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Ameliorative Effects of Methanolic Extract of *Sennaalata*leaves on Streptozotocin (Stz)-Induced Oxidative Stress and Hepatotoxicity in Adult Wistar Albinorats

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Abstract: ***Introduction: Sennaalata leaf is widely available in the tropics and has very important applications in folkloric medicine. It contains several biological properties and antioxidant properties. In the northern part of Nigeria, particularly in Adamawa and Taraba States, the root, stem and leaves are used by practitioners of herbal medicines to treat burns, skin and wound infections, diarrhea, gastrointestinal and upper respiratory tract infections. Objectives: In this work, liver morphology was studied, markers of hepatic oxidative stress and some liver enzymes in STZ-induced rats treated with methanolic extract of Sennaalata leaves. Methods: Oxidative stress was induced in fasted male wistar rats with intraperitoneal injection of Streptozotocin (STZ). A total of 40 wistar rats were used for this study. The animals were randomly divided into four groups of 10 animals per group. The experimental period was three weeks beginning after the induction of STZ-induced oxidative stress. Group A: normal untreated rats. Group B: STZ-induced untreated group (60mg/kg body weight). Group C and D were STZ-induced rats given methanolic extract of Sennaalata leaves (150mg/kg and 300mg/kg body weight) daily using an intragastric tube for three weeks(21 days). Results: The result of this study showed that administration of streptozotocin (STZ) to rats resulted in significant decrease (P <0.05) in the activity of Superoxide dismutase (SOD) and concentration of reduced glutathione (GSH) as well as significant increase (P <0.05) in malondialdehyde (MDA) concentration when compared with the control group. However, simultaneoustreatment of STZ –induced groups(groups C and D) with 150mg/kg and 300mg/kg body weight of methanolic extract of Sennaalata leaves produced a significant increase (P <0.05) in Superoxide dismutase(SOD) activity and reduced gluthathione(GSH) concentration, and a significant decrease (P < 0.05) in malondialdehyde (MDA) concentration as compared with the STZ-induced untreated group. The result of the present study also showed a marked elevation in the activities of serum marker enzymes, alanine transaminase (ALT) and aspartate transaminase(AST) in the STZ – induced group when compared with the normal control group. Nonetheless, treatment of STZ-induced groups(group C and group D) with methanolic extract of Sennaalata leaves significantly decreased (p <0.05) the activities of ALT and AST when compared with STZ-induced untreated group(group B).The histological section of STZ-induced untreated group showed enlarged central vein, periportal fatty infiltration (PFI) with focal necrosis of hepatocytes. Conversely, the histological section of the liver of the rats induced with STZ and simultaneously treated with 300mg/kg methanolic extract of Sennaalataleaves depicted normal hepatocytes with normal morphology and without necrosis. Conclusion: These observations therefore, suggest that methanolicextract of Sennaalata leaves possess antioxidant andhepatoprotective effect against STZ – induced oxidative stress and liver damage in wistar albino rats.***

Keywords: Oxidative stress, Streptozotocin, *Sennaalata* leaves; hepatoprotective effects,wistar albino rats.

1. Introduction

The liver is the principalorgan of metabolism and plays vital roles in many body processes most especially detoxification of chemical compounds (Sunmonu and Oloyede, 2007). The liver is the first processing and purifying station for chemical compounds, most drugs and other xenobiotics. (Mckee and Mckee, 1999; Pamplona-Roger, 2010). The acute, chronic and long-term effects of chemical compounds (including streptozotocin) on living systems could be investigated by assessing the biochemical and histological changes in various organs particularly the liver. Streptozotocin (STZ) is a naturally occurring nitrosourea with molecular weight of 265 and empirical formula of C14 H27 N5 O12 (Dorr and Fritz, 1980). It is an antibiotic often used in the treatment of different types of cancers (Raza and John, 2012). It is also highly cytotoxic to the pancreatic beta-cells and therefore is commonly used to induce experimental type 1 diabetes in animals (Punithavathi*etal.*, 2008; Raza and John, 2012). The diabetogenic action of STZ is the direct result of irreversible damage to the pancreatic beta cells resulting in degranulation and loss of capacity to secrete insulin (Gu*et al.*, 1997).Hyperglycemia in diabetes has been associated with increased formation of reactive oxygen species (ROS)and oxidative damage to tissue compounds (Nakhaee*etal*., 2009). Several hypothesis such as oxidation of glucose, the nonenzymaticand progressive glycation of proteins withconsequently increased formation of glucose-derivedadvanced glycation end products (AGEs) have been proposed to explain the genesis of free radicals indiabetes(Booth *etal*., 1997; VlassaraPalace, 2001). The increased production of reactive oxygenspecies results in oxidative stress, a state where the concentration of reactive intermediates(such as reactive oxygen species, ROS)overwhelms the concentration of anti-oxidants (Maxwell *etal*., 1997; Nwaogu and Onyeze, 2010).Oxidative stress is the result of a redox imbalance between the generation of ROS and the compensatory response from the endogenous antioxidant network. Living organisms have evolved a highly complicated and robust

