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

Sndos, Z.A. Fattiny[1*], Sarah Mesfer Al-Amri[2]

[1]Clinical Nutrition Department, Faculty of Applied Medical Sciences, Umm-Al-Qura University, Makkah, Saudi Arabia.
[2] Nutrition Department, King Khalid University, Abha, Saudi Arabia.

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.*

eIJPPR 2019; 9(6):1-9

HOW TO CITE THIS ARTICLE: Sndos, Z.A. Fattiny and Sarah Mesfer Al-Amri, (2019)."Impact of Ginkgo biloba leaves Extract on Renal Toxicity Induced by Amiodarone in Male Rats", International Journal of Pharmaceutical and Phytopharmacological Research, 9(6), pp.1-9.

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

Corresponding author: Sndos, Z.A. Fattiny
Address:Clinical Nutrition Department, Faculty of Applied Medical Sciences, Umm-Al-Qura University, Makkah, Saudi Arabia.
Relevant conflicts of interest/financial disclosures: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.
Received: 12 June 2019; **Revised:** 20 October 2019; **Accepted:** 29 October 2019

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±10g, 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 GBLE 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 ± 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 aginst AMD-induced nephrotoxicity in rats.

Groups	Creatinine (µmol/L)	Blood Urea Nitrogen (mmol/L)	Uric acid (umol/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± 0.04a	130. 31± 0.03a
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 ± 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

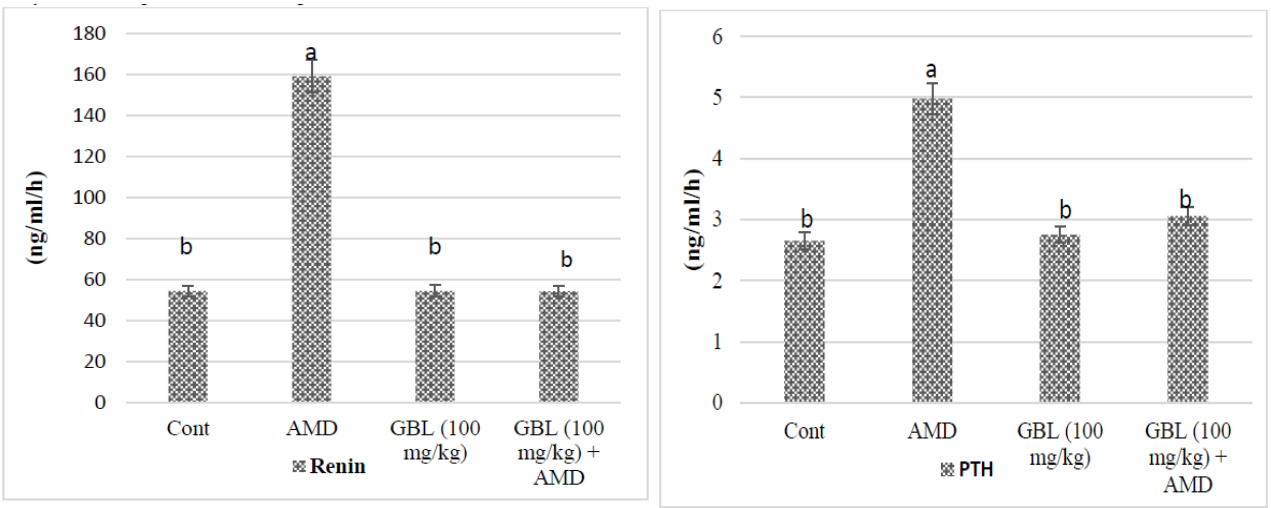
As shown in Figure 1, intoxicated rats by AMD had a significant (P<0.05) increase in rennin and parathyroid hormones in renal tissue relative to the control group. Ingestion of GBL (100 mg/kg) induced significant (P< 0.05) improvement in both hormones relative to the AMD group. There were also significant 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

