



Effect of oral berberine administration on the renal profiles of adenosine deaminase, arginase, and nitric oxide in streptozotocin-induced diabetic nephropathy of rats

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Abstract

Berberine (BBR) administration is effective in the management of diabetic nephropathy, but the full mechanism of action has not been comprehensively established. This study investigated the effect of oral administration of BBR on fasting blood glucose (FBG) level, renal function (serum uric acid, urea, and creatinine) markers, arginase and adenosine deaminase (ADA) activities, and endogenous antioxidant level in streptozotocin (STZ)-induced diabetic nephropathy of rats. Non-diabetic and diabetic (induced with 50 mg/kg body weight (bwt) of STZ) rats were treated with 50 and 100 mg/kg bwt of BBR for 14 days. Thereafter, the rats were sacrificed and the kidney was isolated and homogenized. The supernatant was used for the determination of ADA and arginase activities, nitric oxide (NO), and malonylaldehyde (MDA) levels. Antioxidant status of the kidney (superoxide dismutase (SOD), glutathione peroxidase (GPx) activities, and glutathione (GSH) level) was determined. The result indicated that FBG level, as well as serum biomarkers of kidney function, was drastically reduced in diabetic rats treated with BBR when compared to the untreated diabetic rats. Induction of diabetes led to an increase in ADA and arginase activities, and MDA level, with a concomitant decrease of NO level and antioxidant status, compared with the normal control rats. However, treatment with BBR (50 and 100 mg/kg) reversed these effects. In conclusion, oral administration of BBR decreased FBG, ADA and arginase activities, and MDA level, and increased NO and antioxidant in diabetic rats.

Keywords Berberine · Diabetes · Nephropathy · Nitric oxide · Arginase · Adenosine deaminase

Introduction

Diabetic nephropathy (DN) is one of the severe chronic complications of diabetes mellitus (DM) (Warren et al. 2019). It is characterized by proteinuria, mesangial expansion, glomerular basement membrane (GBM) thickening, persistent fibrosis, and a gradual decrease in renal function, which result from increased extracellular matrix (ECM) deposition at the glomerular level (Kato and Natarajan 2014). Several studies have related the development of kidney damage with the increased activities of arginase and adenosine

deaminase (ADA), as well as increased oxidative stress (Ni et al. 2015; Akinyemi et al. 2014, 2017; Ademiluyi et al. 2014). Although persistent hyperglycemia had been linked to DN (Ni et al. 2015), no study had investigated the activities of arginase and ADA in the DN model.

According to the reports of Bauerle et al. (2011) and Akinyemi et al. (2017), adenosine, which is an endogenous signalling molecule, has been implicated in kidney function. Its production involves enzymatic phosphohydrolysis of ATP and/or AMP. Adenosine is involved in the regulation of glucose homeostasis by improving insulin secretion and also regulating β -cell homeostasis and activity (Antonoli et al. 2015). However, study indicates that the functionality of adenosine could be hampered by its reduction via the activity of adenosine deaminase (ADA), an enzyme that is present in the kidney, and catalyses the irreversible hydrolytic deamination of adenosine to inosine and 2-deoxyadenosine to 2-deoxyinosine, thereby depleting the concentration and functionality of adenosine (Akinyemi et al. 2017). Hence, an attempt to inhibit the activity of ADA has been proposed

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as a therapeutic approach for the prevention of kidney dysfunction in diabetic patients (Quezada et al. 2013; Antonioli et al. 2015).

Nitric oxide (NO) is an active endogenous vasodilator, involved in the regulation of renal function (Obloh et al. 2017). It is produced from catalytic activity of nitric oxide synthase (NOS), using L-arginine as substrate (Obloh et al. 2018). Arginase also competes for L-arginine with NOS to produce urea and L-ornithine, thereby, reducing the production of NO (Obloh et al. 2018). According to the report of You et al. (2015), arginase activity increases during diabetic conditions, but, its inhibition has also been reported to have a therapeutic effect on DN. Studies have also shown that NO level decreases under oxidative stress, a major factor in the pathophysiology of diabetes (Rehman and Akash 2017; Obloh et al. 2018). Free radicals such as superoxide radical (O_2^-) can interact with NO to form peroxynitrite (ONOO), thereby depleting NO bioavailability (Obloh et al. 2016). Therefore, any attempt to alleviate oxidative stress or boost endogenous antioxidant levels could also be a good therapeutic strategy towards the management of DM and DN (Kashihara et al. 2010).