defense system and the body acts against free radical-induced oxidative stress involved by different defense mechanism like preventative mechanisms, repair mechanisms, physical defenses and antioxidant defenses (Valko*etal*., 2007).Under normal circumstances, sufficient amounts of reactive oxygen species are removed by the antioxidant defense systems including enzymes such as superoxide dismutase (SOD), catalase and glutathione peroxidase(Mckee and Mckee, 1999). However, in diabetic condition, the prevailing hyperglycaemia and the consequent excess reactive oxygen species generation can either decrease the activity of these antioxidant enzymes or deplete their metabolites. The decrease in antioxidant enzyme activity under diabetic conditions could be due to glycation of these enzymes, which occurred at persistently elevated blood glucose levels (Taniguchi, 1992). Glycation of SOD reduces its activity, leading to the insufficient dismutation of superoxide anions (O^{-2}) (Taniguchi, 1992; Majithiya and Balaram2005; Ravi *etal*. 2004).The excess formation of ROS, due to a depleted endogenous antioxidant system (Young *etal*., 1995; Baydas*etal*., 2002), for instance, leads to a decrease in reduced glutathione (GSH) concentrationand SOD activity, and an increase in lipid peroxidation (Tavares de Almeida *etal*., 2012).Moreover, chronic hyperglycemia induces carbonyl stress, which in turncan lead to increased lipid peroxidation (Bayanes and Thrope, 1999). The increasedconcentration of lipid peroxidation worsens oxidative damageby increasing peroxy radicals and hydroxyl radicals (Levy *etal*., 1999). The increasedlipid peroxidation in the plasma and tissues of diabetic animalsmay be due to the observed remarkable increase in theconcentration of MDA as a main product of lipidperoxidation in the plasma and liver (Vijayakumar*etal*., 2006). Excessive free radical generation has been linked with abnormal alterations in the dynamic properties of cellular membranes (Lin *etal*., 2000). Free radicals can interact and disrupt the hepatic cell membranes thereby causing the hepatic marker enzymes such as alanine aminotransferase (ALT) and aspartate aminotransferase (AST) to leak out and increase their serum concentrations (Patrick- Iwuanyanwu*etal*., 2011).

The use of bioactive plant-derived compounds is on the rise, because the main preoccupation with the use of synthetic drugs is the side effects which can be even more dangerous than the diseases they claim to cure (Kamboj, 2000). In contrast, plant derived medicines are based upon the premise that they contain natural substances that can promote health and alleviate illness and proved to be safe, better patient tolerance, relatively less expensive and globally competitive. (Kamboj, 2000).*Sennaalata*(L.)Roxb) belongs to the Fabaceae family (subfamily Caesalpinioideae) and commonly known as candle bush, with reference to the shape of its inflorescences, or ringworm tree because of a traditional Use (Abo *etal*., 2008). It is commonly referred to as "Asuwonoyinbo" by the Yoruba ethnic group in Southwestern Nigeria (Awal*et al*., 2004).

