International Journal of Biochemistry Research & Review

21(1): 1-15, 2018; Article no.IJBCRR.40100
ISSN: 2231-086X, NLM ID: 101654445

Nephroprotective Effects of *Vernonia amygdalina* in Alloxan-induced Diabetes in Rats

Adegbolagun T. Adeoye[1], Ademola A. Oyagbemi[2], Temidayo O. Omobowale[3], Aduragbenro D. Adedapo[4], Abiodun E. Ayodele[5], Momoh A. Yakubu[6] and Adeolu A. Adedapo[1*]

[1]Department of Veterinary Pharmacology and Toxicology, Faculty of Veterinary Medicine, University of Ibadan, Nigeria.
[2]Department of Veterinary Physiology and Biochemistry, Faculty of Veterinary Medicine, University of Ibadan, Nigeria.
[3]Department of Veterinary Medicine, Faculty of Veterinary Medicine, University of Ibadan, Nigeria.
[4]Department of Pharmacology and Therapeutics, College of Medicine, University of Ibadan, Nigeria.
[5]Department of Botany, Faculty of Science, University of Ibadan, Nigeria.
[6]Department of Environmental & Interdisciplinary Sciences, College of Science, Engineering & Technology, Texas Southern University, Houston, TX, USA.

Authors' contributions

This work was carried out in collaboration between all authors. Author AAA designed the study, performed the statistical analysis, wrote the protocol, and first draft of the manuscript. Authors ATA, AAO and TOO carried out the studies. Authors ADA and AEA managed the analyses of the study. Author MAY managed the literature searches. All authors read and approved the final manuscript.

Article Information

DOI: 10.9734/IJBCRR/2018/40100
Editor(s):
(1) Muhammad Farhan Jahangir Chughtai, Assistant Professor NUR International University, Lahore, Pakistan.
Reviewers:
(1) Innocent Chidi Anioke, University of Nigeria Enugu Campus, Nigeria.
(2) Marwa Monier Mahmoud Refaie, Minia University, Egypt.
Complete Peer review History: http://www.sciencedomain.org/review-history/23684

Original Research Article

Received 6th January 2018
Accepted 12th March 2018
Published 17th March 2018

ABSTRACT

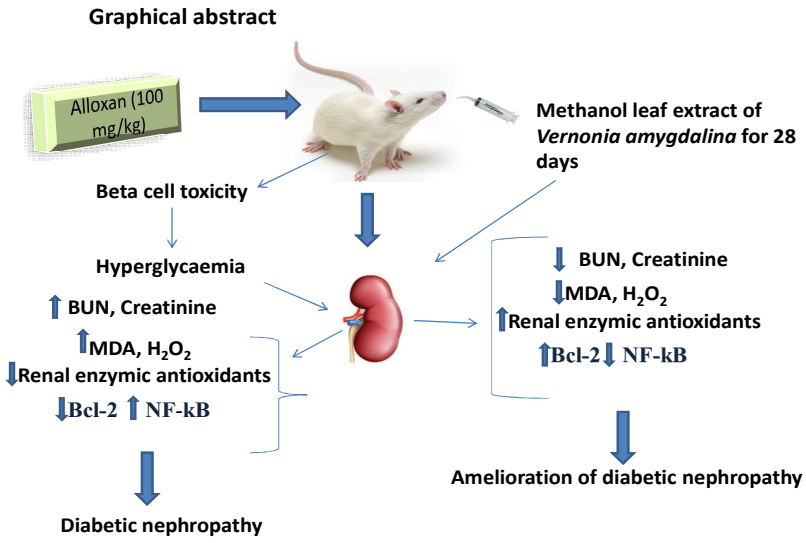
Background: Diabetic nephropathy is a leading cause of end-stage renal failure worldwide. **Purpose:** The methanol leaf extract of *Vernonia amygdalina* (MLVA) was thus investigated for its nephroprotective effects in diabetes.
Materials and Methods: Diabetes was induced in male Wistar rats by a single intraperitoneal (I.P) injection of a freshly prepared solution of Alloxan monohydrate (100 mg/kg). Forty-eight hours after

**Corresponding author: E-mail: adedapo2a@gmail.com;*

alloxan administration, rats with fasting blood glucose levels of 200 mg/dl and above were used for the study. Animals were grouped into five (A-E) of nine animals each. Group A was non-diabetic non treated control; Group B animals were the diabetic untreated control rats while groups C, D and E animals were diabetic and treated with glibenclamide, MLVA 200 mg/kg and MVLA 400 mg/kg respectively. Biochemical changes were evaluated by measuring the serum markers of kidney damage (creatinine and blood urea nitrogen). Markers of oxidative stress and antioxidant activities were measured in renal tissues. Histopathological and immunohistochemical changes were also evaluated.

Results: Four-week administration of MLVA produced significant ($p<0.05$) decrease in serum creatinine, urea, and oxidative markers but it caused a significant increase in enzymic and nonenzymic antioxidant as well as downregulation of Nuclear Factor kappa-light-chain-enhancer of activated B cells (NF-κB) and upregulation of B-cell lymphoma 2 (Bcl-2).

Conclusion: MLVA ameliorates diabetic nephropathy through its antioxidant, anti-inflammatory and antiapoptotic effects.



Keywords: Vernonia amygdalina; nephroprotection; anti-oxidants; anti-inflammatory; Bcl-2; NF-κB.

1. INTRODUCTION

Diabetes mellitus (DM) is one of the most frequent chronic diseases worldwide, being among the top five main causes of death in developed countries. This endocrine disease is also becoming an epidemic in developing countries [1]. The greatest increase in prevalence in the near future, however, is expected to occur in Asia, the Middle East, and Africa, where it is likely that there will be an ~50% increase in diabetes in these parts of the world by 2030 [2].

Diabetic nephropathy (DN) defined as a progressive kidney disease caused by angiopathy of capillaries in the kidney glomeruli, is the leading cause of end-stage renal disease (ESRD) and is a major contributor to the morbidity and mortality of diabetic patients' worldwide accounting for 42% of all patients on renal replacement therapy (RRT) in the USA [3]. Nephropathy is a common consequence of both type 1 diabetes mellitus and type 2 diabetes mellitus (T2DM); occurring in 15% to 25% [4], and 30% to 40% of patients, respectively [5]. The blood glucose levels of a diabetic can be controlled within the normal range using insulin and other oral hypoglycemic agents, but DN develops in 30–40% of the patients [6]. With the rapidly rising number of DM worldwide, DN is becoming one of the leading causes of end-stage renal disease (ESRD).

Hyperglycemia-induced metabolic and hemodynamic derangements, including enhanced production of reactive oxygen species (ROS), elevated levels of inflammatory cytokines, and activation of the renin-angiotensin system (RAS), are considered to be involved in the development and progression of diabetic nephropathy [7,8]. This has initiated increased efforts to reduce or prevent the cumulative damage of ROS/Nitrogen species (NS)-induced injury. Although DN has not been traditionally considered an inflammatory disease, recent studies have shown that kidney inflammation is crucial in promoting the development and progression of DN [9,10]. Nuclear factor (NF)-κB, (which regulates genes encoding pro-inflammatory mediators involved in the inflammatory processes) activation and the transcription of certain proinflammatory chemokines have also been demonstrated as the marker of progressive DN in patients [11].