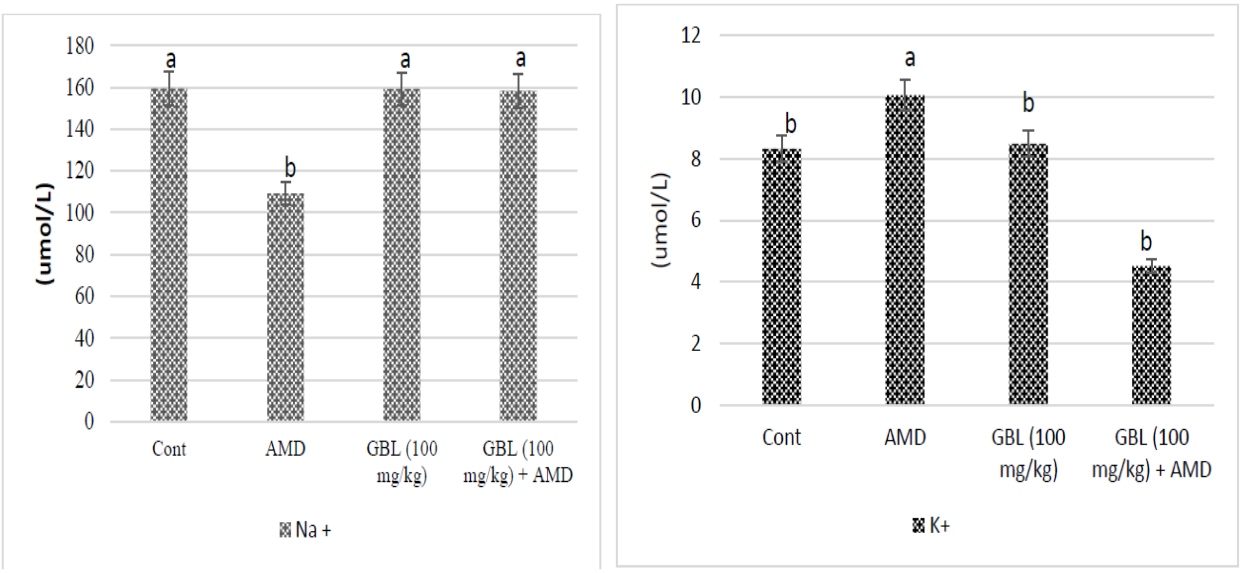


Values are stated as mean ± SMD in each group N = 10.
Values within a column of different superscript letters are substantially different at P<0.05.

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

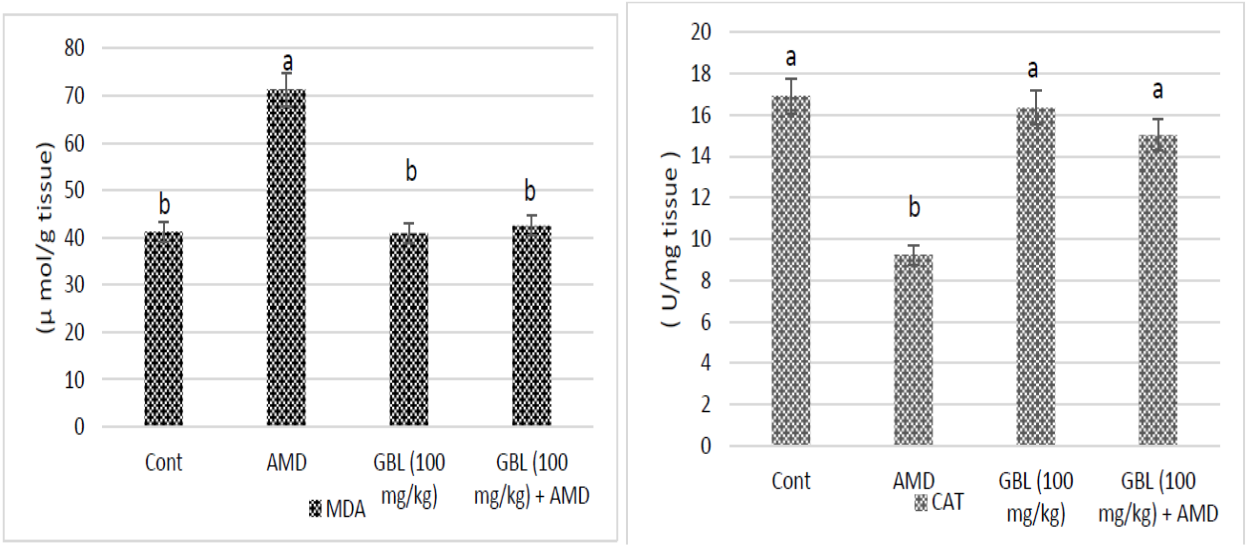
Histopathological studies

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.

