***Musanga cecropioides* attenuates chemical and diabetes induced oxidative stress in experimental animal models.**

Abstract

Chemically induced multiple-organ toxicities and complications of diabetes are linked to oxidative imbalance. This study evaluated the effect of leaf extract and fractions of *M. cecropioides* on both chemical and disease induced oxidative stress. The leaves were extracted with methanol and partitioned into various fractions. Phytochemical analysis and total phenolic content (TPC) were done for the extract and fractions. The DPPH (2,2-diphenyl-1-picrylhydrazyl) and Ferric reducing antioxidant power assay were used to evaluate their in vitro antioxidant potentials. Carbon tetrachloride and STZ-NAD were used to induce chemical and diabetes induced oxidative stress respectively. Liver function tests and lipid peroxidation (LPO) were used to monitor CCL4 induced oxidative stress while serum antioxidant enzymes (catalase and superoxide dismutase), LPO and total plasma antioxidant assays were used to monitor diabetes induced oxidative stress. The ethyl acetate fraction (EAF) showed the highest TPC of 386.06 mgGAE/g. The EAF and butanol fraction (BF) showed better DPPH activity compared to the extract with IC50s of 49.78, 71.57, 98.92 µg/ml respectively. The EAF both at 200 and 400 mg/kg maintained non-significant ($p > 0.05$) difference between pre-induction and post-induction ALT and AST concentrations. Also, only EAF at 400 mg/kg protected against significant rise in CCL4 induced LPO from baseline values which was not achieved with 100 mg/kg silymarin. The EAF at 400 mg/kg was the only treatment that produced significant ($P<0.05$) reduction in blood glucose 24 h post-treatment compared to vehicle control group. The EAF and BF at 400 mg/kg just like 200 mg/kg metformin were able to restore serum catalase almost to their pre-induction values with no significant ($P>0.05$) difference. *M. cecropioides* proved effective in both chemical and disease induced oxidative stress.

Keyword: Oxidative stress; Diabetes; hyperglycermia

1.0 Background

Diabetes mellitus is a metabolic disorder characterized by elevated levels of glucose in blood (hyperglycermia) and insufficiency in production or actions of insulin [1]. Hyperglycermia through glycation of proteins, glucose oxidation and mitochondrial dysfunction contributes to free radical formation. Due to the ability of these free radicals to damage lipids, proteins and DNA, they play great role in the onset and progression of diabetic complications such as coronary artery disease, neuropathy, nephropathy, retinopathy and stroke [2]. Also diabetic hyperglycermia by the process of free radical production causes enzyme protein glycation and oxidative degeneration. Alterations in function and structure of antioxidant protein enzymes may affect the detoxification of free radicals thus enhancing oxidative stress in diabetes [2, 3].

Similar to several other health conditions such as cancer and neurodegenerative disorders, oxidative stress has been widely linked with the incidence of diabetes mellitus [4]. Several studies have shown that oxidative stress is a key element in the development and progression of diabetes and its associated complications [5 – 8]. Oxidative stress has been shown to compromise the two major mechanisms failing during diabetes which are insulin secretion and insulin action [6, 8]. Reduction of hyperglycermia and oxidative stress may therefore be a more holistic approach in the management of diabetes and prevention of its associated complications.

In addition to the association of oxidative stress to disease states like diabetes, oxidative stress has also been implicated in chemical/drug mechanisms of toxicity to organs and macromolecules [9]. The liver, by nature of its physiological role in drug/chemical metabolism is constantly exposed to oxidative damage by reactive metabolites generated in the course of metabolism. The generation of reactive intermediates is a common event in liver damage resulting from a variety of hepatotoxic drugs and solvents [10, 11]. Oxidative damage to liver from reactive metabolites further compromises the detoxification physiological role of the liver and predisposes to further generation of reactive substances capable of causing systemic oxidative stress [12, 13].

Musanga cecropioides (Cecropiaceae) commonly known as African corkwood is a fast growing evergreen tree with umbrella-shaped crown. It is mostly found in the tropical forests of Africa. Traditionally, the plant is used to reduce high blood sugar, induce labour and also reduce elevated blood pressure [14]. Literary reports revealed Oxytocic and hypotensive effect of the leaves and stem bark [14, 15]. A recent study revealed the ethanolic leaf extract of *M. cecropioides* to possess both anti-inflammatory and anti-nociceptive effect. *In vitro* and *in vivo* hypoglycemic properties of the leaf and stem bark have also been reported [16]. Since imbalance in oxidative stress has been implicated in the complications of diabetes and multiple organ failure from chemical/xenobiotics toxicities, this study investigated the effect of the leaf extract and fractions of *M. cecropioides* on both chemical and diabetes induced oxidative stress.

2.0 Materials and method

2.1 Animals

Adult Swiss albino rats of both sex were used for this study. The animals were obtained from the animal house of the Department of Pharmacology, faculty of Pharmaceutical Sciences, Enugu State Unoversity of Science and Technology, Enugu State, Nigeria. The animals were maintained on standard laboratory animal conditions and fed with rodent feed (Guinea Feeds Nigeria Ltd). They were allowed free access to water ad libitum. All animal experiments were conducted in compliance with NIH guide for care and use of laboratory animals and approved by the institution animal ethical committee with approval number ESUT/FPS/PHA/2021/019.

2.2 Extraction and fractionation

The pulverized leaves were cold macerated in methanol for 72 h with intermittent shaking. The resulting solution was filtered and concentrated in vacuo using rotary evaporator at 40^{o}C. One-third of the extract was subjected to liquid–liquid partition successively with 2.5 L of n-hexane, ethyl acetate, butanol and water to obtain different fractions soluble in these fractions. The fractions were concentrated using rotary evaporator and stored at 4°C.

2.3 Phytochemical analysis

Qualitative and quantitative phytochemical analysis of the extract and fractions were determined using standard methods as described by Odoh et al.,[17].

2.4 Total Phenolic content of the extract and fractions by folin ciocalteu's assay

The total phenolic content of the extract and fractions were determined using the method described by Kim *et al*. [18]. The total phenolic content was estimated from the calibrated curve which was made by preparing gallic acid solution and expressed as milligrams of gallic acid equivalent (GAE) per gram of the extracts.

2.5 *In vitro* antioxidant activity

The free radical scavenging activity of the extract and fractions were evaluated by their inhibition of DPPH radical using the method of Patel and Patel [19] while their ferric reducing antioxidant power (FRAP) assay was carried out following the method described by Habibur *et al,* [20]. In both assays, different dilutions (1000, 500, 250, 125 and 62.5 ug/ml) of the extract and fraction were used. A graph of percentage inhibition against concentration was plotted and half maximal inhibitory concentration (IC_{50}) and median effective concentration (EC_{50}) were extrapolated using a regression analyses equation.

2.6 Hepatoprotective Study

2.6.1 *Experimental design*

Thirty-five Swiss Albino rats were divided into seven groups of five animals each and treated with the extract and most active fraction for 10 days. Groups 1 and 2 were treated with 200 and 400 mg/kg of the extract respectively; while groups 3 and 4 - 200 mg/kg and 400 mg/kg of the ethyl acetate fraction; group 5 served as the naïve; group 6 - 100 mg/kg of Silymarin (standard) and the seventh group was treated with the vehicle (5% Tween 80) (5 ml/kg). Six hours after the last day of the treatment oxidative stress was induced with 2% carbon tetrachloride (CCL_4) in olive oil administered intraperitoneally (10 ml/kg) to the animals in all the groups except the naive group. Eighteen hours later blood samples were collected from all the animals through retro-orbital puncture for the estimation of serum liver marker enzymes - ALP, AST, ALT- and lipid peroxidation.

2.6.2 Serum Preparation

The serum was prepared using standard method as described by Yesufu et al. [21]. Briefly, the method used is as follows. Blood was allowed to clot for 30 minutes and then centrifuged at 2500 rpm for 15 minutes and the supernatant was decanted to get the serum.

2.6.3 Quantitative determination of liver function enzymes

Serum alanine transaminase, aspartate transaminase and alkaline phosphatase were enzyme activity were determined using commercial test kit (Span Diagnostics Ltd., India). Manufacturers instruction were followed for all the enzyme quantification.

