



Impact of Ginkgo biloba leaves Extract on Renal Toxicity Induced by Amiodarone in Male Rats

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ABSTRACT

Amiodarone (AMD) is classified as an antiarrhythmic drug. It causes hypothyroidism associated with a rapid decrease in renal functions. Natural plants are perfect remedies as they cost less and are easier to obtain without any troubles. Ginkgo biloba Leaves (GBL) extract had been reported to be a potent antioxidant and free radical scavenger. This research aimed to assess the impact of GBL extract on AMD-induced nephrotoxicity. Male rats (=40) were classified into 4 groups. Group I: negative control (Cont). Group II: AMD; rats injected interpersonally (ip) with MDA in a dose of 50 mg/kg on the 21st day. Group III: GBL100 mg/kg. Group IV: GBL+AMD; rats were injected with AMD in a dose of fifty mg/kg on the day 21st. After 4 weeks, the rats were sacrificed and blood samples were collected. Serum uric acid (UA), blood urea nitrogen (BUN), creatinine (Cr), potassium (K⁺), and sodium (Na⁺) were evaluated. The concentration of malondialdehyde (MDA), catalase (CAT) enzyme activity, kidney hormones (renin and parathyroid) were determined in kidney tissues. In addition, renal tissues of rats from different groups were examined using a light microscope. Results showed that injection with AMD induced significant ($p < 0.05$) increases in the kidney functions, ionic potassium, MDA, and renal hormones with significant ($p < 0.05$) decrease in sodium, as well as CAT enzyme relative to the cont group. The administration of GBL to the intoxicated rats resulted in a significant amelioration ($p < 0.05$) in all tested parameters as well as the histopathological changes of rat kidneys in contrast to the AMD group. The results of the current study revealed that GBL extract attenuated AMD-induced nephrotoxicity via an antioxidant mechanism.

Key Words: Amiodarone, Ginkgo biloba, nephrotoxicity, rats, antioxidant, histopathological changes.

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INTRODUCTION

The kidney has a vital role in the normal physiology of humans. Its disorders have considered a major cause of disability and in worst circumstances lead to death [1]. Drug-induced renal dysfunction considered an essential reason for acute and chronic renal failure diseases [2]. Amiodarone, a benzofuran derivative, has a structural resemblance to thyroid hormones [3]. It was considered to be an ideal antiarrhythmic therapy used for the cardiac dysfunctions and heart arrhythmias treatment because of its convenience, effectiveness, and mild inotropic effects [2,4]. Moreover, it has a week bioavailability and a long half-life [5].

In spite of its therapeutic action, however, it has many

organ side effects. The long term usage of AMD with an accumulative dosage in several organs and tissue resulted in toxicities in these organs [6]. The pulmonary toxicity, alveolar thickening, disruption, fibrosis, hypersensitivity, and inflammatory cellular infiltration have been reported as the AMD-side effects in the lung [7, 8]. Moreover, AMD induced hepatic mitochondrial toxicity, which damaged the electron-transport system [5,9]. Therapy with AMD affects thyroid functions and induced hypothyroidism and thyrotoxicosis. This toxicity was associated with high iodine, autoimmunity, and an increase in free radicals [10, 11]. Treatment with AMD causes dyslipidemia and promotes the formation and

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accumulation of cholesterol [12, 13].

The production and cumulation of free radicals and reactive oxygen species (ROS) enhancing from AMD therapy resulted in damage to the structure and DNA of the cells, thus caused disorders in stress vulnerable organs like kidney, liver, adrenal, and testis [14-16]. Ameliorating these cellular damage antioxidants may decline oxidative stress and phospholipidosis in AMD-induced toxicity [17].

Ginkgo biloba leaves extract used as a nutraceutical herbal [18]. It contains high levels of glycosides, flavonoids, terpenoids, diterpenes, sesquiterpenes, flavonol, and polyphenols which have antioxidant effects, are ROS scavengers and provide protection against oxidative cell damage [19-21]. The extract of GBL has neuroprotective, anti-apoptotic effects, reverse the age-related decrease of neurotransmitter systems [22], and is a cognitive enhancer for Alzheimer's disease [23].

