Bull. Egypt. Soc. Physiol. Sci. Vol. (45), Issue (3), 315-331

Bull. of Egyp. Soc. Physiol. Sci.

(Official Journal of Egyptian Society for Physiological Sciences)

(pISSN: 1110-0842; eISSN: 2356-9514)

Effect of Dipeptidyl Peptidase-4 Inhibitor and angiotensin II type 1 receptor blocker on renal function in D-galactose induced Aging in male rats and possible relation between their effects.

Enas M. Kasem[1], Eman S. Abdelghany[1], Mona A. Said[1], Ahmed I. Agamy[1], Naglaa Y.Nafia[1]*

[1]Department of Physiology, Faculty of Medicine, Benha University, Qalubiya, Egypt.

Submit Date : 21 Jan. 2025
Revised Date : 12 Feb. 2025
Accept Date : 12 Feb. 2025

Keywords

- D-galactose model
- Antioxidants
- Dipeptidyl peptidase-4
- Renin-angiotensin system
- Renoprotective effect

Abstract

Background: Aging induces structural alterations in the kidney and impairs its functionality. The progression of renal aging is intricately influenced by oxidative stress. Research has shown that plasma DPP-4 activity increases across various organs with aging. Additionally, heightened renin-angiotensin system activity during normal aging may contribute to chronic kidney disease (CKD)sequential progression. **Objective:**Toevaluate potential synergistic effects of angiotensin II type 1 (Ang II type 1) receptor antagonists and DPP-4 inhibitors in D-galactose-induced aging in male rats, as well as their potential renoprotective effects.**Materials and Methods:** Thirty-five adult male albino rats were randomly divided into five groups: Group I (control), Group II (D-galactose), Group III (D-galactose + DPP-4 inhibitor), Group IV (D-galactose + Ang II type 1 receptor blocker), and Group V (D-galactose + DPP-4 inhibitor + Ang II type 1 receptor blocker).**Results:**Both of group III and IV provided substantial protection against D-galactose-induced renal damage (group II). Notably, combining both drugs (group V) resulted in enhanced renal protection compared to either drug alone, demonstrating their synergistic effect. Improved renal function was mediated through antioxidant mechanisms, evidenced by significant increase in tissue SOD level($p<0.01$) along with significant decrease serum creatinine level ($p<0.01$).**Conclusion:**DPP-4 inhibitors and angiotensin II blocker combination is a promising therapeutic approach for age-related renal dysfunction.

Corresponding author: Eman Salem Abdelghany Ahmed, Physiology Department, Faculty of Medicine, Benha University, Qalubiya, Egypt. E. Mail: emy21mhmd@gmail.com. Mobile: 002 01061094538

Introduction

Regarding population pyramid, it is noticed increase life expectancy and subsequently increased proportion of elder people. Over the past 60 years, percentage of increased elder people in the world has been elevated from 8% to 10% **[1]**. Various theories have been advanced to explain the underlying mechanisms driving the aging process. One of them is the production of free radicals which can denature proteins, destroy nucleic acids, and some organelles like lysosomes and proteasomes. The crucial role of free radical or reactive oxygen species(ROS) comes from the belief that accumulated cellular damage via these molecules will cumulatively overcome the cell's damage repair mechanisms (mitochondrial antioxidative responses)**[2] [3].**

Another molecular explanation for aging process involves telomere shortening. Telomeres, which are specialized DNA-protein complexes located at termini of human chromosomes, consist of repetitive sequences of (TTAGGG)n. It is important to recognize that telomere shortening occurs under various circumstances, including successive cell divisions, replication of nuclear DNA during mitosis, oxidative stress, and natural processes of senescence and aging. **[4]**.Protective functions of these proteins diminish progressively as telomere length decreases **[5].**

The progressive deterioration of kidney integrity inherently leads to a measurable decline in renal function. Notably, by age of 40, GFR undergoes a decrement of nearly 8 ml/min per 1.73 m^2 with each passing decade. Concurrently, renal blood flow diminishes, and structural alterations in renal architecture become evident **[6].** Several studies have highlighted that prolonged administration of D-galactose (D-gal) can mimic physiological conditions akin to natural aging.D-gal is a normal reducing sugar in the body that can be metabolized into glucose at normal levels; but at high concentrations, D-gal is converted into aldose and hydroperoxides by oxidase enzyme, leading to the formation of the superoxide anion and oxygen-derived free radicals**[7].**

Evidence from prior studies has highlighted renoprotective effects of DPP-4 inhibitors in various animal models, such as unilateral ureteral obstruction, partial nephrectomy, ischemia-reperfusion injury, and nephrotoxicity **[8] [9].** Moreover, kidney serves as a pivotal locus for DPP-4 expression, with its activity distinctly localized to renal vasculature, glomerular structures, and tubular epithelial cells, underscoring its functional significance in these regions **[10].**

DPP-4 inhibitors have been shown to enhance outcomes in CKD by targeting inflammation, oxidative stress, and fibrosis. This therapeutic effect is achieved by reducing production of ROS and promoting activity of superoxide dismutase (SOD) **[11].**Also, DPP-4 inhibitors have been found to decrease the interaction of advanced glycation end products (AGEs) with their receptors (RAGE). This interaction has a pivotal role in the pathogenesis of CKD **[10].**

Extensive research has highlighted renin-angiotensin-aldosterone system (RAAS) as a cornerstone in the progression of CKD in aged mouse models. Clinically, RAAS inhibitors are routinely prescribed for patients with hypertension or nephropathy **[12].**

Although extensive research has underscored the renoprotective properties of angiotensin II type 1 (Ang II type 1) receptor blockers and DPP-4 inhibitors individually, studies exploring their

combined therapeutic efficacy in the context of aging remain relatively limited.

