

Ameliorative Effects of *Ginkgo biloba* Extract on Adenine-Induced Chronic Kidney Disease Model in Male Rats

Amna Shahzadi^{1*}, Fariha Mehmood^{1*}, Saad Abdullah Khan^{1**}, Sajid Khan Tahir¹, Hafiz Faseeh-ur-Rehman²,
Muhammad Imran³, Khalid Abdul Majeed¹

¹Department of Physiology, University of Veterinary and Animal Sciences, Lahore, 54000, Pakistan

²Department of Anatomy and Histology, University of Veterinary and Animal Sciences, Lahore, 54000, Pakistan

³Institute of Biochemistry and Biotechnology, University of Veterinary and Animal Sciences, Lahore, 54000, Pakistan

*These authors contributed equally.

°Deceased

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*Corresponding Author

Dr. Khalid Abdul Majeed,
Department of Physiology, University
of Veterinary and Animal Sciences,
Lahore, 54000, Pakistan,
email: khalid.majeed@gmail.com

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ABSTRACT

Background - Chronic kidney disease (CKD) is an evolving universal health concern with a high prevalence as well as growing morbidity and mortality rates. The present study aims to elucidate the ameliorative effects of *Ginkgo biloba* extract (GBE) in a rat model of adenine-induced CKD. **Methods** - Twenty male albino rats were divided into 4 treatment groups: (A) control, (B) adenine, (C) GBE 1g/kg diet (0.1%), and (D) GBE 2g/kg diet (0.2%). Nutritional determinants, anthropometry, relative organ weights, bone morphometry, serum biochemistry, renal oxidative stress status, and histological parameters were evaluated during the study. **Results** - *Ginkgo biloba* extract was found to effectively attenuate the adenine-induced increase in relative kidney weight and reduction in femur and tibia lengths as well as robusticity index. Increase in serum concentrations of renal health biomarkers, including uric acid, urea nitrogen, and creatinine, as well as hepatic health markers such as alanine and aspartate aminotransferase, gamma-glutamyltransferase, and lactate dehydrogenase induced by adenine was also attenuated by GBE. Additionally, GBE helped in the reversal of renal oxidative stress by restoration of catalase (CAT), in addition to restoration of serum electrolytes that include chloride and bicarbonate. Histological examination of renal and hepatic tissues further verified the beneficial role of GBE. **Conclusions** - GBE has the potential to alleviate adenine-induced CKD and encourages further investigations to optimize its clinical implementation in CKD management.

ONE HEALTH PERSPECTIVE

Chronic kidney disease (CKD) is an important health burden affecting both humans and animals, with several conserved pathological mechanisms, including oxidative stress, inflammation, renal injury, and fibrosis. The present rat model provides insight into these shared mechanisms and evaluates the potential protective effects of *Ginkgo biloba* extract (EGb761). Thus, the findings may contribute to a better understanding of CKD across species and support further translational research in human and animal health.

1. INTRODUCTION

Chronic kidney disease (CKD) is a global health burden, with a rise of 40% in its prevalence and incidence over the past three decades (Xie *et al.*, 2025). Greater than 10% of the general population is affected by the disease worldwide (Kovesdy, 2022). In Pakistan, CKD prevalence is reported to be 12.5-29.9% (Khan *et al.*, 2024). Chronic kidney disease, a condition characterized by progressive loss of renal function, is one of the leading causes of morbidity and mortality worldwide (Mark *et al.*, 2025). An increased number of adverse health outcomes are associated with the disease; the most obvious one is end-stage renal disease (ESRD), which demands uneconomical

procedures, i.e., dialysis and renal transplantation (Lucas and Taal, 2023). Slowing disease progression via effective and economically friendly strategies and thereby reducing the risk of morbidity and mortality has been the subject matter for strategy developers. Rapidly increasing prevalence, morbidity, and mortality elucidate the inadequacy of existing therapeutic approaches. Therefore, studies directed towards the identification of novel remedies are the need of the hour.

Herbs, due to their limited side effect profile as well as potential alleviating traits, have gained significant attention as an alternative treatment for several ailments. In recent years, increasing attention has been given to the potential role of herbal medicines in the prevention and management of CKD (Chen *et al.*, 2021). *Ginkgo biloba*, having a clinical application history of years, is well-known for its therapeutic potential in a variety of health issues such as cardiovascular, neurological, and age-related problems (Chávez-Morales *et al.*, 2017). Various studies have illustrated the nephroprotective role of *Ginkgo biloba* extract (GBE) (Chávez-Morales *et al.*, 2017; Wang *et al.*, 2020; Zhang *et al.*, 2025). *Ginkgo biloba* extract consists mainly of flavonoids and terpenoids, responsible for its antioxidant and anti-inflammatory potential, in a concentration of 24 % and 6%, respectively (Biernacka *et al.*, 2023). One of the critical contributors to the medicinal potency of *Ginkgo biloba* is its capability to scavenge free radical entities (Das *et al.*, 2022). This study aims to investigate the ameliorative potential of GBE at different doses in a rat model of adenine-induced CKD.

