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Preliminary Assessments and Renoprotective Effects of Methanol Extract of *Parquetina nigrescens* (African Parquetina) in Diabetic Wistar Rats

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Authors' contributions

This work was carried out in collaboration between both authors. Both authors designed the study. Author OIA performed the statistical analysis, wrote the protocol and the first draft of the manuscript. Author EOA managed the literature searches. Both authors read and approved the final manuscript.

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ABSTRACT

Aim: Phytochemical screening, toxicity and renoprotective effects of methanol extract of *Parquetina nigrescens* (MEPN) in diabetic Wistar rats were investigated.
Methods: Twenty-five rats divided into five groups (n=5) were used for this study. Groups 1 and 2 served as normal control and diabetic untreated respectively and each received 0.3 ml distilled water. Groups 3-5 served as diabetic groups treated with 100 mg/kg, 200 mg/kg MEPN and 100 mg/kg metformin respectively. Active compounds in MEPN were identified using GC/MS analysis while Phytochemical screening was done following standards procedures. Glucose 6 phosphate dehydrogenase (G6PDH), lactate dehydrogenase (LDH), superoxide dismutase (SOD), glutathione peroxidase (GPx), catalase (CAT), blood urea nitrogen (BUN), creatinine (CRT) and albumin (Alb) levels were determined using randox kits. The kidney histology was done using haemotoxylin-eosin stain. Results were analyzed using one-way ANOVA with statistical significance taken at $P<.05$.
Results: Phytochemical screening showed the presence of alkaloids, cardenoloides,

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anthraquinones, tannins and flavonoids. GC/MS showed the presence of twenty-two active compounds in MEPN. G6PDH, and CAT significantly increased in the diabetic treated with MEPN and metformin compared with normal control. G6PDH, CAT, GPx and Alb levels were significantly increased in diabetic treated with MEPN and metformin groups compared with diabetic untreated. SOD was significantly increased in diabetic treated with 100 mg/kg MEPN compared with normal control. LDH was significantly increased in diabetic treated with metformin compared with diabetic untreated. BUN and CRT significantly decreased in diabetic treated with MEPN and metformin groups when compared with diabetic untreated.

Conclusion: MEPN possesses active components which may be useful in ameliorating the oxidative stress and renal dysfunction in diabetes mellitus.

Keywords: Parquetina nigrescens; oxidative stress; renal dysfunction; diabetes mellitus.

1. INTRODUCTION

Plants have been used for medicinal purposes long before history and many commercially available drugs are medications that were originally from plants [1]. There are synthetic medications produced to treat various forms of illnesses but, because of the cost implications and their side effects, most people prefer alternative medications to meet with their primary health needs [2]. Many medicinal plants have been reported to be useful in treatment of diabetes and its complications [3-5]. Although diet, insulin, and other oral hypoglycemic agents have remained the mainstays of therapy for the diabetic patients for decades [3], many local plants have also been identified and tested for their antidiabetic properties. Among the medicinal plants that have been used in treatment of diabetes mellitus are *Acacia Arabica* [4]*, Aegle marmelos* [5] and *Agrimony eupatoria* [6]*. Acacia arabica* have been shown to cause hypoglycaemic effect in rats by stimulating insulin release [4]. *Aegle marmelos* have been shown to possess antihyperglycemic activities in streptozotozin induced diabetic rats by improving glucose utilization [5]. Similarly, aqueous extract of *Agrimony eupatoria* evoked stimulation of insulin secretion from the BRIN-BD11 pancreatic beta cell line in vitro, an effect which was found to be glucose independent [6]. *Parquetina nigrescens* is an herbaceous, perennial twine belonging to the family Asclepiadaceae. *Parquetina nigrescens* is commonly found in secondary forest and around villages in Senegal and Nigeria. It is a perennial plant with twining stems and a woody base, shortly tapering 10 to 15 cm long, 6 to 8 cm broad, smooth and long stem. In Nigeria, this plant has been shown to be useful in the treatment of anaemia [7]. This plant has also been used in the treatment of fever, inflammatory and painful disorders [8]. It has also been used in the treatment of several other ailments which include diarrhoea, gonorrhea, menstrual disorders, insanity, intestinal worm infections, skin lesions and erectile dysfunction [9-11]. It has also been reported that aqueous extract of *Parquetina nigrescens* caused significant anti-nociception using the hot plate and formalin tests [8] and anti-inflammatory effects by reducing leucocyte migration during the process of inflammation [12].