It is widely available in the tropics and has very important applications in folkloric medicine (Rai and Curr, 1987). In the northern part of Nigeria, particularly in Adamawa and Taraba States, the root, stem and leaves are used by practitioners of herbal medicines to treat burns, skin and wound infections, diarrhea, gastrointestinal and upper respiratory tract infections (El-mahood*etal*., 2008).
Recent researches have shown that the antioxidants ofplant origin with free-radical scavenging properties couldhave great importance as therapeutic agents in severaldiseases caused due to oxidative stress (Ramchoun*etal*., 2009). Preliminary phytochemical study showed the presence of tannins, phlobatannins, alkaloids, anthraquinones, flavonoids, cardiac glycosides and saponins in the chloroform extract of leaves of *Sennaalata*. However, flavonoids (Wegner andFintelmann, 1999) alkaloids (Manjunath, 2006), saponins (Trans *etal*., 2001) and glycoside (Vijayan*etal*., 2003) have been reported to have hepatoprotective activity.Moreover, some researchers have found antioxidant compounds such vitamin E (Tavares de Almeida *etal*., 2012)and quercetin (Coskun*etal*., 2005) , and antioxidant- containing plant *Eucalyptus globules (*Nakhaee*etal*., 1999) to prevent and ameliorate streptozotocin-induced oxidative stress and hepatotoxicity. The aim of this present study was therefore to investigate the possible protective effect of *Sennaalata* on STZ- induced oxidative stress and hepatotoxicity in wistar rats.

2. Materials and Methods

Plant materials
Fresh leaves of *Sennaalata*were collected from underG area, Ogbomoso, Oyo state .The plant was authenticated at the department of Pure and Applied Biology, LadokeAkintola University of Technology, Ogbomoso, Oyo state by Dr.Ogunkunle. The leaves were rinsed severally with clean tap water to remove dust particles and debris and thereafter allowed to completely drain. The collected leaves were then chopped into bits on a chopping board and air dried at room temperature 25^0C -30^0C for three weeks before taking to the experimental site.

Preparation of methanolic extract of *Sennaalata* leaves
Eight hundred and twenty grams of air-dried and pulverized leaves of Sennaalata was immersed in 5L of methanol for five days to ensure sufficient extraction of the active components. The suspension was filtered and the solvent evaporated to dryness in water bath. The stock was then prepared from the residue and the corresponding doses of 100mg/kg and 300mg/kg body weight were calculated and administered to the experimental animals as required.

Animal management
A total number of 40 malewistar rats weighing between 190 -245 g were used for this study. The rats were acclimatized to the experimental room having temperature of 25^oC, controlled humidity conditions (65^oC) and 12:12h light; dark cycle for two weeks. The experimental animals were housed in standard plastic cages, fed with standard diet (pelletized growers mash) obtained from Bovajay Feed Mill at Orita Naira in Ogbomoso and water *adlibitum.*

Induction of Streptozotocin(STZ)
Experimental Streptozotocin (STZ) was induced by intraperitoneal injection of 60mg/kg STZ freshly dissolved in 0.1M Sodium Citrate at p^H buffered at 4.5. Hyperglycemia was confirmed two days after injection by measuring the tail vein blood glucose level with an Accu-

Check Sensor Comfort Glucometer (Roche, Mexico City).Only the animals with fasting blood glucose level ≥ 200mg/dl were considered diabetic.

Experimental design
A total of 40 wistar rats were used for this study. The animals were randomly divided into four groups of 10 animals per group. The experimental period was three weeks beginning after the induction of STZ-induced oxidative stress. Group A: normal control untreated rats. Group B: STZ-induced untreated group (60mg/kg body weight). Group C and D were STZ-induced rats given methanolic extract of *Sennaalata* leaves (150mg/kg and 300mg/kg body weight) daily using an intragastric tube for three weeks(21 days).

Sample preparation
Each clothed blood sample was centrifuged at 2000 revolution per minute for 20minutes to obtain the serum. The supernatant (serum) was siphoned using micropipette.

Tissue Homogenates
The liver was cut into pieces and homogenized in equal volume of chilled 10mM Tris/HCl sucrose buffer pH 7.4 in a mortar and pestle.

Serum Biochemical Assay
Serum alanine aminotransferase (ALT) and aspartate aminotransferase (AST) activities were determined by the colorimetric method of Reitman and Frankel as described by Ochei and Kolhatkar (2005).

Estimation of SOD, MDA and GSH in the Liver

The activity of superoxide dismutase (SOD) was determined by using the method ofMisra and Fridovich(1972). Lipid peroxidationwas determined based on the principle of Varshney and Kale (1990). Estimation of lipid peroxidation was based on the reaction of malondialdehyde (MDA) with thiobarbituric acid (TBA) forming a MDA- TBAR adduct that absorbed strongly a 532nm. Reduced glutathione(GSH) level in the liver was assayed following the method of Ellman(1959), modified by Hissin and Hilf(1973).