Based on this premise, we hypothesized that combined therapeutic approach of Ang II type 1 receptor antagonists and DPP-4 inhibitors would exert a synergistic effect on renal function in male rats subjected to D-galactose-induced aging.

2. Materials and Methods

2.1. Animals

This research was performed on 35 adult male wistar albino rats aged between 10-12 weeks and weighed about 120-170 grams. They were obtained from Laboratory Animals Farm Unit, Veterinary Medicine, Benha University without any previous preparation. Animals were randomly allocated into five groups, with each group comprising seven. Each 3 to 4 rats were placed in a separate cage. Rats were housed in a controlled environment, maintained at 20–22 °C temperature with a regulated 12-hour light/dark cycle, and were provided unrestricted access to food and water. The experimental duration spanned six weeks.No rats died throughout the experiment. Institutional Ethical Committee for Animal Care and Use, Faculty of Medicine, Benha University, authorized the study protocol (Approval No. MS 10-11-2022). After study completion, animals were disposed in the incinerator of the Benha University Hospital.

2.2. Experimental Design:

Following two weeks acclimatization period the groups were then subjected to the following treatment protocols:

Group I (Control group):

Over a six-week period, rats received intraperitoneal injections of saline five days a week.

Group II (D-galactose group):

For a period of six weeks, rats were intraperitoneally administered 300 mg/kg/day of D-galactose to induce aging **[13].**

Group III (D-galactose + DPP-4 inhibitor group):

Rats were administered D-galactose intraperitoneally at a dose of 300 mg/kg/day, five days per week, for six weeks, along with oral administration of DPP-4 inhibitor sitagliptin at 30 mg/kg/daily dose for the same duration**[14]**.

Group IV (D- galactose+Ang II type 1 receptor blocker group):

For six weeks, rats received intraperitoneal injections of D-galactose at a dose of 300 mg/kg/day, five days per week, combined with oral gavage administration of Ang II type 1 receptor antagonist losartan at a dose of 20 mg/kg/day **[15]**.

Group V (D-galactose+DPP-4 inhibitor+Ang II type 1 receptor blocker group):

A 300 mg/kg/day intraperitoneal administration of D-galactose was administered to rats five days a week for a period of six weeks. Concurrently, they received oral treatments with DPP-4 inhibitor sitagliptin at 30 mg/kg/day and Ang II type 1 receptor blocker losartan at 20 mg/kg/day, both administered throughout six-week study period.

2.3. Collection of 24-hour urine

Prior to samples collections and scarification (end of the 6^{th} week), rats were individually placed in a special metallic cage with a tight wire grid floor for 24 hours to calculate urine output (starting from 10:00 am to 10 a.m. next day). A plastic dragger placed underneath the cage was used to obtain urine samples via a collecting bottle. Urine volume was recorded and subsequently centrifuged at a speed of 1500 rpm for a period of five minutes. At a temperature of -20°C, the clear supernatant was stored for further biochemical analysis.

2.4. Blood and tissue sampling

While under anesthesia induced by intraperitoneal administration of urethane (1.5 g/kg), blood and tissue samples were collected. Blood samples were drawn from the abdominal aorta, transferred to test containers, and left to coagulate at room temperature. Using the centrifuge (3000 rpm for 15 minutes) sera were separated. Sera was stored at – 20˚C for further analysis.

The right kidney was meticulously washed with ice-cold 0.9% saline solution and preserved at -80°C to facilitate subsequent analysis of renal tissue. Both kidneys were rapidly dissected from pedicle and removed. The left kidney was fixed in a 10% buffered formalin solution (pH 7.8) for histopathological evaluation, with SOD preserved.

2.5. Biochemical analysis

2.5.1. Based on manufacturing guidelines, the collected sera and urine were analyzed for the following parameters:

(1)Serum and urine creatinine was measured using colorimetric commercial kits

(BioDiagnostic, Egypt)

(2)Serum MDA was measured using the colorimetric commercial kits (BioDiagnostic, Egypt)

(3) Serum and tissue SOD was measured using the colorimetric commercial kits (BioDiagnostic, Egypt)

2.5.2. Part of the right kidney tissue was stored at -80°C and used to detect oxidative stress markers based on the guidelines of manufacturer. Kidney SOD was measured using colorimetric kits (BioDiganostic, Egypt).