The hepatoprotective effect of GBE has been reported against thioacetamide-induced liver fibrosis [24]. The GBL has therapeutic effects in edema and inflammation [25]. The nephroprotective effect of GBL has been reported against methotrexate [26], gentamicin, and cisplatin-induced renal damage and nephrotoxicity [27, 28]. The extract of GBL improved blood flow [29], decreased platelet aggregation, declined proinflammatory functions [30], protected against doxorubicin-induced cardiotoxicity [31], and ameliorated against bleomycin-induced lung fibrosis [32]. It exhibited protective action against oxidative stress and nephrotoxicity induced by vancomycin [33].

To the best of our knowledge, no previous researchers have assessed the effects of GBL on AMD-induced nephrotoxicity. Therefore, this study aimed to explore the protective effect of GBL against AMD-induced nephrotoxicity.

MATERIAL AND METHODS

Plant material

Ginkgo biloba leaves (GBL) were purchased from a local market in Jeddah, Saudi Arabia.

Experimental rats and Diet.

Forty adult male Wister rats, weighing 170 ± 10 g, were purchased from King Fahd Medical Research Center. They were kept in standard conditions for laboratory rats fed on the AIN-93 standard diet [34]. They were kept in compliance with King Fahd Research Center's standard guide.

Chemicals, kits, and drugs

Amiodarone (AMD) was purchased from a local pharmacy (Jeddah). All chemicals and kits with high analytical grade were purchased from Biosystems (Barcelona, Spain).

Induction of nephrotoxicity

A single dose of AMD was injected into rats in a dose of 50 mg/kg to induce nephrotoxicity [35].

Preparation of *Ginkgo biloba* leaf extract

Ginkgo biloba leaves were ground to a fine powder (500 g) and macerated in 1L of ethanol (80%) at room temperature and mixed for 48 hrs by magnetic stirrer at 100 rpm speed. The extract was concentrated under vacuum at 40 °C using a rotary evaporator, and further freeze-drying of the condensed residue [36]. The extract was kept in non-permeable glass containers at 4 °C until used.

Experimental design

Rats were acclimatized under standard laboratory conditions for one week before the experiment started. Rats were distributed into 4 groups (n=10 in each group). Group I (Cont) rats received no treatment; Group II (AMD) rats received distilled water for 3 weeks then were i.p. injected with a single dose of AMD at a dose of 50 mg/kg on the day 21st; Group III received GBL extract 100 mg/kg b.wt [3]; Group IV received GBL extract 100 mg/kg b.wt +AMD. Rats in group IV received GBL extract for twenty-one days, followed by i.p. injections with AMD on day 21st. At the end of the experimental period (4 weeks), rats were anesthetized then blood and renal samples were collected. The collected serum samples were separated and stored at -80 °C until used for biochemical measurements.

Biochemical measurements

Renal function levels (creatinine, BUN, and uric acid), ionic sodium and potassium concentrations were determined using colorimetric kits, purchased from Abcam, USA, following the manufactures' procedure.

Biomarkers estimated in renal tissue

Oxidative stress biomarkers (MDA and CAT) and renal hormones (renin and parathyroid) were estimated in homogenated renal tissue using ELISA kits.

Histopathological studies

The kidneys from all experimental groups were removed after sacrificing the rats. Tissue was placed in 10% formalin, dehydrated, paraffin wax and then stained by routine procedures with hematoxylin-eosin.

Statistical analysis

SPSS Version 24 analyzed the resulting data. Values were expressed as mean \pm SD, and analyzed by one-way variance (ANOVA) followed by t-test. The results at $P \leq 0.05$ were considered statistically significant.