Berberine (BBR) is an isoquinoline alkaloid obtained from numerous herbal plants such as *Coptis chinensis* (Coptis or Goldthread), *Hydrastis canadensis* (goldenseal), *Berberis aquifolium* (Oregon grape), *Berberis aristata* (Tree Turmeric), *Berberis vulgaris* (Barberry), and *Arcangelisia flava* (Singh and Mahajan 2013). BBR is widely known for its anti-oxidative, anti-apoptotic, antidiabetic, anticancer, and anti-inflammatory and cognitive potentials (Ni et al. 2015; Chen et al. 2016; de Oliveira et al. 2016). Numerous studies have documented the protective roles of BBR and its possible mechanism of actions towards the management of DN (Ni et al. 2015; Kumaş et al. 2019; Chen et al. 2016; de Oliveira et al. 2016), but none has ever reported the effect BBR treatment on the activities of ADA and arginase as well as NO level in the kidney of diabetic rats. This study focused on elucidating the therapeutic mechanisms of BBR treatment in the amelioration of DN by investigating the effect of oral BBR administration on the activities of ADA and arginase, as well as NO level in the kidney homogenate of STZ-induced diabetic rats in an experimental model that evaluated hyperglycemia, oxidative stress, and renal dysfunction in relation to nephropathy.

Materials and methods

Chemicals

5,5'-dithio-bis-2-nitrobenzoic acid (DTNB), Tris buffer, reduced glutathione, adenosine, L-arginine, Coomassie brilliant Blue G, streptozotocin (STZ), and berberine

hydrochloride were obtained from Sigma-Aldrich (St. Louis, MO, USA). Acarbose was purchased from Glenmark Generics (Europe) pharmaceutical limited. Thiobarbituric acid (TBA) and trichloroacetic acid (TCA) were procured from Sigma-Aldrich, Inc. (St Louis, MO, USA). Sodium carbonate, ammonium sulphate, sodium nitroprusside, potassium acetate, sodium dodecyl sulphate (SDS), iron (II) sulphate and disodium hydrogen phosphate, and sodium dihydrogen phosphate were sourced from BDH Chemicals Ltd. (Poole, England).

Experimental animals

Fifty-six adult male rats weighing between 200 and 230 g were obtained from the animal house of the Faculty of Veterinary Medicine, University of Ibadan. After 2 weeks of acclimatization, they were randomly divided into 7 groups ($n=8$). The animals were kept in wire mesh cages under a controlled light cycle (12-h light/12-h dark); they were fed with commercial rat chow with free access to water ad libitum. All institutional and national guidelines for the care and use of laboratory animals were followed. All the experimental animals received humane care, according to the criteria outlined in the Guide for the Care and Use of Laboratory Animals (National Institutes of Health 2011).

Induction of diabetes

The rats ($n=48$) were made diabetic via intraperitoneal (i.p.) injection of a single dose (50 mg/kg bwt) of freshly prepared STZ (10 mg/0.2 ml) in citrate buffer (0.1 M, pH 4.5). The dose was reported by Parisa and Siamak (2011) and Kumaş et al. (2019). After 72 h, blood glucose test was conducted, and rats with blood glucose 250 mg/dl were considered diabetic and were used for this study. The ethical regulations are in accordance with national and institutional guidelines for the protection of animal welfare during experiments.

Experimental design

The fifty-six rats (48 diabetes and 8 normal) were, thereafter, divided randomly into 7 treatment groups ($n=8$).

- Group I: Normal control rats received citrate buffer
- Group II: Normal rats treated with 50 mg/kg BBR (10 mg/0.2 ml) through oral gavage
- Group III: Normal rats treated with 100 mg/kg BBR (20 mg/0.2 ml) through oral gavage.
- Group IV: Untreated diabetic rats
- Group V: Diabetic rats treated with 50 mg/kg of BBR (10 mg/0.2 ml) through oral gavage
- Group VI: Diabetic rats treated with 100 mg/kg of BBR (20 mg/0.2 ml) through oral gavage

Group VII: Diabetic rats treated with 25 mg/kg of acarbose (ACA, 5 mg/0.2 ml) through oral gavage

The normal rats were orally treated daily for 14 days with citrate buffer (1 ml/kg bwt of rats) in group I (normal control) and BBR at 50 and 100 mg/ml in group II and group III (treated control) respectively. The diabetic rats were not treated in group IV (negative diabetic control). Group V and VI were diabetic rats treated with 50 and 100 mg/kg of BBR, respectively, to assess the treatment of BBR effect (de Oliveira et al. 2016). Group VII served as positive control and was orally treated with acarbose, which is a standard hypoglycemic drug, having inhibitory effects on α -amylase and α -glucosidase activity (Adefegha et al. 2021a, b). Fasting blood glucose (FBG) levels were monitored on days 1, 4, 7, 10, and 14 during the treatment. At the end of the treatment, on day 15, the rats were anaesthetized with isoflurane, and thorax was opened up to collect the blood via cardiac puncture into the plain bottle. The kidney was isolated, transferred to a clean glass plate on ice, and washed with buffer. The right kidney was used for the biochemical assay, while the left kidney was fixed in 10% formalin for the histological examination.