2.6.4 Determination of the Lipid Peroxidation (LPO) in Serum

The level of thiobarbituric acid reactive substance (TBARS) and malondialdehyde (MDA) production was measured in serum by the modified method as described by Draper and Hadley [22]. The concentration of TBARS was calculated using the molar extinction coefficient of malondialdehyde (1.56×10^5 mol/L/cm) using the formula, $A = \Sigma CL$, where A = absorbance, Σ = molar coefficient, C = concentration, and L = path length. The results were expressed in nmol/ml.

2.7 Diabetes induced systemic oxidative stress

Albino rats (n = 60) were fasted overnight for 12 h and fasting blood glucose levels taken from tail vein using Accu-check Glucometer (Roche, Germany) before the induction of diabetes with 70 mg/kg STZ (i.p) following 30 minis pre-administration with 50 mg/kg NAD (i.p). After 72 h, animals with fasting blood glucose levels > 160 mg/dl were confirmed diabetic and selected for the study. The animals were grouped into 8 groups of six animals each. Groups 1 and 2 received the extract 200 and 400 mg/kg respectively. Groups 3 and 4 ethyl acetate fraction while groups 5 and 6 received butanol fraction at same doses as in extract groups. The control groups (7 and 8) received 5 mg/kg glibenclamide and 5 mL/kg 5% Tween 80 respectively. Treatment with the extract and fractions continued for 21 days with fasting blood glucose determined every 7 days. On the 21 day, blood samples were collected through retro-orbital plexus into a plain tube from the animals 5 h after treatment administration. Blood samples were also obtained before diabetes induction and 72 h post-induction. The sera obtained were used for the determination of antioxidant enzymes, lipid peroxidation and total antioxidant status.

2.7.1 Determination of antioxidant enzymes activity

Serum catalase activity was estimated by visible light method as described by Weydert and Cullen [23] using catalase assay kit (Elabscience Biotechnology Co. Ltd., China) while serum superoxide dismutase activity (SOD) was estimated by hydroxylamine method as described by Weydert and Cullen [23] using SOD assay kit (Elabscience Biotechnology Co. Ltd., China).

2.7.2 Determination of serum total antioxidant capacity

Total antioxidant capacity of the serum was determined using the ABTS radical cation decolorization assay method as described by Re et al., [24]. The assay using Sigma-Aldrich (USA) antioxidant assay kit (Catalog number CS0790) was based on the ability of serum antioxidants to inhibit the formation of ferryl myoglobin radical from metmyoglobin and hydrogen peroxide which oxidizes the 2, 2-Azino-di-3-ethylbenzthiazoline sulphonate (ABTS) to produce a radical cation that can be measured spectrophotometrically at 405 nm. The assay was calibrated with Trolox and total antioxidant capacity expressed in terms of Trolox equivalents (mM).

2.8 Statistical analysis

Results were presented as mean ± Standard error of mean (SEM). The analysis of variance in the outcome of the treatment (one way ANOVA) was done using Statistical Package for Social Science (SPSS, version 20). Multiple comparism for post hoc analysis was done using Turkey's test. Calculation of median inhibitory concentration (IC_{50}) and effective concentration (EC_{50}) of the extracts and fractions were carried out using regression equation in Microsoft Excel, 2010.

3.0 Results

3.1 Phytochemical and total phenolic content

The extract showed presence of many phytocompounds with phenolic compounds like flavonoids and tannins being abundantly present (Tables 1 and 2). These phenolic compounds were differentially distributed in the fractions of the extract with high partitioning in the ethyl acetate and butanol fractions (Table 1). Linear dose response curve was established with the reference standard – gallic acid; at concentrations ranging from 0.01 – 0.06 mg/ml (R^2 = 0.9951). The extract gave total phenolic value of 297.76 mgGAE/g (Figure 1). the order of activity for the fractions was ethyl acetate fraction > butanol fraction >> water fraction >> n-hexane fraction. The ethyl acetate and butanol fractions showed close phenolic content values of 386.04 and 364.88 mgGAE/g respectively.

Table 1: Qualitative Phytochemical Analysis

Phytochemical	Methanol Ext.	n-Hexane F.	Ethyl acetate F.	Butanol F.	Aqueous F.
Alkaloids	+	-	-	+	-
Tannins	++++	-	+++	+++	++
Flavonoids	++++	+	++++	++++	+++
Steroids	+	++	+	+	+
Terpenoids	+	++	+	+	+
Glycosides	+++	-	+++	+++	+
Cardiac glycosides	-	-	-	-	-
Saponins	++	-	++	++	++

Key: ++++ = Abundantly present; +++ = Present in high concentration

++ = Present in moderately high concentration; + = Present in small concentration

– = Not present

Table 2: Quantitative Estimation of Phyto-constituents

Constituent	Result (% w/w)
Alkaloids	1.40
Tannins	2.64
Flavonoids	11.7
Saponins	1.30

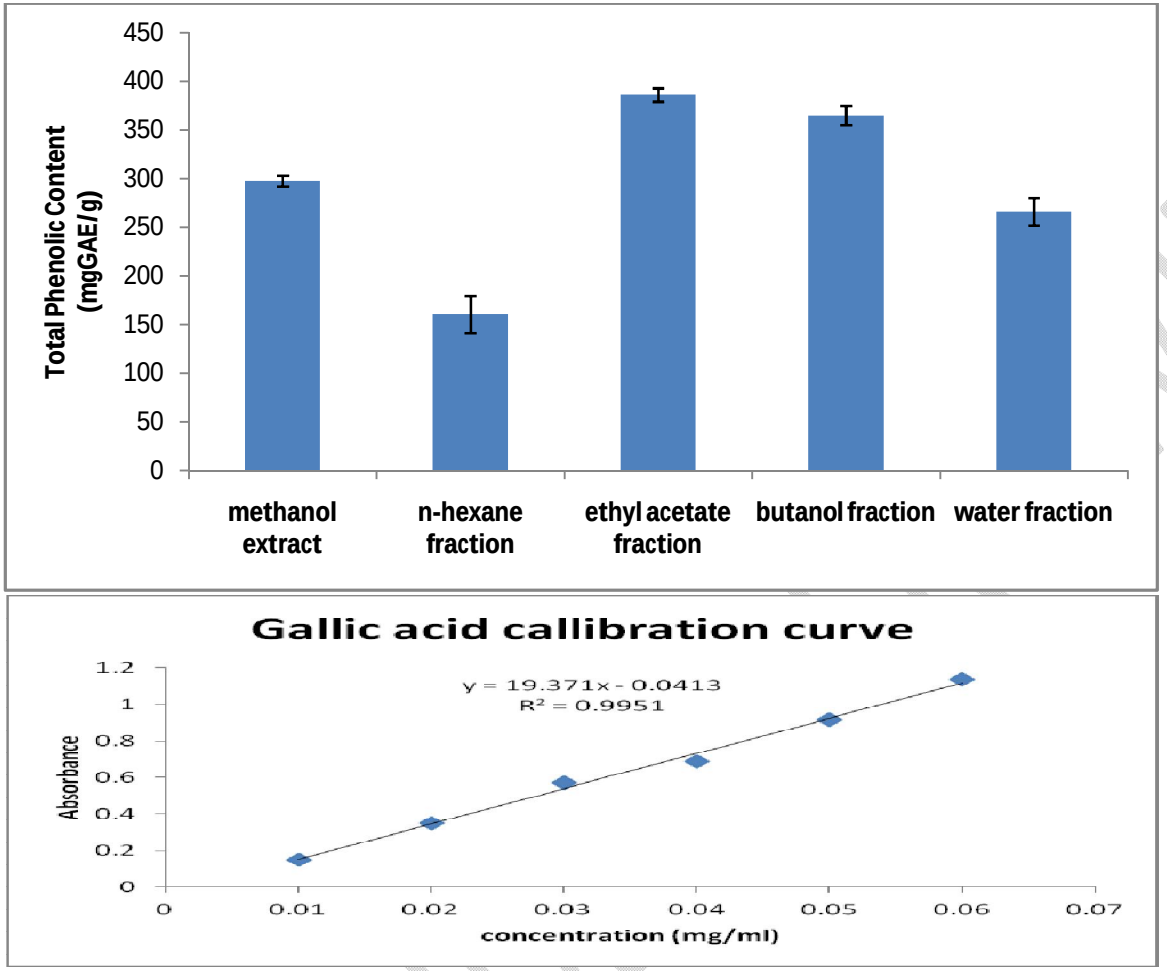

Figure 1: Total phenolic content of the extract and fractions

3.2 DPPH scavenging effect and ferric reducing antioxidant power activity

The extract and fractions showed dose response inhibition of DPPH radical and ferric reducing potentials. The extract showed good inhibition of DPPH radical with IC_{50} less than 100 µg/ml (Figure 2). The potency of the n-hexane fraction when compared to the extract and other fractions was the lowest. The ethyl acetate and butanol fractions showed better potency compared to the extract with IC50s 49.78 and 71.57 µg/ml against 98.92 µg/ml recorded for the extract. The extract showed ferric reducing potentials with EC_{50} value of 169.65 µg/ml (Figure 3). The fractions maintained the same order of activity shown in DPPH scavenging activity; although with lower potency. The ethyl acetate fraction was the only fraction that produced EC_{50} value less than 100 µg/ml similar to the reference standard – ascorbic acid.

