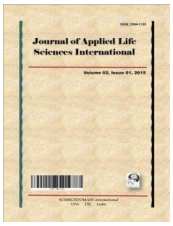

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Betulinic Acid Modulates Dimethylamine-Induced Renal Injury in Male Wistar Rats

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Author's contribution

The sole author designed, analyzed and interpreted and prepared the manuscript.

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ABSTRACT

Dimethylamine (DMA) is a water-soluble basic (pKa=10.73) secondary aliphatic amine. DMA is a common degradation product of trimethylamine oxide, an end product of nitrogen metabolism in fish. At sufficiently high exposure duration, DMA caused severe nasal and lung lesions, irritation of the eyes, skin and respiratory tract in human and animals that was manifested as mild lesion in the nasal mucosa and occasionally lesion of the liver, kidneys, and testes. Betulinic Acid (BA) is a naturally occurring pentacyclic lupane-type triterpenoid which exhibits varieties of medicinal properties such as anti-cancer activities, anti-inflammatory, anti-malarial, anti-human immunodeficiency virus (HIV) etc and is widely distributed throughout the plant kingdom. The aim of this study is to investigate the effects of betulinic acid on dimethylamine through the estimation of lipid peroxidation (LPO) and reduced glutathione (GSH) in kidneys, as well as level of urea and creatinine in both serum and kidneys and the effects of betulinic acid on the histopathology.
Twenty adult male wistar rats were used for this study which was equally divided into four groups. Group A (control) were fed with normal feed and distilled water, Group B (administered with BA 25 mg/kg body weight), Group C (administered with DMA 5 mg/kg body weight), and Group D (administered with BA 25 mg/kg body weight and DMA 5 mg/kg body weight). BA was given by oral gavage for 14 consecutive days, while DMA was administered intra peritoneal on days 7 and 12. After the expiration of the experimental period, the blood samples of the animals were collected by

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ocular bleeding. The sera and the kidneys of the experimental animals were separated and used to determine the level of urea and creatinine, estimation of reduced glutathione (GSH) and lipid peroxidation (LPO) levels. Also, the kidneys were prepared for histopathological analysis. The data presented in this study shows clearly that DMA significantly ($p<0.05$) elevated the renal urea, creatinine and malondialdehyde level. These findings suggest that BA was able to protect the kidney from the effects of DMA treatment.

Keywords: Dimethylamine (DMA); Betulinic acid (BA); Lipid peroxidation (LPO); reduced glutathione (GSH); malondialdehyde(MDA); rats.

1. INTRODUCTION

Dimethylamine (DMA) is a water-soluble, basic (pKa=10.73) secondary aliphatic amine with a smell of ammonia and/or rotting fish. DMA is the major short chain aliphatic amine in human and rat urine [1]. Seafood contains significant quantities of methylamine because DMA is a common degradation product of nitrogen metabolism in fish [2,3,4]. DMA can be formed from dietary choline in a reaction catalyzed by enzymes within the gut of bacteria [5,6]. DMA is present in many foods (e.g cabbage, celery, corn, fish, and coffee) and is also formed endogenously by gut of bacteria from DMA precursors (including trimethylamine-N-oxide). DMA vapors caused irritation of the eyes, skin and respiratory tract in human's animals that was manifested at lower concentrations as lacrimation and a mild lesion in the nasal mucosa.

At sufficiently high concentrations and/or exposure duration, animal studies reported severe nasal and lung lesions and occasionally lesion of the liver, kidneys, and testes. DMA is widely used in industries as a chemical intermediate in organic synthesis, in the manufacture of synthetic rubber and artificial resins, in the pharmaceutical industry, in paint and soap production, in the paper industries and in food processing. DMA can be synthesized by the reaction of methanol and ammonia in the presence of a dehydrating agent, and by catalytic hydrogenation of nitrosodimethylamine. DMA can react with nitrite to form N-nitrosodimethylamine (NDMA), which is hepatotoxic and carcinogenic. It occurs naturally in biological fluids as a result of endogenous metabolic processes. Large amount is found in frozen fish due to the action of trimethylamine oxidase on trimethylamine. It is manufactured by the reaction of ammonia and alcohol and is used as an accelerator in vulcanizing rubber, in leather tanneries, as a dehairing agent, in solvents, surfactants, insecticides, fungicides and many other products.