Sample collection

The cardiac blood collected was allowed to clot and serum was harvested, by centrifuging for 10 min at $5000 \times g$. Right kidney collected was weighed, rinsed with cold saline, and homogenized with five volumes of 0.1 M of phosphate buffer (pH 7.4) (Oboh et al. 2018). The homogenate was subsequently centrifuged at $5000 \times g$ for 10 min to collect the supernatant that was used for the determination of biochemical assays (Oboh et al. 2020).

Biochemical assays

Determination of fasting blood glucose

Tail vein blood, collected after sniping the tail tip, was used in the estimation of FBG with an auto-coding glucose metre and test strip containing glucose oxidase (Finetest, Infopia, South Korea).

Determination of total protein content

The protein content of the supernatant from kidney homogenate was measured by the method of Bradford (1976) using bovine serum albumin (BSA) as standard.

Determination of serum urea, creatinine, and uric acid levels

Serum urea, creatinine, and uric acid levels were estimated by using commercially available kits from RANDOX Laboratories Ltd., Crumlin, County Antrim, UK, and the procedures stated therein were strictly followed.

Determination of malondialdehyde (MDA) level

The MDA level in the supernatant from the kidney homogenate was determined by the method described by Ohkawa et al. (1979) with slight modifications. The reaction mixture contained 200 μ l of the homogenate or standard (MDA 0.03 mM), 200 μ l of 8.1% sodium dodecyl sulphate (SDS), 750 μ l of acetic acid solution (2.5 M HCl, pH 3.5), and 750 μ l of 0.8% TBA was incubated at 100 °C for 60 min. The absorbance was measured at 532 nm and the MDA level was expressed as nmol MDA/mg protein.

Determination of superoxide dismutase and glutathione peroxidase activities, and glutathione (GSH) level

Superoxide dismutase (SOD) activity was determined by the method of Alía et al. (2003) using adrenaline (0.06 mg/ml) as substrate. Glutathione peroxidase (GPx) activity was assayed by the method of Paglia and Valentine (1967), while the glutathione (GSH) level was determined using Ellman's reagent (19.8 mg of 5,5' dithiobisnitrobenzoic acid in 100 mg of 0.1% sodium citrate) (Ellman 1959).

Determination of adenosine deaminase activity

ADA activity was measured in kidney homogenate using the method of Giusti and Galanti (1984), as described by Olasehinde et al. (2020), where 50 μ L of kidney homogenate was added to 21 mM of the substrate (adenosine, pH 6.5) and incubated at 37 °C for 1 h. Absorbance was read at 620 nm.

Determination of arginase activity

Kaysen and Strecker (1973) method as reported by Dada et al. (2020) was adopted for the determination of arginase activity in the kidney homogenate of normal and STZ-induced diabetic rats, using 50 mM L-arginine as the substrate to quantify the produced urea intensity at 450 nm.

Determination of nitric oxide level

Briefly, the mixture of kidney homogenate (150 μ L), distilled water (450 μ L), and Griess reagent (600 μ L) was incubated at room temperature for 10 min and the absorbance was read at 540 nm. The concentration of nitrite in the

supernatant was determined from the sodium nitrite standard curve and expressed as $\mu\text{mol}/\text{mg}$ protein (Green et al. 1982).

Histopathological studies

The kidney that was fixed in formalin was processed for histological sectioning (Avwioro 2002). The thin section (5 microns) of the tissue obtained was mounted on glass slides and stained with haematoxylin and eosin (H&E). Thereafter, the slides were observed in the optical microscope to evaluate possible damage in the kidney tissue.

Statistical analysis

GraphPad (version 5.0) prism was used for statistical analysis of the experimental results. The data were summarized as mean \pm standard error of the mean (SEM). The variation in mean value was analysed for significance level using one-way ANOVA with Tukey post hoc test. Level of significance was accepted at $p < 0.05$, $p < 0.01$, $p < 0.001$.

Results

Effect of oral administration of berberine on fasting blood glucose level of normal and diabetic rats

Increased level of fasting blood glucose (FBG) was observed in untreated diabetic rats ($p < 0.05$) relative to the normal control (Fig. 1). The FBG level of normal rats

