement in most changes that were seen in the old age group despite the kidney section did not return completely to normal appearance, but it showed more preserved kidney architecture and most of the glomeruli appeared normal except few of them show atrophy, hypertrophy, and sclerosis, the tubules looked normal except some tubules still had some vacuolation, few cast formation in this section and small dilatation of the tubules, few inflammatory cells infiltration in the interstitium (Fig. 2).

Microscopic examination of kidney sections from different groups that were stained with Masson's trichrome revealed a mild distribution of collagen fiber around blood vessels and in the interstitium in the control

Table 1. Micro-morphometric study of kidney in Wistar albino rats

Groups	No. of renal corpuscle (4xfield)	Diameter of glomeruli/µm	Diameter of PCT/ µm	Diameter of DCT/ µm
A	21.25 ± 0.75 a	92.10 ± 2.04 a	40.10 ± 1.30 a	42.70 ± 0.90 a
B	6.75 ± 0.47 b	114.15 ± 2.44 b	94.30 ± 2.82 b	66.40 ± 1.72 b
C	115.0 ± 0.50 c	102.00 ± 2.82 c	75.90 ± 1.20 c	53.90 ± 1.45 c

The column contained the same litters means non-significant difference at p>0.05.
The column contained the different litters means there is significant difference at p≤0.05.
PCT=Proximal Convoluted Tubules, DCT=Distal Convoluted Tubules

