Original Research Paper

PRELIMINARY ASSESSMENTS AND RENOPROTECTIVE EFFECTS OF METHANOL EXTRACT OF *Parquetina nigrescens* IN DIABETIC WISTAR RATS

ABSTRACT

Aim: Preliminary studies and renoprotective effects of methanol extract of *Parquetina nigrescens* (MEPN) in diabetic 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 0.3ml distilled water. Groups 3-5 served as diabetic groups treated with 100mg/kg, 200mg/kg MEPN and 100mg/kg metformin respectively. Glucose 6 phosphate dehydrogenase (G6PDH), lactate dehydrogenase (LDH), superoxide dismutase (SOD), glutathione peroxidase (GPx), catalase (CAT) activities and albumen (Alb) level were determined using randox kits. The kidney histology was done using haemotoxylin-eosin stain. Results were analyzed using ANOVA with statistical significance taken at $P<.05$.

Results: Phytochemical screening showed the presence of alkaloids, cardenoloides, anthraquinones, tannins and flavonoids. Gas Chromatography Mass Spectrometry showed twenty-two active compounds. 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 when 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,* active compounds, oxidative stress, renal dysfunction, diabetes mellitus

Comment [j1]: Should be clarified

Comment [j2]: type

Comment [j3]: BUN, CRT?

Comment [j4]: GC/MS

Comment [j5]: Results on diabetic group should be included

Comment [j6]: SOD,LDH?

Comment [j7]: Omit active compounds

1.0 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. 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, Aegle marmelos* and *Agrimony eupatoria. 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 disorder [8]. It has also been used in the treatment of several other ailments

Comment [j8]: disorders

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 results 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.

Figure 1: *Parquetina nigrescens* leaves

2.0 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 (2mm Sieved). About 1823g 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^{o}C for 20 min using a rotatory evaporator (Gallenkamp UK). The residue yielded 53.58g of methanol extract of *Parquetina nigrescens* (MEPN) which was later stored at 2-8^{o}C prior to Physiological investigation.

2.2 Toxicological study and calculation of median lethal dose (LD_{50})

Thirty-five rats weighing between 100-150g were used for this study and were divided into seven groups of five rats per group. Group 1 received 0.3ml distilled water; Groups 2-7 were orally given graded doses of MEPN at 500mg/kg, 1000mg/kg, 2000mg/kg, 3000mg/kg, 4000mg/kg and 5000mg/kg respectively. Rats were placed under continuous observation for 6hours. After 24 hours, rats were sacrificed under mild anesthesia (sodium thiopental 30mg/kg *i.p*) to observe changes in 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 Wister rats weighing (100-150g) 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 (1985) [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 100mg/kg MEPN, 200mg/kg MEPN and 100mg/kg Metformin respectively.

2.4 Induction of diabetes mellitus

Diabetes was induced after a 24hour fast in group 2, 3, 4 and 5 by single intra-peritoneal injection of alloxan monohydrate (Sigma Aldrich, U.S.A) at a dose of 120mg/kg using the method

Comment [j9]: omit

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described by Carvalho *et al*., 2003 [17]. After 72hours of alloxan administration, only rats with fasting blood glucose level of 250mg/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 (30mg/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 albumen level were determined using commercially available randox kits and their absorbance measure using spectrophotometry procedure as described by (Avinash *et al.,* 2017) [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*., 1968 and Khaldun *et al*., 2017 [19-20]

Comment [j16]: albumin

Comment [j17]: measured

Comment [j18]: omit brackets from reference

Comment [j19]: 19,20

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 $P<.05$.

3.0 RESULTS

3.1 Phytochemical screening and GC-MS studies on MEPN

Table 1 and Figure 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).