Despite significant advances in the understanding of the cellular mechanisms that are responsible for the initiation and progression of DN, it remains therapeutically elusive. It is clear that blood glucose and blood pressure control are insufficient to prevent its progression, and that new, more effective treatment options are urgently needed [12].

Medicinal plants possessing antioxidant activity may reduce oxidative stress and improve the functions of various organs affected by hyperglycemia [13]. *Vernonia amygdalina* contain natural antioxidants which have the potential to act as antioxidants against aqueous radicals and reactive species ions [14]. This study is therefore aimed at investigating the effects of *Vernonia amygdalina* in alloxan-induced diabetic nephrotoxicity.

2. MATERIALS AND METHODS

2.1 Plant Collections and Preparation of the Extract

Fresh leaves of *Vernonia amygdalina* were collected from the community around the University of Ibadan, Ibadan, Nigeria. The leaves were identified and authenticated at the Department of Botany University of Ibadan and a voucher specimen deposited in the herbarium with voucher number UIH-22640. The leaves were dried under shade for about 14 days after which they were ground to powder. 600g of the powdered material was soaked in 2.5 litres of methanol and shaken vigorously. The sample was then left for 72 hours with intermittent shaking after which it was filtered and concentrated using a rotary evaporator. The weight of the resulting extract was 32.4 grams.

2.2 Experimental Design

In this study, seventy male Wistar rats with the weight range between 150 and 220 g were used. The rats were housed in rats' cages and were fed with standard rat diet and allowed access to clean water *ad libitum*. After a period of acclimatization of 14 days fasting blood glucose (pre-induction) was measured before induction of diabetes. All experimental procedures were in conformity with the University of Ibadan Ethics Committee on Research in Animals as well as internationally accepted principles for Laboratory animal upkeep and use.

2.3 Induction of Diabetes

Diabetes was induced in rats by a single intraperitoneal (I.P) injection of freshly prepared solution of Alloxan monohydrate (100 mg/kg). Forty eight hours after measuring their blood glucose concentrations in blood samples assessed induction fasting blood glucose level obtained from the tails using ACCUCHEK active blood glucometer and a total of thirty six animals with blood glucose of 200 mg/dl and above were selected for the study.

2.4 Animal Grouping

Rats were randomly allotted to five groups of nine animals each. Group A animals were not diabetic and received vehicle+ normal saline and served as normal control, group B animals were diabetic rats that did not receive any treatment, groups C were diabetic rats that received glibenclamide at 4 mg/kg, group D and E received the methanol leaf extract of *Vernonia amygdalina* at 200 mg/kg and 400 mg/kg respectively. All treatments were done daily via the oral route and lasted 28 days. Blood was collected for serum biochemical assays on days 14 and 28 post-treatment.

2.5 Tissue Preparation

After collection of blood from each of the animals, all rats were sacrificed by cervical dislocation on the 28th day of the treatment and

the kidneys (left and right) were harvested. A portion was then cut into 10% formalin for histological evaluation and the remaining was placed on ice, rinsed and homogenized in aqueous potassium buffer (0.1 M, pH 7.4) and the homogenate was centrifuged at 10,000 rpm (4^{o}C) for 10 min to obtain the post mitochondrial fraction (PMF). The PMF obtained was then subsequently stored at –20 °C until the time of use.

2.6 Biochemical Assays

2.6.1 Serum chemistry profile

Serum markers of kidney damage (creatinine and blood urea nitrogen (BUN)) were determined using commercial kits (Randox laboratories, Ltd. UK) and following standard procedures as outlined by the producer.

2.6.2 Renal antioxidant studies

Superoxide dismutase (SOD) was determined as described by Misra and Fridovich [15] with a little modification from our laboratory [16]. The amount of SOD necessary to cause 50% inhibition of the oxidation of adrenaline to adrenochrome during 1 minute was given as one unit of SOD. Reduced glutathione (GSH) was determined by the method of Jollow et al., [17]. The renal glutathione peroxidase (GPx) activity was measured according to the method described by Beutler et al., [18]. Glutathione-S-transferase activity was estimated by the method of Habig et al., [19]. Thiobarbituric acid reactive substance was quantified as malondialdehyde (MDA) in the renal PMF and was determined according to the method of Varshney and Kale [20]. Lipid peroxidation in units/mg protein was computed with a molar extinction coefficient of 1.56×10^5 M/Cm. Hydrogen peroxide generation was determined according to Woff [21]. The hydrogen peroxide (H_2O_2) generated was extrapolated from the hydrogen peroxide standard curve. Protein concentrations were determined as described by Gornall et al., [22]. The final value for total protein was extrapolated from the total protein standard curve. Non-protein thiol was quantified as described by Ellman [23]. Protein thiol was measured by adding 50 µL of sample to 980 µL of potassium phosphate buffer followed by addition of 30 µL of DTNB and the mixture was incubated for 30 minutes. The absorbance of mixture was read at 412 nm using distilled water as blank [24].

2.7 Histopathology

Small pieces of the kidneys were collected in 10 % formalin for proper fixation. These tissues were processed and embedded in paraffin wax. Sections of 5–6 µm in thickness were made and stained with haematoxylin and eosin for histopathological examination [25].

2.8 Immunohistochemistry

The tissues were processed for immunohistochemistry based on the methods described by Todorich et al., [26]. The immunoreactive positive expression of NF-κB and Bcl-2 regions was viewed starting from low magnification on each slide then with 400× magnifications using a photo microscope (Olympus) and a digital camera.

2.9 Statistical Analysis

Results were expressed as mean ± SD. Statistical analysis was performed by one-way analysis of variance (ANOVA), using GraphPad Prism version 6. The level of statistical significance was considered as $p<0.05$. Student's t-test at 95% level of significance was used to assess significant difference between controls and treated group.

3. RESULTS

3.1 Effect of MLVA on Markers of Kidney Function

BUN and creatinine were significantly increased in diabetic rats when compared with normal control. However MLVA was able to reduce the values significantly in the diabetic treated rats.

3.2 Effect of MLVA on Markers of Oxidative Stress

Diabetic rats exhibited an increase in tissue H_2O_2 level (Fig. 1) and MDA content (Fig. 2) when compared with normal control animals. Treatment with MLVA resulted in a dose dependent reduction in H_2O_2 levels when compared with the diabetic control. MLVA at a lower dose also showed significant ($p<0.05$) lowering of lipid peroxidation when compared with the diabetic control, while a higher dose did not produce such effect. Glibenclamide also lowered H_2O_2 levels (Fig. 1) and lipid

peroxidation (Fig. 2) in the diabetic rats significantly when compared to the diabetic control.