Betulunic acid is a naturally occurring pentacyclic lupane-type triterpenoid which exhibits a variety of biological and medicinal properties such as anti-cancer activities, anti-inflammatory, inhibition of human immunodeficiency virus (HIV), and anti-malarial etc. Betulinic acid is widely distributed throughout the plant kingdom. The birch tree (Betula spp, Betulaceae) is one of the most reported sources of betulinic acid and betulin which can be obtained in considerable quantities. Betulinic acid could also be isolated from various sources including *Zizipus spp* (Rhamnaceae), *Syzygium spp* (Myrtaceae), *Diospyros spp* (Ebenaceae) and *Paeonia spp* (Paeoniaceae). BA is moderately soluble in water and relatively non-toxic, found in many plants especially in tree species. It is a biologically active compound mainly known for its selective inhibitor of human melanoma [7]. The compound gains its name because of its prevalence in the family of betulaceae which include Betula alba, Betula pubescens, Betula platyphylla, Betula maximoviziana, and others. The family still serve as major source of betulinic acid. Also, its congener betulin is one of the first natural products isolated in 1788 from the bark of white birch, Betula alba [8,9].

This study was designed to reveal the modulatory roles of BA against DMA-induced renal injuries.

2. MATERIALS AND METHODS

2.1 Chemicals

DMA and BA were purchased from Sigma Chemical Co. (Saint Louis, Missouri, USA). Potassium Chloride was procured from British Drug House Chemical Ltd. (Poole, UK). Other Chemicals were of analytical grade and purest quality available.

2.2 Preparation of Treatment Solutions

Betulinic Acid= 0.043gBA + 1ml DMSO + 41ml of water

DMSO (Dimethylsulphoxide) was used to dissolve betulinic acid, since BA has a steroid structure that signify that it is insoluble in water. Therefore, little quantity of DMSO is used because it has an excellent anti-oxidant effect. 0.0434 g of BA was dissolved in about 1ml of DMSO and was then dissolved in distilled water.

DMA = 7.4 ml DMA + 4.6 ml of water

2.3 Experimental Animals

Male wistar rats weighing 120-150 g were obtained from Animal House of the Faculty of Basic Medical Sciences, Ladoke Akintola University of Technology, Ogbomoso, Nigeria. They were housed in plastic cages fed on rat pellets and given drinking water ad libitum. The rats were allowed to acclimatized for 14 days before the commencement of the experiment and kept at 12-h light/dark cycle and temperature of 29°C + or - 2°C. The Faculty of Basic Medical Sciences, Ladoke Akintola University of Technology Animal Ethics Committee, approved this study. All animals received humane care in compliance with the institution's guidelines and criteria for humane care as outlined in the National Institution of Health Guidelines for the care and use of laboratory animals [10].

2.4 Experimental Design

Twenty male wistar rats were assigned into four groups of five rats each. The first group (control) received drug vehicle (normal saline), second group (BA) received BA alone (25 mg/kg), third group (DMA) received DMA alone (5 mg/kg) and fourth group received BA and DMA.

BA was administered by oral gavage for 14 consecutive days, while DMA was administered intra peritoneal on days 7 and 12 of the study.

2.5 Collection of Blood and Tissues

The rats were fasted overnight after last dose of BA on day 14 and then sacrificed by cervical decapitation on day 15 under light ether anesthesia. Blood was collected by ocular bleeding, allowed to clot and centrifuged at 3000 g for 10 minutes to obtain serum. The kidneys were quickly excised and washed in ice-cold 1.15% potassium chloride solution to remove blood stains, dried and weighed. A section of the tissue was homogenized (blended using Teflon homogenizer) in tris-ethylenediaminetetraacetic acid (EDTA.TE) buffer at pH 7.4 and centrifuged at 27,000 g for 10 minutes and was used for estimation of biochemical assay while another section was stored in 10% formalin and used for histopathological analysis.

2.6 Biochemical Analysis

Total protein determination: Protein level in serum and kidney was determined according to the method of Lowry et al. [11] using bovine serum albumin as standard.

Urea determination: Serum and kidney urea level were estimated using the Berthelot method [12].

Creatinine determination: The quantitative determination of creatinine level in serum and kidney were based on the assay described by Jaffe [13].

Reduced glutathione (GSH) level estimation: The method of Anderson [14] was followed in estimating the level of total glutathione.