RESULTS

Influence of GBL on renal functions in nephrotoxic rats by AMD

The effect of GBL extract on kidney functions (Cr, BUN, and UA) against AMD-induced nephrotoxicity in male rats was shown in Table (1). The results revealed that acute intoxication of rats by AMD induced a significant elevation in serum levels of BUN, UA, and Cr of AMD group (59.31 ± 1.09 , 17.41 ± 0.04 , and 130.31 ± 0.03 , respectively) compared to control negative group (27.67 ± 1.11 , 8.21 ± 0.12 , and 0.8 ± 0.54 , respectively). The GBL group and GBL+AMD showed significant decreases in kidney function parameters compared to the AMD group.

Table 1: Influence of GBL extract on renal functions against AMD-induced nephrotoxicity in rats.

Groups	Creatinine ($\mu\text{mol/L}$)	Blood Urea Nitrogen (mmol/L)	Uric acid ($\mu\text{mol/L}$)
Cont	27.67 ± 1.11 b	8.21 ± 0.12 b	74.21 ± 0.21 b
AMD	59.31 ± 1.09 a	17.41 ± 0.04 a	130.31 ± 0.03 a
GBL (100 mg/kg)	27.89 ± 1.05 b	8.76 ± 0.03 b	74.59 ± 0.12 b
GBL (100 mg/kg) + AMD	28.05 ± 1.14 b	8.95 ± 0.03 b	75.01 ± 0.23 b

Values are stated as mean \pm SMD in each group (N = 10). Values within a column of different superscript letters are substantially different at $P < 0.05$.

Influence of GBL on renal renin and parathyroid hormones in nephrotoxic rats by AMD

As shown in Figure 1, intoxicated rats by AMD had a significant ($P < 0.05$) increase in renin and parathyroid

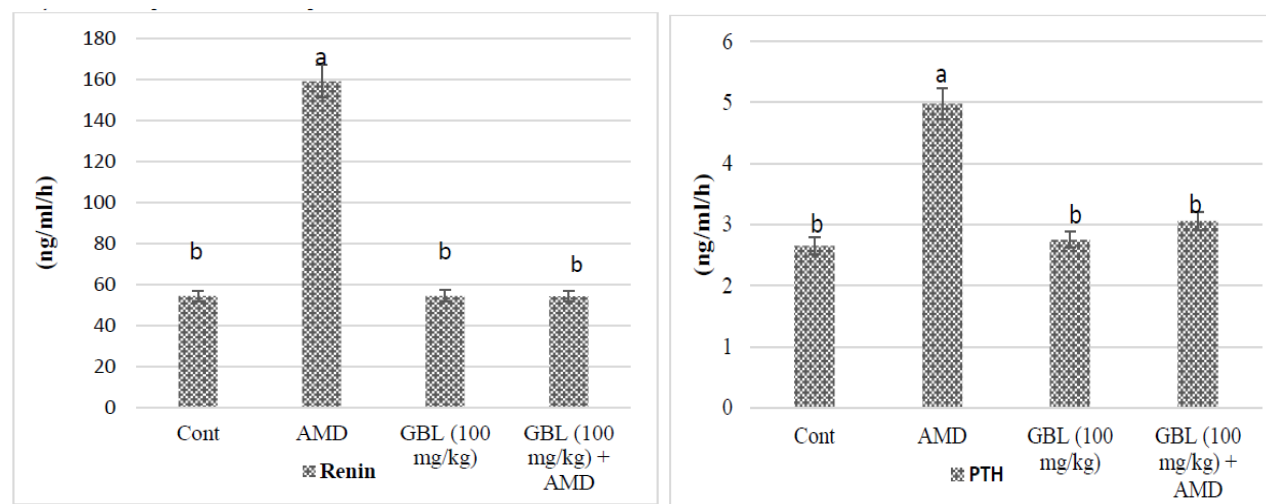
hormones in renal tissue relative to the control group. Ingestion of GBL (100 mg/kg) induced significant ($P < 0.05$) improvement in both hormones relative to the AMD group. There were also significant declines on rennin and parathyroid hormones in the GBL (100 mg/kg) + AMD group relative to the AMD group.