3.3 Effects of MLVA on Non Enzymic Antioxidant Systems

A significant ($p<0.05$) reduction of GSH (Fig. 3), protein thiol (Fig. 4) and non protein thiol (Fig. 5) content was seen in diabetic control rats when compared with normal control while treatment with MLVA at all doses (200 mg/kg and 400 mg/kg) led to a significant increase ($p<0.05$) in the GSH, protein thiol and non protein thiol contents in the renal homogenate of diabetic rats when compared with the diabetic control rats (Figs. 3,4,5).

3.4 Effects of MLVA on the Enzymic Antioxidant Systems

Results showed a statistically non-significant decrease in SOD activity in diabetic control when compared with the normal control (Fig. 6), while the glibenclamide treated group and MLVA treated groups showed significant ($p<0.05$) increase in SOD activity (Fig. 6). Glutathione peroxidase (GPx) activity was significantly reduced in diabetic control in comparison with normal control. Treatment with extract caused a significant increase in the activity of the enzyme in the diabetic rats (Fig. 7). Diabetic control rats showed a statistically insignificant decrease in glutathione-S-transferase activity when compared with normal control; however treatment with MLVA was able to increase enzyme activity significantly when compared with both diabetic and normal controls (Fig. 8).

3.5 Histopathology

Histopathology results showed congestion of glomeruli and hypercellularity of mesangium in diabetic control rats (Fig. 9). Treatment with glibenclamide and MLVA at 200 mg/kg attenuated the pathology to congestion of the glomeruli while treatment with MLVA at 400 mg/kg showed mild hypercellularity of mesangium and congestion of glomeruli (Fig. 9).

3.6 Immunohistochemistry

Immunohistochemistry of the kidney diabetic rats showed higher Nuclear Factor (NF) κB (NF-κB) and a lower expression of Bcl-2 respectively when compared to the control. Treatment with MLVA however resulted in a downregulation of the expression of NF-κB and upregulation of Bcl-2 in the treated rats (Figs. 10 and 11).

4. DISCUSSION

Alloxan (ALX)-induced diabetes model in rodents is very often used to investigate potential drug candidates for DM and its related complications. By producing selective beta cell toxicity, ALX

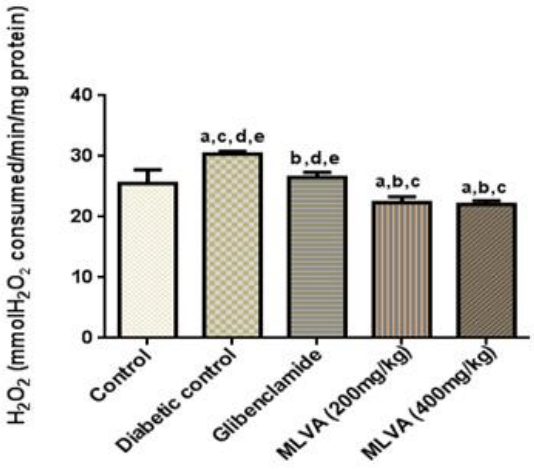


Fig. 1. Effect of MLVA on hydrogen peroxide levels in renal tissue of alloxan induced diabetic rats

*Values are mean ± SD, n =9, , **a** $p<0.05$ compared with control, **b** $p<0.05$ compared with diabetic control, **c** $p<0.05$ compared with glibenclamide, **d** $p<0.05$ compared with MLVA, 200 mg/kg, **e** $p<0.05$ compared with MLVA, 400 mg/kg*

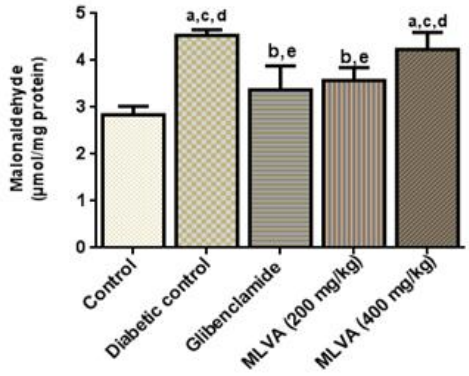


Fig. 2. Effect of MLVA on malonaldehyde content in renal tissue of alloxan induced diabetic rats

*Values are mean ± SD, n =9, , **a** p<0.05 compared with control, **b** p<0.05 compared with diabetic control, **c** p<0.05 compared with glibenclamide, **d** p<0.05 compared with MLVA, 200 mg/kg, **e** p<0.05 compared with MLVA, 400 mg/kg*

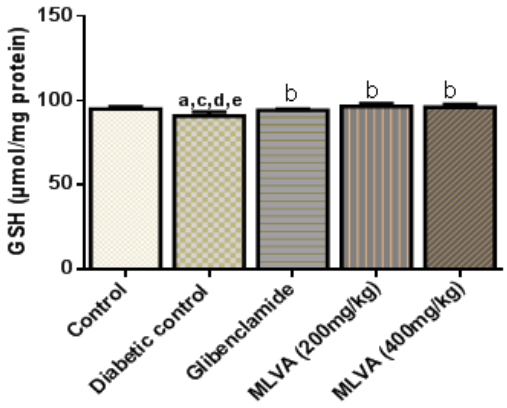


Fig. 3. Effect of MLVA on reduced glutathione (GSH) levels in renal tissue of alloxan induced diabetic rats

*Values are mean ± SD, n =9, , **a** p<0.05 compared with control, **b** p<0.05 compared with diabetic control, **c** p<0.05 compared with glibenclamide, **d** p<0.05 compared with MLVA, 200 mg/kg, **e** p<0.05 compared with MLVA, 400 mg/kg*

exerts its diabetogenic action when intraperitoneally administered to rodents [27, 28]. Studies revealed that progression of diabetes nephropathy is multi-factorial and involves complex biochemical, metabolic and inflammatory processes [29].

The kidneys eliminate metabolic wastes such as urea nitrogen, uric acid, creatinine and ions and thus optimum chemical composition of body fluids is maintained [30]. Hyperglycemia causes renal dysfunction such as acute glomerulonephritis, nephrosclerosis and even tubular necrosis resulting in abnormal excretion of urea and creatinine thereby elevating serum urea nitrogen and creatinine [31, 32, 33]. An elevation in the serum levels of BUN and creatinine in clinical analyses presupposes renal dysfunction [34, 35]. In this study, blood urea nitrogen and creatinine levels were increased following the induction of diabetes indicating renal dysfunction. However, treatment with the methanol leaf extract of *Vernonia amygdalina* resulted in a significant ($p<0.05$) reduction in the values of creatinine and blood urea nitrogen in the rats. This indicates the ability of the extract to protect the kidney from progressive damage in diabetes; this is also in agreement with the report of Atangwho et al. [36].