Lipid peroxidation assessment: Lipid peroxidation was determined by measuring the thiobarbituric acid reactive substances (TBARS) produced during lipid peroxidation as described by Rice-Evans et al. [15].

2.7 Statistical Analysis

The results were expressed in Mean+SD. ANOVA (One-way analysis of variance) method was used for the analysis of data. P-values <0.05 was considered statistically significant.

3. RESULTS

From Table 1, there was a significant ($p<0.05$) decrease in the serum and kidney protein concentration of rats in the group treated with DMA compared with control group. No significant difference was observed in the serum total protein concentration in the group treated with BA supplementation, compared with DMA group. A significant ($p<0.05$) increase in the kidney total protein concentration was observed in the BA supplemented group relative to the DMA-treated rats.

From Fig. 1, there was a significant increase ($p<0.05$) in the serum and kidney urea concentration in the DMA-treated group when compared with control. However, on BA supplementation, there was a significant decrease ($p<0.05$) in serum and kidney urea concentration when compared with the DMA group.

Table 1. Shows the effects of BA on protein concentrations in serum and kidney of rats treated with DMA

Treatment (Kidney)	M±SD (Serum)	M±SD
Control	7.47 ± 2.63	4.92 ± 3.88
BA	9.07 ± 2.24	2.44 ± 0.54
DMA	#5.95 ± 0.21	#1.69 ± 2.39
BA + DMA	*5.92 ± 0.12	*2.41 ± 0.04

BA: betulinic acid, DMA: dimethylamine; Data expressed in mean ± SD, n=5
 # statistic different (p<0.05) compared with control
** statistically different (p<0.05) compared with DMA*

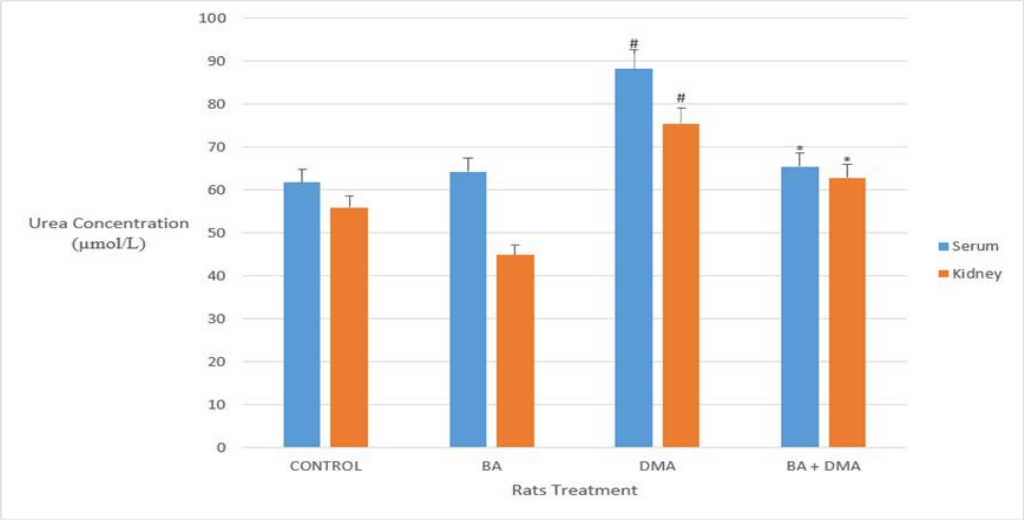


Fig. 1. Shows the effects of BA on urea concentration in serum and kidney of rats treated with DMA

BA: betulinic acid, DMA: dimethylamine;
Data expressed in mean ± SD, n=5
 # statistic different (p<0.05) compared with control
** statistically different (p<0.05) compared with DMA*

From Fig. 2, there was a significant increase (p<0.05) in the serum creatinine concentration in the DMA-treated group compared with control but there existed a significant (p<0.05) decrease in the kidney creatinine concentration in the group treated with DMA compared with the control.

On BA supplementation, a significant (p<0.05) decrease in serum creatinine concentration was observed compared with the DMA-treated group. Also, there was an increase in the kidney creatinine concentration in the BA supplemented group compared with the DMA group.

From Fig. 3, there was a significant (p<0.05) decrease in the kidney GSH level of DMA-treated rats compared with control but upon co-administration of BA, there existed no significant difference between the kidney GSH level of the BA supplemented group compared to the DMA-treated group.