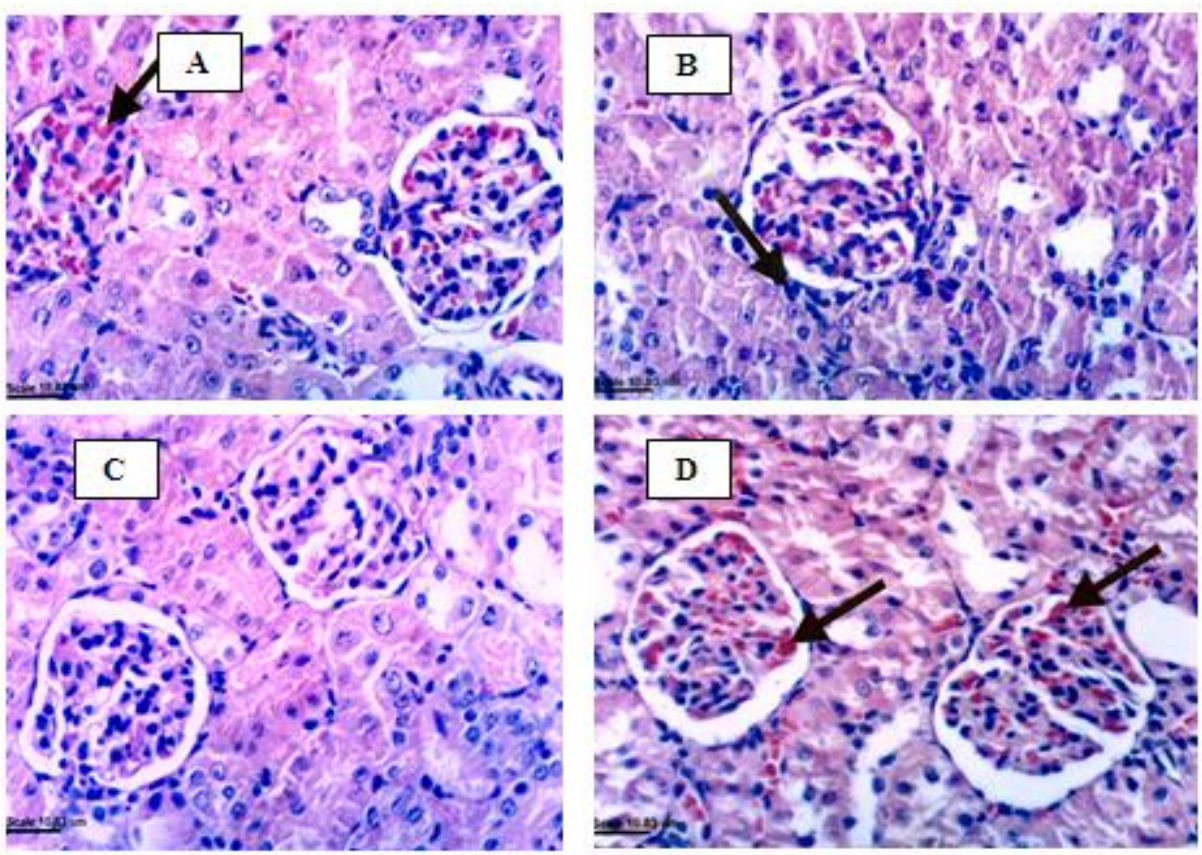


Figure 4: Impact of GBL on the renal tissue histopathological changes detected by H & E staining in AMD-induced nephrotoxicity in rats. Photo A: A healthy rat's kidney that showed normal renal parenchyma (tubules and glomeruli) histological structure. Photo B: The AMD-intoxicated rat kidneys showed pronounced 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 sligh 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 deiodinization 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 excreated 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.

REFERENCES

[1] Sneha, D., Neeru, V. and Sunil, S. Kidney disorders and management through herbs: A Review . J of Phytopharmacol., 2019; 8(1): 21-7.

