Possible amelioration of oxidative stress damage via cyclo- oxygenase pathway by aqueous extract of *Terminalia catappa* leaves in alloxan induced diabetic rats

Ezekiel Etim Ben 1, *, Asuquo Etim Asuquo 1 and Daniel Udofia Owu 2

1 Department of Physiology, Faculty of Basic Medical Sciences, University of Uyo, Akwa Ibom State Nigeria.
2 Department of Physiology, Faculty of Basic Medical Sciences, University of Calabar, Cross River State Nigeria.

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Abstract

Cellular damage due to oxidative stress had been implicated in the pathogenesis of many diseases including diabetes mellitus. This study was to investigate possible amelioration of oxidative stress in diabetes mellitus by aqueous leaf extract of *Terminalia catappa*. Wistar rats weighing 200 - 250g was divided into nine groups with 6 rats per group. The main test group has 5 groups while the group for assessing possible mechanism had 4 groups. Group 1 (control) and group 2 (non-diabetic) received orally per kg body weight; 0.5ml distilled water and 130mg of *Terminalia catappa* respectively. Group 3 (diabetic), group 4 (diabetic + extract) and group 5 (diabetic + insulin) also respectively received 0.5ml distilled water, 130mg *Terminalia catappa* extract and 0.75UI insulin subcutaneously. Groups 6, 7, 8 and 9 administered orally with aspirin; 30mg/kg, meloxicam; 2mg/kg and combination of extract with aspirin and meloxicam respectively. The experiment lasted for 14 days and glucose level ≥ 200mg/dl was considered diabetic following intraperitoneal injection of 150mg/kg body weight of alloxan. Results showed significant (p<0.05) increase in serum low density lipoprotein cholesterol (LDL-c), malondialdehyde (MDA) and a significant (p<0.05) decrease in superoxide dismutase (SOD) in diabetic group compared with control. The LDL and MDA were significantly (p<0.05) reduced while SOD increased significantly (p<0.05) when compared with diabetic group and the control following administration of *Terminalia catappa*, aspirin, meloxicam and insulin. The leaf extract of *Terminalia catappa* possess possible ameliorating potential on oxidative stress induced damages by impeding lipid peroxidation but improved on antioxidant enzyme in diabetic condition.

Keywords: Oxidative stress; Diabetes mellitus; Superoxide dismutase; Malondialdehyde; Aspirin; Meloxicam

1. Introduction

The role of oxidative stress in the development of diabetes mellitus and its complications has been established [1]. It is considered that oxidative stress poses a multifaceted effect on diabetes. The understanding of diabetic complication as it is caused by oxidative stress has led to many researches on natural substances that can attenuate the oxidative damage and cell death [2]. Report has shown that in STZ-induced diabetes, albuminuria was significantly reduced and the renal histopathology was improved by Astragalus membranaceus and Panax notoginseng treatment which gives hope on reduction of one of the major complications of diabetes; diabetic nephropathy and other renal diseases. The oxidative stress driven complications of diabetes mellitus is attributed to alterations of cellular structures with resultant imbalance in physiological processes [3]. Disturbance in lipid profile of the body resulting from diabetes mellitus increases the vulnerability of the cells to lipid peroxidation [4]. Following this existence of abnormality in lipid metabolism, diabetes poses high risk on health which is classified as macrovascular (stroke, peripheral vascular diseases, coronary heart disease) and microvascular (retinopathy, nephropathy and neuropathy) complications and these accounts for the high mortality rate associated with this disease [5].

*Corresponding author: Ezekiel Etim Ben; E-mail: adoofpraise@yahoo.com
Department of Physiology, Faculty of Basic Medical Sciences, University of Uyo, Akwa Ibom State Nigeria.