Influence of GBL on ionic sodium and potassium in nephrotoxic rats by AMD

It is clear from Figure 2 that i.p. injection of AMD induced significant ($P < 0.05$) reduction in serum level of ionic Na^+ along with significant ($p < 0.05$) elevation in the serum ionic K^+ in the AMD group relative to the Cont group. Pretreatment with GBL extract at 100 mg/kg to the AMD-intoxicated group led to a significant ($p < 0.05$) increase in ionic Na^+ level with a significant ($p < 0.05$) decrease in the ionic K^+ level relative to AMD intoxicated group.

Influence of GBL on renal MDA and CAT in nephrotoxic rats by AMD

Figure 3 indicated that rats intoxicated by AMD had significant ($P < 0.05$) elevation in the serum activities of MDA accompanied by a significant reduction in CAT enzyme activity compared to the Cont group. Administration of GBL extract (100 mg/kg) intoxicated with AMD displayed remarkably amelioration the elevation of MDA level and the reduction in CAT enzyme activity when compared with the nephrotoxic rats, thus indicated the antioxidant action of GBL



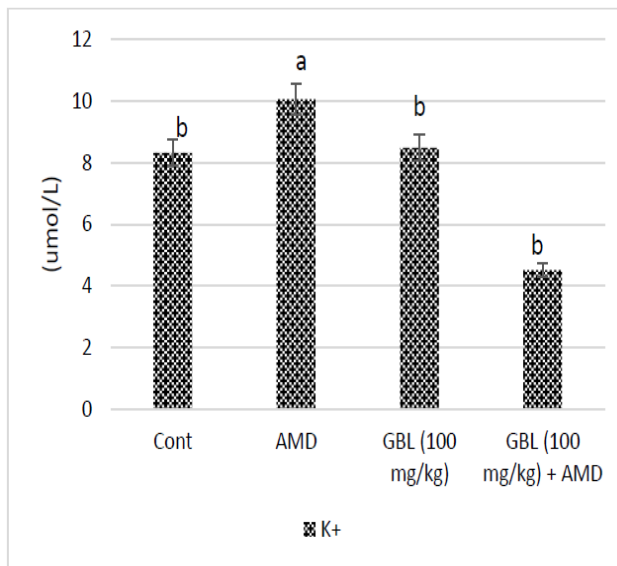
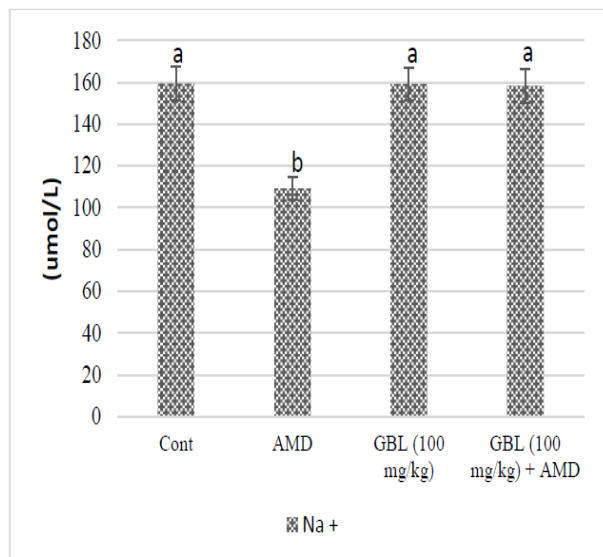
Values are stated as mean \pm SMD in each group N = 10. Values within a column of different superscript letters are substantially different at $P < 0.05$.

Figure 1: Influence of GBL extract on renal renin and parathyroid hormones against AMD-induced nephrotoxicity in rats.