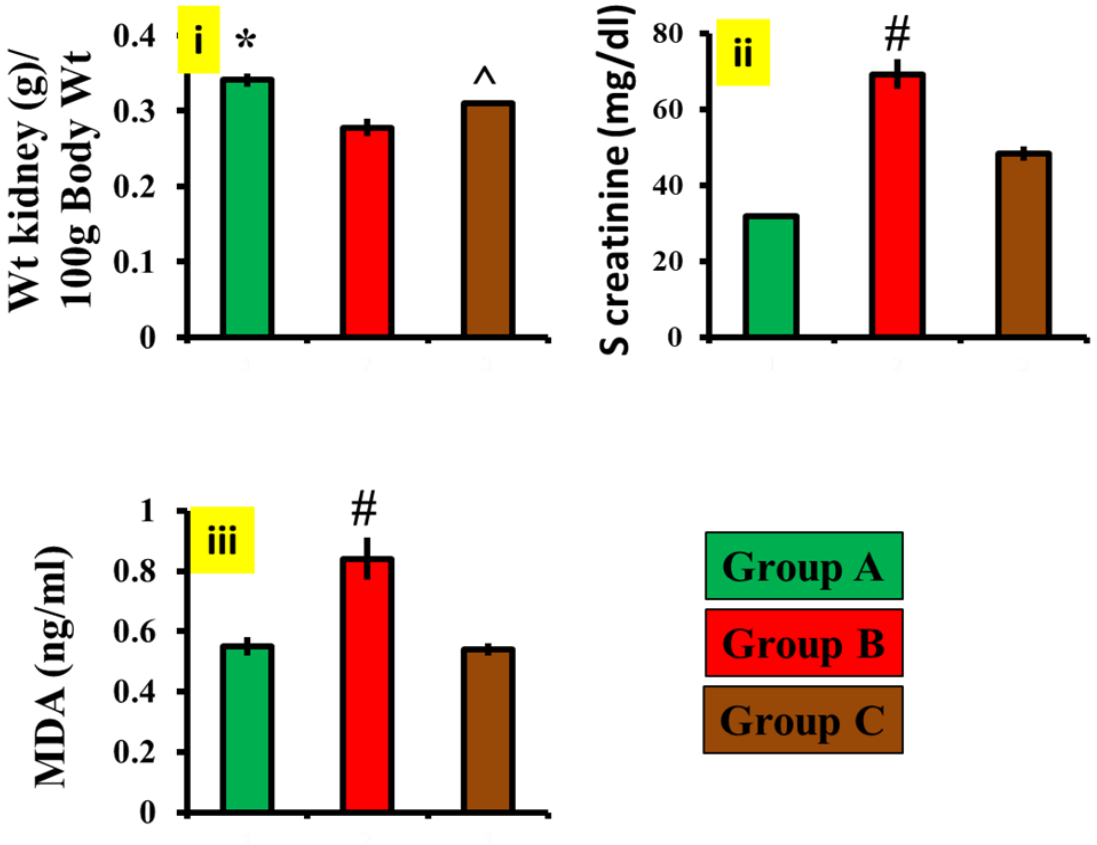

Fig. 1. *Resveratrol modulated important vital parameters in old age rats based on kidney weight(i), serum creatinine(ii), and oxidative stress(iii). Data expressed as mean±SE, n=8 rats per group,*^#p<0.05. *$significant differences as compared to other groups, ^ as compared to group B*

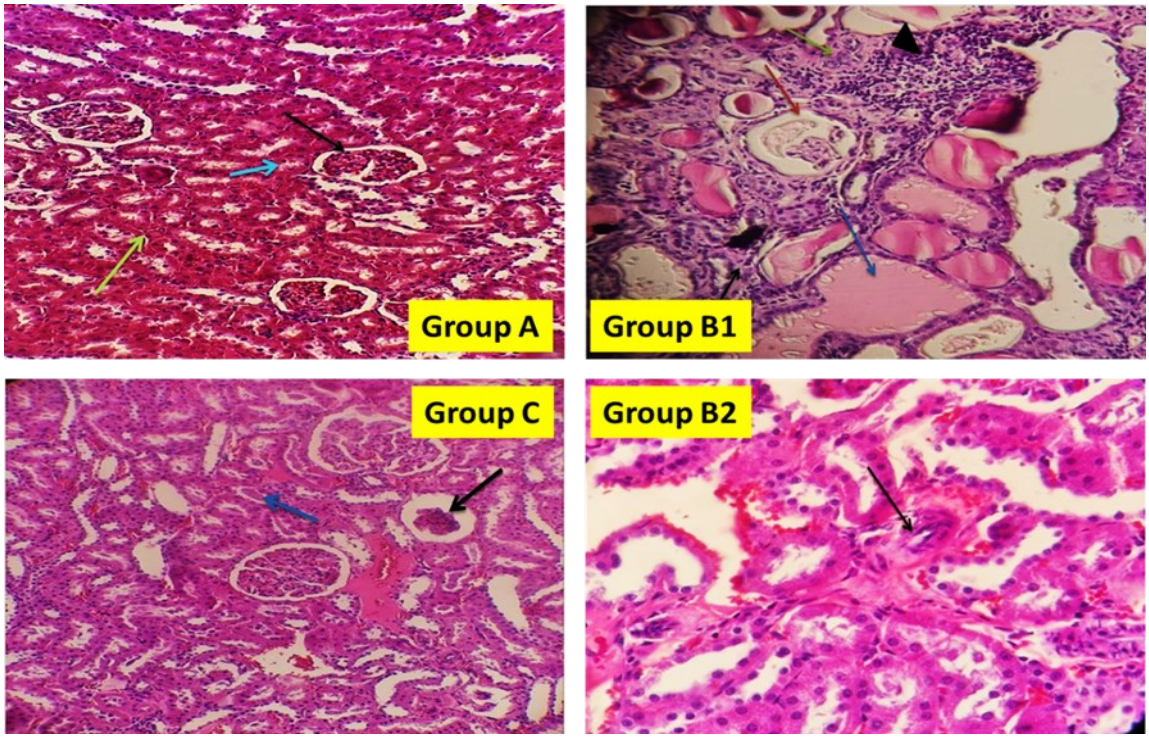

Fig. 2. *Resveratrol slightly preserved kidneys normal architecture of old age rats. Group A: Microphotograph of adult age group rat's kidney group show normal histological architecture normal glomeruli (black arrow), normal proximal and distal convoluted tubules (blue arrow), normal interstitial tissue (green arrow). (H &E X 100). Group B1: Microphotograph of old age group rat's kidney show shows segmental glomerulosclerosis with destroyed atrophied glomerular tuft (red arrow), cystic appearance of the tubules with cast formation inside (blue arrow), peritubular fibrosis and tubular atrophy (green arrow), interstitial tissue changes showed interstitial fibrosis (black arrow) presence of mononuclear cells infiltration (arrow head) (H &E X 100). Group B2: Microphotograph of old age group rat's kidney group show interstitial tissue changes showed thickening in the blood vessels wall with in the interstitial tissue (black arrow) (H &E X 400). Group C: Microphotograph of old age group rat's kidney treated with resveratrol group show most of renal tissue look normal with few glomeruli showed atrophy (black arrow) and some tubules contain hyaline cast (blue arrow) (H &E X 100)*