Although several studies have shown that hyperglycaemia, if uncontrolled may result in several complications, with chronic kidney disease (CKD) being the leading cause of morbidity and mortality [13]. In this present study, the renoprotective effect of methanol extract of *Parquetina nigrescens* was investigated in diabetic Wistar rats.

Fig. 1. *Parquetina nigrescens* leaves [11]

2. MATERIALS AND METHODS

2.1 Plant Collection, Identification and Extract Preparation

The Fresh leaves of *Parquetina nigrescens* were collected from the metropolis of Ibadan. It was identified at the Department of Botany, University of Ibadan with voucher specimen number UIH-22475 deposited in the herbarium. The plant materials were air-dried for a period of six weeks and grinded using Thomas milling machine

(2 mm Sieved). About 1823 g of the grinded materials was soaked in 9 Liters of methanol for 72 hours. The mixture was filtered with cheesecloth and the filtrate concentrated under reduced pressure at 40°C for 20 min using a rotatory evaporator (Gallenkamp UK). The residue yielded 53.58 g of methanol extract of *Parquetina nigrescens* (MEPN) which was later stored at 2-8°C prior to biological investigations.

2.2 Phytochemical Analysis, Toxicological Study and Calculation of Median Lethal DOSE (LD_{50})

Phytochemical screening was done using standard methods [10]. Thirty-five rats with weight varying from 100-150 g were used for toxicity study and were divided into seven groups of five rats per group. Group 1 received 0.3 ml distilled water; Groups 2-7 were orally given graded doses of MEPN at 500 mg/kg, 1000 mg/kg, 2000 mg/kg, 3000mg/kg, 4000mg/kg and 5000 mg/kg respectively. Rats were placed under continuous observation for 6 hours. After 24 hours, rats were sacrificed under mild anesthesia (sodium thiopental 30 mg/kg *i.p*) to observe changes in the internal structure according to OECD method [14]. The median lethal dose was calculated as described by Karber et al*.,* 1931 [15].

2.3 Experimental Design

Twenty-five Wistar rats with weight varying from 100-150 g were obtained from the Central Animal House, College of Medicine, University of Ibadan, Nigeria. They were housed in well-aerated cages, maintained on standard rat chow with free access to drinking water according to the guidelines and regulations of the National Institute of Health [16] and approved by Animal Care And Use Research Ethics committee of the University of Ibadan (Reference no: 7/551/153A). Twenty-five rats were divided into 5 groups of 5 rats per group. Group 1 served as normal control, group 2 served as diabetic untreated, groups 3, 4 and 5 were diabetic treated with 100 mg/kg MEPN, 200mg/kg MEPN and 100 mg/kg Metformin [38] respectively.

2.4 Induction of Diabetes Mellitus

Diabetes was induced after a 24 hour fast in groups 2, 3, 4 and 5 by single intraperitoneal injection of alloxan monohydrate (Sigma Aldrich, U.S.A) at a dose of 120mg/kg using the method described by Carvalho et al., [17]. After 72 hours of alloxan administration, only rats with fasting blood glucose level of 250 mg/kg and above were considered diabetic and selected for this study.

2.5 Blood Sample Collection, Determination of Oxidative Stress Markers and Renal Function Test

Rats were treated orally for 28 days after which rats were mildly exposed to sodium thiopental anesthesia (30 mg/kg *i.p*). Blood samples were obtained from each rat through retro-orbital sinus. The blood samples were centrifuged at 3000 r.p.m to obtain serum which was taken into another plain bottle using pasture pipette. Glucose 6 phosphate dehydrogenase (G6PDH), Lactate dehydrogenase (LDH), Superoxide dismutase (SOD), Catalase (CAT) activities and serum albumin level were determined using commercially available randox kits and their absorbance measured using spectrophotometry procedure as described by Avinash et al*.,* [18]. Blood Urea Nitrogen (BUN) and Creatinine levels were determined using commercially available kits and their absorbance measured using spectrophotometry procedure as described by Evans et al., [19] and Khaldun et al., [20]

2.6 Statistical Analysis

Results obtained were analyzed using one-way analysis of variance (ANOVA) followed by Neuman's keul post-hoc test. Data were expressed as mean ±SEM with the level of statistical significance taken at $P<.05$.