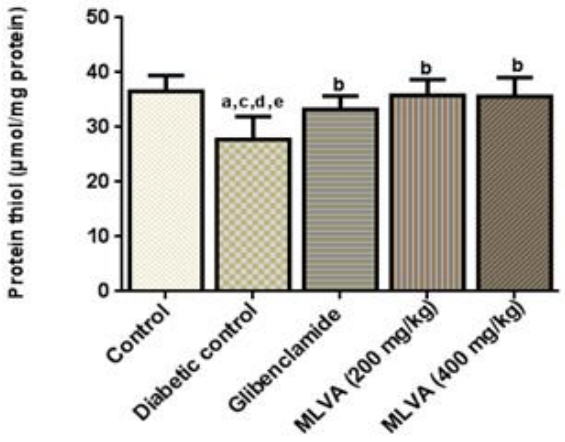


Fig. 4. Effect of MLVA on protein thiol (PT) levels in renal tissue of alloxan induced diabetic rats

*Values are mean ± SD, n =9, , **a** p<0.05 compared with control,*
***b** p<0.05 compared with diabetic control, **c** p<0.05 compared with glibenclamide,*
***d** p<0.05 compared with MLVA, 200 mg/kg,*
***e** p<0.05 compared with MLVA, 400 mg/kg*

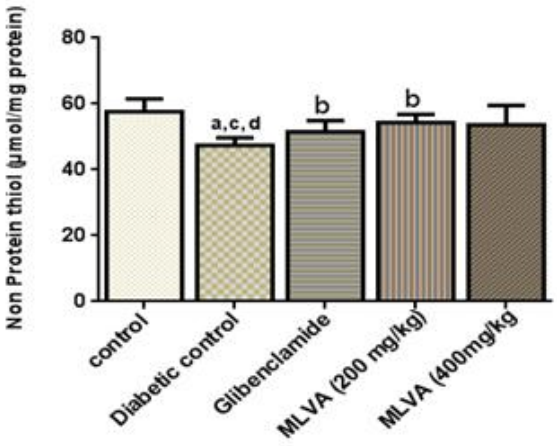


Fig. 5. Effect of MLVA on non-protein thiol (NPT) levels in renal tissue of alloxan induced diabetic rats

*Values are mean ± SD, n =9, **a** p<0.05 compared with control,*
***b** p<0.05 compared with diabetic control, **c** p<0.05 compared with glibenclamide,*
***d** p<0.05 compared with MLVA, 200 mg/kg,*
***e** p<0.05 compared with MLVA, 400 mg/kg*

Diabetic nephropathy progresses in stages, starting with the thickening of the glomerular basement membrane, mesangial cell expansion, and then gradually progressing into glomerulosclerosis and interstitial fibrosis eventually resulting in renal failure [37]. In this study histopathological examination showed hypercellularity of mesangium and congestion of glomeruli in diabetic rats. This is one of the first observations in the diabetic kidney; however treatment with MLVA ameliorated the mesangial hypercellularity.

The pathogenesis of DN is complex, and an increasing body of evidence shows that oxidative stress has a pivotal role in the development and progression of DN [38]. In this study it was observed that H_2O_2 was significantly increased in diabetic control when compared with the normal control this is because hyperglycaemia induces

the over production of O^- which is dismutated by the enzyme Superoxide dismutase (SOD) to form H_2O_2 [39]. Treatment with MLVA reduced the production of H_2O_2 in diabetic rats when compared with the diabetic control and normal control. This observation suggests the free radical scavenging capacity of the extract. Atangwho et al. [40] reported that *Vernonia amygdalina* leaves possess strong electron /hydrogen -donating bioactive compounds, which can serve as effective antioxidants. Several earlier studies have also demonstrated potent antioxidant activities with compounds isolated from this vegetable [41,42].

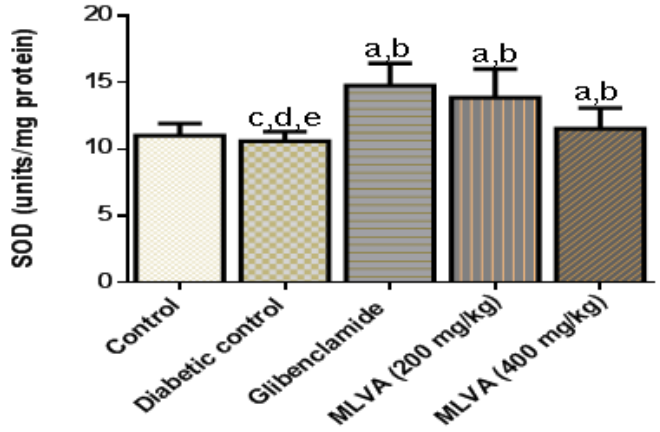


Fig. 6. Effect of MLVA on superoxide dismutase (SOD) activity in renal tissue of alloxan induced diabetic rats

*Values are mean ± SD, n =9, **a** p<0.05 compared with control, **b** p<0.05 compared with diabetic control, **c** p<0.05 compared with glibenclamide, **d** p<0.05 compared with MLVA, 200 mg/kg, **e** p<0.05 compared with MLVA, 400 mg/kg*

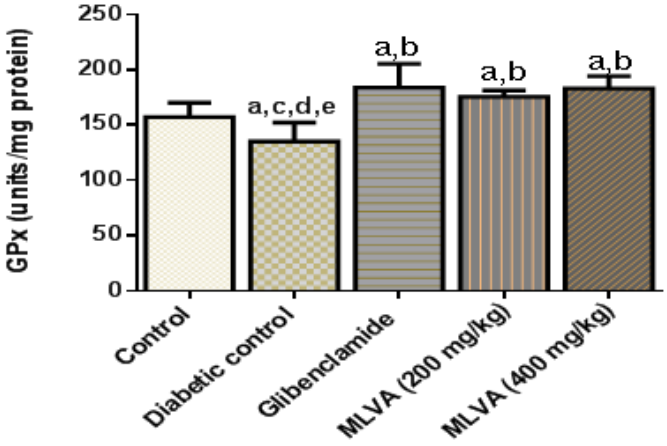


Fig. 7. Effect of MLVA on glutathione peroxidase (GPx) activity in renal tissue of alloxan induced diabetic rats

*Values are mean ± SD, n =9, **a** p<0.05 compared with control, **b** p<0.05 compared with diabetic control, **c** p<0.05 compared with glibenclamide, **d** p<0.05 compared with MLVA, 200 mg/kg, **e** p<0.05 compared with MLVA, 400 mg/kg*