2. MATERIALS AND METHODS

2.1. Experimental Animals

Twenty adult male Sprague-Dawley rats (weighing 130-150g), used in the present study, were housed in standard wire mesh cages (Natsume Seisakusho Co. Ltd., Japan) having wood chip bedding under standard laboratory conditions (alternate 12-hour light/dark cycle, temperature 22±3°C and relative humidity around 60%) at experimental animal house, Department of Physiology, University of Veterinary and Animal Sciences (UVAS), Lahore. Animals had free access to standard pelleted rat chow (nutritional components % by weight: dietary casein 20.5; glucose 20; cellulose 6; cornstarch 39.7; minerals 7; vitamins 1; sunflower oil 5.8) and tap water.

2.2. Experimental Groups

Following an acclimatization period of 1 week, animals were randomly divided into four equal groups (5/group) and treated for a duration of 35 days as follows; (A) offered standard pelleted rat chow throughout the study duration (control), (B) given adenine 5g/kg diet for 3 consecutive weeks and standard pelleted rat chow for another 2 weeks (adenine), (C) offered adenine 5g/kg diet for 3 consecutive weeks and afterwards, *Ginkgo biloba* extract @ 1g/kg diet for an interval of 2 weeks (GBE 0.1%), and (D) treated similarly to group C except for dose of *Ginkgo biloba* extract that was increased to 2g/kg diet (GBE 0.2%).

Adenine (A00684, Lot#C10492151, CAS:73-24-5) from Shanghai Macklin Biochemical Co., Ltd., and *Ginkgo biloba* extract (EGb761) from Hunan Nutramax Inc. were procured. The doses of adenine and *Ginkgo biloba* extract were chosen based on literature analysis (Claramunt *et al.*, 2015; Mansoor *et al.*, 2015; Abd-Eldayem *et al.*, 2016; Diwan *et al.*, 2018).

2.3. Nutritional Determinants, Anthropometric Measurements, and Relative Organ Weights

Feed intake (g/day), energy intake (kJ/day), and water intake (ml/day) were recorded daily. Evaluation of body weight, body length, body mass index (BMI), abdominal circumference (AC), thoracic circumference (TC), and abdominal circumference to thoracic circumference (AC/TC) ratio were carried out once a week. All the measurements and calculations were carried out as described previously (Majeed *et al.* 2017).

2.4. Bone Morphometry

Weight (mg) and length (mm) of bones (femur and tibia), collected at the end of the study, were measured with the help of a digital weighing balance and digital vernier caliper, respectively. Other parameters that include bone robusticity (mm^3/mg) and weight/length (mg/mm) indices were assessed as reported previously (Monteagudo *et al.*, 1997).

2.5. Serum Biochemical Analysis

Blood was collected at the end of the study through cardiac puncture. The blood was centrifuged at 3000 RPM to harvest the serum that was stored at -20°C until analysis. Commercial kits (Human Diagnostics, Wiesbaden, Germany) were used to determine serum concentrations of uric acid, blood urea nitrogen, creatinine, alkaline phosphatase, lactate dehydrogenase, gamma-glutamyltransferase, sodium, potassium, chloride, and bicarbonate. Alanine aminotransferase and aspartate aminotransferase were measured using commercial kits (Diasys Diagnostic Systems GmbH, Holzheim, Germany), while creatine kinase was measured using a commercial kit (Innoline, Merck, France). Absorbance measurements were performed using an Epoch™ microplate spectrophotometer (Biotek Instruments Inc., Winooski, USA).

2.6. Oxidative Stress Markers

Homogenization of the renal tissue samples in saline and then centrifugation at 3000 RPM for a duration of 10 minutes, while keeping the temperature at 4°C, was carried out. Thereafter, the supernatant was obtained and used to measure catalase (CAT) activity as reported previously (Linares *et al.*, 2009).

2.7. Histological Examination

H and E-stained 5 µm thick renal and hepatic tissue sections were examined under a light microscope, and detailed photomicrographs for thorough assessment were captured. Protocols for histomorphometric analysis of both tissues were in accordance with the prior studies (Salman *et al.*, 2019).

2.8. Statistical Analysis

Data were analyzed using One-way analysis of variance (ANOVA) in SPSS software (SPSS Inc., Chicago, USA, Version 20.0) and presented as mean ± standard error. Tukey's multiple-range post hoc test was applied to find out the differences between the groups. A probability value of less than 0.05 was regarded as statistically significant.

3. RESULTS

3.1. Nutritional Determinants, Anthropometric Measurements, and Relative Organ Weights

The effects of adenine and *Ginkgo biloba* extract (GBE) on nutritional determinants, anthropometric measurements, and relative organ weights are shown in Table 1. Among the study groups, no difference ($p = 0.994$) was observed in the feed and energy intake. An increase ($p = 0.024$) in water intake in the GBE 0.1% group was observed compared to the control group. Body length of all the study groups was found to be statistically comparable ($p = 0.121$). However, body weight and body mass index (BMI) of the adenine group were reduced ($p < 0.001$; $p = 0.002$, respectively) compared to the control group. Though the thoracic circumference of all the study groups was similar ($p = 0.184$), the GBE 0.1% as well as GBE 0.2% groups showed reduced ($p = 0.002$) abdominal circumference compared to the control group. A reduction ($p = 0.026$) in the AC/TC ratio of GBE 0.1% rats compared to the control group was discerned as well. An increase in relative kidney weight ($p < 0.001$) was observed in adenine-treated animals compared to the control group. GBE 0.1% effectively attenuated the increase in the relative kidney weight induced by adenine treatment. Relative weights of the spleen, heart, and testis were found to be statistically comparable among all the study groups. Rats in the GBE 0.2% group showed an increase in relative liver weight ($p = 0.004$) compared with the control and adenine groups. No significant difference was found in the relative liver weights of the GBE 0.1% group compared to the other three study groups.