[2] Singh, N.P., Ganguli, A. and Prakash, A. Drug-induced Kidney Diseases. JAPI , 2003; 51 (10): 970-9.

[3] Newman CM, Price A, Davies DW, Gray TA, Weetman AP. Amiodarone and the thyroid: a practical guide to the management of thyroid dysfunction induced by amiodarone therapy. Heart. 1998 Feb 1;79(2):121-7.

[4] Martino E, Bartalena L, Bogazzi F, Braverman LE. The effects of amiodarone on the thyroid. Endocrine reviews. 2001 Apr 1;22(2):240-54.

[5] Babatin, M., Lee, S.S. and Pollak, P.T. Amiodarone hepatotoxicity. Curr Vasc Pharmacol., 2008; 6: 228-36.

[6] Roberts M. Clinical utility and adverse effects of amiodarone therapy. AACN advanced critical care. 2010;21(4):333-8.

[7] Shimizu S, Gabazza EC, Taguchi O, Yasui H, Taguchi Y, Hayashi T, Ido M, Shimizu T, Nakagaki T, Kobayashi H, Fukudome K. Activated protein C inhibits the expression of platelet-derived growth factor in the lung. American journal of respiratory and critical care medicine. 2003 May 15;167(10):1416-26.

[8] Mahavadi, P., Henneke, I., Ruppert, C., Knudsen, L. and Venkatesan, S. Altered surfactant homeostasis and alveolar epithelial cell stress in amiodaroneinduced lung fibrosis. Toxicol Sci, 2014; 4: 1-13.

[9] Vitins AP, Kienhuis AS, Speksnijder EN, Roodbergen M, Luijten M, van der Ven LT. Mechanisms of amiodarone and valproic acid

induced liver steatosis in mouse in vivo act as a template for other hepatotoxicity models. Archives of toxicology. 2014 Aug 1;88(8):1573-88.

[10] Gassanov N, Dietlein M, Caglayan E, Erdmann E, Er F. Amiodarone-induced thyroid gland dysfunctions. Deutsche medizinische Wochenschrift (1946). 2010 Apr;135(16):807-11.

[11] Bogazzi F, Tomisti L, Bartalena L, Aghini-Lombardi F, Martino E. Amiodarone and the thyroid: a 2012 update. Journal of endocrinological investigation. 2012 Mar 1;35(3):340-8.

[12] McCarthy TC, Pollak PT, Hanniman EA, Sinal CJ. Disruption of Hepatic Lipid Homeostasis in Mice after Amiodarone Treatment Is Associated with Peroxisome Proliferator-Activated Receptor-αTarget Gene Activation. Journal of Pharmacology and Experimental Therapeutics. 2004 Dec 1;311(3):864-73.

[13] Mesens N, Desmidt M, Verheyen GR, Starckx S, Damsch S, Vries RD, Verhemeldonck M, Gompel JV, Lampo A, Lammens L. Phospholipidosis in rats treated with amiodarone: serum biochemistry and whole genome micro-array analysis supporting the lipid traffic jam hypothesis and the subsequent rise of the biomarker BMP. Toxicologic pathology. 2012 Apr;40(3):491-503.

[14] Rebrova TY, Afanasyev SA. Free radical lipid peroxidation during amiodarone therapy for postinfarction cardiosclerosis. Bulletin of experimental biology and medicine. 2008 Sep 1;146(3):283.

[15] Aggarwal D, Sharma M, Singla SK. The role of natural antioxidants as potential therapeutic agent in nephrolithiasis. Asian J Pharm Clin Res. 2013;6(3):48-53.

[16] Chakraborty AR, Mondal CH, Sinha SA, Mandal JA, Chandra AK. Amiodarone induced oxidative stress in stress-vulnerable organs of adult male rats. Asian J Pharm Clin Res. 2014;7(4):177-83.

[17] Ágoston M, Örsi F, Fehér E, Hagymási K, Orosz Z, Blázovics A, Fehér J, Vereckei A. Silymarin and vitamin E reduce amiodarone-induced lysosomal phospholipidosis in rats. Toxicology. 2003 Aug 28;190(3):231-41.