2.6. Calculation of GFR:

GFR is calculated by calculating creatinine clearance which was determined using the following formula: Concentration of creatinine in urine (mg/dl) ×Volume of urine (ml/min) /Concentration of creatinine in serum (mg/dl) as described by **Uemura et al.[16].**

2.7. Histopathological examination

Left kidneys were washed and fixed in a formaldehyde solution for 24 hours. They were then dehydrated in ethanol at increasing concentrations (70% for 24 hours, followed by 90% and 100% for 1 hour each) and embedded in paraffin wax. Using a HistoRange microtome (model LKB 2218, LKB-Produkter AB, Bromma, Sweden), tissue samples were sectioned into 5 µm-thick slices. These sections were stained with hematoxylin and eosin (H&E) and examined under a light microscope (Olympus BX50, Tokyo, Japan). A pathologist from the Pathology Department, Faculty of Medicine, Benha University, analyzed H&E-stained samples.

2.8. Statistical analysis.

SPSS software, version 20 (SPSS Inc., Chicago, IL, USA), was utilized for statistical analyses. One-way analysis of variance (ANOVA) was employed to compare differences across multiple groups, followed by post hoc analysis using the least significant difference (LSD) test to identify specific intergroup disparities when ANOVA indicated significance. Results are reported as mean ± standard deviation (SD), with statistical significance defined as a p-value <0.05.

3. RESULTS:

3.1. Renal function tests:

3.1.1 Serum creatinine: Table (1) and figure (1):

Group II exhibited a notable elevation in serum creatinine levels ($p < 0.01$) compared to control (Group I). In contrast, rats treated with D-galactose under the same regimen alongside the oral administration of a DPP-4 inhibitor at 30 mg/kg/day (Group III) or an Ang II type 1 receptor blocker at 20 mg/kg/day via oral gavage (Group IV) demonstrated a protective effect against D-

galactose-induced kidney damage. This protective effect was evidenced by a significant reduction in serum creatinine levels ($p < 0.01$) compared to Group II. However, this protection was partial, as serum creatinine levels in Groups III and IV remained significantly higher than those in the control group.

Group V showed a more protective effect on kidney damage induced by D-galactose compared to giving each drug alone which is evidenced by a significant decrease in serum creatinine level ($p<0.01$) in group V compared to group III or group IV. However, this protection is still partial as the values of serum creatinine in group V still significantly higher than that of control group.

Table (1): Serum creatinine, glomerular filtration rate (GFR), serum malondialdehyde (MDA), serum superoxide dismutase (SOD), and superoxide dismutase in renal tissue in different experimental groups:

	Group I Control	**Group II**	**Group III**	**Group IV**	**Group V**
Serum creatinine (mg/ml)	0.62±0.12	* 1.39±0.13	¥* 1.17±0.09	¥* 1.16±0.06	¥€#* 0.95±0.10
GFR (ml/min)	0.86±0.12	* 0.29±0.03	*¥ 0.51±0.10	*¥ 0.43±0.07	¥€# 0.81±0.09
serum MDA (nmol/ml)	0.67±0.06	* 2.5±0.46	*¥ 1.51±0.22	*¥ 1.43±0.22	#¥€ 0.78±0.11
serum SOD (Mm/l)	4.49±0.25	* 3.14±0.35	*¥ 3.74±0.36	*¥ 3.88±0.27	¥€# 4.46±0.26
SOD in renal tissue (IU/mg. protein)	26.32± 2.64	* 19.37±0.81	*¥ 21.22±1.31	*¥ 21.04±1.15	*¥€# 24.48±1.45

Data are represented as Mean ± SD., n=7. $P < 0.01$ is significant tested by one-way analysis of variance (ANOVA) and post hoc multiple comparison LSD method.
*****Significant difference with Control ($p<0.01$)
¥ Significant difference with D-galactose ($p<0.01$)
€ Significant difference with D- galactose+DPP-4 inhibitor ($p<0.01$)
#Significant difference with D- galactose+ Angiotensin II receptor blocker ($p<0.01$)