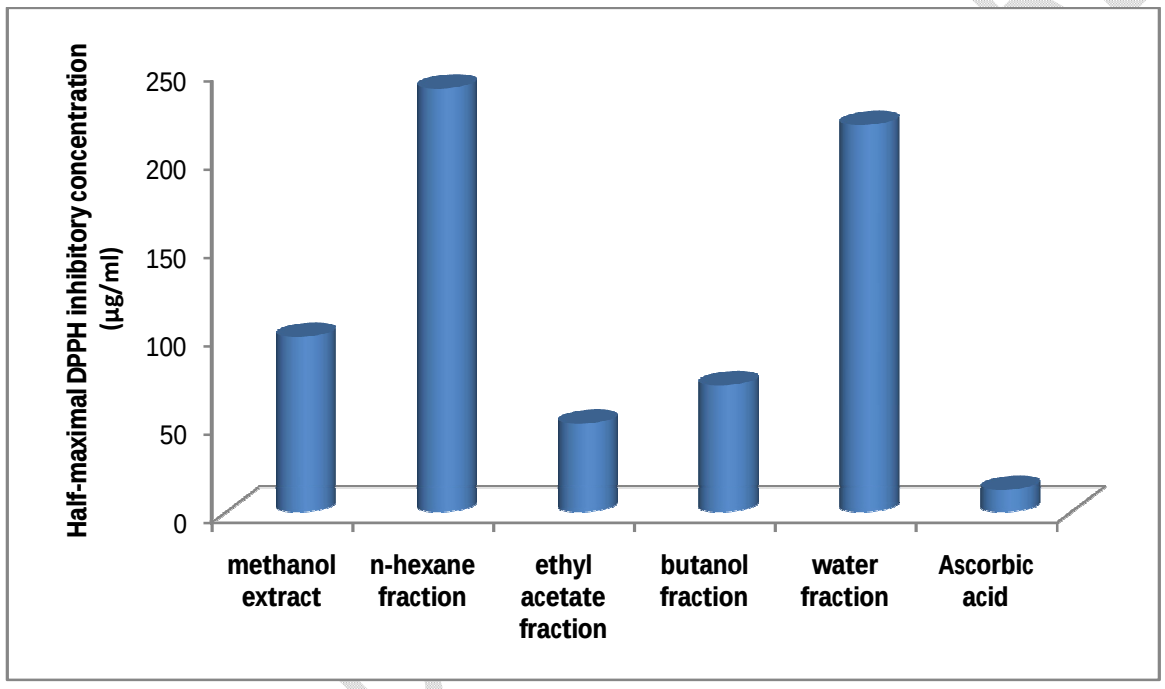

Figure 2: Half maximal DPPH inhibitory concentrations of the extract and fraction

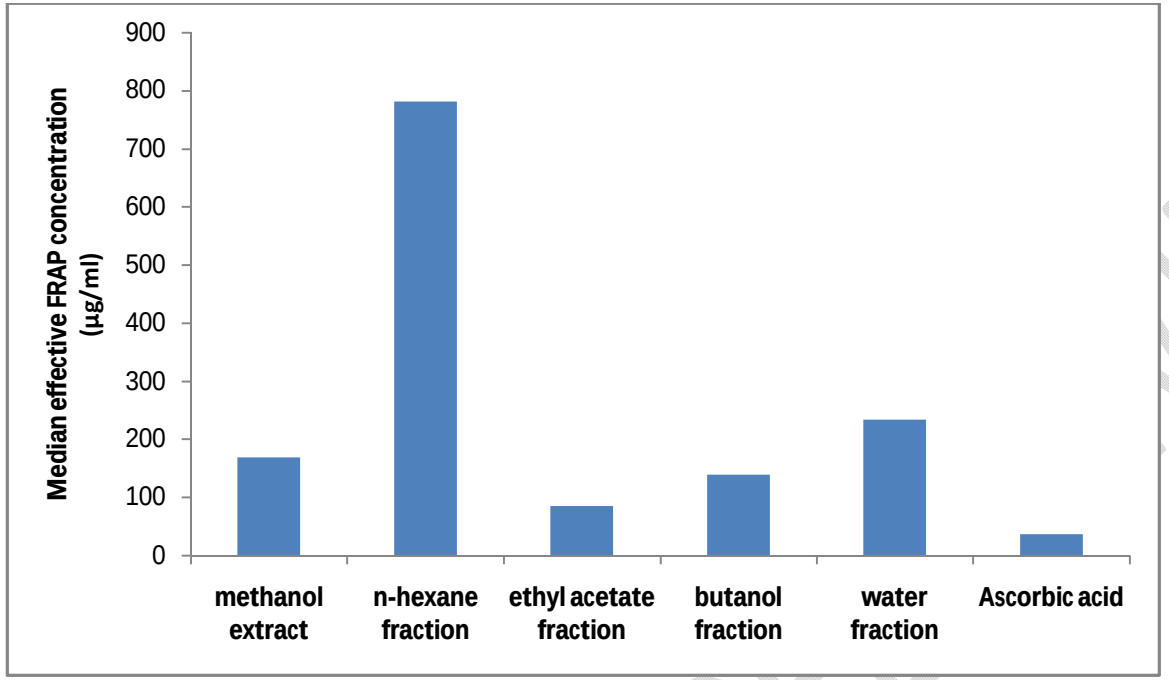

Figure 3: Half maximal FRAP concentrations (EC_{50}) of the extract and fraction

3.3 Hepatoprotective effect of the extract and fraction

Pre-treatment evaluation of the liver function status of the animals for this assay revealed normal liver function as depicted by baseline serum liver function enzyme activity. Acute liver damage was achieved post-induction with CCL_4 as evidenced in significant ($p < 0.05$) increase in serum concentrations of the liver function enzymes – ALT, AST and ALP (Figures 4 and 5). Prophylaxis treatment with the extract at 200 mg/kg showed significant ($p < 0.05$) reduction in serum ALT concentration compared to the vehicle control group just like the ethyl acetate and butanol fractions (Figure 4A). However, the effect of the extract and butanol fraction at 200 mg/kg when compared with baseline value still revealed significant ($p < 0.05$) difference. Unlike the extract and butanol fraction, ethyl acetate fraction at 200 mg/kg protected the liver against damaging effect of CCL_4 with no significant ($p > 0.05$) difference between the pre-induction and post-induction serum ALT concentration. Similar protection was also offered by butanol fraction but at 400 mg/kg (Figure 4B).

For the AST assay, the extract and ethyl acetate fraction at 200 mg/kg showed significant ($p < 0.05$) reduction in in this serum enzyme concentration just like 100 mg/kg silymarin used as reference standard (Figure 4C). The ethyl acetate fraction both at 200 and 400 mg/kg maintained non-significant ($p > 0.05$) difference between pre-induction and post-induction AST concentrations (Figures 4C and D). The extract at 400 mg/kg just as the ethyl acetate fraction at both tested doses and silymarin at 100 mg/kg showed no significant ($p > 0.05$) difference in post-induction ALP serum concentration compared to the baseline value (Figure 5A and B).