Table 1. Effects of adenine and *Ginkgo biloba* extract on nutritional determinants, anthropometry and relative organ weights (%) in male rats.

Parameters	Control	Adenine	GBE 0.1%	GBE 0.2%	p-value
Feed intake (g/day)	30 ± 4	31 ± 7	32 ± 7	31 ± 7	0.994
Energy intake (kJ/day)	350 ± 47	364 ± 81	380 ± 81	359 ± 82	0.994
Water intake (ml/day)	35 ± 6 ^b	40 ± 2 ^{ab}	51 ± 2 ^a	44 ± 3 ^{ab}	0.024
Body weight (g)	284 ± 12 ^a	202 ± 8 ^b	207 ± 14 ^b	210 ± 7 ^b	<0.001
Body length (cm)	19.70 ± 0.25	19.10 ± 0.18	19.20 ± 0.12	19.20 ± 0.12	0.121
BMI (g/cm ²)	0.73 ± 0.03 ^a	0.55 ± 0.01 ^b	0.56 ± 0.03 ^b	0.57 ± 0.01 ^b	0.002
Abdominal circumference (cm)	13.3 ± 0.25 ^a	12.4 ± 0.24 ^{ab}	11.4 ± 0.40 ^b	11.9 ± 0.18 ^b	0.002
Thoracic circumference (cm)	12.3 ± 0.12	11.8 ± 0.20	11.5 ± 0.31	11.9 ± 0.29	0.184
AC/TC ratio	1.08 ± 0.01 ^a	1.05 ± 0.01 ^{ab}	0.99 ± 0.02 ^b	1.00 ± 0.02 ^{ab}	0.026
Kidney	0.66 ± 0.01 ^c	1.19 ± 0.08 ^a	0.86 ± 0.01 ^c	1.01 ± 0.07 ^{ab}	<0.001
Liver	2.82 ± 0.12 ^b	2.54 ± 0.12 ^b	3.02 ± 0.15 ^{ab}	3.37 ± 0.13 ^a	0.004
Spleen	0.23 ± 0.02	0.29 ± 0.04	0.20 ± 0.01	0.23 ± 0.01	0.071
Heart	0.43 ± 0.08	0.33 ± 0.01	0.34 ± 0.02	0.36 ± 0.04	0.508
Testis	1.37 ± 0.09	1.56 ± 0.12	1.34 ± 0.07	1.50 ± 0.07	0.346

Data are presented as mean ± standard error. Within the row, lowercase letters a, b, and c are indicative of significant differences ($p < 0.05$).

3.2. Bone Morphometry

There were no differences ($p = 0.207$) in the femur weight of rats from different groups. However, the adenine treatment resulted in decreased ($p = 0.008$) femur length compared with the control group. A decrease ($p = 0.002$) in the femur robusticity index of the adenine group compared to the control group was also observed. However, rats consuming a *Ginkgo biloba*-supplemented diet did not show any significant difference in their femur robusticity index compared to the control group. It was found that the adenine treatment did not alter the femur weight/length index compared to the control group. No significant changes ($p = 0.200$) in tibia weight among the groups were observed. Adenine treatment induced a reduction ($p = 0.004$) in tibia length compared to the control group. Tibia robusticity index was also reduced ($p = 0.002$) in the adenine group compared to the control group. However, tibia weight/length index was statistically comparable ($p = 0.059$) among the groups (Table 2).

Table 2. Effects of adenine and *Ginkgo biloba* extract on bone morphometry in male rats.

Parameters	Control	Adenine	GBE 0.1%	GBE 0.2%	p-value
Femur weight (mg)	495 ± 20	450 ± 10	465 ± 5	435 ± 15	0.207
Femur length (mm)	34 ± 0.57 ^a	29 ± 0.46 ^b	33 ± 1.12 ^a	33 ± 0.36 ^a	0.008
Femur robusticity index (mm/ ³ √mg)	4.30 ± 0.02 ^a	3.75 ± 0.03 ^b	4.31 ± 0.12 ^a	4.34 ± 0.02 ^a	0.002
Femur weight/length index (mg/mm)	15 ± 0.38 ^{ab}	16 ± 0.10 ^a	14 ± 0.31 ^{ab}	13 ± 0.31 ^b	0.032
Tibia weight (mg)	390 ± 20	365 ± 5	340 ± 15.80	358 ± 10.10	0.200
Tibia length (mm)	38 ± 0.63 ^a	34 ± 0.45 ^b	37 ± 0.54 ^a	36 ± 0.35 ^a	0.004
Tibia robusticity index (mm/ ³ √mg)	5.21 ± 0.07 ^a	4.70 ± 0.04 ^b	5.31 ± 0.06 ^a	5.13 ± 0.06 ^a	0.002
Tibia weight/length index (mg/mm)	10.23 ± 0.43	10.84 ± 0.10	9.16 ± 0.35	9.82 ± 0.28	0.059

Data are presented as mean ± standard error. Within the row, lowercase letters a and b are indicative of significant differences ($p < 0.05$).