Tissue processing
The tissues were allowed to fix in 10% formol saline for 48hours, tissues were grossed and cut into smaller pieces of 3mm thick in prelabelled tissue cassette. They were processed using Automatic tissue processor (LEICA TP1020) where they passed through various reagents including Alcohol (of various concentrations starting from 70%, 80%, 90%, and two 100% or absolute alcohol) for dehydrations, two changes of xylene and three changes of molten paraffin wax set at 65 degree centigrade. The processing time was 12hours(Carleton, 1967).

Statistical analysis
Data were expressed using Graphpad prism 6. Data were expressed as Mean ± Standard error of mean (Mean ± S.E.M). Values were compared using one-way analysis of variance (ANOVA) followed by student t-test.P- Values less than 0.05 were taken to be statistically significant and insignificant at P>0.05.

3. Results

Table 1 and figure 1 showed that malondialdehyde concentration in the liver was significantly higher in the STZ –induced untreated group (group B) when compared with the normal control (group A) at P <0.05 but treatment with 150mg/kg and 300mg/kg of methanolic extract of *Sennaalata leaves* significantly reduced the MDA levels in both group C (STZ- induced +ML) & group D(STZ-induced +MH) with higher reduction in group D(STZ-induced +MH).

The result showed in table 1 and figure 1 also indicated a significant decrease in GSH level in the STZ-induced untreated group as compared with the normal control group (group A) at P <0.05.However, treatment with methanolic extract of *Sennaalata leaves* significantly increased the reduced glutathione levels in dose dependent manner respectively.

Table 1 also representsthe results of the oxidative status of the rats. The activity of superoxide dismutase was significantly (P <0.05) lowered in Streptozotocin induced untreated group (Group B) when compared with the normal control group A. However, treatment with 150mg/kg and 300mg/kg of methanolic extract of *Sennaalata* significantly increased superoxide dismutase activity in dose dependent manners in group C (STZ-induced +ML) and group D (STZ-induced + MH) respectively.

The results from table 2 and figure 2 below showed significantly (P < 0.05) higher activity of serum biomarker enzymes, Alanine aminotransferase(ALT) and Aspartate aminotransferase(AST) in the STZ-induced untreated group (Group B)when compared with the normal control group (Group A). However, treatment of rats with methanolic extract of *Sennaalata leaves* significantly (P<0.05) reduced the activity of ALT and AST when compared with the STZ-induced untreated group. The reduction in the activity of ALT was not dose dependent.

Table1: Effect of methanolic extract of *Sennaalata* leaves on malondialdehyde (MDA) level, reduced gluthathione(GSH) level and Superoxide dismutase activity of the streptozotocin induced oxidative stress in rats.

PARAMETERS	*GROUP A (Control)*	*GROUP B (STZ-induced untreated)*	*GROUP C (STZ-induced+ML)*	*GROUP D (STZ-induced+MH)*
MDA(nmol/gtissue)	7.08±0.19	13.82±1.08[a]	11.05±0.22[a,b]	9.61±0.10[a,b]
GSH(µmol/gtissue)	23.70±0.58	11.29±0.31[a]	13.71±0.33[a,b]	19.92±0.40[a,b]
SOD(µmol/min)	88.80±3.07	23.14±1.38[a]	30.90±0.90[a,b]	56.90±1.79[a,b]

ML = methanolic low dose & MH = methanolic high dose. Values are expressed as Mean ± S.E.M (Standard Error of Mean);MDA,GSH and SOD in liver of STZ- induced oxidative stress in wistar rats using student t- test.[a]Represents

significant increase at P<0.05when compared with Group A(control),[b]Represents significant decrease at P<0.05 when compared with Group B(STZ- induced) .