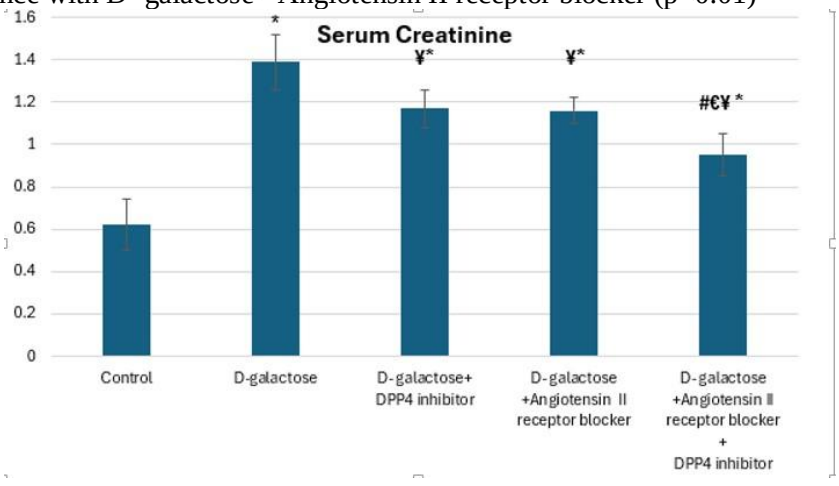

Figure 1: Effects of D-Galactose, DPP4 Inhibitor, and Angiotensin II Receptor Blocker on Serum Creatinine Level in D-galactose treated rats.
Data are represented as mean±SEM and analysis was done by one-way ANOVA followed by LSD post-hoc test at $P < 0.01$.
(*) Significant difference compared to the Control group ($p < 0.01$).
(¥) Significant difference compared to the D-galactose group ($p < 0.01$).
(€) Significant difference compared to the D-galactose + DPP-4 inhibitor group ($p < 0.01$).
(#) Significant difference compared to the D-galactose + Angiotensin II receptor blocker group ($p < 0.01$).

3.1.2GFR: Table (1) and figure (2):

Group II showed a notable decrease in GFR ($p<0.01$) compared to group I. Group III and group IV (Rats injected by D-galactose plus Ang II type 1 receptor blocker) showed a significant increase in GFR ($p<0.01$) compared to group II which proves the protective effect of DPP4 inhibitor and Ang II type 1 receptor blocker on kidney damage induced by D-galactose. However, this protection is partial as the values of GFR in groups III and IV still significantly lower than that of the control group.

Group V (Rats that injected by D-galactose plusDPP4 inhibitor and Ang II type 1 receptor blocker) showed a more protective effect on kidney damage induced by D-galactose compared to giving each drug alone (groups III and IV) which is evidenced by a significant increase in GFR ($p<0.01$) compared to group III or group IV. It has to be noted that combination of the two drugs (group V) results nearly a complete protection effects regarding GFR value, this is because the value of GFR in group V showed no significant change when compared with group I.

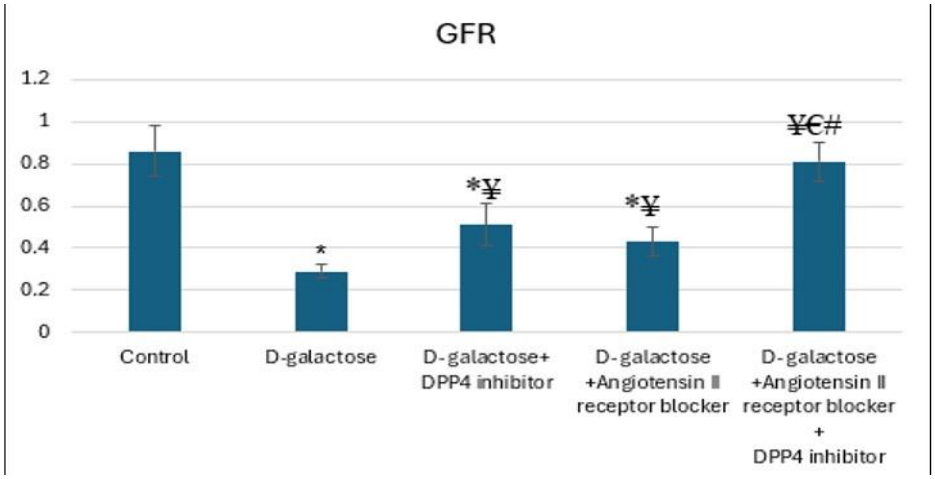

Figure 2: Effects of D-Galactose, DPP4 Inhibitor, and Angiotensin II Receptor Blocker on GFR in D-galactose treated rats.

Data are represented as mean±SEM and analysis was done by one-way ANOVA followed by LSD post-hoc test at $P < 0.01$.

(*) Significant difference compared to the Control group ($p < 0.01$).

(¥) Significant difference compared to the D-galactose group ($p < 0.01$).

(€) Significant difference compared to the D-galactose + DPP-4 inhibitor group ($p < 0.01$).

(#) Significant difference compared to the D-galactose + Angiotensin II receptor blocker group ($p < 0.01$).

3.2 Markers of oxidative stress and antioxidants:

Our results showed that both DPP-4 inhibitors and Ang II type 1 receptor blockers decreased serum MDA (one of the main oxidative stress markers) which was increased by D-galactose pretreatment together with increasing SOD both in serum and renal tissue, which is one of the main antioxidants. This proves that one of the main mechanisms of the protective effect of DPP-4 inhibitors and Ang II type 1 receptor blocker is the protection of renal tissue against oxidative stress by ROS.

3.2.1Serum MDA: Table (1) and figure (3)

Injecting rats intraperitoneally by D-galactose (group II) results in a significant increase in serum MDA (($p<0.01$) compared to the control group. This increase in serum MDA was significantly reduced when rats takeDPP4 inhibitor together

with D-galactose (group III)($p<0.01$) or they take Ang II type 1 receptor blocker together with D-galactose (group IV)($p<0.01$). However, serum MDA in groups III and IV still significantly higher than that of the control group ($p<0.01$) denoting that protection induced by either DPP4 inhibitor or Ang II type 1 receptor blocker alone is partial. Combined using of DPP4 inhibitor and Ang II type 1 receptor blocker together with D-galactose (group V) leads to more significant decrease in serum MDA ($p<0.01$) compared to groups III and IV. Nevertheless, control group and group V exhibited no significant difference in serum MDA, suggesting that combination of the two medications provides nearly complete protection of the kidney against oxidative stress injury.