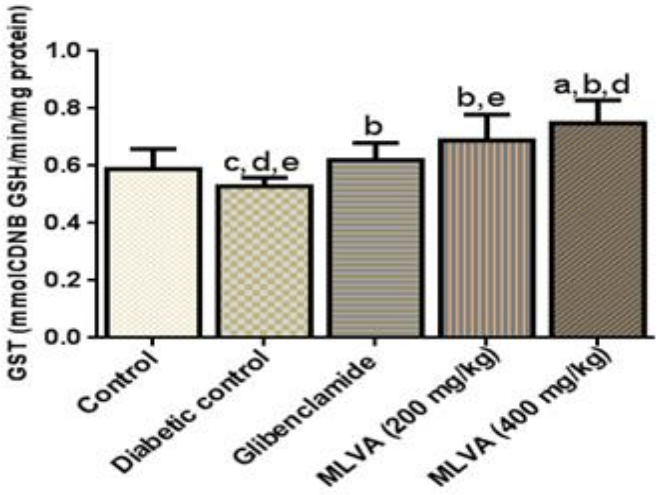


Fig. 8. Effect of MLVA on glutathione-S-transferase activity in renal tissue of alloxan induced diabetic rats

*Values are mean ± SD, n =9, **a** p<0.05 compared with control, **b** p<0.05 compared with diabetic control, **c** p<0.05 compared with glibenclamide, **d** p<0.05 compared with MLVA, 200 mg/kg, **e** p<0.05 compared with MLVA, 400 mg/kg*

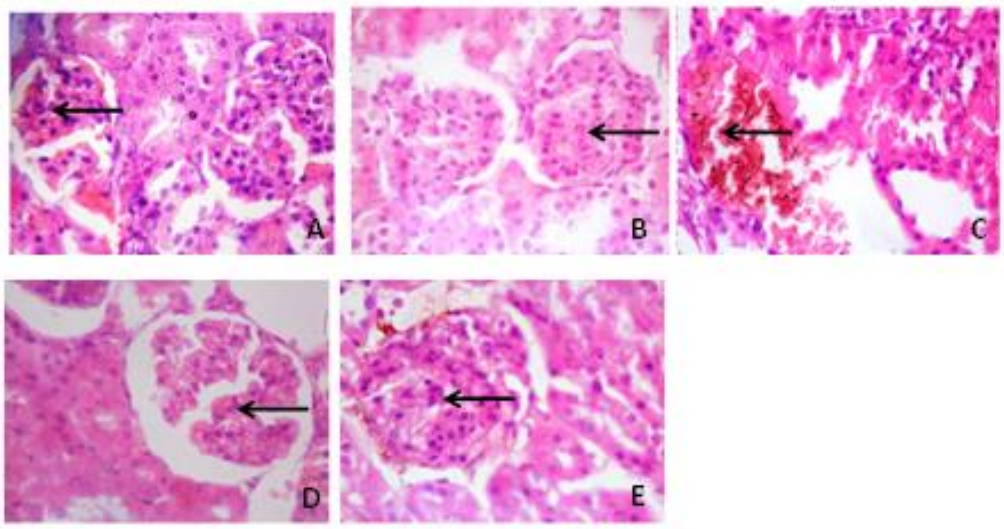


Fig. 9. Photomicrograph showing effects of MLVA on kidneys of alloxan induced diabetic rats

Rat in control group show congestion of the glomeruli (A) whereas the diabetic control rats showed hypercellularity of the messangium as well as congestion of the glomeruli (B). Treatment with glibenclamide and MLVA at 200 mg/kg attenuated the pathology to congestion of the glomeruli (C and D). Treatment with MLVA at 400 mg/kg showed mild hypercellularity of mesangium and congestion of glomeruli (E). The tissues are stained with H & E (X400 objectives)

Malondialdehyde, an end product of lipid peroxidation and a marker of oxidative stress was found to increase in diabetes mellitus as a result of increased production of free radicals [43] and a reflection of enhanced oxidative damage to lipids. This was also observed in this study in the diabetic control. However treatment with MLVA caused a significant reduction in MDA at the lower dose used in this study, which was comparable with the MDA content of normal

control, and the glibenclamide treated group. The inability of the extract to reduce the MDA content of diabetic rats administered a higher dose could mean that further studies need to be done on the inhibition of lipid peroxidation in diabetic kidney by the extract at that dose.

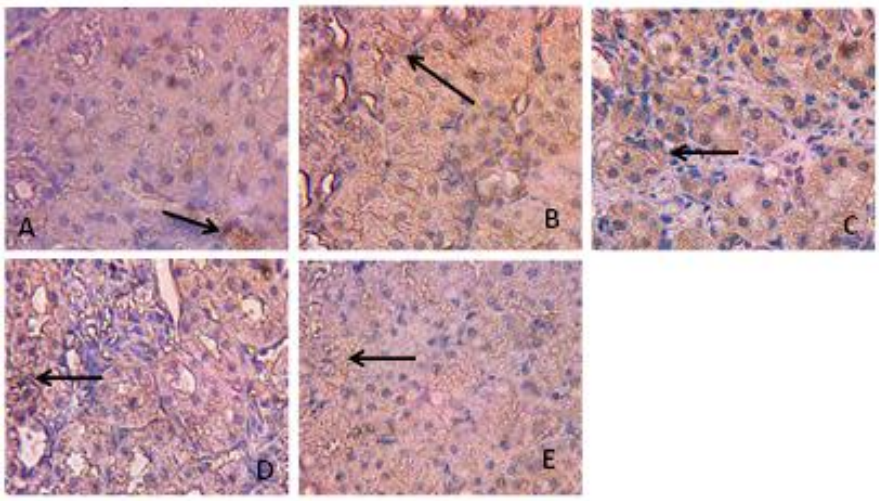


Fig. 10. Immunohistochemistry of nuclear factor (NF) κB (NF-κB) in the kidney of rats

Group A (normal control) shows low expression of NF-κB; Group B (Diabetic control) shows a higher expression of NF-κB than control; Group C (Glibenclamide treated diabetic group) shows a lower expression of NF-κB when compared to diabetic control (B), and higher expression when compared with normal control (A); Group D (200 mg/kg of MLVA) and Group E (400 mg/kg of MLVA) shows a lower expression of NF-κB when compared with diabetic control and glibenclamide treated group similar to normal control. The slides were counterstained with high definition haematoxylin and viewed 400× objectives

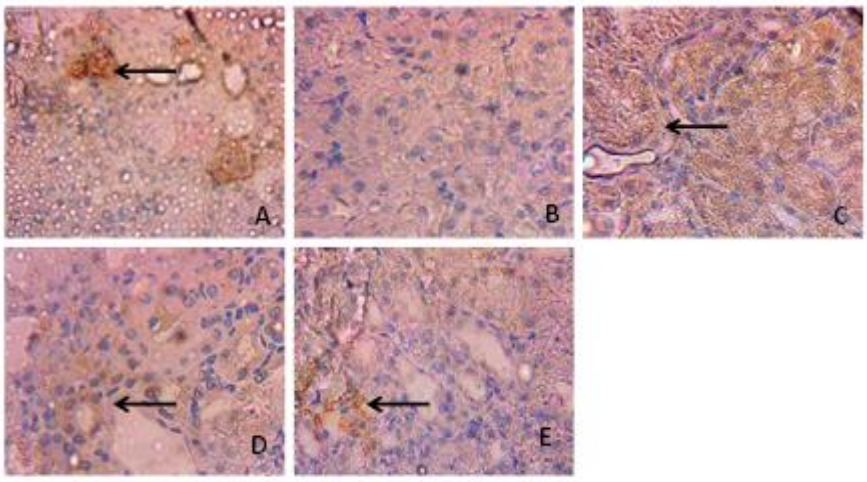


Fig. 11. Immunohistochemistry of Bcl-2 (anti-apoptotic protein) in the kidney of rats

A (normal control) shows positive and high expression of Bcl-2 ; B (Diabetic control) shows low and almost no expression of Bcl-2 when compared with the control; C (Glibenclamide treated diabetic group) shows a higher expression of Bcl-2 when compared to diabetic control (B), similar to the normal control (A); D (200 mg/kg of MLVA) and E (400 mg/kg of MLVA) shows a higher expression of Bcl-2 when compared with diabetic control and similar to normal control. The slides were counterstained with high definition haematoxylin and viewed 400× objectives.