3.3. Serum Renal and Hepatic Biochemicals

A rise ($p < 0.001$) in serum uric acid, blood urea nitrogen, and creatinine concentrations of adenine-treated rats compared to the control ones was observed. Both doses of GBE made a notable contribution in bringing back these elevated parameters to near-control group levels. The adenine group showed an elevation ($p = 0.009$) in serum alanine transaminase compared to the control group. However, aspartate transaminase levels, though high, were not different ($p = 0.150$) from those of the control group. There were no significant differences in the serum levels of alanine and aspartate transaminases of rats receiving diet supplemented with GBE compared to the control group rats. Serum alkaline phosphatase and creatine kinase levels were found to be non-significantly different ($p = 0.995$) and ($p = 0.627$), respectively, among the groups. Adenine exposure resulted in an elevation ($p < 0.001$) in serum gamma-glutamyltransferase and lactate dehydrogenase compared to the control group. *Ginkgo biloba* supplementation significantly ameliorated the alterations in these serum biochemical indices (Table 3).

Table 3. Effects of adenine and *Ginkgo biloba* extract on serum renal and hepatic biochemical parameters in male rats.

Parameters	Control	Adenine	GBE 0.1%	GBE 0.2%	p-value
Uric acid (mg/dl)	5.40 ± 0.09 ^d	9.10 ± 0.13 ^a	6.14 ± 0.06 ^c	6.73 ± 0.07 ^b	<0.001
Blood urea nitrogen (mg/dl)	23 ± 0.59 ^d	95 ± 2.36 ^a	64 ± 4.28 ^c	78 ± 3.47 ^b	<0.001
Creatinine (mg/dl)	0.77 ± 0.04 ^c	2.14 ± 0.09 ^a	0.89 ± 0.03 ^c	1.83 ± 0.08 ^b	<0.001
Alanine aminotransferase (U/L)	30 ± 0.34 ^b	35 ± 2.37 ^a	29 ± 0.35 ^b	29 ± 0.17 ^b	0.009
Aspartate aminotransferase (U/L)	57 ± 1.80	79 ± 14.70	58 ± 0.93	57 ± 0.75	0.150

Alkaline phosphatase (U/L)	152 ± 8.46	152 ± 8.63	152 ± 8.61	152 ± 8.45	0.995
Creatine kinase (U/L)	438 ± 11	432 ± 4	428 ± 15	445 ± 5	0.627
Gamma-glutamyl transferase (U/L)	3.04 ± 0.32 ^c	6.43 ± 0.56 ^a	4.08 ± 0.33 ^b	3.03 ± 0.28 ^c	<0.001
Lactate dehydrogenase (U/L)	485 ± 4 ^c	546 ± 4 ^a	469 ± 5 ^c	512 ± 3 ^b	<0.001

Data are presented as mean ± standard error. Within the row, lowercase letters a, b, c and d are indicative of significant differences ($p < 0.05$).

3.4. Serum Electrolytes

All four groups showed no statistically significant differences in serum sodium ($p = 0.890$) or potassium ($p = 0.070$) levels. However, compared to the control group, serum chloride ($p = 0.029$) and bicarbonates ($p = 0.014$) were significantly different in rats receiving adenine only. GBE 0.1% and 0.2% efficiently attenuated the abnormalities (Table 4).

Table 4. Effects of adenine and *Ginkgo biloba* extract on serum electrolytes in male rats.

Parameters (mmol/L)	Control	Adenine	GBE 0.1%	GBE 0.2%	<i>p</i> -value
Sodium	147 ± 1.15	147 ± 1.41	147 ± 0.92	148 ± 1.53	0.890
Potassium	4.58 ± 0.08	4.90 ± 0.08	4.88 ± 0.059	4.98 ± 0.159	0.070
Chloride	103 ± 0.66 ^b	112 ± 0.58 ^a	108 ± 0.66 ^{ab}	107 ± 3.76 ^{ab}	0.029
Bicarbonate	31 ± 0.40 ^a	27 ± 0.58 ^b	28 ± 0.58 ^{ab}	28 ± 1.00 ^{ab}	0.014

Data are presented as mean ± standard error. Within the row, lowercase letters a and b are indicative of significant differences ($p < 0.05$).

3.5. Renal Oxidative Status

The adenine group displayed a reduction ($p = <0.001$) in renal catalase activity compared to the control group animals. Treatment with GBE at both doses resulted in a remarkable recovery of renal CAT activity (Table 5).

Table 5. Effects of adenine and *Ginkgo biloba* extract on catalase in renal tissue in male rats.