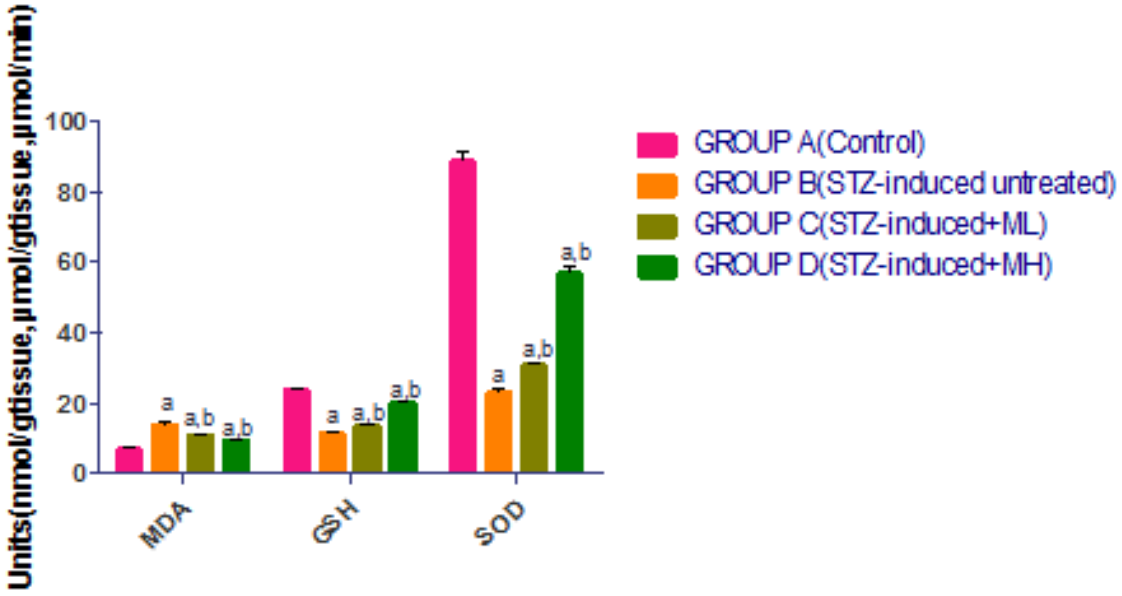

Figure 1: Effect of methanolic extract of *Sennaalata* leaves on MDA and GSH Concentration and SOD activity of STZ-induced oxidative stress in Wistar rats

Table 2: Effect of methanolic extract of *Sennaalata* leaves on the activity of Alanine aminotransferase (ALT) and Aspartate aminotransferase (AST) in Serum of wistar rats after treatment.

PARAMETERS	GROUP A (Control)	GROUP B (STZ-induced untreated)	GROUP C (STZ-induced+ ML)	GROUP D (STZ-induced+ MH)
ALT(U/L)	55.71±8.29	198.60±10.30[a]	128.30±1.18[a,b]	60.90±3.29[a,b]
AST(U/L)	22.53±2.35	55.61±2.42[a]	47.88±0.74[a,b]	41.88±0.79[a,b]

ML = methanolic low dose, MH = methanolic high dose. Values are expressed as Mean ± S.E.M (Standard error of Mean) on serum analysis of Alanine Aminotransferase (ALT) and Aspartate Aminotransferase (AST) in liver of STZ – induced oxidative stress in wistar rats using student t- test. [a]Represents significant decrease at P<0.05when compared with Group A(control),[b]Represents significant increase at P<0.05 when compared with Group B(STZ- induced).

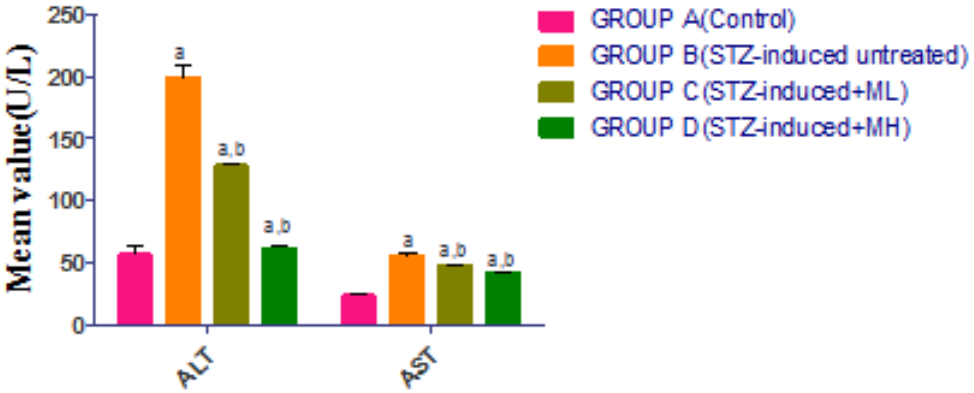

Figure 2: Effect of methanolic extract of *Sennaalata* leaves on serum ALT and AST activities of STZ-induced oxidative stress in Wistar rats.