From Fig. 4 there was a significant (p<0.05) increase in the kidney lipid peroxidation level of rats in the group treated with DMA compared with the control. However, a significant (p<0.05) decrease in the lipid peroxidation level was observed in the BA supplemented group compared with the DMA-treated group. BA supplementation suppressed the kidney lipid peroxidation level.

4. DISCUSSION

DMA is a common degradation product of trimethylamine oxide, an end product of nitrogen metabolism in fish [2,3,4]. DMA can be formed from dietary choline, in a reaction catalyzed by enzymes within the gut [5,6]. Betulinic acid is a

naturally occurring pentacyclic lupane-type triterpenoid which exhibit a variety of biological and medicinal properties. It is a biological active compound, mainly known as selective inhibitor of human melanoma [7].

In this study, administration of DMA resulted in serum and kidney protein concentration. This result suggested the administration of DMA alone has ultimately led to the decrease in total protein concentration in serum and kidney which may reflect renal dysfunction or disease. Pretreatment with BA significantly increased the level of total protein concentration. In other word, BA pretreatment was able to attenuate the induced effects of DMA.

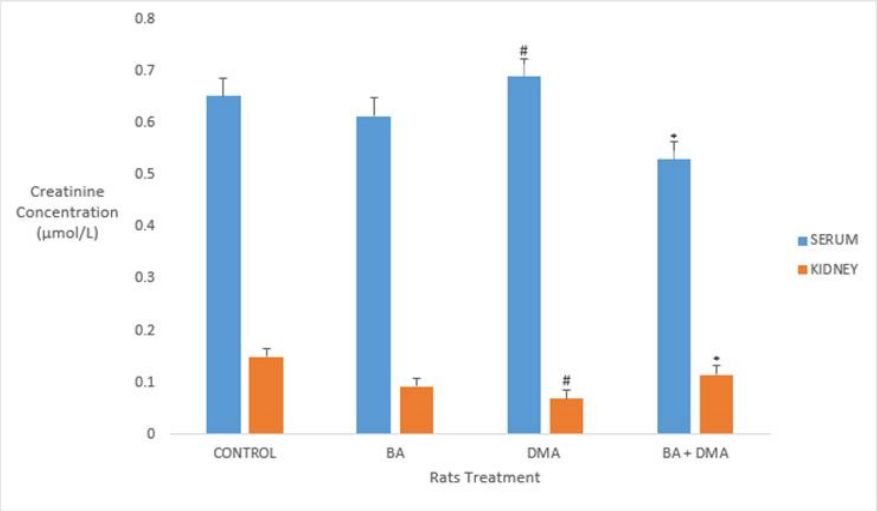


Fig. 2. Shows the effects of BA on creatinine concentration in serum and kidney of rats treated with DMA

BA: betulinic acid, DMA: dimethylamine;
Data expressed in mean ± SD, n=5
 # statistic different (p<0.05) compared with control
** statistically different (p<0.05) compared with DMA*

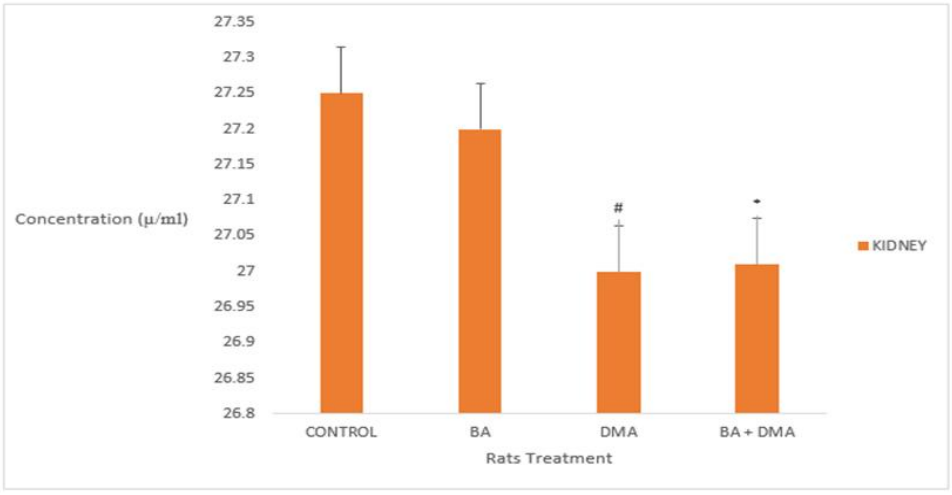


Fig. 3. Shows the effects of BA on GSH level in kidney of rats treated with DMA

BA: betulinic acid, DMA: dimethylamine;
Data expressed in mean ± SD, n=5
 # statisc different (p<0.05) compared with control
** statistically different (p<0.05) compared with DMA*