Parameter	Control	Adenine	GBE 0.1%	GBE 0.2%	<i>p</i> -value
Catalase	67 ± 3.70 ^a	43 ± 2.90 ^b	66 ± 3.61 ^a	67 ± 3.06 ^a	<0.001

(Units/g tissue)

Data are presented as mean ± standard error. Within the row, lowercase letters a and b are indicative of significant differences ($p < 0.05$).

3.6. Renal Histological Parameters

For histomorphometric analysis, several cortical parameters were studied using H and E-stained renal sections (Table 6). An increase ($p = <0.001$) was observed in cortical thickness, renal corpuscular, mesangial tuft diameter, capsular space width, and parietal layer thickness of adenine rats compared to the control ones. In addition, the adenine group revealed a decrease ($p = <0.001$) in glomerular number compared to the control group. *Ginkgo biloba* 0.1% was found to effectively shift the reported indices towards the control ones. Apart from capsular space width and parietal layer thickness, GBE 0.2% performed similarly to GBE 0.1% in this regard. Histological examination of

the control group exhibited intact renal architecture with no obvious pathological change (Figure 1A). On the other hand, marked renal structural injuries characterized by tubular dilatation, proximal convoluted tubules dilatation with destroyed brush border, irregular Bowman's space, and fewer glomeruli were observed in rats subjected to adenine treatment alone (Figure 1B). Renal architecture was comparable to that of the control group, with mild cellular infiltration observed in the GBE 0.1% group and slight tubular dilatation in the GBE 0.2% group (Figure 1C and D).

Table 6. Effects of adenine and *Ginkgo biloba* extract on renal histological parameters in male rats.

Parameters (μm)	Control	Adenine	GBE 0.1%	GBE 0.2%	<i>p</i> -value
Cortical thickness	820 ± 12^b	889 ± 10^a	832 ± 12^b	841 ± 16^b	0.001
Renal corpuscular diameter	87 ± 1.24^b	92 ± 1.28^a	84 ± 0.97^b	87 ± 1.31^{ab}	<0.001
Mesangial tuft diameter	72 ± 1.14^b	76 ± 0.75^a	69 ± 0.95^b	69 ± 0.91^b	<0.001
Capsular space width	10 ± 0.14^b	14 ± 0.27^a	11 ± 0.13^b	13 ± 0.26^a	<0.001
Parietal layer thickness	1.96 ± 0.04^{ab}	2.11 ± 0.04^a	1.95 ± 0.04^b	2.10 ± 0.04^a	0.005
Glomerular number (/3mm ²)	14 ± 0.92^a	9 ± 0.86^b	15 ± 0.51^a	14 ± 1.05^a	<0.001

Data are presented as mean $\pm$ standard error. Within the row, lowercase letters a and b are indicative of significant differences ($p < 0.05$).