[18] Tousson E, Keshta AT, Hussein Y, Fekry RM, Abo-Ghaneima WK. Renal protective effect of Ginkgo biloba and L-carnitine extracts against pentylenetetrazol induced toxicity, oxidative stress, injury and proliferation alternation in epileptic rats. Annual Research & Review in Biology. 2019 Jun 28:1-3.

[19] van Beek TA, Bombardelli E, Morazzoni P, Peterlongo F. Ginkgo biloba L. Fitoterapia. 1998;68:195-244.

[20] Guan H, Qian D, Ren H, Zhang W, Nie H, Shang E, Duan J. Interactions of pharmacokinetic profile of different parts from Ginkgo biloba extract in rats. Journal of ethnopharmacology. 2014 Aug 8;155(1):758-68.

[21] Escárcega-González CE, Reynoso-Andeola IG, Jaramillo-Juárez F, Martínez-Ruvalcaba H, Posadas del Rio FA. The Ginkgo biloba extract reverses the renal effects of titanium dioxide nanoparticles in adult male rats. Biochemistry research international. 2016;2016. DOI: 10.1155/2016/5781579

[22] Williams B, Watanabe CM, Schultz PG, Rimbach G, Krucker T. Age-related effects of Ginkgo biloba extract on synaptic plasticity and excitability. Neurobiology of aging. 2004 Aug 1;25(7):955-62.

[23] Das A, Shanker G, Nath C, Pal R, Singh S, Singh HK. A comparative study in rodents of standardized extracts of Bacopa monniera and Ginkgo biloba: anticholinesterase and cognitive enhancing activities. Pharmacology Biochemistry and Behavior. 2002 Nov 1;73(4):893-900.

[24] Al-Attar AM. Attenuating effect of Ginkgo biloba leaves extract on liver fibrosis induced by thioacetamide in mice. BioMed Research International. 2012 Oct 2;2012. doi:10.1155/2012/761450.

[25] Diamond BJ, Shiflett SC, Feiwel N, Matheis RJ, Noskin O, Richards JA, Schoenberger NE. Ginkgo biloba extract: mechanisms and clinical indications. Archives of physical medicine and rehabilitation. 2000 May 1;81(5):668-78.

[26] Tousson E, Atteya Z, El-Atrash E, Jeweely OI. Abrogation by Ginkgo Byloba leaf extract on hepatic and renal toxicity induced by methotrexate in rats. Journal of Cancer Research and Treatment. 2014;2(3):44-51.

[27] Öztürk G, Anlar Ö, Erdoğan E, Kösem M, Özbek H, Türker A. The effect of Ginkgo extract EGb761 in cisplatin-induced peripheral neuropathy in mice. Toxicology and applied pharmacology. 2004 Apr 1;196(1):169-75.

[28] Gulec M, Iraz M, Yilmaz HR, Ozyurt H, Temel I. The effects of ginkgo biloba extract on tissue adenosine deaminase, xanthine oxidase, myeloperoxidase, malondialdehyde, and nitric oxide in cisplatin-induced nephrotoxicity. Toxicology and industrial health. 2006 Apr;22(3):125-30.

[29] Dubey AK, Shankar PR, Upadhyaya D, Deshpande VY. Ginkgo biloba--an appraisal. Kathmandu University medical journal (KUMJ). 2004;2(3):225-9.

[30] Gurley BJ, Gardner SF, Hubbard MA, Williams DK, Gentry WB, Carrier J, Khan IA, Edwards DJ, Shah A. In vivo assessment of botanical

supplementation on human cytochrome P450 phenotypes: Citrus aurantium, Echinacea purpurea, milk thistle, and saw palmetto. Clinical Pharmacology & Therapeutics. 2004 Nov;76(5):428-40.

[31] Naidu, M.U., Kumar, K.V., Mohan, I.K., Sundaram, C. and Singh S. Protective effect of gingko biloba extract against doxorubicin-induced cardiotoxicity in mice. Indian J. Exp Biol., 2002; 40(8):894-900.