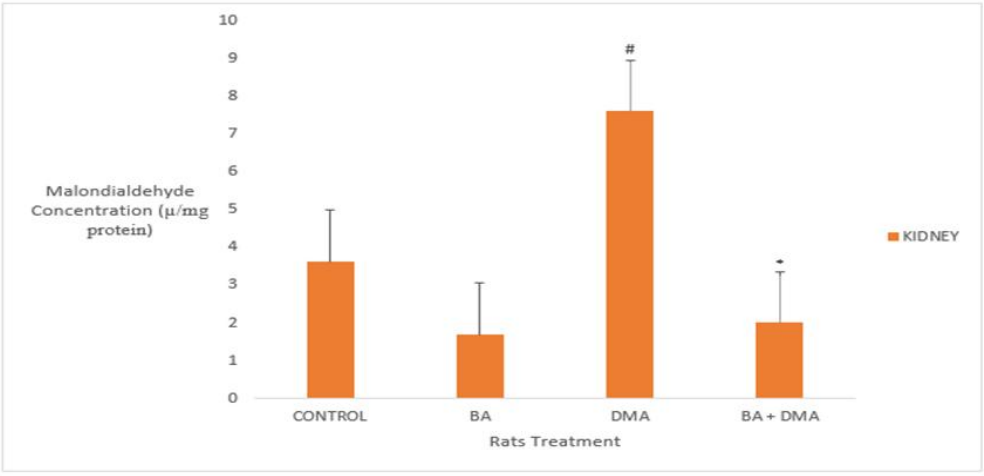
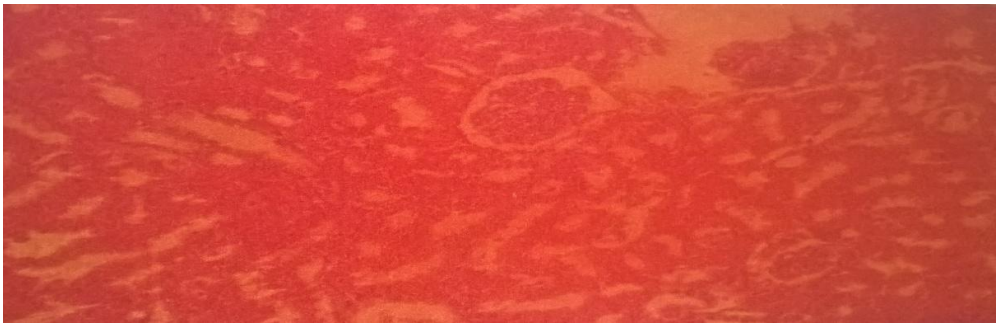


Fig. 4. Shows the effects of BA on Malondialdehyde concentration in kidney of rats treated with DMA

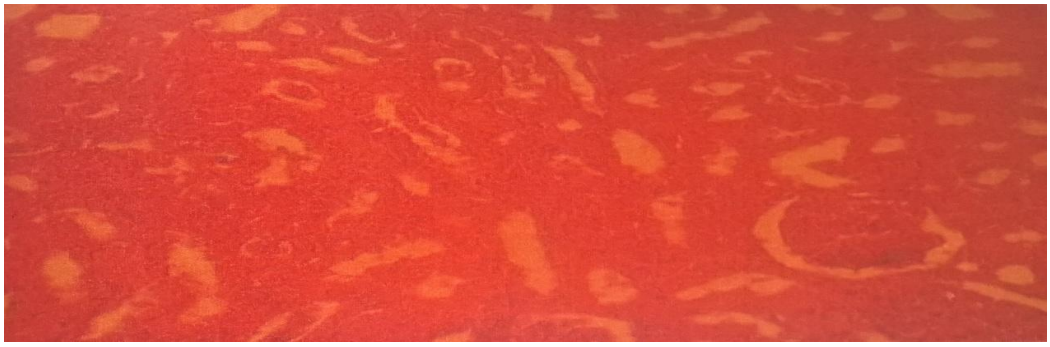
BA: betulinic acid, DMA: dimethylamine;
Data expressed in mean ± SD, n=5
 # statistic different (p<0.05) compared with control
** statistically different (p<0.05) compared with DMA*