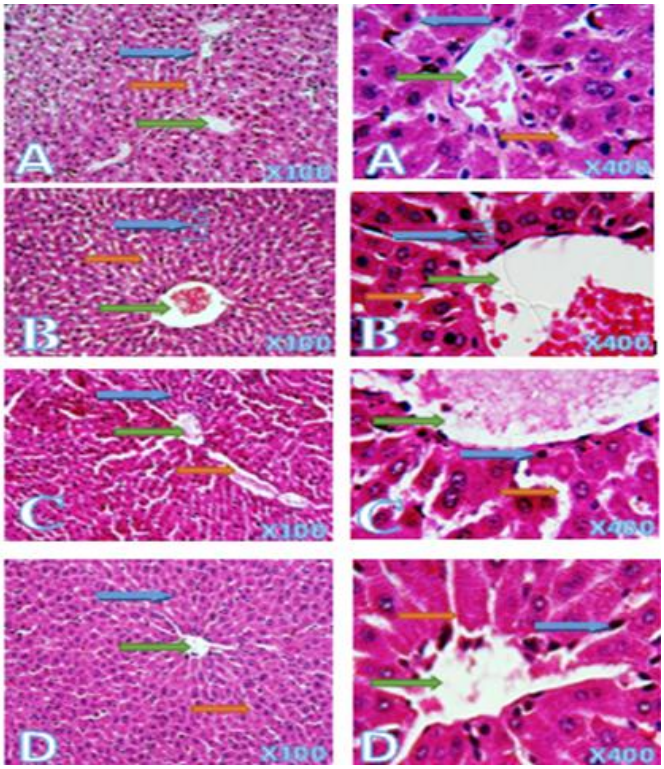

Plate 1: Hepatic histology after three weeks of treatment (H&E stain)
A: Normal control; B: STZ-induced untreated; C: STZ-induced + ML D: STZ-induced + MH

A photomicrogragh of liver sections of the normal control group (group A) showed normal liver microarchitecture, central vein(green arrow), sinusoids (red arrow), hepatocytes(blue arrow), no haemorrhage and there is no infiltration of inflammatory cells.A photomicrograph of liver sections of group B induced with 60mg/kg STZ showed peroportal fatty infiltration(PFI) with focal necrosis of hepatocytes(blue arrow), enlarged sinusoids(red arrow) and central vein(green arrow). A photomicrograph of liver sections of group C induced with 60mg/kg STZ and treated with 150mg/kg of methanolic extract of *Sennaalata* leaves showed normal central vein(green arrow), reduced distortion in the histology of the hepatocytes(blue arrow), enlarged sinusoids with perivenous fatty infiltration (red arrow). A photomicrograph of liver sections of group D induced with 60mg/kg STZ and treated with 300mg/kg of methanolic extract of *Sennaalata* leaves showed normal liver microarchitecture featured hepatocytes(blue arrow), sinusoids(red arrow) and central vein(green arrow) no haemorrhage and there is no infiltration of inflammatory cells compared with normal control group.
STZ: streptozotocin; ML: methanolic low dose; MH: methanolic high dose

4. Discussion

The result obtained in this study indicated that exposure of rats to STZ resulted in significant decrease ($P < 0.05$) in the activity of superoxide dismutase (SOD) and concentration of reduced glutathione (GSH) as well as significant ($P < 0.05$) increasein malondialdehyde (MDA) concentration compared with the control group. This is consistent with the results of previous researchers on the effect of STZ on the oxidative status of STZ- exposed rats (Nakhaee*etal*., 1999; Coskun*etal*., 2005; Tavares de Almeida *etal*., 2012). The observed significant decrease ($P < 0.05$) in the activity of superoxide dismutase (SOD) and concentration of reduced glutathione (GSH) along sides significant ($P < 0.05$) increase in malondialdehyde (MDA) concentration indicates oxidative stress in STZ-induced rats (Ujowundu*etal*., 2012).SOD catalyses the consumption of superoxide anion (O_2) which peroxidises cell membrane. The activity of SOD in the cell is therefore a predictor of oxidative status of that cell. The significantly low SOD activity recorded in the liver of the STZ- induced untreated rats could mean the tissue was undergoing oxidative attack occasioned by superoxide anions (Table 1 and Figure 1). However, treatment with 150mg/kg and 300mg/kg of methanolic extract of *Sennaalata leaves* significantly increased superoxide dismutase activity in dose dependent manner in group C (STZ-induced +ML) and group D (STZ-induced + MH).