3. RESULTS

3.1 Phytochemical Screening and GC-MS Studies on MEPN

Table 1 and Fig. 1 showed Phytochemical screening and Gas Chromatography Mass Spectrometry (GC-MS) analysis of MEPN respectively. The phytochemical screening showed positive results for alkaloids, cardenoloides, anthraquinones, tannins and flavonoids. GC-MS analysis of MEPN showed the presence of twenty-one (22) bio-active compounds which include Alpha-phellandrene (5.819), Cymene (6.117), Beta-curcumene (12.005), Alpha-begarmotene (12.126), Beta-bisabolene (12.262), Naphthalene (12.455), Diethylphthalate (12.995), 1,12-tridecadiene (15.033), 5-ethyl-2-furaldehyde (15.283), 7-hexadecyne (15.478), Pyranos (15.576),

decanoic acid (15.867) Mannos (16.275), Thiophene (16.438), Hexadecanoic acid (16.813), Octadecanoic acid (17.710), Catrienoate (17.772) Phytol (17.878) Octadecatrienoate (18.274), Octadecanoic (18.388), 9-Octadecenamide (20.222), Squalene (28.028).

3.2 Toxicological Study of Different Doses of MEPN Administered Orally in Rats

Table 1 showed the effect of graded doses of MEPN in normal rats. There were no cases of mortality in the experimental rats and this means that the LD_{50} of MEPN was greater than 5000 mg/kg. However, fecal materials and urine were found in group administered with 5000 mg/kg MEPN.

Table 1. Phytochemical analysis of methanol extract of *Parquetina nigrescens*

Phytochemicals compounds	Methanol extract of *Parquetina nigrescens* MEPN
Alkaloids	+
Cardenloids	+
Anthraquinones	+
Saponins	-
Tannins	+
Flavonoids	+

+ = Present - = Absent

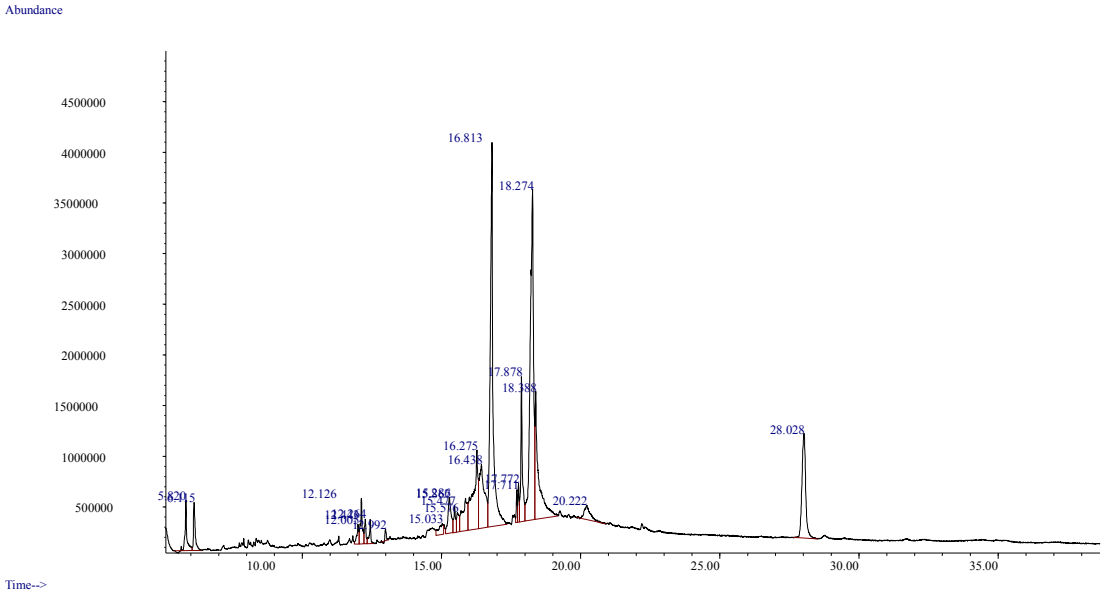

Fig. 2. Gas Chromatography Mass Spectrometry (GC-MS) analysis of MEPN

Table 2. Toxicological study of different doses of MEPN administered orally in rats