Urea is a major end product of protein and amino acid catabolism produced by liver and has been widely used as one of the suitable indicators of renal function in human and animal models [16,17]. DMA intoxication elevated serum urea level. This is in consonance with the findings of Adeleke and Adaramoye (2016). Urea elevation is an indication of abnormal renal function. Urea has also been reported to increase in acute and chronic renal dysfunction [18]. Interestingly, BA pretreatment significantly reduced serum and kidney urea level.

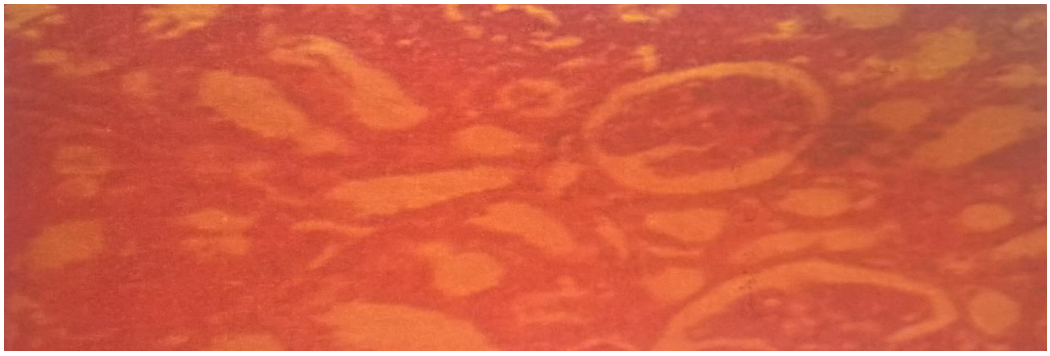


(a) Control (kidney)- Normal renal corpuscles and tubules x100

(b) BA (kidney)-Normal renal corpuscles and tubules x100



(c) DMA (kidney)- Mild cellular hypertrophy (x100)



(d) BA + DMA (kidney)- Mild cellular degeneration (x100)

Fig. 5. Shows the effects of BA on Histology of kidney of rats treated with DMA

Creatinine is a breakdown product of creatine phosphate in muscle [19]. Creatinine level has also been widely used as one of the suitable indicators of renal function in human and animal models [16,17]. DMA intoxication increased serum creatinine level. This observation is consistent with the findings of Adeleke and Adaramoye (2016) who reported that N-nitrosodimethylamine elevated serum creatinine of male rats. The elevation of creatinine in DMA-treated rats may be a sign of renal impairment which may be due to tubular damage or induced glomerular filtration [20]. Creatinine elevation has also been implicated in diseases such as acute kidney injury [18]. Interestingly, pretreatment with BA significantly decreased the serum creatinine level. This showed the renal protective effect of BA.

Administration of DMA significantly lowered the level of GSH concentration in the kidney. However, BA pretreatment could not reverse or improve this effect.

DMA intoxication in the present study significantly increased the level of malondialdehyde (MDA). Similar findings were reported by Adeleke and Adaramoye (2016). Lipid peroxidation has been shown to be one of the basic mechanisms of cellular damage caused by free radicals. Lipid peroxidation is due to the reaction of free radicals with lipids (polyunsaturated fatty acids) which results in the release of malondialdehyde (MDA), hydrogen peroxide H_2O_2 and hydroxyl radicals. Increase in the level of malondialdehyde (MDA) is a well-established biomarker of tissue damage [21]. Exposure of experimental animals to the toxicant has been found to induce lipid peroxidation leading to oxidative stress. However, pretreatment with BA significantly decreased malondialdehyde (MDA) level and was able to ameliorate the DMA-induced toxicity in the experimental rats.

Histological examination revealed that DMA caused mild cellular hypertrophy of renal corpuscles and tubules of the kidney but on supplementation with BA, mild cellular degeneration was observed.

5. CONCLUSION

In conclusion, from the data presented in this present study, it could be concluded that DMA elevated renal urea, creatinine and malondialdehyde (MDA) levels. However, BA protected the kidney from the effects of DMA treatment.

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COMPETING INTERESTS

Author has declared that no competing interests exist.

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