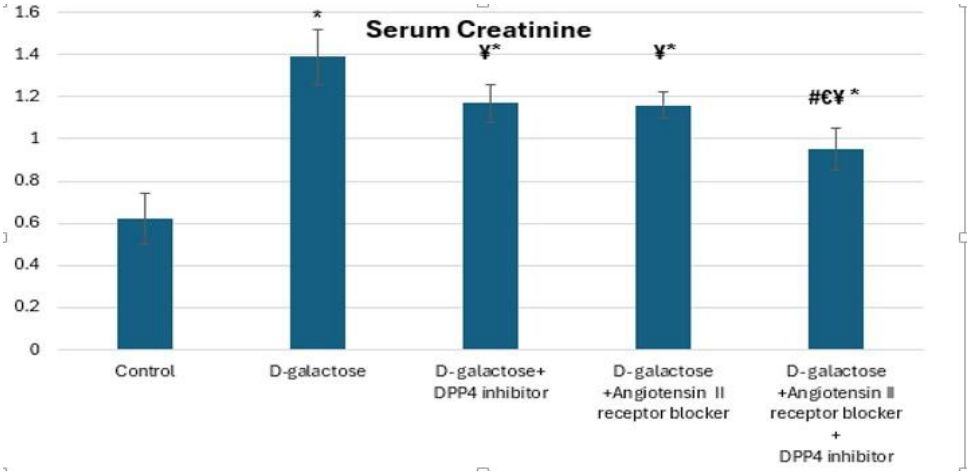

Figure 3: Effects of D-Galactose, DPP4 Inhibitor, and Angiotensin II Receptor Blocker on Serum MDA Level in D-galactose treated rats.

Data are represented as mean±SEM and analysis was done by one-way ANOVA followed by LSD post-hoc test at $P < 0.01$.

(*) Significant difference compared to the Control group ($p < 0.01$).

(¥) Significant difference compared to the D-galactose group ($p < 0.01$).

(€) Significant difference compared to the D-galactose + DPP-4 inhibitor group ($p < 0.01$).

(#) Significant difference compared to the D-galactose + Angiotensin II receptor blocker group ($p < 0.01$).

3.2.2.Serum SOD: Table (1) and figure (4)

Group II exhibited a significant reduction in serum SOD levels ($p<0.01$) compared to Group I. Conversely, Groups III and IV demonstrated a marked increase in serum SOD levels ($p<0.01$) relative to Group II, highlighting the renoprotective effects of DPP-4 inhibitor and Ang II type 1 receptor blocker against D-galactose-induced kidney damage via the activation of antioxidant mechanisms. However, this protective effect was only partial, as serum SOD levels in Groups III and IV remained significantly lower than those in the control group ($p<0.01$).

Group V showed a more protective effect on kidney damage induced by D-galactose compared to giving each drug alone which is evidenced by a marked increase in serum SOD($p<0.01$) compared to group III or group IV. Notably, the combination of the two medications (Group V) conferred nearly complete protection, as evidenced by the serum SOD levels in Group V showing no significant difference when compared to control group.

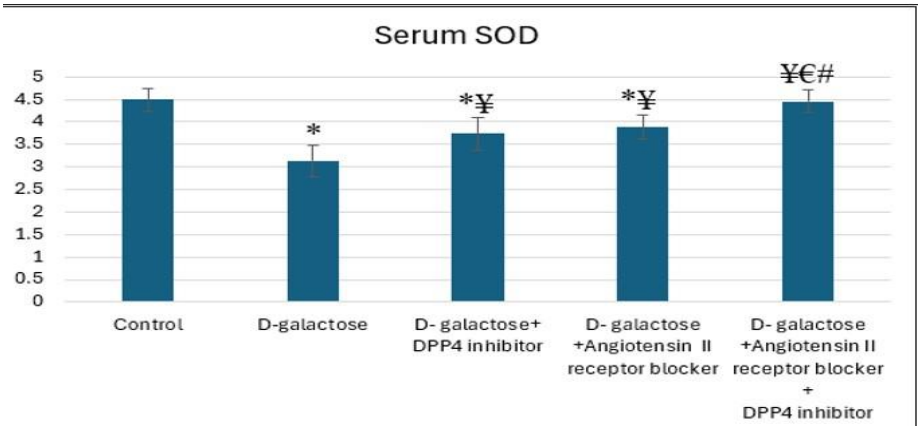

Figure 4: Effects of D-Galactose, DPP4 Inhibitor, and Angiotensin II Receptor Blocker on Serum SOD Level in D-galactose treated rats.

Data are represented as mean±SEM and analysis was done by one-way ANOVA followed by LSD post-hoc test at P < 0.01.
(*) Significant difference compared to the Control group (p < 0.01).
(¥) Significant difference compared to the D-galactose group (p < 0.01).
(€) Significant difference compared to the D-galactose + DPP-4 inhibitor group (p < 0.01).
(#) Significant difference compared to the D-galactose + Angiotensin II receptor blocker group (p < 0.01).