S/n	Groups	Mortality x/N	Symptoms (0-6 hrs)
Group 1	0.3 ml	0/5	Nil
Group 2	500 mg/kg	0/5	Nil
Group 3	1000 mg/kg	0/5	Nil
Group 4	2000 mg/kg	0/5	Nil
Group 5	3000 mg/kg	0/5	Nil
Group 6	4000 mg/kg	0/5	Nil
Group 7	5000 mg/kg	0/5	Frequent Defecation and urination

3.3 Anti-oxidative Parameters in Normal, MEPN and Metformin Treated Groups

Table 3 showed changes in oxidative stress parameters in normal and treated rats. There was significant increase (P<.05) in G6PDH activities in normal control, diabetic treated with 200 mg/kg MEPN and 100 mg/kg metformin groups when compared with diabetic untreated and diabetes treated with 100 mg/kg MEPN respectively. There was also significant increase in G6PDH activities in diabetes treated with 100 mg/kg metformin when compared with normal control and diabetes + 200 mg/kg MEPN. Lactate dehydrogenase (LDH) was significantly lower (P<.05) in diabetes treated with 100 mg/kg MEPN when compared with diabetes treated with 100 mg/kg metformin.

Catalase (CAT) activities significantly increase (P<.05) in normal control, diabetes + 100 mg/kg MEPN, diabetes + 200mg/kg MEPN, diabetes + 100 mg/kg Metformin when compared with diabetic untreated. There was significant increase in CAT activities in normal control, diabetes + 200 mg/kg MEPN, diabetes + 100 mg/kg Metformin when compared with diabetes treated with 100 mg/kg MEPN. A significant increase was also observed in CAT activities in normal control and diabetes + 200 mg/kg MEPN when compared with diabetes treated with 100 mg/kg meformin.

Glutathione peroxidase (GPx) significantly increased (P<.05) in normal control, diabetes treated with 200 mg/kg MEPN and 100 mg/kg Metformin groups when compared with diabetic untreated and diabetes treated with 100 mg/kg MEPN respectively.

The level of albumen was significantly higher (P<.05) in normal control, diabetes treated with 100 mg/kg, 200 mg/kg MEPN and diabetes treated with 100 mg/kg Metformin when compared with diabetic untreated.

3.4 Blood Urea Nitrogen in Normal, MEPN and Metformin Treated Groups

Fig. 3 showed blood urea nitrogen (BUN) in normal, diabetes treated with MEPN and metformin rats. BUN significantly decreased (P<.05) in normal control, diabetes treated with 100mg/kg and 200 mg/kg MEPN when compared with diabetic untreated. BUN significantly decreased (P<.05) in diabetes treated with 100 mg/kg and 200 mg/kg MEPN when compared with diabetes treated with metformin. There was no significant difference in MEPN treated groups when compared with normal control but, BUN significantly increased in metformin treated group when compared with normal control.

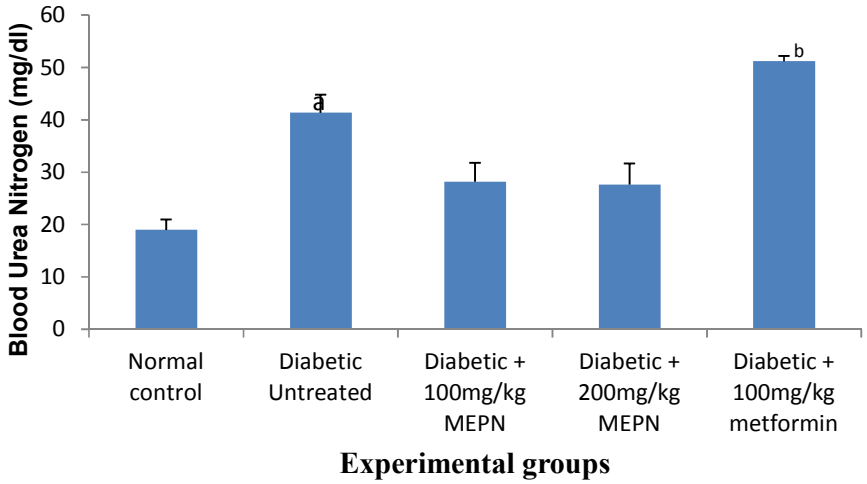

Fig. 3. Blood urea nitrogen in normal, diabetes treated with MEPN and Metformin groups.
Data were expressed as Mean ± SEM; P<.05. [a] indicates value significantly different from the normal control, diabetes + 100mg/kg MEPN, diabetes + 200mg/kg MEPN, diabetes + 100mg/kg Metformin. [b] indicates value significantly different from normal control, diabetes + 100mg/kg MEPN, diabetes + 200mg/kg MEPN (n=5).