CCL_4 induction produced marked increase in lipid peroxidation as evidenced in significant ($p < 0.05$) elevation of malondialdehyde – a product of lipid peroxidation. The extract and fractions at 200 mg/kg produced significant ($p < 0.05$) protection against CCL_4 induced lipid peroxidation compared to vehicle control group (5 ml/kg 5% Tween 80) (Figure 5C). When compared to baseline pre-induction values, the extract and fractions at 200 mg/kg were unable to produce non-significant difference. At higher concentrations of the extract and fraction, significant differences ($p < 0.05$) were still maintained when compared with vehicle control group. However, only ethyl acetate fraction at 400 mg/kg protected against significant rise from baseline values which was not even achieved with 100 mg/kg of the standard treatment (100 mg/kg silymarin) (Figure 5D).

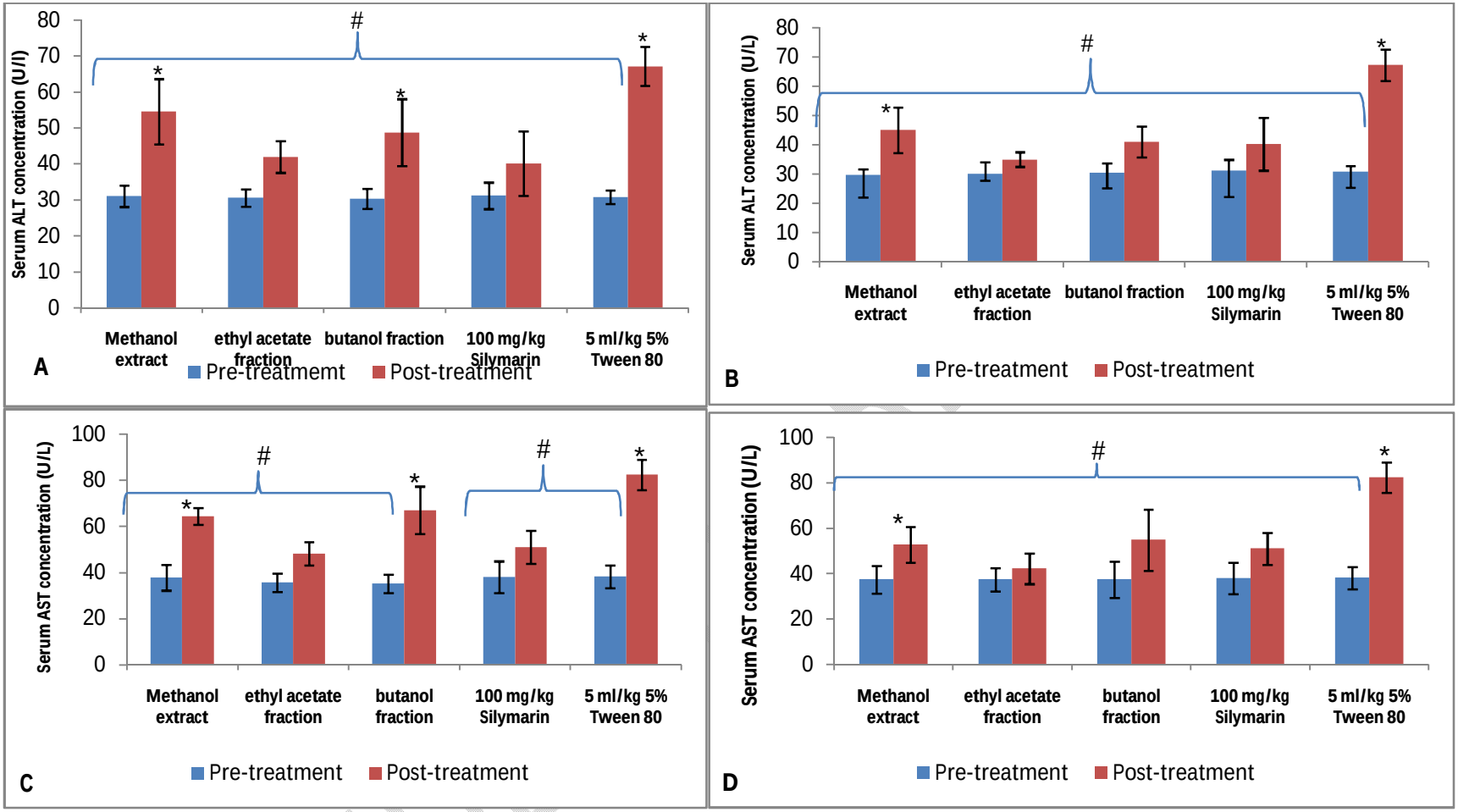

Figure 4: Effect of extract and fractions on serum ALT and AST concentrations

* P<0.05 compared to Pre-treatment values; # P<0.05 compared to 5% Tween 80 vehicle control post treatment value

A = ALT for 200 mg/kg extract and fractions; B = ALT for 400 mg/kg extract and fractions; C = AST for 200 mg/kg extract and fractions; D = AST for 400 mg/kg extract and fractions

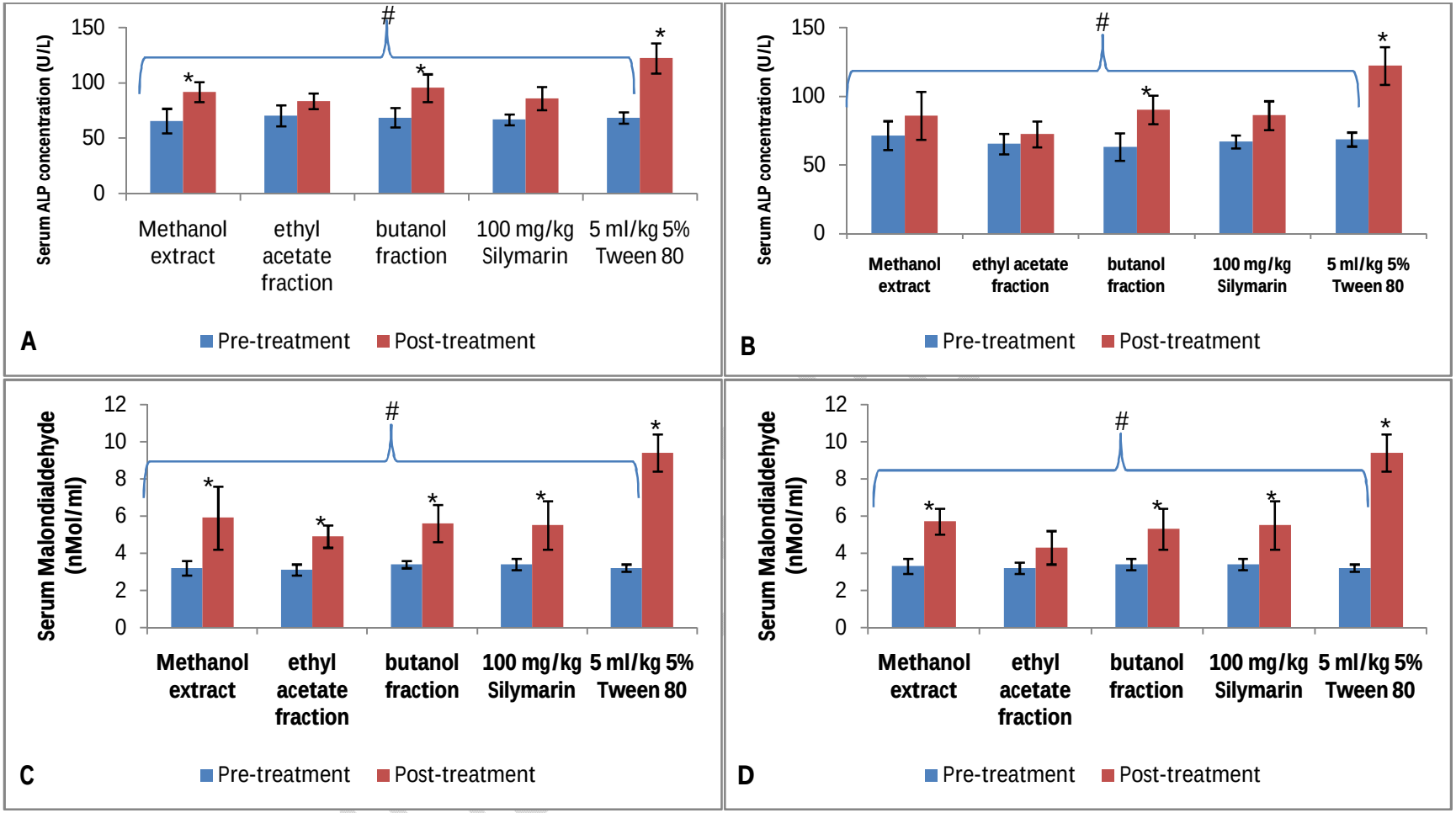

Figure 5: Effect of extract and fraction on serum ALP and lipid peroxidation

* P<0.05 compared to Pre-treatment values; # P<0.05 compared to 5% Tween 80 vehicle control post treatment value

A = ALP for 200 mg/kg extract and fractions; B = ALP for 400 mg/kg extract and fractions; C = lipid peroxidation for 200 mg/kg extract and fractions; D = lipid peroxidation for 400 mg/kg extract and fractions.