3.2.3. Renal tissue SOD: Table (1) and figure (5)

Similar to serum SOD, renal tissue SOD was significantly lower in Group II (p<0.01) compared to control group. However, Group III and group IV showed a significant increase in renal tissue SOD (p<0.01) compared to group II which again proves the protective effect of DPP4 inhibitor and Ang II type 1 receptor blocker on kidney damage induced by D-galactose is through stimulating the antioxidant mechanisms. However, this protection is partial as the values of renal tissue SOD in groups III and IV still significantly lower than that of the control group (p<0.01).

The combination of the two drugs (group V) stimulates more antioxidant mechanisms as the value of renal tissue SOD in group V was significantly higher (p<0.01) when compared with its values in groups III and IV. However, the value of renal tissue SOD in group V still significantly lower (p<0.01) when compared with its values in group I indicating that the protection regarding renal tissue SOD is partial.

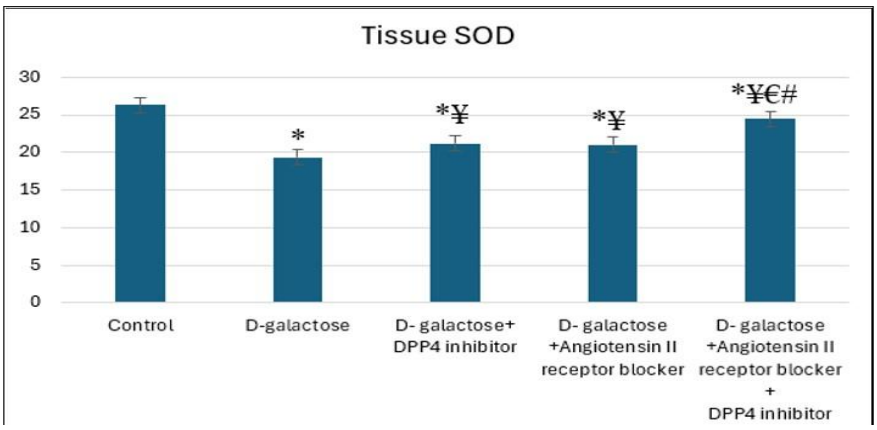

Figure 5: Effects of D-Galactose, DPP4 Inhibitor, and Angiotensin II Receptor Blocker on renal tissue SOD Level in D-galactose treated rats.

Data are represented as mean±SEM and analysis was done by one-way ANOVA followed by LSD post-hoc test at P < 0.01.
(*) Significant difference compared to the Control group (p < 0.01).
(¥) Significant difference compared to the D-galactose group (p < 0.01).
(€) Significant difference compared to the D-galactose + DPP-4 inhibitor group (p < 0.01).
(#) Significant difference compared to the D-galactose + Angiotensin II receptor blocker group (p < 0.01).

3.3-Histopathological changes of renal tissues: Figure (6).

H&E, stained kidney specimens of control group revealed normal renal architecture, glomerular size (yellow arrow) and tubular width (green arrow)**(Figure 6-a)** while rat received D-galactose (group II), renal specimens showed marked shrinkage of the glomerular tufts (yellow arrow), thickening of basement membrane, increase of Bowman's space and dilations of renal tubules (green arrow) **(Figure 6-b)**. However, rats which received D-galactose plus sitagliptin (group III) and those received losartan (group IV), their kidney specimens' examination revealed mild glomerular shrinkage **(Figure 6-c and 6-d respectively)**. On the other hand, rats which received D-galactose plus sitagliptin and losartan (group V) showed better results regarding their preservation of glomerular architecture (yellow arrow) and width of renal tubules (green arrow)**(Figure 6-e)**.