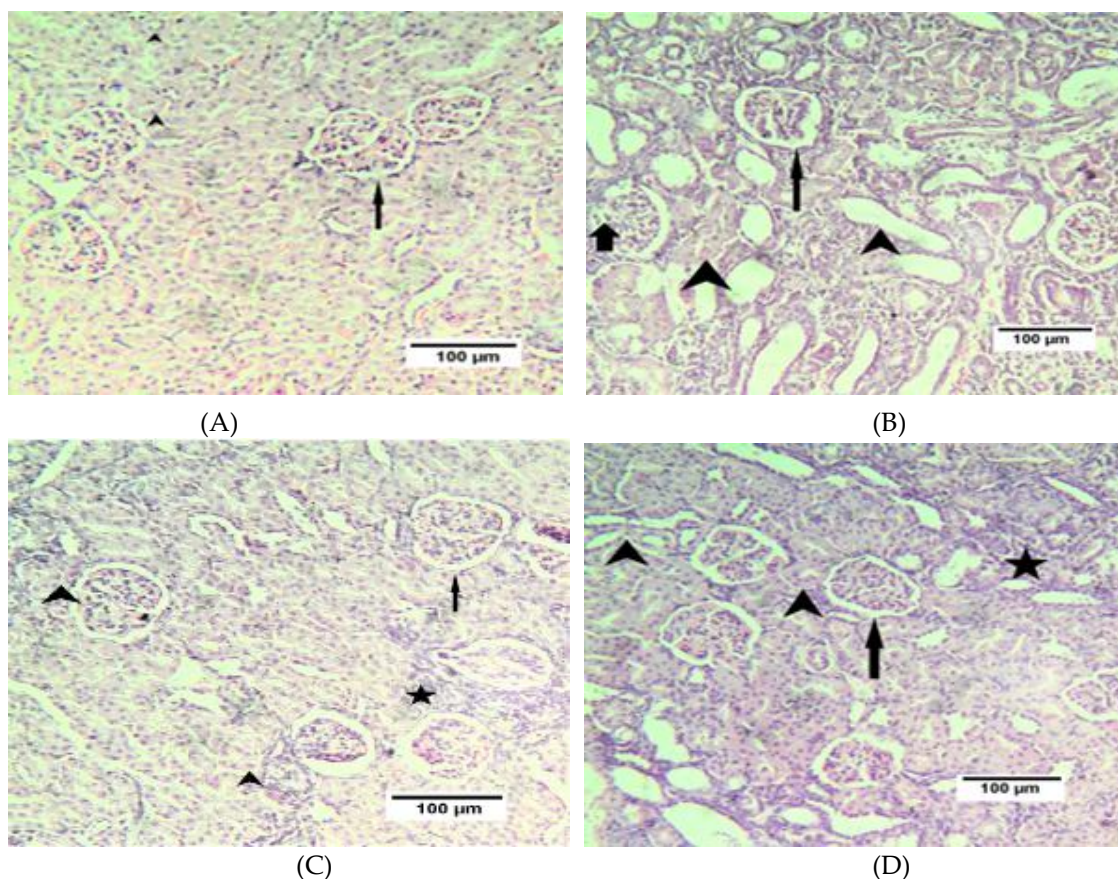


Figure 1. Histomicrograms of rat renal tissue (H and E 100x). A: Control; B: Adenine; C: GBE 0.1%; D: GBE 0.2%. Long arrows (corpuscle); Short arrows (mesangial tuft); Arrowheads (convoluted tubules); Asterisks (cellular infiltrations).

3.7. Hepatic Histological Parameters

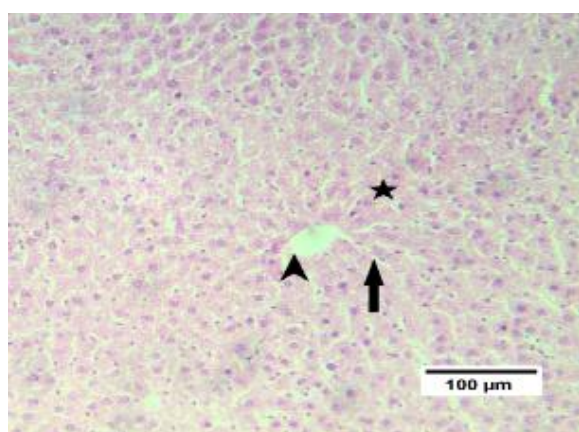
Histomorphometric examination of the liver revealed clear structural differences among the studied groups (Table 7). In contrast to the control group, central vein and sinusoidal dilatation along with decreased hepatocyte width were observed in rats receiving adenine ($p < 0.001$). Histomorphometric observations were similar among the GBE-treated rats and the control group, thereby reflecting the ameliorative role of the supplement.

Histopathological examination of the liver sections of the adenine group exhibited pronounced structural disorganization defined by hepatocyte necrosis with hepatic cord degeneration, central vein congestion, and sinusoidal dilatation (Figure 2B). Normal hepatic architecture with hepatocytes radially arranged around the central vein without any congestion or dilatation was observed in the control group (Figure 2A). Hepatic structural organization in GBE-treated rats was comparable to the control except for slight central vein congestion (Figure 2C and D).

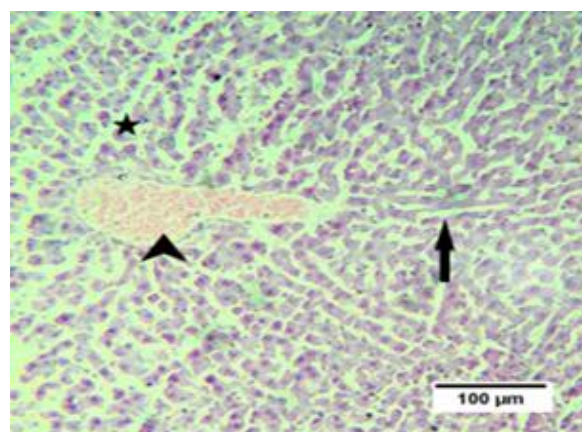
Table 7. Effects of adenine and *Ginkgo biloba* extract on hepatic histological parameters in male rats.

Parameters (μm)	Control	Adenine	GBE 0.1%	GBE 0.2%	<i>p</i> -value
Central vein long diameter	59 ± 3.06^b	84 ± 7.95^a	73 ± 7.93^{ab}	61 ± 5.37^b	0.006
Central vein short diameter	42 ± 1.64^b	56 ± 1.62^a	50 ± 3.08^{ab}	44 ± 2.48^b	0.001
Hepatocytes width	14 ± 0.18^a	11 ± 0.20^b	14 ± 0.19^a	14 ± 0.28^a	<0.001
Sinusoids short diameter (max)	7 ± 0.19^b	12 ± 0.48^a	8 ± 0.23^b	7 ± 0.20^b	<0.001
Sinusoids short diameter (min)	4 ± 0.12^b	7 ± 0.28^a	4 ± 0.12^b	4 ± 0.15^b	<0.001

Data are presented as mean $\pm$ standard error. Within the row, lowercase letters a and b are indicative of significant differences ($p < 0.05$).