3.4 Effect of the extract and fractions on Diabetes induced hyperglycemia

Injection of STZ-NAD elicited more than double fold increase in plasma blood glucose concentration 72 h post-injection (Figures 6 and 7). The hyperglycemia state was maintained throughout the 21 days of the study. Treatment with the extract and fractions of *M. cecropioides* showed a dose dependent reduction in hyperglycemia that became significant ($P<0.05$) from the 7^{th} day of treatment when compared to vehicle control (5ml/kg 5% Tween 80) as well as 72 h post induction values. Continuous rise in plasma blood glucose for the vehicle control animals was recorded till the 7^{th} day post induction contrary to the extract and fraction treated groups that started showing reduction in blood glucose from the 7^{th} day. Compared to the 72 h post diabetes induction blood glucose values, the ethyl acetate fraction just like metformin at 200 mg/kg showed significant ($P<0.05$) reduction in blood glucose from Day 7 (Figure 6). However, the extract and butanol fraction at same dose produced significant ($P<0.05$) reduction from Day 14. The ethyl acetate fraction at 400 mg/kg was the only treatment that produced significant ($P<0.05$) reduction in blood glucose 24 h post-treatment compared to vehicle control group (Figure 7). Similarly, higher dose (400 mg/kg) of ethyl acetate fraction from day 14 showed higher reduction in blood glucose compared to 200 mg/kg metformine although the differences in both treatment were not significant ($P>0.05$).

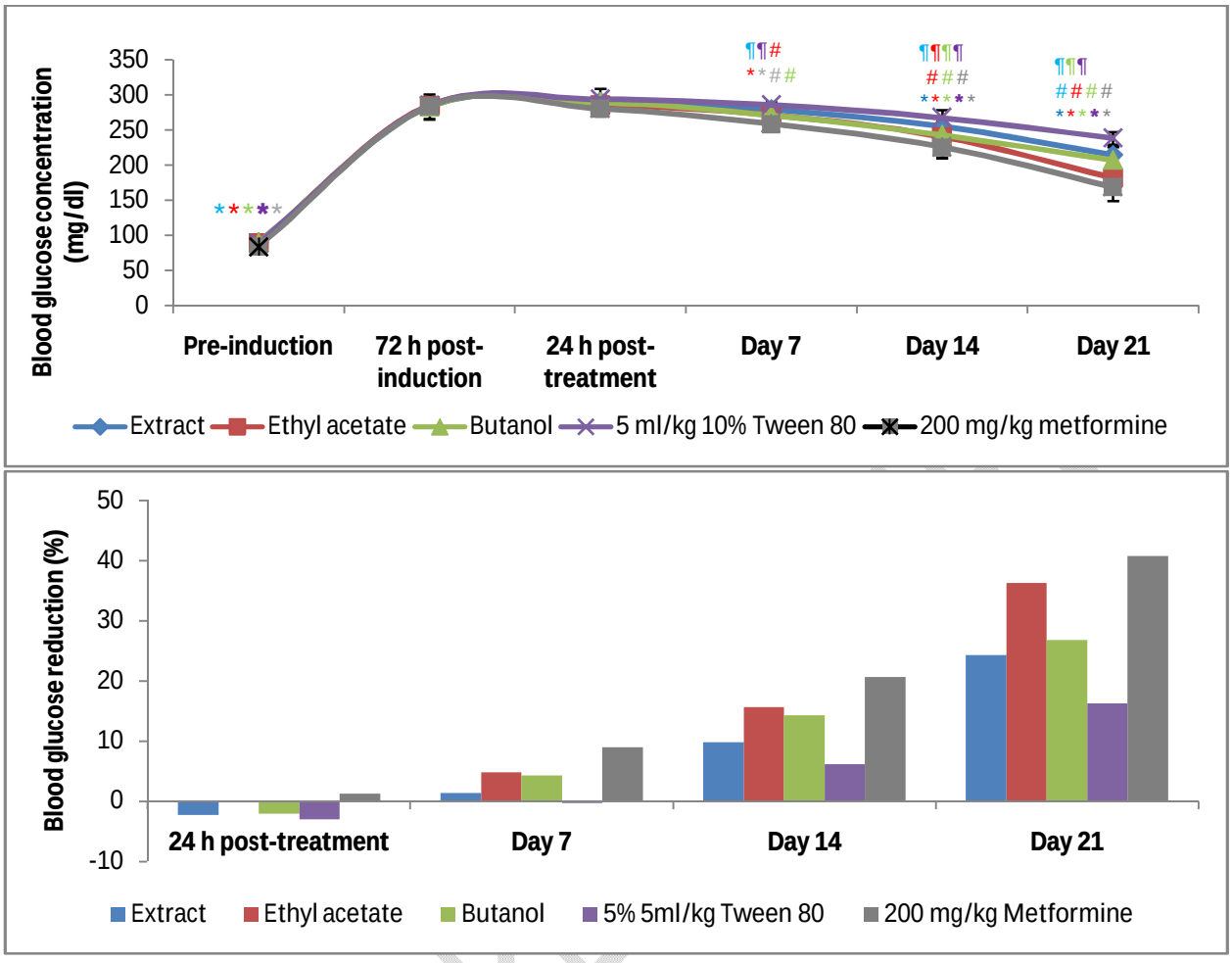

Figure 6: Effect of 200 mg/kg extract and fractions on blood glucose concentration

* = P<0.05 compared to 72h post-induction; # = P<0.05 compared to 5% Tween 80 (vehicle); ¶ = P<0.05 compared to 200 mg/kg metformine