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The characteristic persistent hyperglycaemia associated with diabetes mellitus [6] facilitates the increased production of free radicals especially reactive oxygen species (ROS), for all tissues from glucose auto-oxidation and protein glycosylation [7, 8, 9]. The increase in reactive oxygen species level in diabetes mellitus can also be attributed to a reduction/destruction in antioxidant enzyme and the resultant increase in the ROS population is in part responsible for the development of diabetic complication [10]. Cellular enzymatic components like superoxide dismutase (SOD), catalase (CAT), and glutathione peroxidase (GSHPxs) are inherent cellular protective mechanism against ROS induced damage [11,12] and are often referred to as biological antioxidant. These are classified as chain breaking antioxidant and preventive antioxidant depending on the mechanism of action [13]. Therefore, changes in the level of these antioxidant enzymes is a major determinant factor in the said tissue vulnerability to oxidative stress and range of diabetic complications [14].

But oxidation being a normal and imperative body process is continuous. Thus, an imbalance in pro oxidant (free radicals) versus antioxidant activity in favour of the pro oxidant can set in leading to oxidative stress [15]. In the face of a significant reduction in the antioxidant level, a proportionate increase in the ROS occur thereby enhancing lipid peroxidation. Consequently, self-enhanced and uncontrolled alteration/disruption of lipid blayer cell membrane is triggered which results in a chain of reaction that damages various cellular molecules [16]. Numerous products formed depending on the type of lipid oxidized and the location of the electron are malondialdehyde (MDA), 4-hydroxynonenal (4-HNE), lipid hydroperoxide (LOOH), isoprostanes, conjugated dienes, lipid-DNA adduct, lipid-protein adduct, lipofuscin pigments and exhaled gases [17].

Destruction of DNA, proteins, lipids, and other macromolecules [18] due to oxidation is implicated in the pathogenesis of numerous diseases notably diabetes mellitus, heart disease and cancer [19, 20].

From research findings, the protection of the human body from the scavenging effect of free radicals by natural agents had been reported. Thus, antioxidant agents of natural origin have attracted special interest [21, 22]. As research into the natural agent progresses, medicinal plants have shown the ability to synthesize numerous chemical compounds that are essential in healthcare as therapeutic remedies hence serving as alternative source to conventional drugs. Recent studies have shown that the leaf extract of Terminalia catappa possesses anti-inflammatory effects [23, 24] in addition to other usefulness earlier reported. Terminalia catappa is a tropical trees of the combretaceae family whose leaves and barks are often used because of its diaphoretic, anti-infection, hepatoprotective effect [25, 26], nephroprotective effect [27] and anti-tumor effect [28]. Therefore, this study seeks to evaluate the possible intervention of oxidative stress in diabetes mellitus by Terminalia catappa leaf extract.

2. Material and methods

2.1. Preparation of Extract

Terminalia catappa leaves (fresh) were obtained from the University of Uyo Premises and was free of contamination. The leaves were authenticated by the botanist at the Department of Botany and Ecological Studies, University of Uyo with herbarium voucher number UUPH 22(a). The leaves were furthered washed with clean water devoid of debris and dirt. The water was blotted out and kept overnight at room temperature to dry up. The clean leaves were pulverized and 5000g of the pulverized leaves were soaked in litres of deionized water and allowed to stand for 18 hours. Thereafter, the mixture was filtered using muslin cloth and evaporated to dryness using a thermostatic water bath at 45°C until a semi solid paste is gotten which weighed 204.18g of the extract upon evaporation, this represents the percentage yield of 4.08%. The extract was stored in the refrigerator for later use.

2.2. Experimental Animal

Thirty (30) Wistar rats of weight 150-200g were obtained from the animal house of the Department of Physiology, Faculty of Basic Medical Sciences, and University of Uyo, Nigeria and were used for the study. The animals were allowed to acclimatize for two weeks in a well-ventilated cage in the animal house. The animals were fed with standard pellets (from Guinea feeds, Plc Nigeria) and had access to water ad libitum.