[32] Daba MH, Abdel-Aziz AA, Moustafa AM, Al-Majed AA, Al-Shabanah OA, El-Kashef HA. Effects of L-carnitine and ginkgo biloba extract (EG b 761) in experimental bleomycin-induced lung fibrosis. Pharmacological research. 2002 Jun 1;45(6):461-7.

[33] Celik I, Cihangiroglu M, Ilhan N, Akpolat N, Akbulut HH. Protective effects of different antioxidants and amrinone on vancomycin-induced nephrotoxicity. Basic & clinical pharmacology & toxicology. 2005 Nov;97(5):325-32.

[34] Reeves, P.G., Nielsen, F.H. and Fahey,G.C. AIN-93 purified diets for laboratory rodents: final report of the American Institute of Nutrition ad hoc writing committee on the reformulation of the AIN-76A rodent diet. J Nutr., 1993; 123 (11):1939-51.

[35] Morales AI, Barata JD, Bruges M, Arévalo MA, González de Buitrago JM, Palma P, Branco P, Pérez-Barriocanal F. Acute renal toxic effect of amiodarone in rats. Pharmacology & toxicology. 2003 Jan;92(1):39-42.

[36] Vanitha, R. Effect of ginkgo biloba extract against the behavioral changes induced by dementia in Wistar rats. Inter J of Med Sci Current Res., 2019; 2(1):293-9.

[37] Reiffel JA, Waldo AL, Prystowsky EN, DiBianco R. A consensus report on antiarrhythmic drug use. Clinical cardiology. 1994 Mar;17(3):103-16.

[38] Şener G, Sehirli Ö, Tozan A, Velioğlu-Övunç A, Gedik N, Omurtag GZ. Ginkgo biloba extract protects against mercury (II)-induced oxidative tissue damage in rats. Food and chemical toxicology. 2007 Apr 1;45(4):543-50.

[39] Pollak PT, Sharma AD, Carruthers SG. Creatinine elevation in patients receiving amiodarone correlates with serum amiodarone concentration. British journal of clinical pharmacology. 1993 Aug;36(2):125-7.

[40] Sakr SA, El-Gamal EM. Effect of grapefruit juice on amiodarone induced nephrotoxicity in albino rats. Toxicology and industrial health. 2016 Jan;32(1):68-75..

[41] Bartalena LU, Grasso LU, Brogioni SA, Aghini-Lombardi FA, Braverman LE, Martino E. Serum interleukin-6 in amiodarone-induced thyrotoxicosis. The Journal of Clinical Endocrinology & Metabolism. 1994 Feb 1;78(2):423-7.

[42] Pitsiavas V, Smerdely P, Li MU, Boyages SC. Amiodarone induces a different pattern of ultrastructural change in the thyroid to iodine excess alone in both the BB/W rat and the Wistar rat. European Journal of Endocrinology. 1997 Jul 1;137(1):89-98.

[43] Ágoston M, Cabello RG, Blázovics A, Fehér J, Vereckei A. The effect of amiodarone and/or antioxidant treatment on splenocyte blast transformation. Clinica chimica acta. 2001 Jan 1;303(1-2):87-94.

[44] Serviddio G, Bellanti F, Giudetti AM, Gnoni GV, Capitanio N, Tamborra R, Romano AD, Quinto M, Blonda M, Vendemiale G, Altomare E. Mitochondrial oxidative stress and respiratory chain dysfunction account for liver toxicity during amiodarone but not dronedarone administration. Free Radical Biology and Medicine. 2011 Dec 15;51(12):2234-42.

[45] El Golli-Bennour E, Bouslimi A, Zouaoui O, Nouira S, Achour A, Bacha H. Cytotoxicity effects of amiodarone on cultured cells. Experimental and toxicologic pathology. 2012 Jul 1;64(5):425-30.

[46] Ray SD, Patel D, Wong V, Bagchi D. In vivo protection of dna damage associated apoptotic and necrotic cell deaths during acetaminophen-induced nephrotoxicity, amiodarone-induced lung toxicity and doxorubicin-induced cardiotoxicity by a novel IH636 grape seed proanthocyanidin extract. Research communications in molecular pathology and pharmacology. 2000;107(1-2):137-66.