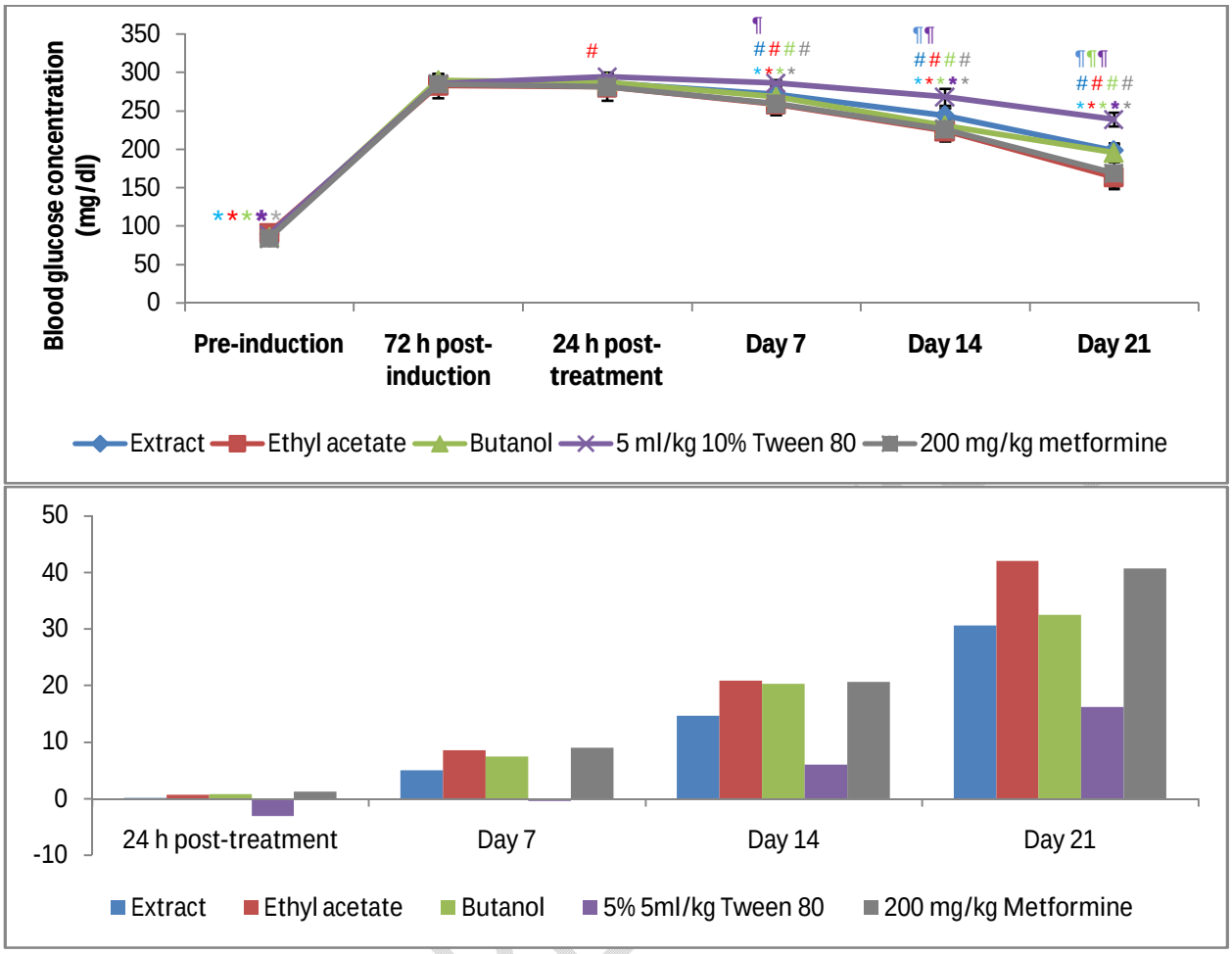

Figure 7: Effect of 400 mg/kg extract and fractions on blood glucose concentration

* = P<0.05 compared to 72h post-induction; # = P<0.05 compared to 5% Tween 80 (vehicle); ¶ = P<0.05 compared to 200 mg/kg metformine

3.5 **Effect of the extract and fractions of Diabetes induced oxidative stress**

Hyperglycemia associated with STZ-NAD induced diabetes was accompanied by significant ($P<0.05$) increase in MDA serum concentration compared to pre-induction values (Figure 8). Treatment with the extract and fraction for 21 days at both 200 and 400 mg/kg produced marked reduction in hyperglycemia induced lipid peroxidation as evidenced by significant ($P<0.05$) reduction in MDA – a lipid peroxidation by-product – compared to 72 h post-induction values as well as when compared to vehicle control group (5ml/kg 5% Tween 80). Ethyl acetate fraction at both 200 and 400 mg/kg and butanol fraction at 400 mg/kg produced similar lipid peroxidation reductive effect ($P>0.05$) when compared to 200 mg/kg metformin (reference antidiabetic drug).

Serum antioxidant enzymes SOD and CAT were also significantly ($P<0.05$) depleted 72 h post-induction of diabetes (Figures 9 and 10). The extract and the fractions at both 200 and 400 mg/kg produced significant ($P<0.05$) increase in catalase enzyme activity when compared to 72 h post-induction values (Figure 9). These treatments also showed significant ($P<0.05$) increase in catalase enzyme activity when compared to vehicle control group except for lower (200 mg/kg) dose of the extract. The ethyl acetate and butanol fraction at 400 mg/kg just like 200 mg/kg metformine were able to restore serum catalase almost to their pre-induction values with no significant ($P>0.05$) difference recorded between these treatments and their pre-induction values. Conversely, the ethyl acetate and butanol fractions at 400 mg/kg were unable to restore superoxide dismutase enzyme activity to pre-induction values (Figure 10). However, they were able to produce significant ($P<0.05$) increase when compared to vehicle control group and 72 h post induction values.

The total antioxidant status of the serum was not spared by diabetes induction. Significant ($P<0.05$) reduction was also recorded 72 h post-induction of diabetes with STZ-NAD (Figure 11). The reduction in total antioxidant activity of the serum was sustained throughout the study. However, treatment with the extract and fractions just like metformin produced significant ($P<0.05$) boost in serum total antioxidant activity compared to 72 h post-induction values and vehicle control group.

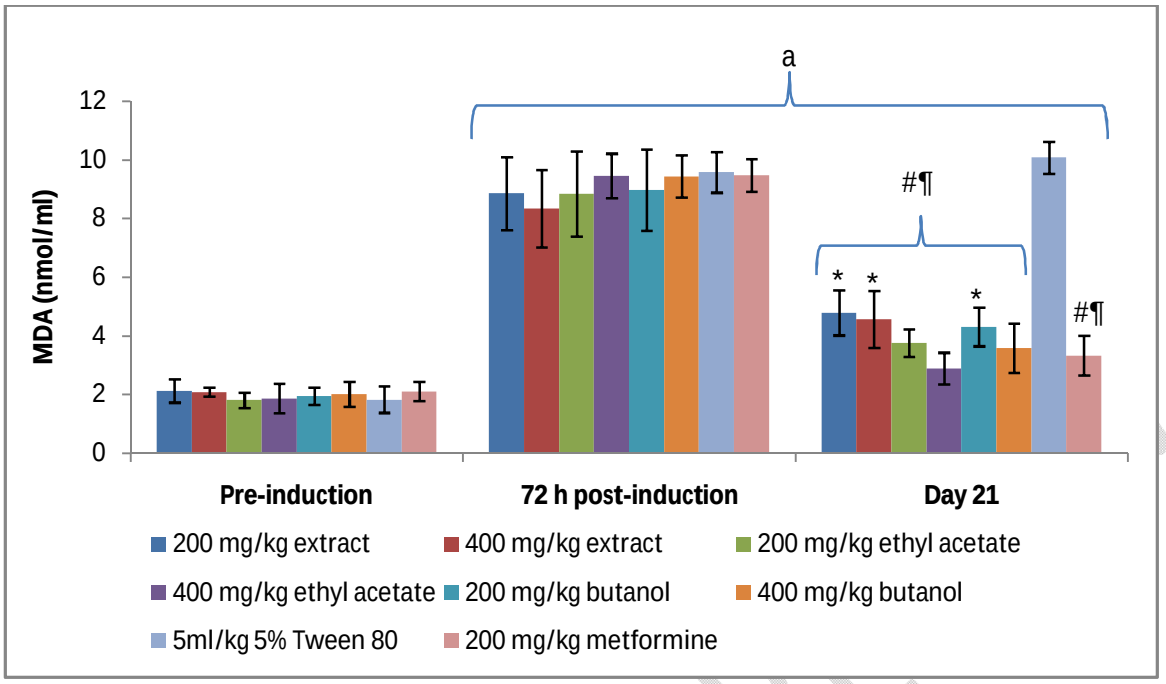

Figure 8: Effect of extract and fraction on diabetes induced changes in lipid peroxidation

Where a = P<0.05 compared to pre-induction values; # = P<0.05 compared to 72h post-induction; ¶ = P<0.05 compared to 5ml/kg 5% Tween 80; * = P<0.05 compared to metformine