Histopathological studies

Histological examination of healthy rat kidneys showed normal renal parenchyma (glomeruli and tubules) histological structure. The AMD-intoxicated rat kidneys showed pronounced renal tubular necrosis in their lumens

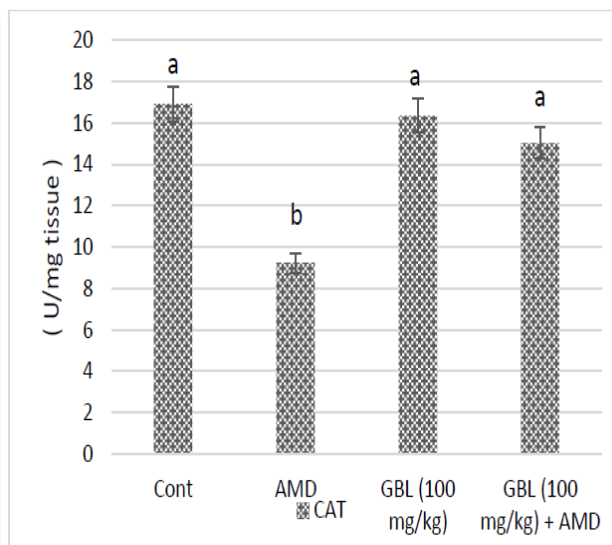
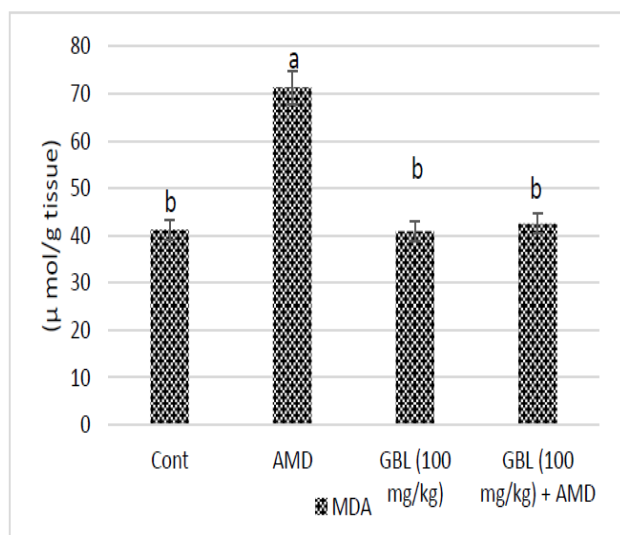
consistent with protein casts. Examination of rat kidneys orally given 100 mg/kg GBL extracts showed the normal appearance of the renal parenchyma. Simultaneous administration of the GBL extract together with AMD revealed slight intertubular blood capillary swelling.



Values are stated as mean ± SMD in each group (N = 10).

Values within a column of different superscript letters are substantially different at P<0.05.

Figure 2: Influence of GBL extract on serum ionic Na⁺ and K⁺ levels against AMD-induced nephrotoxicity in rats.



Values are stated as mean ± SMD in each group (N = 10).

Values within a column of different superscript letters are substantially different at P<0.05.

Figure 3: Influence of GBL extract on oxidative stress biomarkers against AMD-induced nephrotoxicity in rats.