Bcl-2 – B-cell lymphoma 2

Table 1. Effects of methanol leaf extract of *Vernonia amygdalina* blood urea nitrogen and creatinine levels of alloxan induced diabetic rats

Parameter	Groups	Pre-induction	14 days post induction	28 days post induction
Blood urea nitrogen (bun) (mg/dl)	A	16.50±1.29	15.75±0.50	16.00±0.00
	B	16.20±0.45	16.75±0.45^{ad}	18.25±0.50^{acde}
	C	16.25±0.96	16.80±0.45^{ad}	15.33±1.15^{b}
	D	16.00±0.89	15.80±0.45^{bc}	15.42±0.53^{b}
	E	17.25±0.96	16.50±0.58	15.33±0.52^{b}
Creatinine (mg/dl)	A	0.66±0.05	0.58±0.08	0.48±0.05
	B	0.60±0.07	1.17±0.15^{acde}	1.45±0.17^{acde}
	C	0.58±0.09	0.85±0.10^{b}	0.57±0.06^{b}
	D	0.56±0.05	0.76±0.05^{b}	0.52±0.08^{b}
	E	0.68±0.05	0.78±0.05^{b}	0.53±0.08^{b}

*Values are mean ± SD, n =9, **a** p<0.05 compared with control, **b** p<0.05 compared with diabetic control, **c** p<0.05 compared with glibenclamide, **d** p<0.05 compared with MLVA, 200mg/kg, **e** p<0.05 compared with MLVA, 400mg/kg*

Oxidative stress acts on signal transduction and affects gene expression of antioxidant enzymes thereby reducing the expression of antioxidant enzymes or hyperglycemia can simply inactivate existing enzymes by glycating these proteins [44].

In this study a decrease in antioxidant enzymes (SOD, GPx and GST) was observed in diabetic rats group when compared with the normal control although the decrease in SOD and GST were not significant this could be as a result of adaptive mechanism by the animals to cope with the hyperglycaemia induced free radical generation. Also in literature with regards to the effect of oxidative stress on antioxidant enzyme activities a lot of variations have been reported from no changes [45] decrease [46,47] and increase [48] depending on the duration of the experiments and the age of animals. MLVA administration in this study resulted in an increase in the activity of SOD and GST to levels that were significantly higher than the diabetic control and also the normal control. In experimental mouse models of types 1 and 2 diabetes, overexpression of SOD-1 suppressed increases in urinary albumin excretion, glomerular volume, glomerular content of collagen IV, TGF-β and mesangial matrix volume compared with the corresponding values in diabetic wild-type mice. Overexpression of SOD-1 in the diabetic mice was associated with decreases in renal cortical accumulation of MDA and/or higher GSH levels which are indices of reduced renal oxidative stress [49,50].

Glutathione peroxidase (GPx), a selenium containing enzyme, catalyses the reduction of hydrogen peroxide into water using glutathione as substrate, thereby preventing cells against oxidative stress. Results showed significant decrease in GPx activity in diabetic controls as compared to normal controls. This is in agreement with results reported by Singh and Singh, [43]. GSH is an efficient antioxidant present in almost all living cells and is also considered as a biomarker of redox imbalance at cellular level [51]. GSH level was reduced in diabetic rats in this study similar to reports by [52, 53]. Decreased GSH level may be one of the factors in the oxidative DNA damage in type 2 diabetics [54]. Treatment with MLVA restored the GSH levels to near normal values indicating the ability of the plant to restore GSH synthesis and lower oxidative stress and oxidant damage in the face of persistent hyperglycemia [55].

The transcription factor NF-κB is induced by various cell stress-associated stimuli including growth factors, vasoactive agents, cytokines, and oxidative stress. NF-κB in turn controls the regulation of numerous genes activated during inflammation, such as cytokines, chemokines, growth factors, cellular ligands, and adhesion molecules [56]. The activation and nuclear translocation of NF-κB has been demonstrated in diabetic kidneys from human and rodents [11, 57]. NF-κB has been recognized as an important inflammatory cytokine in DN and the inhibition of NF- κB expression enhances DN treatment [58]. Therefore, NF-κB has been proposed as an intervention target for DN treatment.

In diabetic nephropathy, high-glucose is one of the major contributors to free radical- and ROS-induced cell death in these cells [59] and pathogenic lesions are characterized by initial hypertrophy followed by a gradual loss of renal mass, sclerosis, and fibrosis. Apoptosis is known to contribute to the later process in both humans and animals [60,61] and it includes the upregulation of cell surface markers which encourages phagocytosis (removal) by resident and circulating immune cells and changes in internal pro-apoptotic proteins such as BASP1, Bcl-2 family proteins, and p53 [61]. The Bcl-2 family of proteins is highly conserved in diabetic nephropathy, and is involved in the regulation of both pro-survival and pro-apoptotic signaling. The numerous family members interact downstream of p53 and NF-κB to regulate cell survival. The anti-apoptotic protein Bcl-2 has been shown to play an important role in diabetic nephropathy [62] as it antagonizes the pro-apoptotic Bax and Bak proteins that together control mitochondrial cell death.

Cipollone et al., [62] reported that Bcl-2 down-regulation in diabetic patients with poor glycemic control results in the activation of the NF-κB pathway leading to the development of nephropathy. This is similar to what was observed in this study where the expression of Bcl-2 was seen to be downregulated and NF-κB expression was upregulated in the diabetic control group.

Treatment of experimental diabetic nephropathy using *Vernonia amygdalina* leaf extract, in this study downregulated the expression of the NF-κB and upregulated the expression of Bcl-2 (anti apoptotic protein). *Vernonia amygdalina* might provide a novel form of therapy for prevention of nephropathy in diabetic patients in which an acceptable glycemic control is difficult to achieve despite insulin therapy.