Table 3. Antioxidative parameters in normal, MEPN and Metformin treated groups

Experimental groups	G6PDH (U/L)	LDH (U/L)	SOD (U/L)	CAT (U/ml)	GPx (U/L)	ALB (mg/dl)
Normal control (0.3 ml distilled water)	37.78 ± 3.29	27.17 ± 1.19	149.2±7.98	166.6±5.65	7.49±0.94	2.38±0.12
Diabetic untreated (0.3 ml distilled water)	20.52 ± 0.52[a]	23.30 ± 3.84	143.0±27.09	113.1±3.48[b]	0.78±0.26[a]	1.32±0.04[b]
Diabetes + 100 mg/kg MEPN	20.80 ± 2.18[a]	17.49 ± 1.09[c]	165.0±8.52	131.5±7.884[a]	0.99±0.39[a]	2.35±0.16
Diabetes +200 mg/kg MEPN	30.46 ± 0.61	23.36 ± 3.50	140.0±20.31	168.4±2.36	4.53±0.72	2.48±0.14
Diabetes + 100 mg/kg Metformin	45.14 ± 2.98[#]	32.18 ± 3.24	141.0±13.45	186.2±4.85[#]	5.43±1.46	2.58±0.03

Data were expressed as Mean ± SEM; P<0.05. [a] indicate values significantly different from normal control, diabetes + 200 mg/kg MEPN, diabetes + 100 mg/kg Metformin. [b] indicate values significantly different from normal control, diabetes + 100 mg/kg MEPN, diabetes + 200 mg/kg MEPN, diabetes + 100 mg/kg Metformin. [#] indicate values significantly different from normal control, diabetes + 200 mg/kg MEPN. [c] indicates value significantly different from diabetes + 100 mg/kg metformin (n=5)

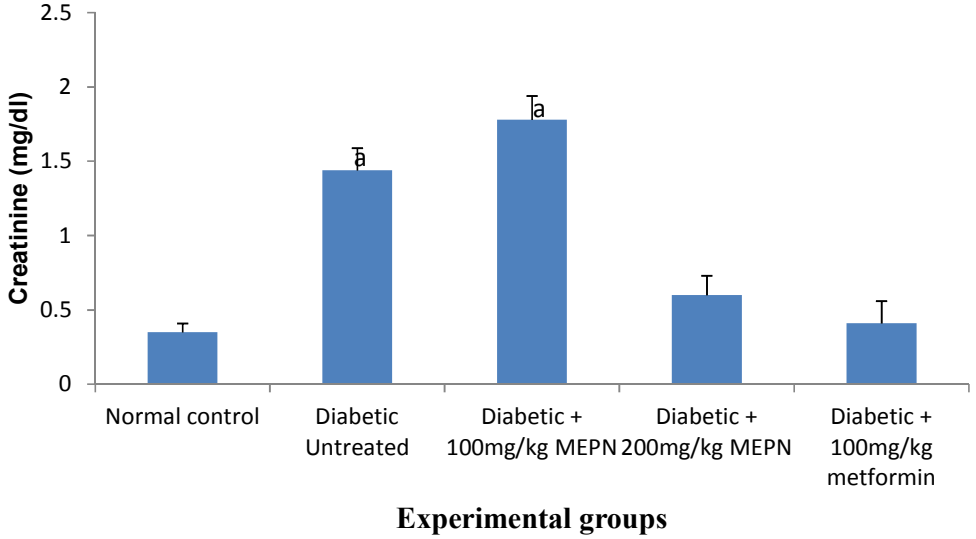

Fig. 4. Creatinine level in normal, MEPN and Metformin treated groups.

Data were expressed as Mean ± SEM; P<.05. [a] indicate values significantly different from the normal control, diabetes+200 mg/kg MEPN, diabetes+100 mg/kg metformin (n=5)

3.5 Creatinine Level in Normal, MEPN and Metformin Treated Groups

Fig. 4 showed creatinine level in normal, MEPN and Metformin treated groups. There was significant increase (P<.05) in creatinine level in diabetic untreated group when compared with normal control. Similarly, creatinine level significantly increased in 100 mg/kg MEPN treated group when compared with normal control. However, there was significant decrease (P<.05) in creatinine level in diabetes treated with 200 mg/kg MEPN and 100mg/kg metformin when compared with diabetic untreated and diabetes treated with 100 mg/kg MEPN.