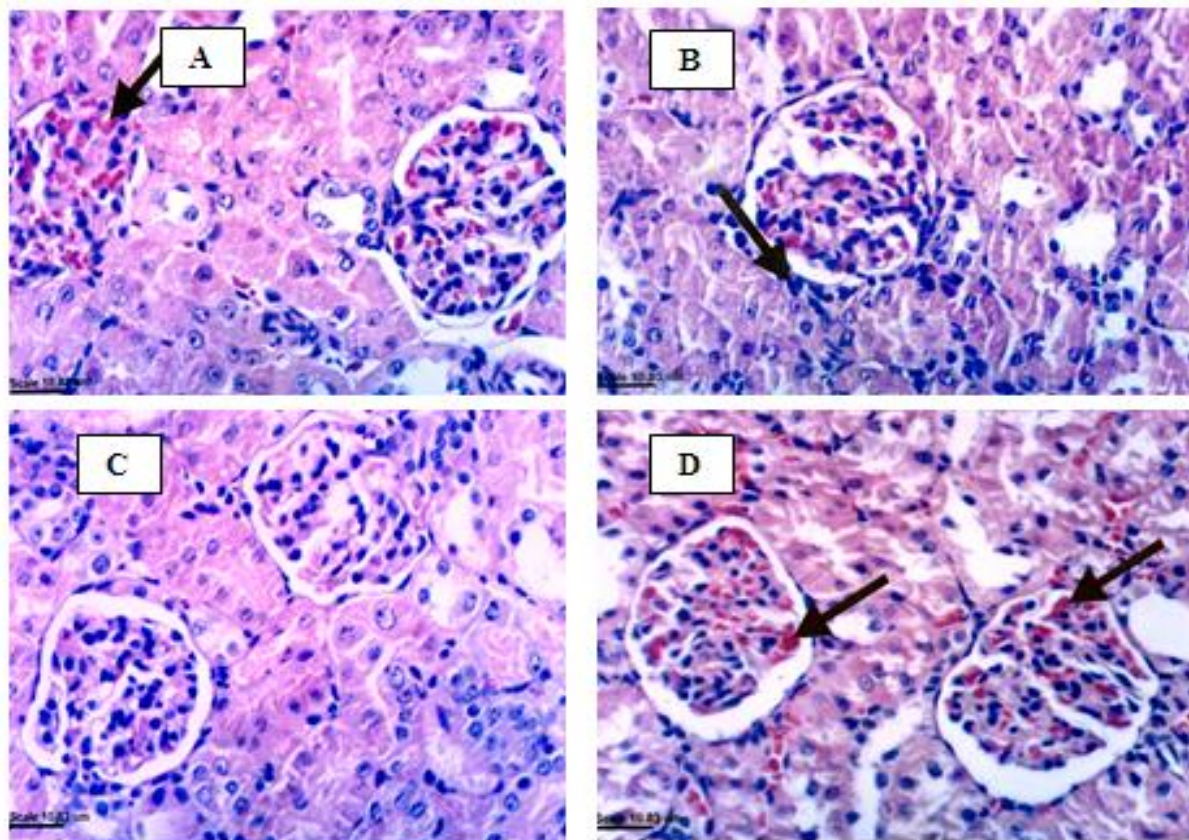


Figure 4: Impact of GBL on the renal tissue histopathological changes detected by H & E staining in AMD-induced nephrotoxicity in rats. Photo A: A healthy rat's kidney that showed normal renal parenchyma (tubules and glomeruli) histological structure. Photo B: The AMD-intoxicated rat kidneys showed pronounced renal tubular necrosis in their lumens consistent with protein casts. Photo C: An examination of rat kidneys orally given 100 mg/kg GBL extracts showed the normal appearance of the renal parenchyma. Photo D: Simultaneous administration of the GBL extracts together with AMD revealed slight intertubular blood capillary swelling.

DISCUSSION

Amiodarone is a common effective therapy for atrial fibrillation, atrial flutter, ventricular arrhythmias, and supraventricular tachycardia [37]. However, AMD therapy showed severe side effects in time- and dose-dependent manner. Today, several plants, plant extracts, or their derived have commonly used for the protection and treatment of several diseases. Extract of GBL exerted potent antioxidant activities [38]. Therefore, this study aimed to assess the potential renal protective effect of GBL against AMD in rats.

The obtained results revealed that acute intoxication of rats by AMD induced a significant elevation in renal function parameters, renin, and parathyroid hormones, renal MDA, and ionic K^+ , accompanied by a significant reduction in renal CAT enzyme activity and serum ionic Na^+ relative to the Cont group. Moreover, renal histopathological alteration including renal tubular

necrosis in their lumens consistent with protein casts was showed in the AMD group.