Put together the findings from this study establishes that hyperglycaemia causes oxidative injury resulting into activation of transcription factor NF-κB and apoptotic cell death in the renal tissue of alloxan induced diabetic rats. Treatment with the leaf extract of *Vernonia amygdalina* mitigated these effects by increasing the synthesis of antioxidant GSH, increasing the activity of antioxidant enzymes (SOD, GPx and GST), down regulating the activation of NF-κB and up regulating the expression of the anti apoptotic protein Bcl-2 in renal tissues of diabetic rats.

5. CONCLUSION

In conclusion this study shows that *Vernonia amygdalina* a household plant in Nigeria could serve as an effective protection against the progression of diabetic nephropathy via its antioxidant, antinflammatory and anti apoptotic effects.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

ACKNOWLEDGEMENT

The authors acknowledged that the study was supported with a grant received from the National Research Foundation of the Tertiary Education Trust Fund (TETFUND), Abuja, Nigeria. The grant number is TETFUND/DESS/NRF/UI IBADAN/STI/VOL/I/B2.20.11.

REFERENCES

1. World Health Organisation. Available:http://www.who.int/diabetes/en (accessed on 10 August 2017).
2. Shaw JE, Sicree RA, Zimmet PZ. Global estimates of the prevalence of diabetes for 2010 and 2030. Diabetes Res Clin Pract. 2010;87:4–14.
3. Akmal M. Hemodialysis in diabetic patients. Am J Kidney Dis. 2001;38(4 Suppl 1):S195–S199.
4. Steinke JM, Mauer M. International Diabetic Nephropathy Study Group. Lessons learned from studies of the natural history of diabetic nephropathy in young type 1 diabetic patients. Pediatr Endocrinol Rev. 2008;5(Suppl 4):958-963.
5. Koro CE, Lee BH, Bowlin SJ. Antidiabetic medication use and prevalence of chronic kidney disease among patients with type 2 diabetes mellitus in the United States. Clin Ther. 2009;31(11):2608-2617.
6. Valk EJJ, Bruijn JA, Bajema IM. Diabetic nephropathy in humans: Pathologic diversity. Curr Opin Nephrol Hypertens. 2011;20:285–289
7. Kanwar, YS, Sun L, Xie P, Liu FY, Chen S A. Glimpse of various pathogenetic mechanisms of diabetic nephropathy. Annu. Rev. Pathol. 2011;6:395–423
8. Sun YM, Su Y, Li J, Wang LF. Recent advances in understanding the biochemical and molecular mechanism of

diabetic nephropathy. Biochem. Biophys. Res. Commun. 2013;433:359–361
9. Lim AK, Tesch GH. Inflammation in diabetic nephropathy. Mediators Inflamm. 2012;146-154.
10. Wada J, Makino H. Inflammation and the pathogenesis of diabetic nephropathy. Clin Sci (Lond). 2013;124:139-152
11. Mezzano S, Aros C, Droguett A, Burgos ME, Ardiles L, Flores C, Schneider H, Ruiz-Ortega M, Egido J. NF-kappaB activation and overexpression of regulated genes in human diabetic nephropathy. Nephrol Dial Transpl. 2004;19:2505-2512.
12. Lindblom R, Higgins G, Coughlan M, de Haan JB. Targeting mitochondria and reactive oxygen species-driven pathogenesis in diabetic nephropathy. Rev Diabet Stud. 2015;12(1-2):134-156.
13. Singh R, Bhardwaj P, Sharma P. Antioxidant and toxicological evaluation of *Cassia sopherain* streptozotocin-induced diabetic Wistar rats. Pharmacognosy Research. 2013;5(4):225-232.
14. Owolabi MA, Jaja SI, Oyekanmi OO, Olatunji OJ. Evaluation of the antioxidant activity and lipid peroxidation of the leaves of *V. amygdalina*. J. Compl. Integr. Med. 2008;5:1152.
15. Misra HP, Fridovich I. The role of superoxide anion in the autoxidation of epinephrine and a simple assay for superoxide dismutase. J Biol Chem. 1972;217:3170–3175.
16. Oyagbemi AA, Omobowale TO, Akinrinde AS, Saba AB, Ogunpolu BS, Daramola O. Lack of reversal of oxidative damage in renal tissues of lead acetate-treated rats. Environ Toxicol. 2015;15(30):1235–1243
17. Jollow, DJ, Mitchell JR, Zampaglione N, Gillette JR. Bromobenzene induced liver necrosis. Protective role of glutathione and evidence for 3,4–bromobenzene oxide as the hepatotoxic metabolite, Pharmacology. 1974;11(3):151–169.
18. Beutler EO, Duron B, Kelly M. Improved method for the determination of blood glutathione', J Lab Clin Med. 1963;61:882–888.
19. Habig, WH, Pabst MJ, Jacoby WB. Glutathione-S-transferase activity: the enzymic step in mercapturic acid formation J. Biol. Chem. 1974;249:130–139.
20. Varshney R, Kale RK. Effect of calmodulin antagonists on radiation induced lipid peroxidation in microsomes. Int J Biol. 1990;158:733–741.
21. Woff SF. Ferrous ion oxidation in the presence of ferric ion indicator, xylenol orange for measurement of hydrogen peroxides. Methods Enzymol. 1994;233: 182–189.
22. Gornall, AG, Bardawill CJ, David MM. Determination of serum proteins by means of Biuret reaction. J. Biol. Chem. 1949; 177:751–766.
23. Ellman, GL. Tissue sulfhydryl groups. Archives of Biochemistry and Biophysics. 1959;82:70-77.
24. Sedlak J, Lindsay RH. Estimation of total, protein-bound, and nonprotein sulfhydryl groups in tissue with Ellman's reagent. Analytical Biochemistry. 1968;25(1):192-205.
25. Drury RA, Wallington EA, Cancerson R. Carlton's histopathological techniques, 4th ed. Oxford University Press, Oxford, London, New York; 1976.
26. Todorich B, Olopade JO, Surguladze N, Zhang X, Neely E, Connor JR. The mechanism of vanadium-mediated developmental hypomyelination is related to destruction of oligodendrocyte progenitors through a relationship with ferritin and iron. Neurotox Res. 2011; 19(3):361-73
27. Manickam D, Periyasamy L. Antidiabetic effect of methanolic extract of *Decalepis hamiltonii* root (wight and Arn) in normal and alloxan induced diabetic rats. J Pharm Res. 2013;6:166–72.
28. Rashid K, Das J, Sil, PC. Taurine ameliorate alloxan induced oxidative stress and intrinsic apoptotic pathway in the hepatic tissue of diabetic rats. Food Chem Toxicol. 2013;51:317–29.
29. Kikkawa R, Koya D, Haneda M. Progression of diabetic nephropathy. Am J Kidney Dis. 2003;41:S19–21
30. Shokeen P, Anand P, Murali YK, Tandon V. Antidiabetic activity of 50% ethanolic extract of *Ricinus communis* and its purified fractions. Food and Chemical Toxicology. 2008;46(11):3458-66
31. Cheng H, Liu W, Ai X. Protective effect of curcumin on myocardial ischemial reperfusion injury in rats. Zhong Yao Cai. 2005;28:920-922.
32. Stambe C, Atkins RC, Tesch GH, Kapoun AM, Hill PA, Schreiner GF, Nikolic-Paterson DJ. Blockade of p38 {alpha} MAPK ameliorates acute inflammatory renal injury in rat anti-GBM glomerulonephritis. Journal of the

American Society of Nephrology. 2003; 14(2):338–351.