In organ and tissue damage, GSH makes up the first line of defense against free radicals resulting from the ingestion of xenobiotics (Ujowundu*etal*., 2012). The significantly low GSH level in the Streptozotocin induced untreated group compared with the normal control group might have confirmed damage to the liver presumably by the oxidant (Table 1 and Figure 1). Nonetheless, treatment with chloroform extract of *Sennaalata also* significantly increased the reduced glutathione concentration in dose dependent manner. The observed increase in malondialdehyde (MDA) concentration in STZ-induced untreated group as compared to the controlindicates increased lipid peroxidation which could have resulted from depletion of GSH concentration. Again treatment with 150mg/kg and 300mg/kg of methanolic extract of *Sennaalata leaves* significantly reduced the MDA levels in both group C (STZ- induced +ML) and group D(STZ-induced +MH), with higher reduction in group D(STZ-induced +MH).

The observed significant increase in SOD activity and reduced glutathione concentration with corresponding significant decrease in MDA level in rats induced with STZ and treated with methanolic extract of *Sennaalata leaves*indicates the ameliorative effect of methanolic extract of *Sennaalata leaves* on STZ-induced oxidative stress and hepatotoxicity.Methanolic extract of *Sennaalata* leaves may have conferred protection against oxidative damage of the hepatocytes by the antioxidant activity of its phytoconstituents as the preliminary phytochemical screening of methanolic extract of *Sennaalata leaves revealed* the presence of antioxidants such as flavonoids, alkaloid, glycosides and saponins(Wegner andFintelmann, 1999, Manjunath, 2006, Trans *etal*., 2001,Vijayan*etal*., 2003). Previous studies have established antioxidant compounds such as vitamin E (Tavares de Almeida et al., 2012) and quercetin (Coskun*etal*., 2005) , and antioxidant-containing plant *Eucalyptus globules* (Nakhaee*etal*., 1999) to prevent and ameliorate streptozotocin-induced oxidative stress and hepatotoxicity. Moreover,flavonoids,alkaloids,saponins,glycosidehave been reported to possesshepatoprotective activity(Wegner andFintelmann, 1999, Manjunath, 2006, Trans*etal*., 2001,Vijayan*etal*., 2003).

The result of this present study also indicated hepatic injury as evident by a significant increase ($P<0.05$) in the activities of serum marker enzymes namely serum alanine transaminase (ALT) and serum aspartate transaminase (AST) in the group treated with streptozotocin only when compared with the normal control (Table 2 and Figure 2). Increased levels of serum ALT and AST have been reported to be sensitive marker of hepatic damage(Ochei, Kolhatkar, 2005;Achuba and Ogwumu, 2014). Thismay be due to leakage from the cells through peroxidative damage of the membrane. However,the reduction in the levels of these marker enzymes in groups administered with methanolic extract (Groups C andD) is suggestive of regeneration process and repair of hepatic damage induced by streptozotocin.

Histological findings showed normal histoarchitecture in the normal control group. Photomicrograph plates of Group A(Plate 1) reflects normal hepatocytes separated by sinusoids. The central veins show normal histoarchitecture, no haemorrhage and there is no infiltration of inflammatory cells seen within the liver parenchyma. The histological section of STZ –induced untreated group showed enlarged central vein, periportal fatty infiltration (PFI) with focal necrosis of hepatocytes. The sinusoids were enlarged with fatty infiltration (Plate 1).

However, treating rats with methanolic extract of *Sennaalata*leaves improved the histological features of the liver cells. Although the liver of rats treated with 150mg/kg(group C) still featured some lesionsas seen in group B, the liver of the group treated with 300mg/kg(group D) showed normal histological features as observed in the normal control group. This suggests the antioxidativeactions and hepatoprotective potential of *Sennaalata leaves* extract is dose-dependent and more efficient at higher doses.

5. Conclusion

This study demonstrated that methanolic extract of *Sennaalata leaves* through its marked antioxidant properties salvagedstreptozotocin-induced oxidative stress and hepatic damage. Therefore,*Sennaalata*leaves can be used for the treatment of oxidative stress-induced hepatic disorders.

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