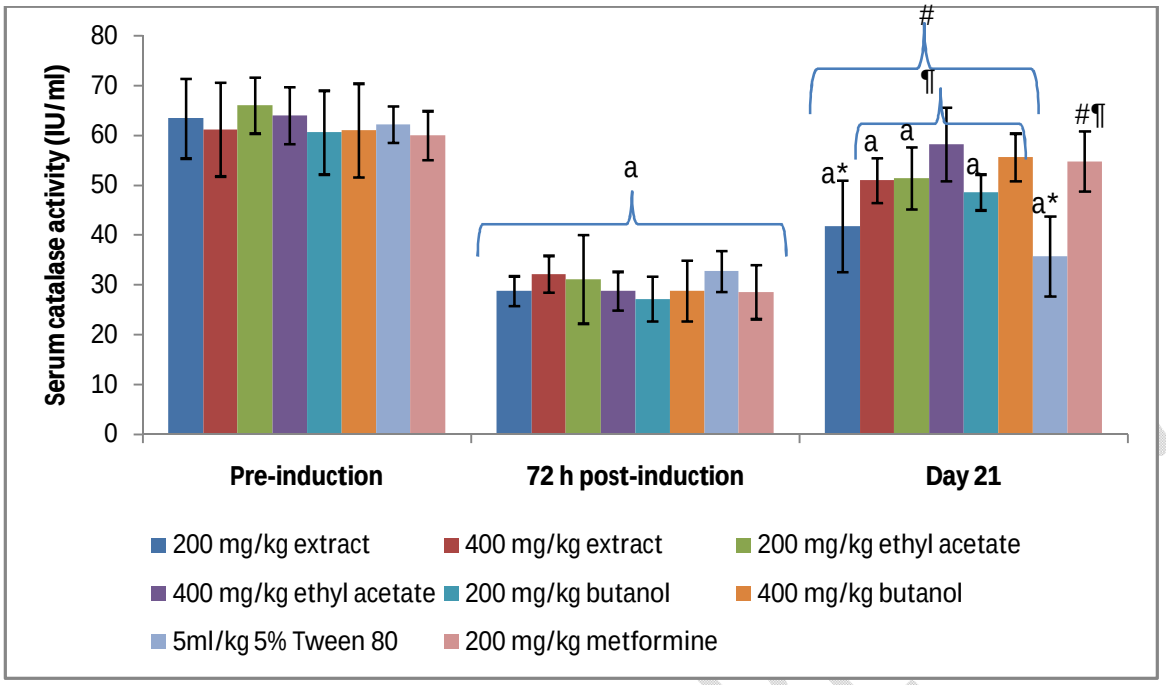

Figure 9: Effect of extract and fraction on diabetes induced changes in serum catalase enzyme activity

Where a = P<0.05 compared to pre-induction values; # = P<0.05 compared to 72h post-induction; ¶ = P<0.05 compared to 5ml/kg 5% Tween 80; * = P<0.05 compared to metformine

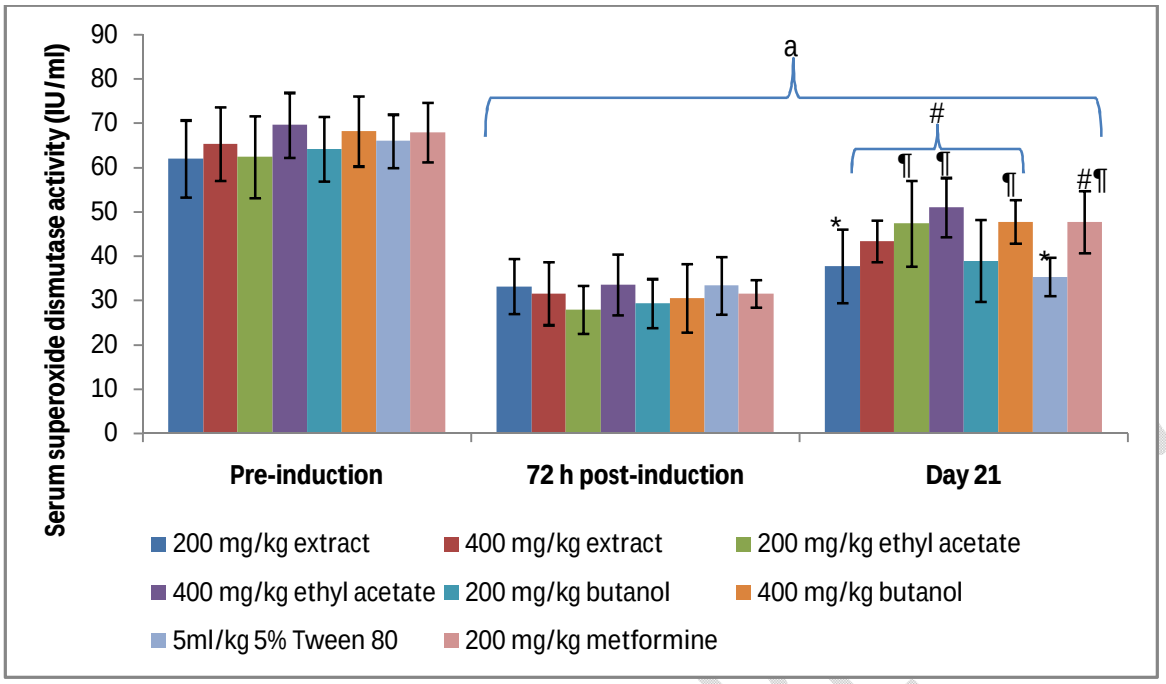

Figure 10: Effect of extract and fraction on diabetes induced changes in serum superoxide dismutase enzyme activity

Where a = P<0.05 compared to pre-induction values; # = P<0.05 compared to 72h post-induction; ¶ = P<0.05 compared to 5ml/kg 5% Tween 80; * = P<0.05 compared to metformine

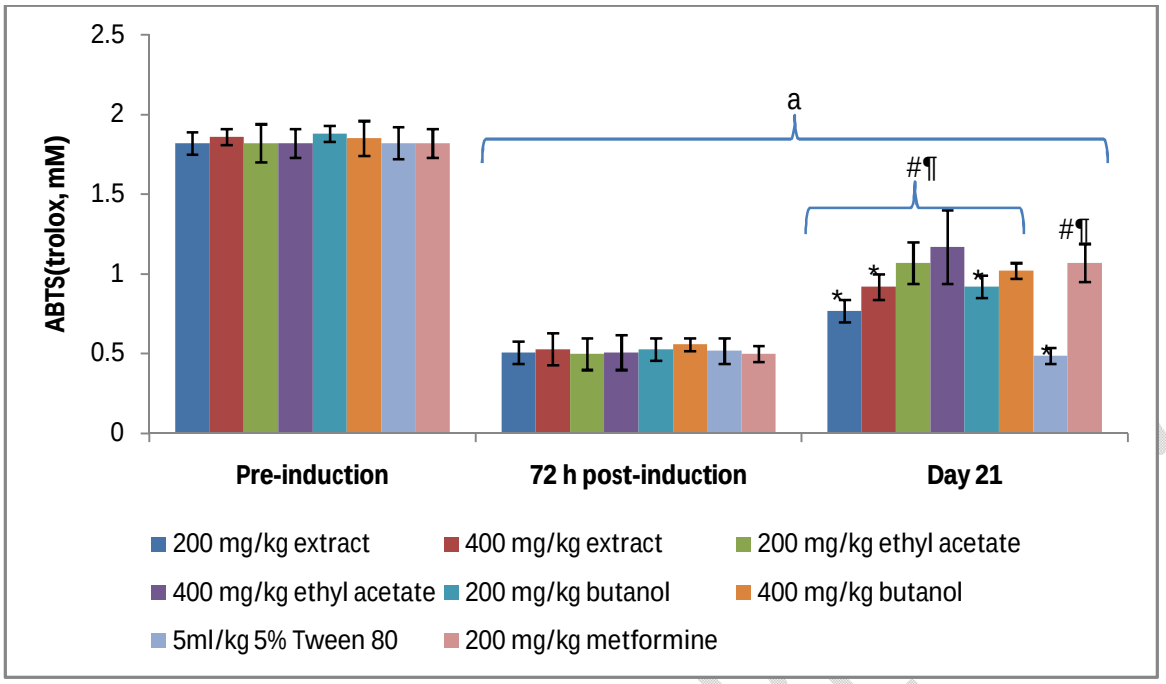

Figure 11: Effect of extract and fraction on diabetes induced changes in serum total antioxidant activity

Where a = P<0.05 compared to pre-induction values; # = P<0.05 compared to 72h post-induction; ¶ = P<0.05 compared to 5ml/kg 5% Tween 80; * = P<0.05 compared to metformine

4.0 Discussion

The role of the liver in the biotransformation of CCL4 to reactive metabolites makes it susceptible to CCL4 induced oxidative damage. Carbon tetrachloride is a known hepatotoxicants and best characterized model of xenobiotic and oxidative stress induced hepatotoxicity [25, 26]. Elevation of serum liver function enzymes and lipid peroxidation product – malondialdehyde are evidences of oxidative stress induced hepatotoxicity [27, 28]. This damage must have led to loss of functional integrity of the cell membrane of the liver cells leading to cellular leakages of liver function enzymes. Pretreatment with the extract and fractions of *M. cecropioides* protected the liver against CCL4 induced oxidative damage. Their protective potentials was according to their total phenolic content which suggested that phenolic phytochemicals present in the extract and fractions may have been responsible for their hepatoprotective effect against oxidative damage. Strong relationship between the phenolic content and free radical scavenging potentials was also established by the vitro antioxidant activities of the extract and fraction. Several other studies have also reported hepatoprotective effect of phenolic compounds particularly against damage mediated through oxidative or reactive radical attacks [12, 29, 30].