group; the section of the old rat group showing increased collagen deposition around blood vessels and in the interstitium, as well as increased collagen fiber deposition around the glomeruli and inside the glomeruli causing segmental glomerulosclerosis and around the tubules causing tubular atrophy . The section of kidney in the old age group treated with resveratrol revealed normal histological appearance with a mild increase of collagen fiber deposition in the interstitium and around the blood vessels but less prominent than old age group and still not returned to normal as the control group (Fig. 3).

The Periodic Acid Schiff (PAS) staining of the control group kidney sections showed a mild PAS +ve reaction of the basement membrane of the glomerular capillaries, the glomerular capsule, and the tubular basement membrane with a thick brush border . While in the older age group, the PAS staining revealed a strong PAS stain reaction as demonstrated in the thickened lamellated basement membrane of the capillary tuft of glomeruli and the basement membrane of Bowman's capsule, as well as a thickening in the renal tubules basement membrane and a thinning and damaging of the brush borders. Some sections of old rats showed mesangial expansion with increased mesangial cells and matrix; also, some glomeruli revealed microaneurysmal dilatation and their lumens were filled with a fibrous material, which is pink in color by PAS stain . the kidney section of old age treated group with RES showed moderate PAS reaction, normal basement membrane of the glomerular capillary and capsule, and most of the tubules look normal basement membrane and normal thick brush border when compared with the old age group, except a few numbers of the tubules and glomeruli showed thickening of the basement membrane, no dilatation of the glomerular capillary no mesangial matrix expansion as seen in old age group (Fig. 4).

The study on TUNNEL apoptosis assay showed that the kidney sections in the control group, there were a small number of apoptotic cells that appeared dark brown in color , whereas there was a significant rise in the rate of apoptosis in the group of elderly rats, especially around the glomeruli and tubules, with cells that appeared dark brown in color with deeply stained fragmented nuclei . on the other hand the treated old age