3.6 Photomicrographs of Kidney in Normal, MEPN and Metformin Treated Rats

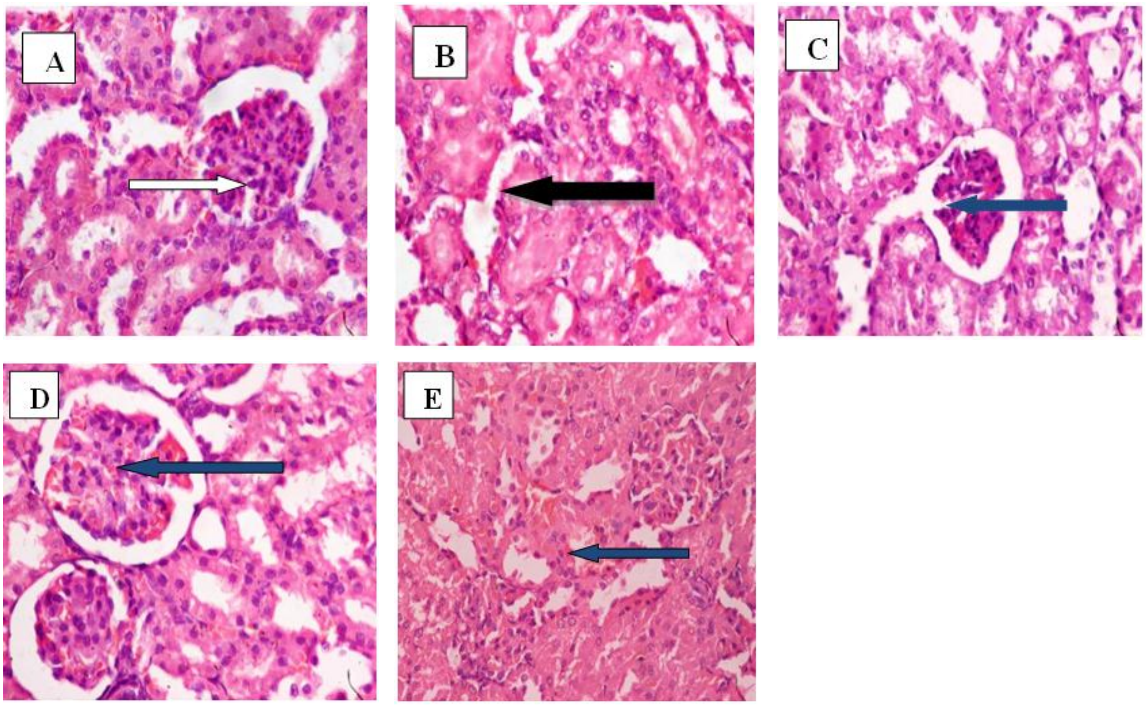

Plate 1 (A – E). Shows sections stained with H & E showing architecture of the Kidney in A (Control), B (Diabetic untreated), C (Diabetes + 100mg/kg MEPN), D (Diabetes + 200mg/kg MEPN), E (Diabetes + 100mg/kg Metformin). Plate 1A showed the architecture of the kidney with normal glomerulus and capsular space (white Arrow), Plate 1B showed the architecture of the kidney with degenerated glomerulus and Bowman's capsule with inflammatory cells (Black Arrows). Plate 1C& E showed architecture of the kidneys with distorted glomerulus. Plate 1D showed architecture of the kidney with glomerulus and capsular space comparable with the normal control (Plate 1A) (Blue Arrow) X 400

4. DISCUSSION AND CONCLUSION

This present study investigates the renoprotective effects of methanol extract of *Parquetina nigrescens* (MEPN) in alloxan-induced diabetic rats. The acute toxicity test performed in the experimental rats using MEPN showed no case of mortality in various groups treated and this suggests that MEPN is non-toxic even at higher doses administered. According to Hodge and Sterner toxicity scale [21], a plant extract with an LD_{50} < 2000 mg/kg is said to be toxic and since the LD_{50} of MEPN from our study was >5000 mg/kg therefore, MEPN is said to belong to a non-toxic category of medicinal plants. However, this finding is not in agreement with the report of Lyon et al., [22] who had earlier reported that the latex part of the plant is toxic and even used in making poison.