These results agree with several studies that revealed a significant increase in serum creatinine and urea accompanied by a tubular alteration in renal tissue associated with AMD therapy relative to the control, thus confirmed renal damage in rats induced by AMD [35, 39, 40]. The AMD has been proved to induce histoarchiterural alterations in the kidney, liver, and testis and induced cytokine production [16, 41, 42]. The obtained results were explained through AMD-induced deterioration of tubular epithelial cells, a decline in renal tubule reabsorption, and atrophy of glomeruli, which decrease glomerular filtration [40].

The AMD-induced toxicity by the production of ROS leads to oxidative damage. Our results are on the same line with the findings that AMD induced depletion of antioxidant enzymes, which decline *via* oxidative stress and resulting cytotoxicity [43]. The renal oxidative stress induced by AMD could be explained through AMD elevated mitochondrial synthesis of hydrogen peroxide,

which induced peroxidation [44]. Moreover, AMD therapy induced an elevation in urinary iodine, hypertrophied cortex, and cortisol level. Increased lipid peroxidation developed stress for deiodination and metabolism of AMD which caused kidney toxicity [45]. The AMD has the ability to generate free radicals, phospholipase inhibition, and membrane destabilization which involved in the pathogenesis of its toxicity [17,46]. In addition, AMD after metabolism, when deiodinized, releases excess iodine in circulation, which generates oxidative stress and free radicals in kidney and liver that evidenced by the elevation in MDA levels [47-49].

Renal is vulnerable to damage as a result of AMD administration which elevated levels of excreted compounds and perfusion that occur in peri-tubular cells [40]. Increasing levels of renin and PTH obtained in this study could be explained through AMD-induced tubulointerstitial damage showed in experimental nephropathies [50]. The elevation of PTH further induced tubular damage, fibrosis, and kidney function deterioration post nephrotoxic injury in experimental animals [51, 52]. Renal is the main target organ for thyroid hormone, which stimulates the secretion of the renin hormone by juxtaglomerular cells and affecting renal functions. In this concern, Abd El-Ghany [53] reported that gentamicin-induced nephrotoxicity showed a significant increase in serum renin with a significant decrease in vitamin D relative to the control. However, in rats administered with gentamicin and celery or ginger, there was a significant increase in vitamin D with significant decrease in the renin hormone.

Recently, the use of natural antioxidant products in attenuation of drug-induced nephrotoxicity gained attention. In this study ingestion of GBL (100 mg/kg) to rats displayed remarkably amelioration against AMD-intoxication. It induced significant decreases in kidney function parameters, renin, and parathyroid hormones, renal MDA, and serum ionic K^+ levels, accompanied by significant increases in renal CAT enzyme activity, and ionic Na^+ relative to the AMD group; it overcame the histopathological changes in renal tissue. The GBL extract protected and improved renal function in the AMD group. These results confirmed nephroprotective effect of GBL extract. The GBL reversed the oxidative damage with its antioxidant effects.

The obtained results were in harmony with several researches that revealed amelioration of kidney function with GBL extract in diabetic nephropathy model [54], as well as adriamycin [55], methotrexate [56], and cisplatin [57]-induced renal toxicity models. This effect could be explained through GBL containing several natural compounds that regulate the balance between the antioxidants and oxidants [56]. Moreover, Hsu et al. [58]

reported that GBL extract had therapeutic mechanisms and cytoprotective effects against apoptosis and oxidative damage. There are many antioxidant constituents in GBL extract like flavonoids, diterpene terpenoids, catechins, steroids, quercetin, kaempferol, flavone glycosides, etc. [59, 60]. Moreover, GBL extract contains proanthocyanidins and flavonol glycosides, which have antioxidant and free radical scavenging properties, thus protecting and improving many diseases resulting from oxidative damage [61].

CONCLUSION

GBL extract exerted a renal protective effect against AMD-induced renal damage through its antioxidant mechanisms. Clinical studies are recommended to validate these results in humans.

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