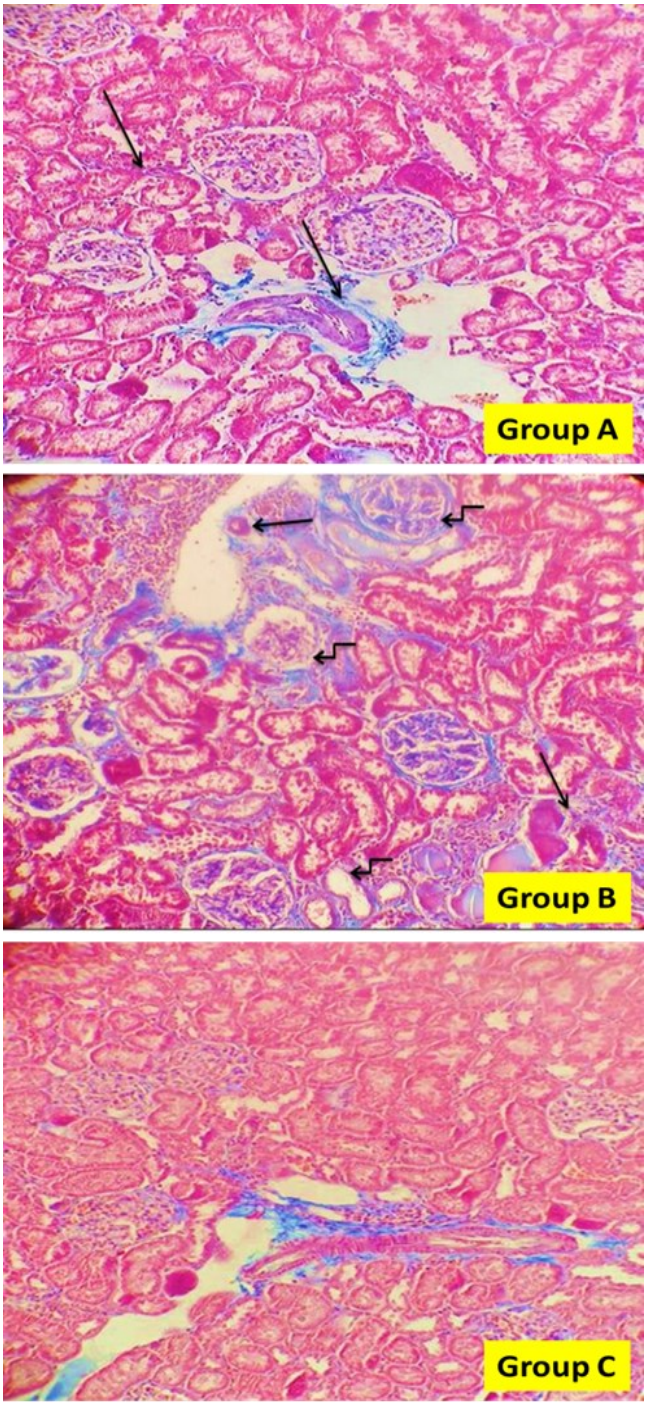

Fig. 3. *Resveratrol reduced the reaction to Masson's trichrome stain in old age. Group A: Microphotograph of control adult age group rat's kidney show mild distribution of collagen fiber in the interstitium and around the blood vessels (black arrows) (Masson´ s trichrome X 100). Group B: Microphotograph of old age group rat's kidney showing increase in the deposition of collagen fiber in the interstitial tissue and around blood vessels (black arrows), also around glomeruli and tubules (curved arrows). Group C: Microphotograph of old age group rat's kidney treated with RES group kidney section looks normal except discrete small area of increase collagen fiber deposition in the interstitial tissue and around blood vessels (black arrows) (H &E X 100)*

groups with RES showed decrease in the rate of apoptosis when compared to old age group (Fig. 5).

Quantitative morphometric study of the kidney

The morphometric assessment of the number of glomeruli showed a significant decrease in the mean number of glomeruli in the old age group (B) compared to the control group (A) at $p \leq 0.05$. and the mean of the number of glomeruli in the RES old-aged group (C) is significantly increased at $p \leq 0.05$ when compared to the old-age group (B) . The morphometric study revealed glomerular diameter (GD) significantly rose in the old age group (B) at $p \leq 0.05$ when compared to the control group (A). However, GD decreased in the RES-treated old age group (C) compared to the old age group (B) at $p \leq 0.05$. Diameter of proximal convoluted tubules (PCT): The morphometric investigation of PCT diameter showed a rise in the PCT diameter mean in the old age group (B) compared to the control group (A) at $p \leq 0.05$, with the reduction in PCT diameter in RES treated old age group (C) compared to old age group. Diameter of distal convoluted tubules (DCT): Morphometric analysis of DCT diameter revealed a highly significant increase in the diameter of the DCT in the old age group (B) compared to the control group (A) and a decrease in the diameter in RES-treated group (C) compared to old age group at $p \leq 0.05$ (Table 1)

DISCUSSION

In the present study, RES moderately preserved kidney function compared to control group, confirmed via measurement of creatinine, kidney weight, and MDA levels. The ageing process led to a significant drop in kidney weight in the old age group compared with the control group (Figure 1i). This is in agreement with Denic *et al.* (2016), who reported that the reduction in the weight of the kidney during aging process started to appear in the fifth decade of life and caused a reduction in kidney weight by about 10–30% in the eighth decade of life. This could be attributed to tubule and glomerular atrophy and decreased renal epithelial proliferation, which leads to the loss of renal tissue with aging (Abdel-Rahman and Okusa, 2014).

Renal function estimated by the serum creatinine measurement (Nankivell, 2001) showed that there were significantly higher serum creatinine levels in the old age group than in the control group, meaning renal function and GFR were reduced with aging (Figure 1ii). This is in agreement with Tiao *et al.* (2002). Moreover, Glassock and Rule (2012) and Schmitt and Melk (2017) have shown that GFR declined by about 5–10% per decade after age 35. This reduction in the GFR may be attributed to nephron loss with aging.