Diabetes mellitus is a multi-factorial disease in which increased oxidative stress plays an

important pathogenic role [23]. A low Glucose-6-phosphate dehydrogenase (G6PDH) activity has been considered to play a role in increased oxidative stress because of less NAPDH produced during pentose phosphate pathway [24]. The significant decrease in G6PDH activities observed in diabetic untreated is consistent with the reports of Wan et al., [25] who showed that positive correlation exists between reduced G6PDH activities and diabetes mellitus. In diabetic untreated, less NADPH is likely produced to reduce the oxidized glutathione in the pentose phosphate pathway leading to altered glucose tolerance [26]. The increase in G6PDH observed in 200 mg/kg MEPN group indicates that more reduced glutathione may have been produced to mop up free radicals that may have been generated in diabetes mellitus [27]. The increase in G6PDH observed in metformin-treated group suggests that metformin may also have the potential of reducing oxidative stress as well [26].

The decrease observed in Catalase (CAT) activities in diabetic untreated may indicate that more hydrogen peroxide is produced and if this is allowed to accumulate may be potentially toxic at high concentration [28]. However, the increase observed in the treatment groups indicates that more hydrogen peroxide may have been broken down to harmless water and oxygen [28].

Albumin is a multifunctional protein with 585 amino acids and one reduced cysteine residue (Cyst 34) and a molecular weight of 66 kDa [29]. The significant decrease in albumin in diabetic untreated may likely indicates a decrease in the quantity of the reduced Cyst 34 residues in albumin to scavenge hydroxyl radicals [30]. However, the increase observed in albumin level in the treatment groups suggests an increase in reduced cyst 34 in albumin which is converted to sulfenic acid that is important in redox modulation of reactive species [31]. It is also likely that MEPN may have reversed methionine sulphoxide that may have been produced in diabetic untreated back to methionine residue in albumin which is highly susceptible to oxidative damage [32] and these effects observed in 100 mg/kg and 200 mg/kg MEPN were comparable to metformin-treated group.

Creatinine is a metabolic waste product produced from muscle creatine and excreted through the kidneys [33]. The significant increase in creatinine level in diabetic untreated rats is an indication of kidney damage, sclerosis, inflammation and decreased glomerular filtration rate [34] as shown by the photomicrograph (Plate 1B). This observation is consistent with previous reports which identified creatinine as a basic marker of renal dysfunction in diabetes mellitus [35]. However, the significant decrease in creatinine level in 200 mg/kg MEPN was comparable to that of normal control and this implies that MEPN may have the ability to improve kidney functions in diabetic condition so that more waste products can be filtered from the blood and excreted in urine as shown by the photomicrograph (Plate 1D) [36].

Blood urea nitrogens (BUN) are nitrogenous waste products produced from the breakdown of protein into ammonia which undergoes deamination by liver enzymes to produce urea which are excreted through the kidneys. The increase in a BUN in diabetic untreated rats may be due to damage to the glomerulus of the renal tubular cells from uncontrolled hyperglycemia [37] as shown by the photomicrograph (Plate 1B). However, oral administration of MEPN at 100mg/kg and 200 mg/kg significantly decrease urea nitrogen and this implies that MEPN could also repair the damaged renal tubular cells and increase excretion of urea nitrogen in the urine as shown by the photomicrograph (Plate 1C& D).

In summary, administration of MEPN caused increased G6PDH activities; an effect which may be due to its ability to reduce oxidized glutathione. MEPN caused increased Catalase activities, an effect which may result from the decreased formation of hydrogen peroxide. Similarly, MEPN caused increased albumin level; which may be due to the availability of more cysteine 34 amino acid residues for redox modulation of free radicals. MEPN also caused a decreased level of creatinine and blood urea nitrogen, an effect which may be due to its ability to repair the damaged renal tubular cells, reduce thickening of the glomerulus and improve ultrafiltration. This various effects of MEPN may be due to the presence of phytochemicals and active compounds present in the plant extract as shown by the results of phytochemical screening and Gas chromatography-mass spectrometry analysis.

CONSENT

It is not applicable.

ETHICAL APPROVAL

As per international standard or university standard, written approval of Ethics committee

has been collected and preserved by the authors.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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