2.3. Induction of Diabetes

Diabetes was induced by intraperitoneal injection of alloxan monohydrate at a dose of 150mg/Kg body weight [29, 30, 31]. The animals were assessed for development of diabetes after 72 hours [32] by obtaining blood sample from the tip of the tail. The blood sample was dropped into glucose strip to measure the glucose level using a glucometer (One Touch Ultra, Life Scan Inc, U.S.A). Blood glucose of ≥200mg/dl was considered diabetic (normal range of blood glucose in rat is 80 – 120mg/dl) and were used for the experiments [30, 32].
3. Experimental Design

3.1. Experiment 1

The test experiment for oxidative markers

The experimental animals were randomly distributed into five (5) groups of six (n=6) rats per group as follows:

- Group 1 (Control) Non diabetic rats administered with only distilled water orally at a dose of 5ml/kg body weight.
- Group 2 Non diabetic rats administered orally with aqueous leaf extract of *Terminalia catappa* at a dose of 130mg/kg body weight.
- Group 3 Diabetes only (Diabetic control) administered with only distilled water orally at a dose of 5ml/kg body weight.
- Group 4 Diabetic rats treated with aqueous leaf extract of *Terminalia catappa* at a dose of 130mg/kg body weight by oral administration.
- Group 5 Diabetic rats treated with exogenous Insulin at a dose of 0.75U/kg body weight by subcutaneous administration

3.2. Experiment 2

Determination of possible mechanism of action

The second part of the experiment involved random distribution of animals into four (4) groups as follows:

- Group 6 Diabetic rats treated with aspirin at a dose of 30mg/kg body weight orally
- Group 7 Diabetic rats treated with meloxicam at a dose of 2mg/kg body weight orally
- Group 8 Diabetic rats treated with combination of extract and aspirin at a dose of 130mg/kg and 30mg/kg body weight respectively
- Group 9 Diabetic rats treated with combination of extract and meloxicam at a dose of 130mg/kg and 2mg/kg body weight respectively.

3.3. Determination of low-density lipoprotein cholesterol (LDL-c)

The low-density lipoprotein cholesterol was obtained by calculation according to the formula of Friedewald [33] as follows

\[ \text{LDL (mmol/L)} = \text{TC} - \text{HDL (mmol/L)} - \frac{\text{TG (mmol/L)}}{2.2} \]

3.4. Superoxide dismutase (SOD) and malondialdehyde (MDA) assay

The assay for oxidative stress markers; superoxide dismutase and malondialdehyde was carried out with rat specific commercial kits using sandwich-ELISA method. Standards or samples were added to the appropriate micro elisa strip plate wells and combined to specific antibody. The horseradish peroxidase (HRP) – conjugated antibody specific for SOD and MDA was respectively added to each micro elisa strip plate and incubated. Wells with test substance and HRP antibody appears blue and then turn yellow after the addition of stop solution. The optical density was measured spectrophotometrically at wavelength of 450nm and values obtained using microplate reader.

3.5. Statistical Analysis

The data obtained from the result was subjected to statistical testing using oneway ANOVA followed by Tukey test using Graph Pad Prisms software 6.0. Data were expressed as mean ± standard error of mean (SEM). Results with values of \( p < 0.05 \) were considered significant when compared to untreated diabetic group and control group respectively.

Ethical Approval for the research was given by the Animal Research ethics committee of the University of Uyo. Uyo, Nigeria.
4. Results and discussion

Diabetes induced oxidative stress and possible intervention was assessed by determining the lipid peroxidation biomarker; Malondialdehyde (MDA) and superoxide dismutase (SOD) respectively in addition to low density lipoprotein (LDL) cholesterol level using leaf extract of *Terminalia catappa*, aspirin, meloxicam and insulin.

The result of this research showed significant increase in serum level of low density lipoprotein (LDL) cholesterol in diabetic untreated group compared to the non-diabetic control group. This result is in line with many research findings from similar work done by researchers like Singh and Kumar [34], Mona [35] and Shankarprasad [36]. This alteration could be attributed to the hyperglycaemia induced derangement in lipid metabolism such as altered LDL catabolism [37]. According to American Health Association, diabetes tends to increase triglyceride and bad cholesterol; LDL level but lowers HDL cholesterol leading to diabetes dyslipidemia [38]. It is known that reaction of LDL cholesterol with free radicals causes oxidation of LDL [39].