With the progress of age, the finding in the older age

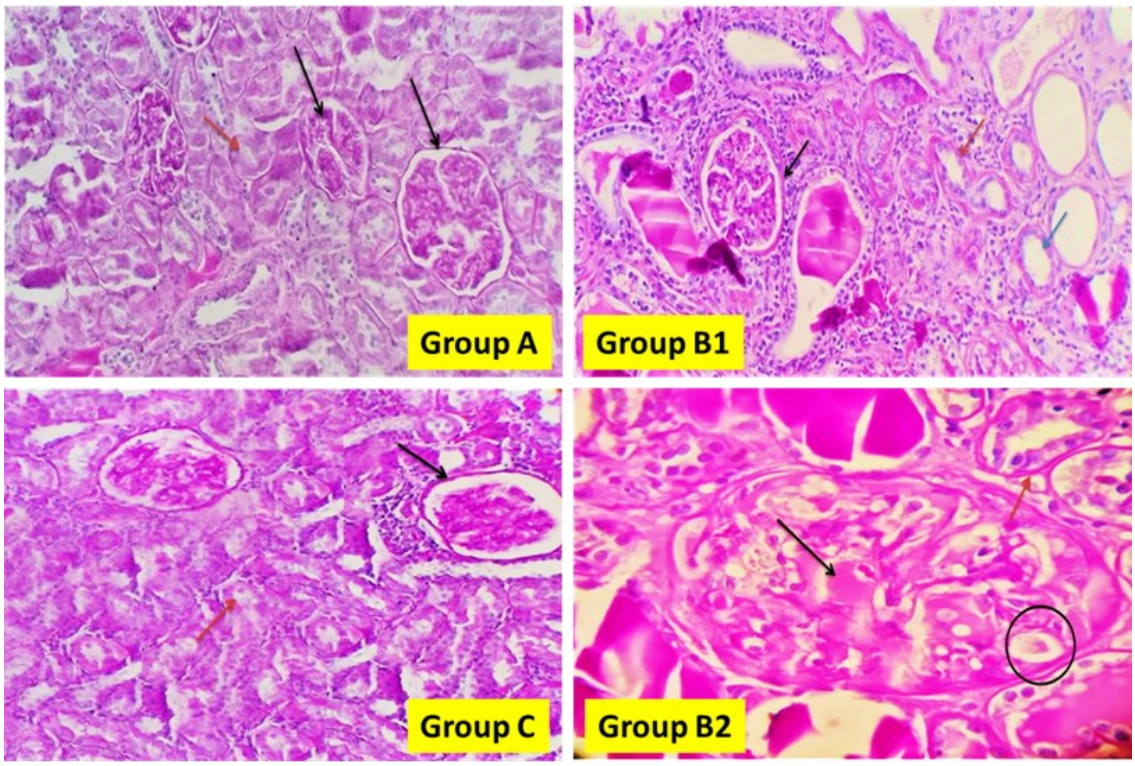

Fig. 4. *Resveratrol reduced the reaction to PAS stain in old age rats. Group A: Microphotograph of control group kidney section showing mild PAS +ve reaction of basement membrane of the glomerular capillary and of the glomerular capsule (black arrows). Normal renal tubule with thick brush border (red arrow) PAS, 100X. Group B1: Microphotograph of old age group kidney sections showing strong PAS + ve reaction, thickened lamellated basement membrane of Bowman's capsule (black arrow), thickened lamellated basement membrane of the renal tubules (red arrows), thin and absence of brush border (blue arrow). PAS, 100X. Group B2: Microphotograph of old age group kidney section showing strong + ve PAS reaction, mesangial matrix expansion(arrow), microaneyrisymal dilatation of glomerular capillary (circle), thickened basement membrane of the renal tubules (red arrow). PAS, 400X. Group C: Microphotograph of kidney of old age treated with RES group showing moderate + ve PAS reaction of basement membrane of the glomerular tuft capillary and of the glomerular capsule (red arrow), some glomeruli show thickened basement m. (arrows). PAS, 100X*

group became more evident in this experiment, which showed asymmetry in the size and shape of the glomeruli: some decreased in size with atrophy of the glomeruli and increased the Bowman's space, while the other glomerular tuft hypertrophied with obliteration of the Bowman's space. The increase in glomerular diameter in morphometric study might be a compensatory mechanism for glomerular atrophy and loss of the nephron (Table 1). The expansion of the mesangial matrix, glomerular basement membrane thickening, and sclerosed glomeruli was observed in this research. These changes were also observed by Glassock and Rule (2012) and Schmitt and Melk (2017) studies.