33. Jaramillo-Juarez F, Rodriguez-Vazquez ML, Rincon-Sanchez AR, Consolacion-Martinez M, Ortiz GG. Acute renal failure induced by carbon tetrachloride in rats with hepatic cirrhosis. Annals Hepatology. 2008;7(4):331-338.
34. Sood R. Medical Laboratory Technology, Jaypee Brothers Medical Publishers Limited, New Delhi; 2006.
35. Mehrdad M, Mozhgan GP, Sayed AT, Alireza J. Study of histopathologic changes of zingiber extract on mice kidneys. International Conference on Food Engineering and Biotechnology – IPCBEE. 2011;9:16–20.
36. Atangwho IJ, Ebong PE, Egbung GE, Eteng MU, Eyong EU. Effect of *Vernonia amygdalina* Del. on liver function in alloxan-induced hyperglycaemic rats. Journal of Pharmacy & Bioresources. 2007;4(1):1-7
37. Karihaloo A. Anti-fibrosis therapy and diabetic nephropathy. Current Diabetes Reports. 2012;12(4):414–422
38. Giacco, F, Brownlee M. Oxidative stress and diabetic complications. Circ Res. 2010;107:1058–1070.
39. Brownlee M. Biochemistry and molecular cell biology of diabetic complications. Nature. 2001;414:813-20
40. Atangwho IJ, Egbung GE, Ahmad M, Yam MF, Asmawi MZ. Antioxidant versus anti-diabetic properties of leaves from *Vernonia amygdalina* Del. growing in Malaysia. Food Chem. 2013;141(4):3428-34.
41. Farombi, EO, Owoeye O. Antioxidative and chemopreventive properties of *Vernonia amygdalina* and Garcinia biflavonoid. Int. J. Environ. Res. Public Health. 2011;8(6): 2533-2555.
42. Ho WY, Liang WS, Yeap SK, Beh B, Yousr AHN, Alitheen NB. *In vitro* and *in vivo* antioxidant activity of *Vernonia amygdalina* water extract. African Journal of Biotechnology. 2012;11(17):4090-4094.
43. Singh K, Singh G. Alterations in some oxidative stress markers in diabetic nephropathy. J Cardiovasc Disease Res. 2017;8(1):24-762.
44. Wiernsperger NF. Oxidative stress as a therapeutic target in diabetes: Revisiting the controversy. Diabetes Metab. 2003;29: 579-82.
45. Kakkar, R, Mantha SV, Radhi J, Prasad K, Kalra J. Lipid peroxidation and activity of antioxidant enzymes in diabetic rats. Mol Cell Biochem. 1995;151:113-119.
46. Hussein HK, Abu-Zinadah OA. Antioxidant effect of curcumin extracts in induced diabetic wistar rats. Inter J Zool Res. 2010; 13:266-276
47. Arun N, Nalini N. Efficacy of turmeric on blood glucose and polyol pathway in diabetic albino rats. Plant Foods Hum Nutr. 2002;57:41-52.
48. Limaye PV, Raghuram N, Sivakami S. Oxidative stress and gene expression of antioxidant enzymes in the renal cortex of streptozotocininduced diabetic rats. Mol Cell Biochem. 2003;243:147-152.
49. Craven, PA, Melhem MF, Phillips SL, DeRubertis FR. Overexpression of Cu2+/Zn2+ superoxide dismutase protects against early diabetic glomerular injury in transgenic mice. Diabetes. 2001;50:2114–2125.
50. DeRubertis, FR, Craven PA, Melhem MF, Salah EM. Attenuation of renal injury in db/db mice overexpressing superoxide dismutase: Evidence for reduced superoxide-nitric oxide interaction. Diabetes. 2004;53:762–768.
51. Rizvi SI, Chakravarty S. Day and night GSH and MDA levels in healthy adults and effects of different doses of melatonin on these parameters," International Journal of Cell Biology. Article ID 404591. 2011;5.
52. Calabrese V, Cornelius C, Leso V, Trovato-Salinaro A, Ventimiglia B, Cavallaro M et al. Oxidative stress, glutathione status, sirtuin and cellular stress response in type 2 diabetes, Biochimica et Biophysica Acta. 2012;1822 (5):729–736.
53. Rahigude A, Bhutada P, Kaulaskar S, Aswar M, Otari K. Participation of antioxidant and cholinergic system in protective effect of naringenin against type-2 diabetes-induced memory dysfunction in rats. Neuroscience. 2012;226:62–72.
54. Dincer, Y, Akcay T, Alademir Z, Ilkova H. Assessment of DNA base oxidation and glutathione level in patients with type 2 diabetes," Mutation Research. 2002;505 (1-2):75–81.
55. Sekhar RV, Mckay SV, Patel SG, Guthikonda AP, Reddy VT, Balasubramanyam A, Jahoor F. Glutathione synthesis is diminished in patients with uncontrolled diabetes and

restored by dietary supplementation with cysteine and glycine, Diabetes Care. 2011; 34(1):162–167.
56. Karin, M, Greten, FR. NF-kappa B: Linking inflammation and immunity to cancer development and progression. Nat Rev Immunol. 2005;5(10):749-759.
57. Liu WH, Zhang XY, Liu PQ. Effects of berberine on matrix accumulation and NF-kappa B signal pathway in alloxan-induced diabetic mice with renal injury. Eur J Pharmacol. 2010;638:150–155.
58. Wada J, Makino H. Innate immunity in diabetes and diabetic nephropathy. Nat Rev Nephrol. 2016;12:13-26.
59. Habib, SL. Diabetes and renal tubular cell apoptosis. World J Diabetes. 2013;4(2): 27-30.
60. Kumar D, Robertson S, Burns KD. Evidence of apoptosis in human diabetic kidney. Mol Cell Biochem 2004;259(1-2): 67-70.
61. Sanchez-Nino MD, Sanz AB, Lorz C, Gnirke A, Rastaldi MP, Nair V et al. BASP1 promotes apoptosis in diabetic nephropathy. J Am Soc Nephrol 2010; 21(4):610-621.
62. Cipollone, F, Chiarelli F, Iezzi A, Fazia ML, Cuccurullo C, Pini B, De Cesare D, Torello M, Tumini S, Cuccurullo F, Mezzetti A. Relationship between reduced BCL-2 expression in circulating mononuclear cells and early nephropathy in type 1 diabetes. Int J Immunopathol Pharmacol. 2005;18(4):625-635.

Peer-review history:
The peer review history for this paper can be accessed here:
http://www.sciencedomain.org/review-history/23684