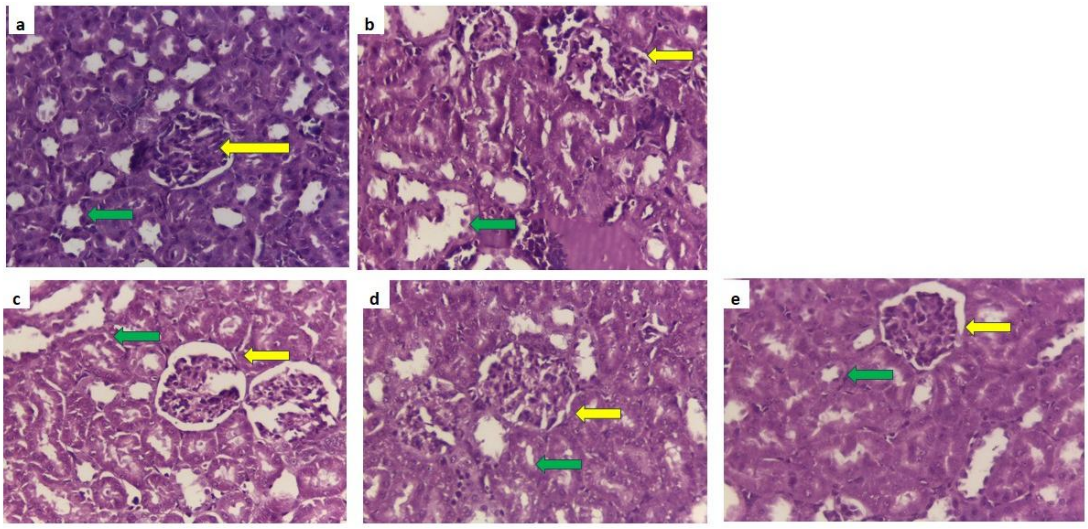

Figure (6) Light microscopy of H & E-stained renal specimens. (Magnification X400). **(a)** Control group, **(b)** D-galactose induced aging group, shrinking of the glomerular tufts (yellow arrow), and dilations of kidney tubules (green arrow), **(c)** D-galactose plus sitagliptin group, **(d)** D-galactose plus losartan group, both c and d groups showed mild disrupted renal architecture **(e)** D-galactose plus sitagliptin plus losartan group, revealed improvement of glomerular and tubular structures.

4.Discussion

Aging is a multifactorial physiological process stimulated by various stressors that can lead to accumulation of physiological and molecular deficits at different rates within different tissues and organs, leading to a gradual decrease in biological events. While aging itself does not directly induce kidney injury, it is inherently linked to physiological alterations that progressively impair renal function, reflecting the natural course of aging process. **[17]**

This work aims to assess the possible protective role of DPP-4 inhibitor and Ang II type 1 receptor blocker on kidney function in D-galactose induced aging in male rats and their possible synergistic action.

Our results find out that rats in which aging was induced by D-galactose showed a significant increase in serum creatinine level($p<0.01$) and a significant decrease in GFR measurements ($p<0.01$) compared with control group. These findings are consistent with those reported by

Glassock et al. [18],Hakimizadeh et al. [19], Marquez-Exposito et al. [20] andLan et al.[21].

Aging process involves a complex and multifaceted mechanism that remains incompletely understood. However, the accumulation of ROS has been proposed as a pivotal factor contributing to advancement of aging and the development of chronic degenerative conditions **[2],[3].** In our study the aged rats showed a significant increase in serum MDA($p<0.01$) and a significant decrease of both serum and tissue SOD which signifies a state of an imbalance between antioxidants and pro-oxidants attributed to the process of aging. In accordance with these results **Zbroch et al.[22]Harun et al. [23] and Wan et al. [24]**who reported increased levels of serum MDA in elderly patients which was attributed to aging, and this explains the elevation in oxidative stress and free radicals leading to cell damage and subsequent aging. Additionally, in support of these results **Marquez-Exposito et al.[20]** showed down-regulation of SOD gene expression in 12 months aged rats compared to 3 months aged ones. Also, **Yang et al. [25]** reported in their studies that D-galactose decreased the activities of SOD level in both serum and tissue.

Interestingly, histopathological analysis of renal tissues in rats which received D-galactose confirmed the renal impairment in the form of shrinkage of renal glomeruli, widening in renal tubules together with the appearance of thrown epithelium in their lumen. These findings are in agreement with those of **Fang et al. [26]** and **Marquez-Exposito et al.[20]**

Meanwhile rats receiving D-galactose and sitagliptin showed improved renal function, evidenced by a significant decrease in serum creatinine($p<0.01$) and a significant increase in GFR($p<0.01$). However, these values did not fully normalize, indicating partial recovery. Aligning with findings **Alam et al. [27]** showed the renoprotective effect of sitagliptin on rat model with unilateral stenosis of renal artery by clipping. Also **Ban et al.[28]** stated in their studies that DPP-4 inhibitors had a protective effect on kidneys in rat model of ischemia/reperfusion injury. However, **Kawanami et al. [29]** found that GFR didn't markedly differ in cardiac patients taking sitagliptin compared to those receiving placebo. This may be explained by the fact that the present study was carried out on rats while in humans there might be associated comorbidities that explain the difference in results.

Additionally, these rats (which were administered D-galactose plus a DPP4 inhibitor) exhibited a significant decrease in MDA levels ($p<0.01$) along with a significant increase in serum and tissue SOD levels ($p<0.01$) compared to the rats that received only D-galactose. However, these values did not reach the levels observed in the control group, indicating that the improvement was partial. These outcomes aligned with **Zhang et al. [30] and Guo et al. [17]** who reported the renoprotective effect of sitagliptin via an increase in antioxidant markers leading to attenuation of glomerular lesion. Also, **Liu et al.[31], Civantos et al.[32] and Wang et al.[33],** declared in their studies that sitagliptin (DPP4 inhibitor)decreased level of ROS in different tissues by decreasing of the expression of NOX4 (nicotinamide adenine dinucleotide phosphate oxidase 4) which is a major source of ROS. Moreover, **Chung, S., and Kim, G. H. [34]** reported in their study that DPP-4 inhibitors exhibit renoprotective effects in non-

diabetic renal disease. These protective mechanisms, which include anti-inflammatory and antioxidative actions, are mediated through the inhibition of NADPH activity.