Oxidative stress may consider the main factor of the renal aging process as evidenced by elevation in MDA in the present study (Figure 1iii); it may cause alterations in the arterial system in the kidney. The most essential arterial wall sclerosis was observed in this study also, with time caused hypoxic and ischemic damage that led to aging-associated changes in renal component (glomerulosclerosis, tubular atrophy and interstitial fibrosis), leading to insufficient renal function. This caused feedback activation of the renin-angiotensin-aldosterone system causing a further elevation in hypertension that makes a cycle of arterial damage and ischemic renal injury. These outcomes harmonized with Zhou *et al.* (2008) and Fang *et al.* (2020), who said that arteriosclerosis triggers age-related renal changes. During aging, there was an arterial intimal thickening due to the deposition of collagen, with decreasing amounts of the smooth muscle being replaced by fibrous tissue in addition to the thickening in the tunica media, which causes arteriosclerosis, glomerulosclerosis, and interstitial fibrosis (Harkema *et al.*, 2016).

The observed atrophied, sclerosed glomeruli and reduction of glomerular number in the morphometric study can be attributed to the ischemic lesion that caused glomerular tuft collapse, thickened glomerular basement membrane, and intracapsular fibrosis, which are precursors to glomerulosclerosis. With time, the glomerular tuft became shrinkage with subsequent widening of the Bowman's space plus deposition of collagen in the space, caused glomerulosclerosis**,** and led to decreased number of glomeruli, nephron loss, tubular