Comment [j20]: /

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

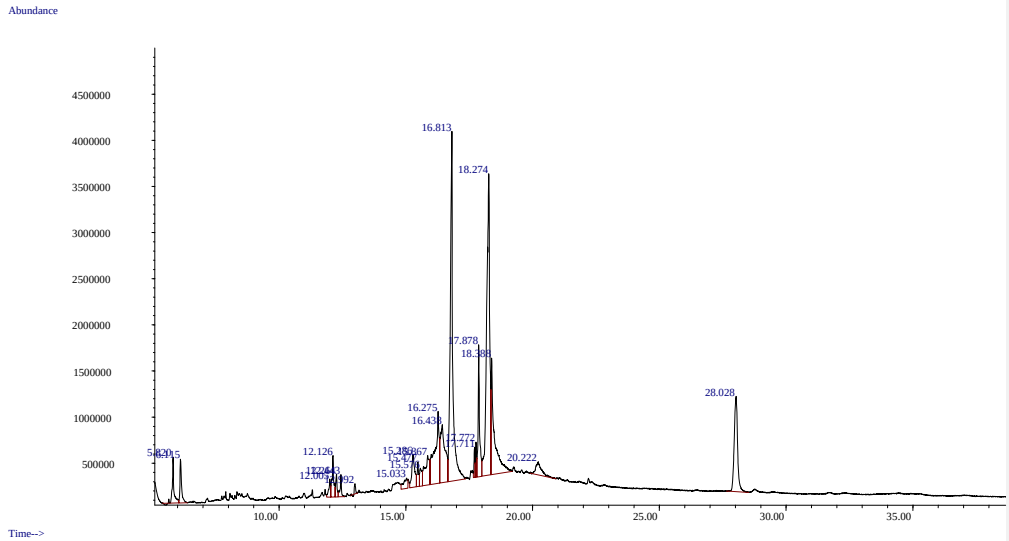

Figure 2: Gas Chromatography Mass Spectrometry (GC-MS) analysis of MEPN

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 5000mg/kg. However, fecal materials and urine were found in group administered with 5000mg/kg 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	0.3ml	0/5	Nil
Group 2	500mg/kg	0/5	Nil
Group 3	1000mg/kg	0/5	Nil
Group 4	2000mg/kg	0/5	Nil
Group 5	3000mg/kg	0/5	Nil
Group 6	4000mg/kg	0/5	Nil
Group 7	5000mg/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 200mg/kg MEPN and 100mg/kg metformin groups when compared with diabetic untreated and diabetes treated with 100mg/kg MEPN respectively. There was also significant increase in G6PDH activities in diabetes treated with 100mg/kg metformin when compared with normal control and diabetes + 200mg/kg MEPN. Lactate dehydrogenase (LDH) was significantly lower (P<.05) in diabetes treated with 100mg/kg MEPN when compared with diabetes treated with 100mg/kg metformin.

Catalase (CAT) activities significantly increase (P<.05) in normal control, diabetes + 100mg/kg MEPN, diabetes + 200mg/kg MEPN, diabetes + 100mg/kg Metformin when compared with diabetic

untreated. There was significant increase in CAT activities in normal control, diabetes + 200mg/kg MEPN, diabetes + 100mg/kg Metformin when compared with diabetes treated with 100mg/kg MEPN. A significant increase was also observed in CAT activities in normal control and diabetes + 200mg/kg MEPN when compared with diabetes treated with 100mg/kg meformin

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

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

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 (0.3ml 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 (0.3ml 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 + 100mg/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 +200mg/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 + 100mg/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 + 200mg/kg MEPN, diabetes + 100mg/kg Metformin. [b] indicate values significantly different from normal control, diabetes + 100mg/kg MEPN, diabetes + 200mg/kg MEPN, diabetes + 100mg/kg Metformin. [#] indicate values significantly different from normal control, diabetes + 200mg/kg MEPN. [c] indicates value significantly different from diabetes + 100mg/kg metformin (n=5)

3.4 Blood Urea Nitrogen in normal, MEPN and Metformin treated groups

Figure 2 showed blood urea nitrogen (BUN) in normal, diabetes treated with MEPN and metformin rats. BUN significantly increased (P<.05) in normal control, diabetes treated with 100mg/kg, 200mg/kg MEPN and 100mg/kg Metformin when compared with diabetic untreated. BUN significantly increased (P<.05) in diabetes treated with MEPN and metformin treated groups when compared with diabetic untreated. 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.

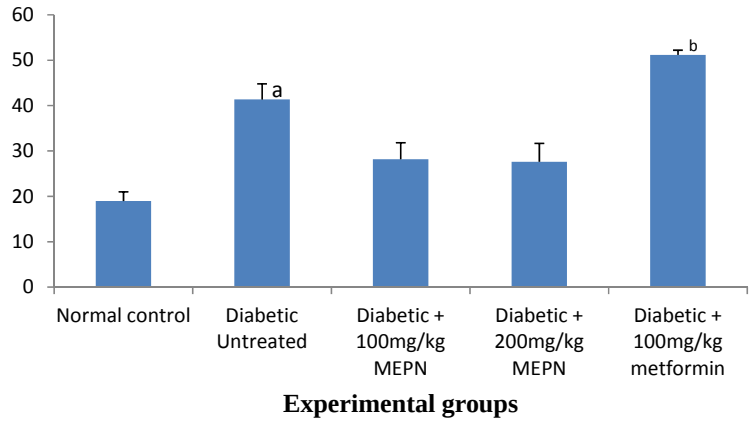

Figure 3: Blood Urea Nitrogen in normal, diabetes treated with MEPN and Metformin groups.

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).

3.5 Creatinine level in normal, MEPN and Metformin treated groups.

Figure 3 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 100mg/kg MEPN treated group when compared with normal control. However, there was significant decrease (*P*<.05) in creatinine level in diabetes treated with 200mg/kg MEPN when compared with diabetes untreated and diabetes treated with 100mg/kg MEPN.