(A)



(B)

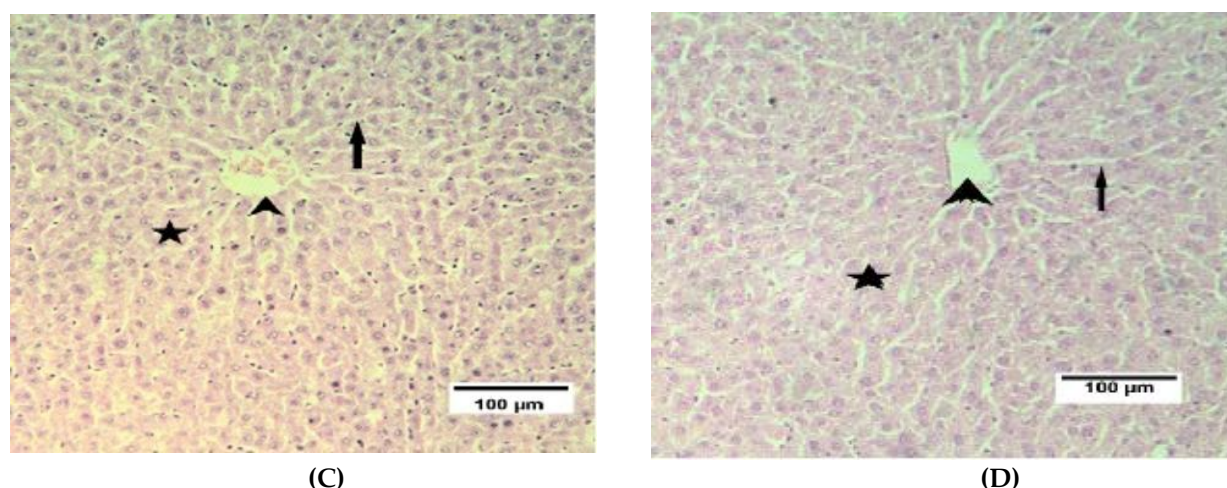


Figure 2. Histomicrograms of rat hepatic tissue (H and E 100x). A: Control; B: Adenine; C: GBE 0.1%; D: GBE 0.2%. Arrows (sinusoids); Arrowheads (central vein); Asterisks (hepatocytes).

4. DISCUSSION

Although Ginkgo biloba extract has demonstrated nephroprotective effects in several experimental models of renal injury (Chávez-Morales *et al.*, 2017; Abdel-Zaher *et al.*, 2017; Sherif *et al.*, 2019), evidence regarding its effects specifically against adenine-induced chronic kidney disease remains limited. Given this, our study was designed to elucidate the ameliorative effects of GBE in a rat model of adenine-induced CKD. Because of limitations associated with other developed animal models, the rodent (rat) model of adenine-induced CKD has been recognized as an authentic model to understand human CKD by many authors (Al Za'abi *et al.*, 2018; Diwan *et al.*, 2018; Yang *et al.*, 2024).

In the present study, adenine treatment did not influence feed and energy intake. Our data on feed intake are consistent with previous reports (Nguy *et al.*, 2012; Ali *et al.*, 2015). No statistically significant difference in water intake was observed between the adenine-treated and control groups, although water intake was numerically higher in the adenine group. Several studies have reported significantly increased water intake in rats treated with adenine (Nguy *et al.*, 2016; Al Za'abi *et al.*, 2018; Zhu *et al.*, 2018). Feed intake of the GBE 0.1% group, though not significantly, was increased compared to the control, and increased water intake of the GBE 0.1% group might be due to this increase in feed intake. Body weight as well as body mass index of the adenine group were significantly reduced compared to the control group. A significant reduction in body weight of rats treated with adenine is also evident from the previous work (Ali *et al.*, 2018; Al Za'abi *et al.*, 2018). Ginkgo biloba extract at either dose failed to attenuate these parameters. This behavior of Ginkgo biloba might be attributed to its role as an anti-obesity agent, possibly due to its lipolytic activity (Aziz *et al.*, 2018; Hirata *et al.*, 2019a). Significant reduction in abdominal circumference of GBE 0.1% as well as GBE 0.2% groups compared to the control group rats can be explained by the fact that Ginkgo biloba has a role in the reduction of retroperitoneal fat pads and thereby visceral adiposity (Hirata *et al.*, 2019b).

In our study, a significant increase in relative kidney weight of adenine-fed animals, indicative of renal enlargement attributable to damaged renal architecture in addition to functional disability, compared to control ones, is supported by other authors (Thakur *et al.*, 2018; Abdelrahman *et al.*, 2025; Baro *et al.*, 2026). Ginkgo biloba extract 0.1% effectively attenuated the increase in relative kidney weight induced by adenine treatment. A significant increase was found in relative liver weight of the GBE 0.2% group when compared to the control and adenine groups. Although the liver histology and most hepatic biochemical parameters did not indicate marked hepatocellular injury in this group, the increase in relative liver weight at the higher GBE dose may represent a dose-related adaptive response or a potential adverse effect. Therefore, this finding warrants further investigation, particularly through dedicated dose-response and toxicological studies. A significant reduction in femur and tibia lengths, as well as in the robusticity index, was observed in the adenine group. The reduction in bone length may not have resulted from a direct effect of adenine on the skeleton but rather from compromised overall growth in the adenine-treated rats. Notably, this growth-related alteration was not observed in either of the GBE-treated groups. Nevertheless, as the present study was not specifically designed to assess skeletal growth or bone metabolism, the mechanisms underlying

these findings cannot be established from the current data and warrant further investigation. The femur weight/length index of the GBE 0.2% group was found to be statistically lower compared to the adenine group. The reason for this is unclear and demands further evaluation. No relevant studies have been reported to compare our results regarding bone morphometry.