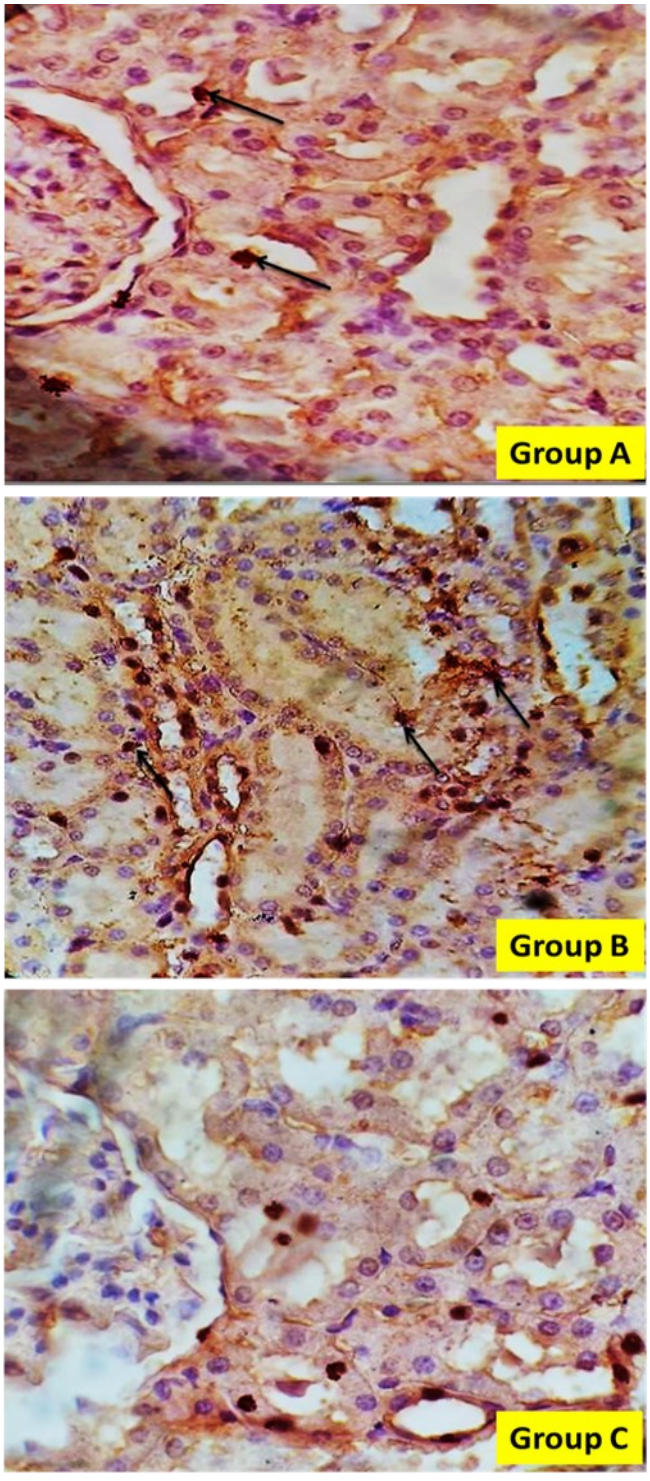

Fig. 5. *Resveratrol reduced apoptotic cells expression in old age rats using tunnel apoptotic assay (tunnel stain X400) . Group A: Microphotograph of adult age group rat's kidney shows few number of apoptotic cells (dark brown) around tubule and glomeruli (black arrows). Group B: Microphotograph of old age group rat's kidney show increase in apoptotic cells number (dark brown) around tubule and glomeruli (black arrows). Group C: Microphotograph of old age group rat's kidney treated with RES group show decrease in apoptotic cells number (dark brown) around tubule (black arrows)*

atrophy, and interstitial fibrosis (Karakaya-çimen and Esrefoglu, 2022).

This glomerular hypertrophy could be considered as a means to compensate for the glomerular atrophy and vascular adaptation to the nephron loss to preserve the GFR by shifting perfusion to the adjacent functioning glomeruli and causing hyperperfusion and hyperfiltration in a normal functioning nephron, which results in hypertrophy and enlargement of the glomeruli, an increase in the glomerular diameter with the expansion of mesangial matrix and deposition of mesangial material around glomerular capillary, that end with glomerulosclerosis (Kotob *et al.*, 2021, Zhou *et al.*, 2008).

Other important histological changes observed in this study were the degenerative changes in the tubular epithelium, peritubular fibrosis, and tubular atrophy. Other tubules that were dilated, increased in diameter and filled with hyaline cast material appear as cystic dilatation of renal tubules as observed by morphometry in this study, and the tubular epithelium that flattened showed various degrees of degeneration and apoptosis, as well as widening of interstitial tissue and fibrosis. These results were also reported by and Nankivell (2001). The degenerative changes in the renal tissue are attributed to the ischemic changes resulting from the degeneration of the afferent and efferent arterioles. The formation of direct channels between them will decrease the blood supply to the renal cortex (Nitta *et al.*, 2014). The dilatation of the tubules and the cast formation due to variations in the structure of the glomeruli and damage to the glomerular filtration barrier, in addition to the deterioration of the tubular function and diminished tubular reabsorption and decrease of renal blood flow, cause the crossing of large quantities of proteins from the barrier and the formation of hyaline cast in the tubules (Zhou *et al.*, 2008).

The tubular atrophy and peritubular and interstitial fibrosis seen by Masson´s trichrome in this study were also observed by other researchers in their studies, such as Kotob *et al.* (2021); Karakaya-çimen and Esrefoglu (2022) and Zhou *et al.*, (2008). It may be attributed to the loss of peritubular capillaries, which can be observed in ageing kidneys secondary to glomerulosclerosis and nephron loss. In addition, the ageing process also leads to changes in capillary health; there is a decline in nitric oxide production and a rise in the production of endothelin-1, leading to an autoregulation impairment with great vasoconstrictor responsiveness. These changes lead to chronic hypoperfusion, ischemia, hypoxia, the loss of nephron and peritubular capillaries, and the development of glomerulosclerosis with ageing (Schmitt and Melk, 2017).