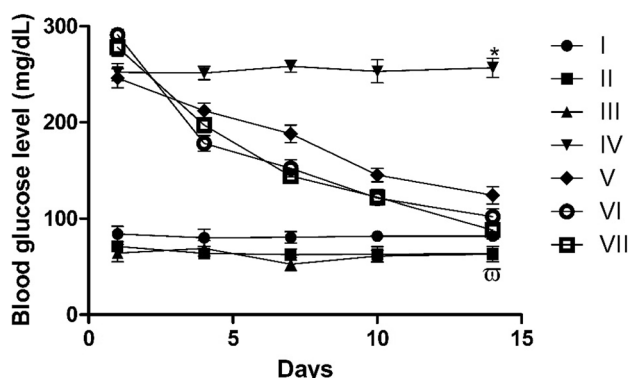


Fig. 1 Blood glucose level of normal and STZ-induced diabetic rats treated with oral administration of berberine (BBR). Key: Group I: Normal control rats received citrate buffer. Group II: Normal rats treated with 50 mg/kg BBR through oral gavage. Group III: Normal rats treated with 100 mg/kg BBR through oral gavage. Group IV: Untreated 50 mg/kg STZ-induced diabetic rats. Group V: Diabetic rats treated with 50 mg/kg of BBR through oral gavage. Group VI: Diabetic rats treated with 100 mg/kg of BBR through oral gavage. Group VII: Diabetic rats treated with 25 mg/kg of acarbose (ACA) through oral gavage

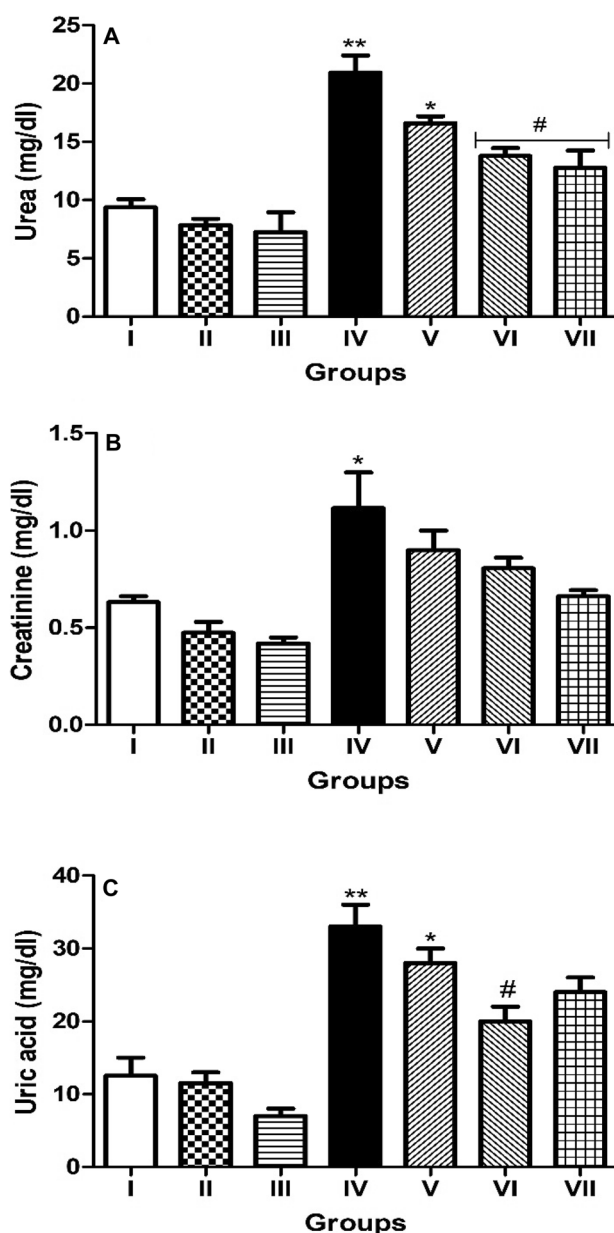


Fig. 2 Effect of oral administration of berberine (BBR) on urea (A), creatinine (B), and uric acid (C) levels in the serum of normal and STZ-induced diabetic rats. Bars represent mean \pm SEM ($n = 8$). Values are statistically different at ** $p < 0.001$, * $p < 0.05$ versus normal control rats; ## $p < 0.01$, # $p < 0.05$ versus STZ-induced diabetic rats. Key: Group I: Normal control rats received citrate buffer. Group II: Normal rats treated with 50 mg/kg BBR through oral gavage. Group III: Normal rats treated with 100 mg/kg BBR through oral gavage. Group IV: Untreated 50 mg/kg STZ-induced diabetic rats. Group V: Diabetic rats treated with 50 mg/kg of BBR through oral gavage. Group VI: Diabetic rats treated with 100 mg/kg of BBR through oral gavage. Group VII: Diabetic rats treated with 25 mg/kg of acarbose (ACA) through oral gavage

treated with 50 and 100 mg/kg BBR, respectively, ranged between 62 and 69 mg/dl, which was significantly lower ($p < 0.05$) when compared to normal control rats (80 and