*Significant change compared to control group (p<0.05); a significant change compared to diabetic untreated group (p<0.05); Values are expressed as mean ± sem, n=6*

**Figure 1** Comparison of low-density lipoprotein cholesterol concentration in different experimental group

*Significant change compared to control group (p<0.05); a significant change compared to diabetic untreated group (p<0.05); Values are expressed as mean ± sem, n=6*

**Figure 2** Comparison of Malondialdehyde concentration in different experimental groups
Increased level of ROS have been established in diabetes mellitus [40] and the ROS-LDL reaction chemically damages LDL to generate ox-LDL which directly delivers lipid oxides and hydroperoxides to target cells [39]. The delivered substances thereby act as cytotoxins, monocyte chemo-attractants and stimulators of cholesterol esters accumulation by macrophages and inhibitors of macrophage movement [41]. The oxidized LDL becomes more reactive with the surrounding tissues to cause tissue damage which include cell membrane destruction and ion channel deformation. Research has it that the level of ox-LDL is increased by diet, metabolic syndrome and diabetes mellitus [39]. Regarding diabetes mellitus, in vitro studies have shown that ox-LDL present in type 1 diabetes mellitus is associated with auto-antibodies generated against it in plasma of diabetes patient [42].

Figure 3 Comparison of superoxide Dismutase concentration in different experimental groups

Figure 4 Malondialdehyde concentration in combined treatment of Aspirin and Meloxicam
Consequently, the ox-LDL is taken up by macrophages as it is no longer recognized by the LDL receptor but by scavenger receptors present in microphages resulting in the formation of foam cells leading to atherosclerotic plaques [43] and related macrovascular complications common in diabetes mellitus. It is established that atherosclerotic cardiovascular disease is the main source of morbidity and mortality in patients with diabetes [44].

Moreover, lipoprotein serves as substrate for lipid peroxidation by both radical and non-radical oxidant [45]. Lipid peroxidation is a chain reaction involving ROS on polyunsaturated fatty acid [46] which results in alteration on cell membranes integrity and fluidity [47]. The by-products of lipid peroxidation include lipid peroxide (LOOH), advanced glycosylation end products (AGEs) and malondialdehyde (MDA). Formation of a powerful oxidant; peroxynitrite (-ONOO) is particularly from LDL [48] but being very unstable is immediately converted into MDA. Malondialdehyde (MDA) is a stable product of lipid peroxidation, therefore its serum concentration is a useful biomarker of oxidative stress [49]. It was observed that the serum level of MDA increased significantly in the diabetic untreated group in this experiment when compared to the control. This finding was in consonance with previous findings by many researchers [50, 51, 52, 53, 54]. The high reactivity of MDA to proteins of the vascular system, collagen and elastin via its intermolecular cross-linking contributes to the stiffening of the cardiovascular tissue thereby implicating MDA in diabetic complications like atherosclerosis [55]. Further interactions like MDA-DNA cross-link results in the mutagenic and carcinogenic effects of MDA [56, 57].

Elevation of oxidative stress biomarkers especially MDA with concomitant reduction in antioxidant defense system generates a vicious circle that worsens complications associated with diabetes mellitus [58]. The body’s mechanism to curb the excess of ROS must be first overcome before the expression of oxidative stress. Such body mechanism includes superoxide dismutase (SOD), catalase (CAT) and glutathione peroxidase (GSHPx) as major enzymes of the cellular antioxidant defence system that detoxify the free radicals in the body. The result of this experiment showed significant reduction in SOD level in diabetic untreated rats compared to the control group. The deleterious effect of an increased toxic radical is due to an increase in the formation of superoxide radicals within the cells which causes inactivation of superoxide dismutase enzyme in hyperglycemic condition. The low level of SOD as observed in this study agrees with previous findings [59, 60, 61, 62]. Various studies had shown association between reduction in SOD level and diabetic complications like diabetic foot ulcer [63], nephropathy [64], albuminuria [65] and cardiovascular diseases [66]. Therefore, improving SOD level may be a promising target in reducing oxidative stress and its related diabetic complications [67].