PAS staining of the studied section showed thickening of glomerular capillaries and tubular basement membrane, in addition to thickening in Bowman's capsule

with a decrease in the thickness of the tubular brush border, which is in harmony with Nitta *et al.* (2014), and. These changes could be clarified that the kidneys of old age rats showed changes in the extracellular matrix composition (ECM), which contain elevated levels of thrombospondin, laminin chains B1, and S-laminin, which cause an increase in the basement membrane (Abrass, *et al.*, 1995). On the other hand, the thin brush border in the tubules due to degenerative changes and the increased rate of apoptosis in the epithelium is considered the main causal factors in the decreased absorptive function of the tubules in the aged kidney (Kotob *et al.*, 2021).

The MDA level increased in the old age group of this study; this indicated a state of oxidative stress during ageing that had a damaging effect on aging. This is in agreement with Jose, who mentioned that the index of oxidation in old age animals was higher than in young adult animals and showed oxidized lipid accumulation, protein oxidation, and oxidized DNA, and these free radicals may responsible for the damaging effect in old age animals (Viña *et al.*, 2007).

Inflammation or low-grade inflammation was observed clearly in this study. Especially in an old-age group, it is a fundamental characteristic of the ageing process. It is the sterile, low-grade, chronic state of inflammation usually referred to as "inflammaging." that was in agreement with Sarkar and Fisher (2006) and Costello-White *et al.* (2015). Franceschi *et al.* (2007) mentioned in their research that inflammation is a hallmark of ageing and it is associated with increasing the level of inflammatory cytokines such as IL-6 (interleukin 6) and CRP (C-reactive protein), associated with the activation of the innate immune system and increasing morbidity and mortality in the elderly.

The use of RES in this study caused improvement in kidney weight and serum creatinine level compared to the old-age group. This may be due to the antioxidant effect of RES and its role in improving the histological structure of the kidney tissue, and this result is in agreement with Al Dera (2016), who revealed that using 20 mg/kg of RES for 40 days ameliorates AlCl3 induced nephrotoxicity by improving levels of endogenous antioxidants and reducing inflammatory biomarkers.

The use of RES reduces glomerulosclerosis, tubulointerstitial fibrosis, infiltration of inflammatory cells and apoptosis and reduces the MDA level. This is in accordance with Kim *et al.* (2018), who mentioned that in old-age mice resveratrol treatment (40 mg/kg for 6 months) decreased extracellular matrix collagen IV expressions and the pro-fibrotic growth factor TGF-β1, as well as a decrease in the pro-apoptotic factor BAX and an increase in the anti-apoptotic factor. Furthermore, RES increased SOD antioxidant protein expression and decreased oxidative stress markers as lipid peroxidation markers in old mice treated with RES. These protective effects were mediated by the activation of the Nrf2 and SIRT1 signaling pathways which ameliorated oxidative stress, fibrosis, inflammation, and apoptosis in this mouse model of age-related renal injury, thereby reducing the pathologic changes of aging in the kidney. Elbe *et al.* (2015) and their colleague found that the use of 10mg/kg/day RES given i.p for 30 days in diabetic nephropathy can reduce glomeruli sclerotic changes, brush border loss, and increase SOD and CAT activities, as well as the reduction of MDA. So RES improves diabetic nephropathy by preventing oxidative stress.

The use of RES in old age rats alleviated basement membrane thickness and hyaline cast formation following Qiao *et al.* (2017), who found that in diabetic rats, 20mg/kg/day for 4 weeks resulted in lower serum creatinine and lower glomerular basement membrane thickness, decreased interstitial fibrosis, degenerative epithelial changes and reduced hyaline casts formation.

Conclusion

In conclusion, the study's findings indicated that RES has a potential prophylactic and therapeutic application for aging-related renal changes. The improvement in creatinine levels and decreased MDA levels, along with the improvement in histological changes of the aged kidney, demonstrate the effectiveness of RES in mitigating the negative effects of aging on the kidney. Aging was characterized by various histological variations that can cause physiological dysfunction of the kidney and increase the risk of renal illnesses. Therefore, using RES as a preventative measure can improve the quality of life in aging individuals and reduce the burden of renal illnesses on healthcare systems. Further research is necessary to explore the long-term effects of RES on aging-related renal changes and to determine the optimal dosage for its clinical application.

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Conflict of interest

The authors declare that they have no conflict of interest.

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