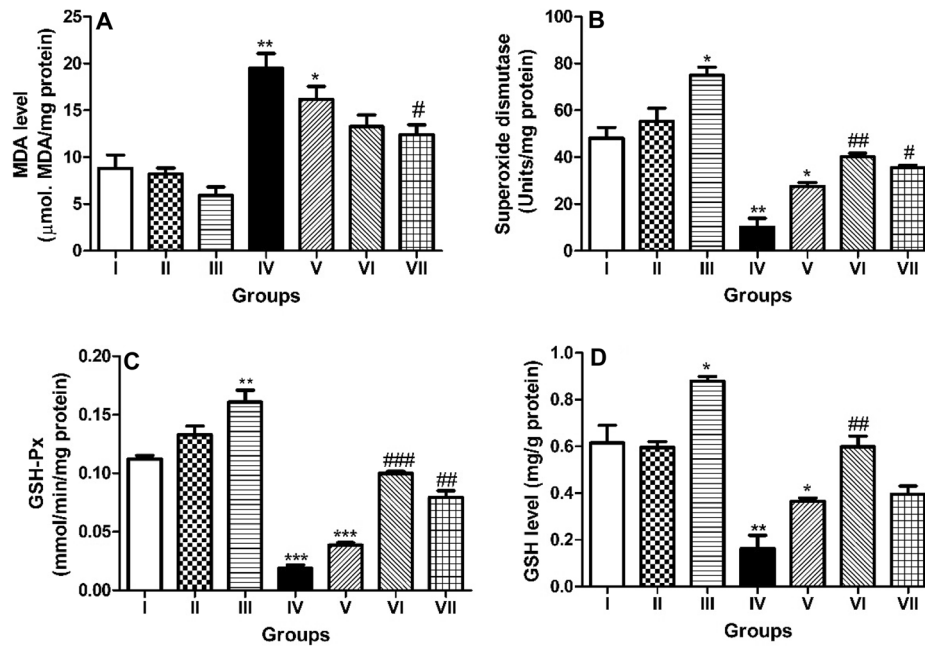


Fig. 3 Effect of oral administration of berberine (BBR) on malonyl-aldehyde (MDA) level (A), superoxide dismutase activity (B), glutathione peroxidase (GPx) activity (C), and glutathione (GSH) level (D) in the kidney homogenate of normal and STZ-induced diabetic rats. Bars represent mean \pm SEM ($n=8$). Values are statistically different at *** $p < 0.001$, ** $p < 0.01$, * $p < 0.05$ versus normal control rats; ### $p < 0.01$, ## $p < 0.01$, # $p < 0.05$ versus STZ-induced diabetic rats. Key: Group I: Normal control rats received citrate buffer. Group

II: Normal rats treated with 50 mg/kg BBR through oral gavage. Group III: Normal rats treated with 100 mg/kg BBR through oral gavage. Group IV: Untreated 50 mg/kg STZ-induced diabetic rats. Group V: Diabetic rats treated with 50 mg/kg of BBR through oral gavage. Group VI: Diabetic rats treated with 100 mg/kg of BBR through oral gavage. Group VII: Diabetic rats treated with 25 mg/kg of acarbose (ACA) through oral gavage

84 mg/dl). Reduced FBG level was observed in diabetic rats treated with ACA (278.0 to 88 mg/dl) at the end of the experiment. That of the diabetic rats treated with 50 mg/kg of BBR had 124 mg/dl, while that of 100 mg/kg BBR treated diabetic rats was reduced from 291.0 to 102.2 mg/dl at the end of the experimental period. Both FBG levels of the normal rats treated with 50 mg/kg BBR and that of the 100 mg/kg BBR treated normal rats were not different ($p > 0.05$) when compared, but were lower ($p < 0.05$) relative to the FBG level of the normal control rats. Interestingly, the FBG level of diabetic rats treated with ACA was not different ($p > 0.05$) compared to the FBG level of normal control rats at the end of the experimental period.

The effect of oral administration of berberine on serum urea, creatinine, and uric acid levels in the diabetic rats

Figure 2A–C reveal the levels of urea, creatinine, and uric acid, respectively, in the serum of STZ-induced diabetes in rats. On the other hand, diabetic rats treated with BBR (50 and 100 mg/kg) and ACA (25 mg/kg) reversed these effects.

The effect of berberine on MDA level, SOD and GPx activities, and GSH level in the kidney of diabetic rats

In Fig. 3A, MDA level was increased in the kidney homogenate in untreated diabetic rats ($p < 0.01$) when compared with the normal control rats. In normal rats, administration of BBR (50 and 100 mg/kg) inhibited MDA production but was not different from that of normal control rats. As evidenced in Fig. 3B–D, the diabetic rats had a significant ($p < 0.01$) decrease in the activities of SOD and GPx, and GSH level, when compared with the control rats. Upon treatment with BBR (50 and 100 mg/kg) and ACA (25 mg/kg), activities of antioxidant enzymes, and level of GSH were increased with varying significant differences in comparison with untreated diabetic rats.