Oxidative stress is associated with many diseases linked with metabolic disorders [4, 31]. Numerous studies have demonstrated that diabetes represent an ideal metabolic disease condition

for studying the significance of oxidative stress in disease initiation, progression and complications [2, 5]. Oxidative stress mediated by hyperglycemia induced generation of free radical contributes to the development and progression of diabetes as well as its associated complications [5]. It is expected that comprehensive approach aimed at preventing hyperglycemia induced generation of these free radicals as well as scavenging of already formed radical due to this disease condition may be an effective strategy for the management of diabetes and its complications [2, 32].

In this study, STZ-NAD induced oxidative stress may be as a result of hyperglycemia associated increase in reactive oxygen species generation. Glucose can undergo autoxidation and generate hydroxyl radical. In addition, glucose can also react with proteins in a non-enzymatic manner leading to the formation of advanced glycation end product (AGEs) [33, 34]. Experimental data supports the generation of oxygen free radicals in all glycation steps. Other pathways involved in the flux of high level glucose like polyol pathway, hexosamine pathway, protein kinase C activation pathway and glyceraldehyde auto-oxidation pathway have all been linked to ROS production, oxidative stress and the pathogenesis of diabetes and its complications [5, 35]. Hypoglycemic effect of the extract and fractions of *M. cecropioides* may partly account for its oxidative stress reductive potentials in addition to their established free radical scavenging activities.

Several studies using in vitro cell culture and in vivo animal models including this study have suggested that STZ treatment causes an increase in oxidative stress and an alteration in antioxidant defense system [2, 34, 36, 37]. In the present study, the animals were pretreated with NAD to prevent STZ induced total destruction of pancreatic beta cell and to mimic type II diabetes disease condition. The STZ-NAD induced-diabetes is associated with impairment of basal insulin secretion by the beta cell and STZ associated hepatic oxidative effect affecting both hepatic insulin receptors and sensitivity. Since the liver plays a pivotal role in regulating glucose homeostasis during fed-fasting transition through gluconeogenesis and glycogenolysis, deregulation of these important insulin regulated mechanisms contributes to impaired fasting blood glucose which is a risk factor for diabetes [34, 38]. Oxidative stress in diabetes is particularly relevant and dangerous for the islet which is among tissues that have lowest level of intrinsic antioxidant defense [39]. Preventing the oxidative damage to the pancreatic beta cells as well as insulin receptor may have contributed to the mechanisms of the extract and fraction mediated reduction in diabetes induced oxidative stress. It is expected that through this effect, *M. cecropioides* may play a critical role in prevention of diabetes mellitus disease initiation as well as its progression.

Lipids are reported as one of the primary targets of ROS. Peroxidation of lipids produces highly reactive aldehydes such as MDA which has been documented as major biomarker of free radical mediated lipid damage [40]. Oxidative damage to lipids structure and functions has been reported in diabetes particularly diabetic patients with vascular complications [3]. This finding is consistent with the result from this study and further confirms the ability of diabetes to induce oxidative stress. Increased level of MDA in diabetes suggests that peroxidative injury may be involved in the development of diabetic complications. The increase in lipid peroxidation is also an indication of decline in defense mechanism of enzymatic antioxidants. Many studies have shown a strong and positive correlation between the phenolic compound contents and the antioxidant potentials of fruits and vegetable [39, 41]. Antioxidant activities of the extract and

fraction also showed strong relationship with their total phenolic content. The antioxidant activity of phenolic compounds is attributed to their molecular structure, particularly the number and position of the hydroxyl groups and the nature of substitutions on the aromatic rings. Differential quantitative distribution of phenolic compounds in the fractions of the extract may have accounted for the corresponding degrees of antioxidant activity.

Oxidative stress certainly plays a central role in the development of diabetes and diabetic complications [5, 42]. Indeed, it has been reported that ROS can induce insulin resistance [43, 31], impair insulin synthesis [44], and impair beta cell insulin secretion [45]. Additionally, oxidative stress biomarkers have been shown to be increased in individuals who exhibit insulin resistance [46] or insulin secretion impairment [46], indicating a positive correlation between oxidative stress and insulin resistance and insulin secretion impairment. Moreover, numerous studies have also established that ROS are involved in the etiology of diabetic complications including retinopathy, neuropathy, cardiomyopathy, and nephropathy [3,5,33,47]. Given these established relationships, *M. cecropioides* may also be useful in the prevention of diabetes complications by virtue of its interference in oxidative stress which has been reported to be an important mechanism that mediates diabetes complications.

Superoxide dismutase (SOD) is the antioxidant enzyme that catalyzes the dismutation of superoxide anion into hydrogen peroxide and molecular oxygen [48]. SOD plays important protective roles against cellular and histological damages that are produced by ROS. It facilitates the conversion of superoxide radicals into hydrogen peroxide, and in the presence of other enzymes it converted into oxygen and water [48]. SOD, a major defender against superoxide, in the kidneys during the development of murine diabetic nephropathy and downregulation of renal SOD (SOD 1 and SOD 3) may play a key role in the pathogenesis of diabetic nephropathy [49].The ability of the extract and fractions of *M. cecropioides* to stimulate increase in SOD enzyme activity further supports our hypothesis that this plant attenuates oxidative stress mediated by diabetes.

Catalase is an antioxidative enzyme present nearly in all living organisms. It plays an important role against oxidative stress-generated complications such as diabetes [2]. Catalase acts as main regulator of hydrogen peroxide metabolism. Excessive concentration of hydrogen peroxide may cause significant damages to proteins, DNA, RNA, and lipids. Catalase enzymatically processes hydrogen peroxide into oxygen and water and thus neutralizes it. Increased risk of diabetes has been documented in patients with catalase deficiency [39]. The deficiency of this enzyme leads, in the β-cell, to an increase in oxidative stress and ultimately to a failure of this cell type [2]. β-cells are rich in mitochondria, and thus this organelle might be a source of ROS [50]. The catalase restorative effect of M. cecropioides following diabetes induction is a strong indication of its ability to complement the antioxidative effect of SOD enzyme as well as restoration of normal oxidative status disrupted by diabetes induction.

Antioxidant capacity of plasma is the primary measure and marker to evaluate the status and potential of oxidative stress in the body [51]. Plasma contains many compounds which function against the oxidative stressors in the body thus protecting the cell and cellular biomolecules from being damaged. The combined action of all the antioxidant molecules in the plasma represents the antioxidant capacity of the plasma. Prevalence of oxidative stress is reported in processes

where reduced/depleted plasma antioxidant potential is reported [52]. Reduction in antioxidant capacity of the plasma following STZ-NAD administration is another indication of diabetes induced oxidative stress established in this study. The increase in total antioxidant status of the plasma exhibited by extract and fractions of *M. cecropioides* further indicated their counter effect against diabetes induced oxidative stress. This effect may be connected to their phenolic content, a class of phytocompounds already known for their antioxidant activity.

5.0 Conclusion

M. cecropioides showed antioxidant activity via scavenging free radicals, increase in antioxidant enzymes, elevation of plasma total antioxidant capacity and inhibition of lipid peroxidation. It proved effective in both chemical and disease induced oxidative stress. It showed protective effect against liver oxidative damage and counters diabetes induced hyperglycemia and oxidative stress. There is a strong relationship between the total phenolic content of its extract and fractions and their antioxidant activity which suggested that the presence of these phytocompounds may be responsible for its antioxidant and antioxidative effect.

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