Histological analysis further supported these observations, revealing mild glomerular shrinkage. This came in accordance with **Ren et al. [35]** who proved the ability of sitagliptin to decrease renal tubulointerstitial fibrosis**.** Conversely, **Sarker et al. [36]** observed a notable reduction in glomerular size in DPP4-deficient rats, whereas an enlargement was reported in diabetic rats. The observed divergence in results may be attributable to heterogeneity in the underlying progression and severity of renal pathology inherent to the distinct animal models utilized in the investigations.

As regards the group of rats which received D-galactose plus Ang II type 1 receptor blocker (losartan), the administration of Ang II type 1 receptor blocker is proven to have a protective effect on kidney against the hazardous effect of D-galactose on renal tissues. This is exhibited by significant decrease in serum creatinine level ($p<0.01$)and a significant increase in GFR values($p<0.01$)when compared with that of rats which received D-galactose only, however these values did not reach the values of control group which indicates that improvement was partial. These findings are consistent with **MOETY et al.[37]** and **Rodriguez-Romo et al. [38].**On the other hand, **Schmidt et al. [39] and Ohkuma et al. [40]** found an increase in serum creatinine level (by 30% or more) after the start of angiotensin converting enzyme inhibitor/angiotensin receptor blocker treatment in elder patients with cardio-renal comorbidity.

Compared to those treated with D-galactose alone, the administration of an Ang II type 1 receptor blocker significantly reduced MDA levels ($p<0.01$)while markedly made a significant increase in serum and tissue SOD levels. These findings align with those of **Rincón et al. [41]** and **MOETY et al. [37]**, who reported a marked enhancement in antioxidant capacity of renal tissue in groups treated with Ang II type 1 receptor blockers. This improvement was evidenced by a significant elevation in serum SOD levels accompanied by a notable reduction in serum MDA levels.

Interestingly, all these effects of Ang II type 1 receptor blocker were confirmed by improvement in the histopathological characteristics, examination of kidney specimens showed better result regarding their preservation of glomerular architecture and width of renal tubules. This came in accordance with **MOETY et al.[37]** who proved the protective power of Ang II type 1 receptor blocker in maintaining the normal physiological function of podocytes in rat model with diabetic nephropathy through its inhibitory effects on RAAS and ROS and **Rodriguez-Romo et al. [38]**who proved the protective effect of this blocker on preserving renal architecture before ligation of renal arteries.

Interestingly, the result of present study showed that the combination of DPP-4 inhibitor with Ang II type 1 receptor blocker produced a greater improvement in renal functions as provided by making a significant reduction in values of serum creatinine levels ($p<0.01$)and a significant increase in GFR measures in group V when compared with aged rats which received DPP-4 alone or angiotensin blocker alone. Also, the

combined therapy produced more protection against oxidative stress damage provided by a significant decrease in MDA level($p<0.01$)along with a significant increase in serum and tissue SOD level($p<0.01$) in group V when compared with groups of aged rats which received each drug alone. These results were not beyond expectations since increased activity of DPP-4 and RAAS activation are age-related causes of renal injury, and both can lead to CKD. In this context, **Qiu et al. [42]** demonstrated in their study that the combination therapy of a DPP-4 inhibitor and an Ang II type 1 receptor blocker offers greater benefits compared to monotherapy with either agent alone. This finding aligns with the more pronounced reduction in proteinuria and the slower decline in GFR observed in patients with diabetic nephropathy over a two-year follow-up period. It has to be noted that, in our results, combined therapy results in nearly complete improvement in three parameters (reduced GFR, elevated serum MDA and reduced serum SOD as there is no significant difference between their values in group V and control group while in the other two parameters (Serum creatinine and SOD in renal tissue) the improvement is only partial because their values in group V still significantly different from control group.

Conclusion

This study demonstrates that the combination therapy of DPP-4 inhibitors and Ang II type 1 receptor blocker is an appropriate candidate for treating renal injury resulting from aging which may be attributed to its antioxidant effect. Enhancement of antioxidant defense mechanism acts as a barrier against progression of renal deterioration as a result of aging process.

Some limitations to our study and recommendations:

1) We used a single dose of either DPP-4 inhibitors or Ang II type 1 receptor blocker. However, it is much better to use gradually increasing doses to prove the effect is dose dependent and to find out the optimum dose that gives the maximum effect.
2) This investigation demonstrated that the combination therapy of both DPP-4 inhibitors or Ang II type 1 receptor blocker leads nearly complete improvement in some parameters (namely GFR, elevated serum MDA and reduced serum SOD) while in other parameters it leads only to partial improvement (namely serum creatinine and SOD in renal tissue). More studies are needed to explain this. Would a higher dose more than the dose we used will lead to nearly complete improvement in all parameters or there are other explanations.
3) Are there any potential off-target effects of DPP-4 inhibitors or Ang II blockers in aging kidneys?
4) Could fibrosis markers (e.g., TGF-β, collagen deposition) provide additional insights?

Author contributions:

All authors contribute equally to the study design, collection of samples, data analysis, and manuscript writing.

Conflict of interest:

None

Funding:

None

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