The effect of berberine on ADA and arginase activities, and NO level in the kidney of diabetic rats

Figure 4A depicts the effect of BBR on ADA activity. Untreated diabetic rats have increased ADA activity compared to normal control. The normal rats treated with 50 mg/kg BBR as well as 100 mg/kg BBR treated normal rats had

reduced ADA activity compared to normal control rats. However, there was a significant ($p < 0.05$) reduction in ADA activity of 100 mg/kg BBR-treated normal rats when compared to the normal control rats. Diabetic rats treated with BBR (50 and 100 mg/kg bwt) and acarbose had reduced ADA activity, but diabetic rats treated with 100 mg/kg bwt had the highest ($p < 0.01$) reduction of ADA activity. From Fig. 4B, arginase activity was higher ($p < 0.05$) in untreated diabetic rats compared to normal control rats. This effect was reversed significantly ($p < 0.01$) in diabetic rats treated with 100 mg/kg of BBR and ACA. All the normal rats treated with BBR (50 and 100 mg/kg bwt) have reduced arginase activity, but not significantly different from normal control rats. Figure 4C reveals that NO level was boosted in normal rats, most especially in 100 mg/kg treated normal rats ($p < 0.05$) compared to normal control rats. Untreated diabetic rats have a depleted level of NO ($p < 0.05$) relative to normal control rats; however, the diabetic rats treated with BBR, most especially that of 100 mg/kg treated rats, had a significant ($p < 0.05$) increase of NO level.

The effect of oral administration of berberine on the histopathology of the kidney

Figure 5 reveals the photomicrograph of the kidney where group I (a) showed normal histoarchitecture of the kidney with the glomeruli and the tubules well delineated. Group II (b), which is normal rats treated with 50 mg/kg BBR, also revealed good histology when compared with (group III) normal rats treated with 100 mg/kg BBR (c) and diabetic rats treated with ACA (group VII), where the tubules are not distinct enough. Group IV (e) on the other hand shows signs of necrosis, especially within the glomeruli (yellow outlines). Groups V (f), group VI (g), and group VII (h) also show improved histology, but a better one is seen in group VI with the tubules clearly revealing better nephroprotective property of oral administration of BBR.

Discussion

The present study evaluates the renoprotective mechanism of oral administration of BBR in the management of DN model rats. Accordingly, the fasting blood glucose (FBG) level was high in the untreated diabetic rats than in the normal control (NC) rats. This suggested that the diabetic model was successful. After oral administration of BBR, the FBG level was reduced closer to that of normal control. Interestingly, diabetic rats treated with 100 mg/kg of BBR had reduced ($p < 0.05$) FBG compared with that of 50 mg/kg BBR-treated diabetic rats. This suggests that oral administration of BBR at 50 mg/kg can reduce FBG, but 100 mg/kg dosage had a