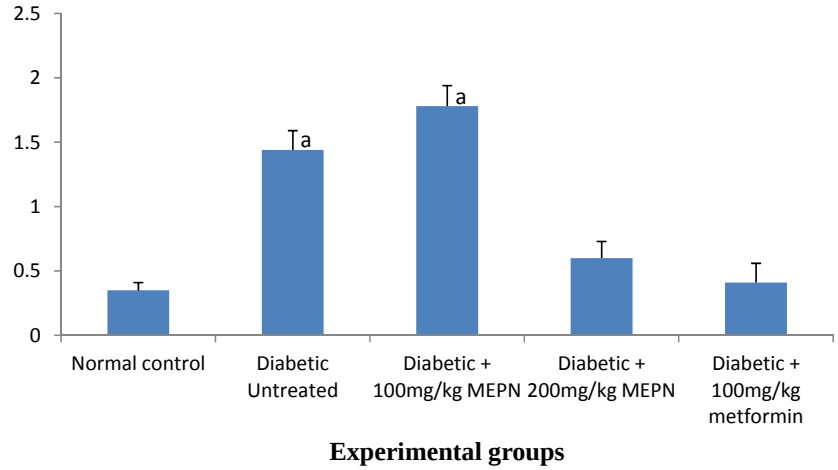

Figure 4: Creatinine level in normal, MEPN and Metformin treated groups.

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

3.6 Photomicrographs of kidney in normal, MEPN and Metformin treated rats

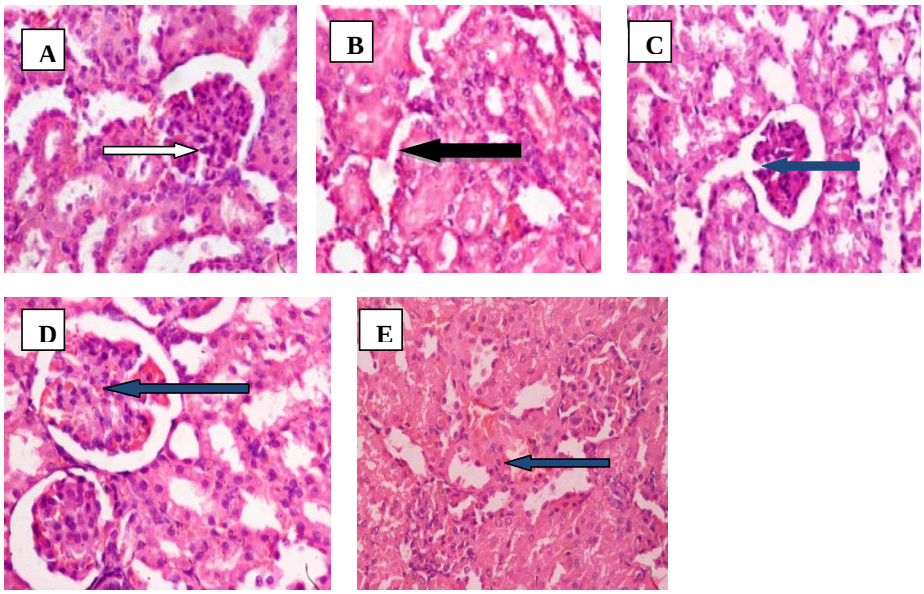

Plate 1 (A – E): Shows sections stained with H & E showing architecture of the Kidney in A (Control), B (Diabetic untreated), C (Diabetes + 100mg/kg MEPN), D (Diabetes + 200mg/kg MEPN), E (Diabetes + 100mg/kg Metformin). Plate 1A showed architecture of the kidney with normal glomerulus and capsular space (white Arrow), Plate 1B showed 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.

4.0 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 (2005) toxicity scale [21], a plant extract with an LD_{50} < 2000mg/kg is said to be toxic and since the LD_{50} of MEPN from our study was >5000mg/kg therefore, MEPN is said to belong to a non toxic category of medicinal plant. 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.

Comment [j21]: plants

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*., 2002 [25] who showed that positive correlation exist between a 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 200mg/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].

Albumen is a multifunctional protein with 585 amino acids and one reduced cysteine residue (Cyst 34) and a molecular weight of 66kDa [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 100mg/kg and 200mg/kg MEPN were comparable to metformin treated group.

Comment [j22]: albumin

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 100mg/kg and 200mg/kg MEPN was comparable to that of metformin treated group 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 1C&D) [36].

Blood urea nitrogens (BUN) are nitrogenous waste products produced from breakdown of protein into ammonia which undergoes deamination by liver enzymes to produce urea which are excreted through the kidneys. The increase in 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 200mg/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 decreased formation of hydrogen peroxide. Similarly, MEPN caused increased albumen level; which may be due to availability of more cysteine 34 amino acid residues for redox modulation of free radicals. MEPN also caused 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 glomerulus and improve ultrafiltration. This various effects of MEPN may be due 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.

Comment [j23]: albumin

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