Renal status evaluation via common indicators, blood urea nitrogen, creatinine, and uric acid, was carried out. Adenine-induced nephrotoxicity resulted in a significant increase in blood urea nitrogen, uric acid, and creatinine levels, and this is in harmony with other studies (Abdelrahman *et al.*, 2025; Baro *et al.*, 2026). An increase in blood urea nitrogen and creatinine generally indicates filtration insufficiency, whereas raised uric acid levels are possibly due to alterations in metabolic handling as well as renal excretion. A noticeable improvement in renal function indicators, suggesting restoration of renal health, was brought about by GBE treatment (Sherif *et al.*, 2019; Sndos and Al-Amri, 2019). It was noticed that following GBE administration, adenine-induced disturbances in alanine and aspartate transaminases were considerably improved. Adenine-induced rise in liver enzymes (alanine transaminase and aspartate transaminase) has been reported earlier (Al Za'abi *et al.*, 2017). The elevation of hepatic enzymatic activity is suggestive of hepatic dysfunction possibly due to renal impairment. *Ginkgo biloba's* role in restoration of alanine and aspartate transaminases is evident from the work of others (Pu *et al.*, 2024). *Ginkgo biloba* administration also triggered a remarkable correction regarding elevation of gamma-glutamyltransferase and lactate dehydrogenase, in this case suggestive of hepatic tissue damage in association with compromised renal function, because of adenine intoxication. As kidneys play a crucial role in maintaining serum electrolyte homeostasis, assessment of renal status by evaluation of an electrolyte panel was also performed. It was found that GBE helps in returning the changes in serum chloride and bicarbonate levels produced by adenine. The data thus demonstrate renal recovery as a regulator of electrolyte balance.

Investigations concerning the key antioxidant CAT were carried out. Adenine treatment produced a significant decrease in CAT concentration suggestive of compromised defensive antioxidant status. *Ginkgo biloba* extract, due to its high antioxidant profile, was found to be effective in ameliorating alterations in renal catalase activities (Chávez-Morales *et al.*, 2017; Sndos and Al-Amri, 2019).

Histological evaluation of renal sections helped us in comprehension of renal damage caused by adenine-induced CKD and ameliorative potential of GBE in this context. A well-organized renal architecture without any noticeable pathology, thereby serving as a baseline to assess other experimental groups, was observed in the control group. Marked histomorphometric and histopathological abnormalities that include glomerular expansion, tubular dilatation, brush border degeneration, and low glomerular number were observed because of adenine exposure. The reported alterations reflect unstable renal tissue integrity and are characteristic of chronic renal damage. Overall, *Ginkgo biloba* intervention resulted in substantial improvement in these pathologies and subsequently promoted restoration of renal tissue organization (Sndos and Al-Amri, 2019; Dawood *et al.*, 2025). Histomorphometric and histopathological observations of hepatic sections provided an important insight into hepatic injury, more likely linked to adenine-induced CKD as well as the therapeutic potential of GBE. A clear hepatic architectural disruption comprising hepatocyte necrosis, hepatic cord degeneration, sinusoidal dilatation, and central vein congestion was noticed in the adenine group versus the control group that showed a well-maintained structural framework. This pattern is characteristic of hepatopathy and reflects marked impairment of hepatocellular integrity. *Ginkgo biloba* also exhibited a mitigating role in preservation of hepatic histological features and thereby promotion of hepatic health status (Al Kury *et al.*, 2020).

5. CONCLUSIONS

In conclusion, GBE attenuated several biochemical, oxidative, and histological alterations associated with adenine-induced CKD in male rats. GBE treatment improved renal biochemical indicators, renal catalase activity, electrolyte disturbances, and renal histological alterations. However, the high dietary doses used in the present study, together with the increase in relative liver weight observed at 0.2%, warrant further evaluation of dose-response relationships and safety.

6. LIMITATIONS

This study has some limitations. The relatively small sample size and use of an adenine-induced CKD model in male rats may limit the generalizability of the findings to naturally occurring CKD and other species. Furthermore, the molecular mechanisms underlying the nephroprotective effects of *Ginkgo biloba* extract were not investigated.

Further studies using larger sample sizes and mechanistic approaches are warranted to validate and extend these findings.

CRedit AUTHORSHIP CONTRIBUTION - **Conceptualization:** KAM; **Methodology:** AS, FM, and SAK; **Software:** SKT and HFR; **Validation:** KAM, SKT, and MI; **Formal Analysis:** KAM and MI; **Investigation:** AS, FM, and SAK; **Resources:** KAM and HFR; **Data Curation:** AS, FM, and SAK; **Writing – Original Draft Preparation:** AS, FM, and KAM; **Writing – Review & Editing:** SKT, HFR, and MI; **Supervision:** KAM.

INSTITUTIONAL REVIEW BOARD STATEMENT - Experimental protocol was approved by the Ethical Review Committee for Care and Use of Laboratory Animals, UVAS (DR NO. 211).

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