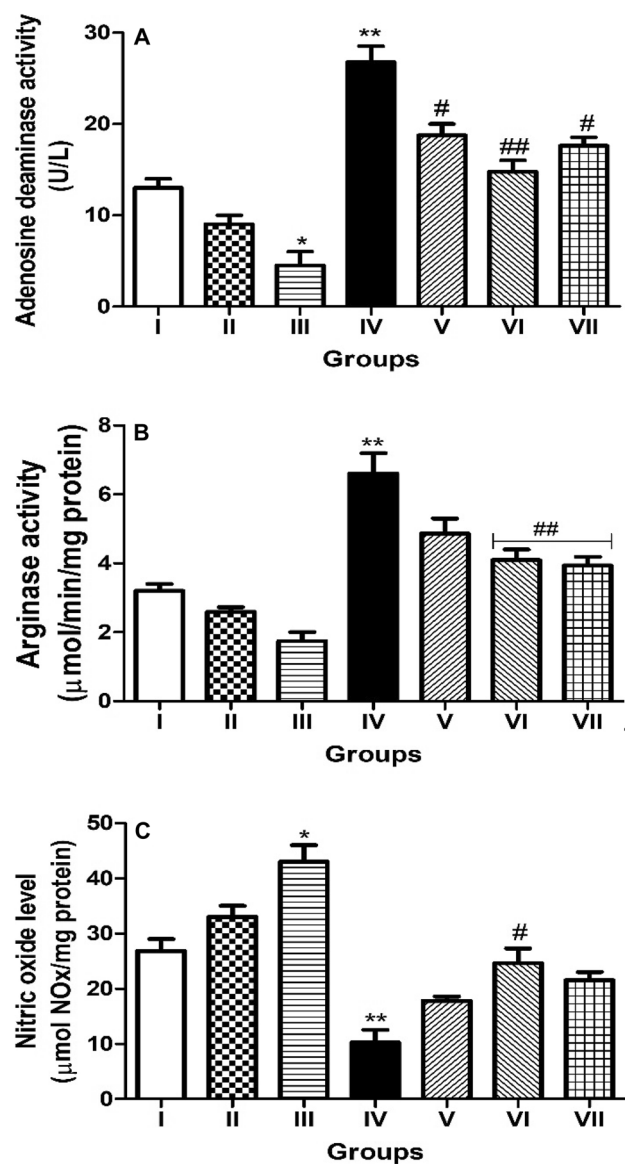


Fig. 4 Effect of oral administration of berberine (BBR) on adenosine deaminase activity (A), arginase activity (B), and nitric oxide (NO) level (C) in the kidney homogenate of normal and STZ-induced diabetic rats. Bars represent mean \pm SEM ($n = 8$). Values are statistically different at ** $p < 0.001$, * $p < 0.05$ versus normal control rats; ## $p < 0.01$, # $p < 0.05$ versus STZ-induced diabetic rats. Key: Group I: Normal control rats received citrate buffer. Group II: Normal rats treated with 50 mg/kg BBR through oral gavage. Group III: Normal rats treated with 100 mg/kg BBR through oral gavage. Group IV: Untreated 50 mg/kg STZ-induced diabetic rats. Group V: Diabetic rats treated with 50 mg/kg of BBR through oral gavage. Group VI: Diabetic rats treated with 100 mg/kg of BBR through oral gavage. Group VII: Diabetic rats treated with 25 mg/kg of acarbose (ACA) through oral gavage

better effect and, thus, could prevent the progression of DN (Zhang et al. 2010; Liang et al. 2019).

A significant increase in serum urea, creatinine, and uric acid levels of diabetic rats pointed towards kidney

damage/dysfunctions (Ademiluyi et al. 2014; Kumaş et al. 2019). The increased levels of these biomarkers confirm that diabetes-induced damage to the kidney. However, oral administration of BBR significantly improved kidney function by lowering urea, creatinine, and uric acid levels, when compared with the untreated diabetic rats. This indicates that BBR possesses antidiabetic properties and is effective towards the management of DN; the observation agreed with the study reported by Wu et al. (2012).

Oxidative stress triggers chronic kidney disease via redox imbalance and free radical production, thus leading to a low antioxidant defence mechanism (Chirino and Pedraza-Chaverri 2009; Daenen et al. 2019). The mechanism of diabetes-induced oxidative stress involves an overwhelming production of reactive oxygen species (ROS) in tissues and cellular components, and/or decreased antioxidants, thereby, causing damage to membranes, DNA, and proteins (Kashihara et al. 2010). This effect could partly be responsible for reduced NO level in the diabetic rats, as radicals such as superoxide radical (O_2^-) react with NO to form peroxynitrite ($ONOO^-$) and other peroxy radicals such as MDA (Ademiluyi et al. 2016; Adefegha et al. 2021a, b). This is evidently observed in this study, as the elevated MDA level was observed in the kidney of untreated diabetic rats. MDA has been regarded as a major biomarker of oxidative stress (Sodipo et al. 2021). Also, a large number of oxidized lipids, such as hydroperoxide, have been linked to the progression of DN (Zwolińska et al. 2006). The observed decrease in endogenous antioxidant

(SOD and GPx activities, and GSH level) in the kidney tissue of untreated diabetic rats as against that of the control and treated normal rats, further, confirmed the occurrence of oxidative damage and renal injury, possibly caused by the excessive production of ROS. However, treatment with BBR (50 and 100 mg/kg) reversed these effects and also decreased MDA level with a concomitant increase in the antioxidant defence mechanism via an increase in the activities of SOD and GPx, and GSH level, when compared with the untreated diabetic rats. Interestingly, the improved antioxidant defence system is significant in diabetic rats treated with oral administration 100 mg/kg BBR.

Increased activity of ADA in the kidney had been ascribed to kidney toxicity as well as defects in the DNA of cells and immune system (Kumar et al. 2015). In addition, increased ADA activity in this study could mean a decreased level of adenosine, a renoprotective molecule. Extracellular adenosine primarily functions as a signalling molecule and mediates diverse physiological functions (Adefegha et al. 2021a, b). Several studies have established the level of adenosine in hypoxia, inflammation, or acute renal injury (Yap and Lee 2012; Bowser et al. 2017). In this study, treatment with BBR prevented increased ADA activity in the kidney of diabetic rats. This apparently proves that BBR has a nephroprotective role against DN, and the ability to inhibit/reduce ADA activity could be an added mechanism of the renoprotective ability of BBR.

Several studies have reported that the inhibition of arginase activity improved kidney function in DN (You et al.