[47] Joanta AE, Filip A, Clichici S, Andrei S, Cluj-Napoca Romania CN. Iodide excess exerts oxidative stress in some target tissues of the thyroid hormones. Acta Physiologica Hungarica. 2006 Dec 1;93(4):347-59.

[48] Shoyinka SV, Obidike IR, Ndumnego CO. Effect of iodine supplementation on thyroid and testicular morphology and function in euthyroid rats. Veterinary research communications. 2008 Dec 1;32(8):635-45.

[49] Gutowski M, Kowalczyk S. A study of free radical chemistry: their role and pathophysiological significance. Acta Biochimica Polonica. 2013 Mar 19;60(1).

[50] Esbrit P, Santos S, Ortega A, Fernández-Agulló T, Vélez E, Troya S, Garrido P, Peña A, Bover J, Bosch RJ. Parathyroid hormone-related protein as a renal regulating factor. American journal of nephrology. 2001;21(3):179-84.

[51] Ortega A, Rámila D, Izquierdo A, González L, Barat A, Gazapo R, Bosch RJ, Esbrit P. Role of the

Renin-Angiotensin System on the Parathyroid Hormone–Related Protein Overexpression Induced by Nephrotoxic Acute Renal Failure in the Rat. Journal of the American Society of Nephrology. 2005 Apr 1;16(4):939-49.

[52] Ortega A, Rámila D, Ardura JA, Esteban V, Ruiz-Ortega M, Barat A, Gazapo R, Bosch RJ, Esbrit P. Role of parathyroid hormone–related protein in tubulointerstitial apoptosis and fibrosis after folic acid–induced nephrotoxicity. Journal of the American Society of Nephrology. 2006 Jun 1;17(6):1594-603.

[53] El-Ghany A, Ramadan AM, Ghozy SF. Nutraceutical effects of curcuma, ginger, celery, yeast and honey on side effects of gentamicin induced nephrotoxicity in rats. World Applied Sciences Journal. 2012;10(1):01-11.

[54] Lu Q, Zuo WZ, Ji XJ, Zhou YX, Liu YQ, Yao XQ, Zhou XY, Liu YW, Zhang F, Yin XX. Ethanolic Ginkgo biloba leaf extract prevents renal fibrosis through Akt/mTOR signaling in diabetic nephropathy. Phytomedicine. 2015 Nov 15;22(12):1071-8.

[55] Abd-Ellah MF, Mariee AD. Ginkgo biloba leaf extract (EGb 761) diminishes adriamycin-induced hyperlipidaemic nephrotoxicity in rats: association with nitric oxide production. Biotechnology and applied biochemistry. 2007 Jan;46(1):35-40.

[56] Tousson E, Atteya Z, El-Atrash E, Jeweely OI. Abrogation by Ginkgo Byloba leaf extract on hepatic and renal toxicity induced by methotrexate in rats. Journal of Cancer Research and Treatment. 2014;2(3):44-51.

[57] Song J, Liu D, Feng L, Zhang Z, Jia X, Xiao W. Protective effect of standardized extract of Ginkgo biloba against cisplatin-induced nephrotoxicity. Evidence-Based Complementary and Alternative Medicine. 2013;2013.

[58] Hsu CL, Wu YL, Tang GJ, Lee TS, Kou YR. Ginkgo biloba extract confers protection from cigarette smoke extract-induced apoptosis in human lung endothelial cells: role of heme oxygenase-1. Pulmonary pharmacology & therapeutics. 2009 Aug 1;22(4):286-96.

[59] Vasseur, M., Jean, T., DeFeudis, F.V. and Drieu, K. Effects of repeated treatments with an extract of Ginkgo biloba (EGb 761), bilobalide and ginkgolide B on the electrical activity of pancreatic beta cells of normal or alloxan-diabetic mice: an ex vivo study with intracellular microelectrodes. Gen Pharmacol., 1994; 25(1):31-46.

[60] Brown DJ. Phytotherapy: Herbal medicine meets clinical science. NARD J. 1996 May;118:41-52.

[61] Joyeux M, Lobstein A, Anton R, Mortier F. Comparative antilipoperoxidant, antinecrotic and scavanging properties of terpenes and biflavones from Ginkgo and some flavonoids. Planta medica. 1995 Apr;61(02):126-9.