However, the raised LDL and MDA were reduced in diabetic rats treated with aqueous extract of *Terminalia catappa*, aspirin, meloxicam and insulin respectively. This could point to the fact that the extract can ameliorate the diabetes induced dyslipidemia and lipid peroxidation as observed in this experiment. Investigation on the possible mechanism employed by the extract was considered by the administration of non-steroidal anti-inflammatory drugs (NSAIDs); aspirin and meloxicam. These conventional drugs are known blockers of cyclooxygenase I and II (COX1 and COX2) respectively. The reductions in LDL and MDA levels in the aspirin and meloxicam treated diabetic groups were similar.
to that of the extract and this may be suggestive that cyclooxygenase (COX1 and COX2) pathway(s) is utilized in the mitigation of oxidative stress by aqueous extract of *Terminalia catappa*. The result of combined extract with aspirin and meloxicam in a way is supportive of this assertion. The non-significant difference in the levels of the biomarkers especially MDA in combined administration compared with that of individual substances might be related to competitive action of the two substances (extract and aspirin, extract and meloxicam) on similar route instead of separate pathways. However, in-depth study is required to substantiate this inference.

On the other hand, the SOD level was significantly increased when diabetic rats were administered with aqueous leaf extract of *Terminalia catappa* as compared with diabetic untreated rats. The observed elevation in SOD level towards normal is indicative of the potency of the extract and its possible usefulness in supporting the anti-oxidant system thereby combating oxidative stress although the level was not as high as that of control. Report on progressively decreased SOD in later stage of diabetes [68, 69] was explained in relation to glycation of enzymes in hyperglycemia [70, 71, 72, 73]. Thus, it could be suggested that the observed increase in SOD level may be associated with interruption of the ROS enzyme/protein glycation process [74, 75] besides the reduction in blood glucose earlier reported [24].

Insulin used as a standard anti-diabetic drug in this research presented results similar to those obtained by the extract. The serum levels of LDL and MDA were reduced while SOD level was increased in diabetic rats treated with insulin. Inhibition of hormone-sensitive lipase and activation of lipoprotein lipase by insulin are the mechanisms involved in regulation of lipid metabolism [76] and these might equally be utilized by the extract.

The observed effect of the extract depends on the phytochemicals present in *Terminalia catappa*. Research findings had shown that anti-scavenging potential of *T. catappa* are attributed to the actions of polyphenols [77]. Phytochemicals like tannins and flavonoid may be responsible for the LDL lowering activity [78, 79] as well as reduction in the oxidation of LDL [80]. Thus, multiple antioxidant effects of tannin and polyphenols components of *Terminalia catappa* leaf extract is capable of preventing lipid peroxidation and reduce formation of superoxide as well as their free radical scavenging activity [81, 82] hence impeding the arterogenesis of lipid peroxidation. Polyphenolic compounds have been reported to function through COX pathway [83, 84, 85]. This further lends credence to the suggestion of inhibition of COX as the possible mechanism of action in the mitigation of oxidative stress by extract of *Terminalia catappa*.

5. Conclusion

In conclusion, it is established that a poorly managed diabetes mellitus is associated with lipid peroxidation and oxidative stress induced cell damage. This is implicated in the disruption of structural integrity of cell membranes, inactivation of membrane bound enzymes and surface receptor molecules resulting in overall cell signaling errors and various disease complications.

Therefore, the leaf extract of *Terminalia catappa* shows potential of ameliorating oxidative stress induced damages by impeding lipid peroxidation as evidenced by lowering of elevated LDL and MDA level and improvement of serum SOD level in diabetic condition.

**Compliance with ethical standards**

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**Disclosure of conflict of interest**

The author(s) declare that there is no conflict of interest and the research was self-sponsored by the author(s).

**Statement of ethical approval**

Ethical Approval for the research was obtained from the Animal Research Ethics Committee with reference number FAREC/PA/021PY30417.
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