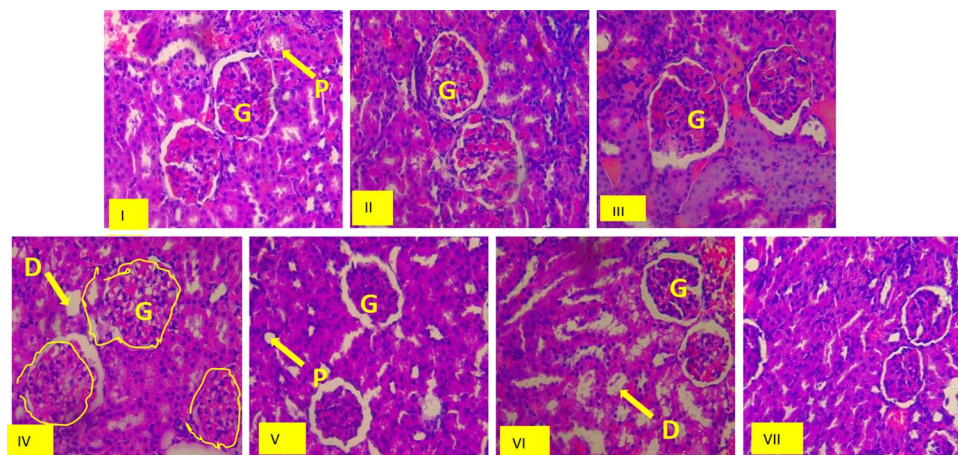


Fig. 5 Haematoxylin and eosin of kidney tissue of groups I, II, III, IV, V, VI, and VII respectively. The group I shows normal histoarchitecture of the kidney with the glomeruli and the tubules well delineated. Group II also reveals good histology when compared with group III and group VII where the tubules are not distinct enough. Group IV the other hand shows sign of necrosis especially within the glomeruli (yellow outlines). Groups V, VI, and VII, respectively, also show improved histology but a better one is seen in group VI with the tubules clearly revealed indicating its better protective property. G, glomerulus; P, proximal convoluted tubule; D, distal convo-

luted tubule; stain, haematoxylin and eosin; magnification, $\times 400$; STZ, streptozotozin; BER, berberine; ACA, acarbose. Key: Group I: Normal control rats received citrate buffer. Group II: Normal rats treated with 50 mg/kg BBR through oral gavage. Group III: Normal rats treated with 100 mg/kg BBR through oral gavage. Group IV: Untreated 50 mg/kg STZ-induced diabetic rats. Group V: Diabetic rats treated with 50 mg/kg of BBR through oral gavage. Group VI: Diabetic rats treated with 100 mg/kg of BBR through oral gavage. Group VII: Diabetic rats treated with 25 mg/kg of acarbose (ACA) through oral gavage

2013; Akinyemi et al. 2017). Significant elevation of arginase activity was observed in the kidney of untreated diabetic rats compared with that of normal control rats. This effect could result in low availability of L-arginine, as a substrate for nitric oxide synthase (NOS) activity, hence, induce reduced NO level. Therefore, pharmacological inhibition of renal arginase activity in BBR-treated rats implies that BBR could mediate renal tissue protection. Hence, this study revealed an important role of arginase activity in the pathogenesis of renal injury and provided evidence for arginase inhibition as a potential therapeutic modality for treating DN. In this study, diabetic rats treated with BBR (50 and 100 mg/kg) had a significant reduction of arginase activity compared with that of untreated diabetic rats. Inhibition of arginase activity could increase the availability of L-arginine for the production of NO by NOS activity.

The changes in the architecture observed in the histological study of kidney tissue of diabetic rats further revealed the renal damage caused by oxidative stress as a result of diabetes. Also, the observed necrosis within the glomeruli of the diabetic rats is an indication of DN compared with the normal control rats, which showed normal histoarchitecture of the kidney with the glomeruli and the tubules well delineated. However, treatment with BBR (50 and 100 mg/kg) ameliorated histological distortion compared with that of the untreated diabetic rats. Overall, histological examination showed that BBR (50 and 100 mg/kg) caused reconstruction, repair, and recovery of damaged kidneys in diabetic rats. Interestingly, diabetic rats treated with 100 mg/kg BBR showed a better protective effect.

Conclusions

This study revealed that oral administration of BBR normalized fasting blood glucose level and kidney function (urea, creatinine, and uric acid) makers in diabetic rats. The ability of oral administration of BBR to inhibit ADA and arginase activities, and MDA production and improve antioxidant status with increasing NO level could be a mechanism against DN.

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Declarations

Ethical approval The investigation was performed according to the Animal Ethics Committee Guidelines for the use of experimental animals (Ethic code FUTA/ETH/2020/014).

Informed consent Informed consent was obtained from all individual participants in this study.

Conflict of interest The authors declare no competing interests.

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