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PROTECTIVE EFFECTS OF MOMORDICA BALSAMINA LINN ON HEPATIC OXIDATIVE STRESS IN STREPTOZOTOCIN-INDUCED DIABETIC RATS

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ABSTRACT

Oxidative stress has been reported as one of the heterogeneous etiologies that produces the multiple biochemical sequelaes of diabetes mellitus. Hence the urgent need for the development of antidiabetic drugs that can target the multiple etiologies of diabetes. The current study evaluates the protective effects of the aqueous leaf extract of Momordica balsamina Linn (MB) on hepatic oxidative stress in streptozotocin (STZ)-induced diabetic rats. Oxidative stress status of STZ-induced diabetic rats treated orally (28 days) with 200 mg/kg

Introduction:

Momordica balsamina Linn is commonly used for dietary and traditional herbal medicinal purpose in Northern Nigeria. *M. balsamina* belongs to the family Cucurbitaceae known to have different subspecies exhibiting different pharmacological properties. The leaves, fruits, seeds, and bark of *M.balsamina* have been

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of the aqueous leaf extract of MB was evaluated using some hepatic oxidative stress biomarkers. STZ induction resulted in a significant decline (p<0.05) in the levels of antioxidant enzymes; superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GPx) and glutathione s transferase (GST) and reduced glutathione (GSH) compared to the untreated diabetic rats. In addition, significant increase (p<0.05) in the degree of lipid peroxidation measured as TBARS was observed. However, significant elevation (p<0.05) of the levels of antioxidant enzymes and significant decrease (p<0.05) in the degree of lipid peroxidation after repeated oral treatment with MB was observed compared to diabetic controls. These findings demonstrate the protective potentials of MB against oxidative stress induced liver damage and its probable potency as an antioxidant and antidiabetic agent.

Keywords: *Momordica balsamina, Streptozotocin, Oxidative stress, Antioxidant, Diabetes.*

reported to contain resins, alkaloids, flavonoids, glycosides, steroids, terpenes, cardiac glycoside and saponins which are attributed to the plant's numerous medicinal importances (Bhardwaj *et al*., 2010). In Northern Nigeria, *M. balsamina* has long been utilized as a non-pharmacological intervention alone or as a polyherbal formulation for treatment of diabetes by the traditional herbal healers. The activity based review of *M. balsamina* indicated that it possess activities like antimicrobial, antispasmodic, anti-inflammatory, analgesic, anti-HIV, anti-diahorrial, hepatoprotective, anti-malarial, anticancer and wound healing properties (Karumi *et al*., 2003; Otimeyin *et al*., 2008; Thakur *et al*., 2009). The plant is a rich source of triterpenoids and phenolic compounds reported to possess antioxidant activities (Nagarani *et al*, 2014).

Streptozotocin, an accepted model for the induction of diabetes, has been shown to damage islet cells of the pancreas through its nitric oxide donor property. Diabetes mellitus; characterized by persistent hyperglycemia; is a metabolic disorder of heterogeneous etiologies that produces multiple biochemical sequelae. Changes in oxidative stress biomarkers, majorly enzymatic and non enzymatic antioxidants have been observed as major contributors to hyperglycemic-induced complications of diabetes (Martim *et al.,* 2003). Elevated generation of free radicals resulting in the consumption of antioxidant defense and peroxidation of lipid components is associated with the symptoms and progression of diabetes resulting in oxidative stress (OS). During diabetes-induced OS, free radicals are formed disproportionately by glucose oxidation through the mitochondrial transport chain, non-enzymatic glycation of proteins, and the subsequent oxidative degradation of glycated proteins (Mahdi, 2002; Nishikawa *et al*., 2000).Validating the antioxidant property of traditional antidiabetic plants could thus pave way for developing novel antidiabetic agents that can target therapeutic pathways which do not depend on blood glucose level thereby reducing mortality and morbidity due to the disease.
This study was therefore aimed to evaluate the effect of the aqueous leaf extract of *M. balsamina* on hepatic oxidative stress in STZ-induced diabetic rats in an attempt to understand its underlying mechanism of action.

Materials and Methods

Preparation of Aqueous Crude Extracts

Fresh plant leaves of *M. balsamina* were collected from Kumbotso Local Government Area of Kano State, Nigeria in March 2013. The leaves were authenticated at the Herbarium unit, Biological science department, Ahmadu Bello University, Zaria; Voucher number (No.

900220). The leaves were separated from undesirable plant parts, washed, air dried in the shade and grounded. A portion of dried leaf powder (500 grams) was extracted with distilled water using maceration method. The extract was filtered with Whatman No 1 filter paper, evaporated to dryness using water bath preset at 60°C and was later stored at 4˚C.

Experimental Animals and Induction of Diabetes

Apparently healthy albino Wistar rats of both sexes weighing averagely 250 grams were purchased from the animal house of the Department of Biological sciences, Bayero University, Kano. The animals were housed in a wire meshed laboratory cage and fed with commercial feeds (Vital feeds®, Jos, Nigeria) and water *ad libitum*. They were kept at 25ºC±2 at natural cycle of light and darkness throughout the experimental period. The animals received human care in accordance with the requirements of the guide for care and use of laboratory animals (National Research Council, 1996). Diabetes was induced in the experimental animals by a single intramuscular injection of freshly prepared solution of streptozotocin (60 mg/kg body weight) in 0.1 M cold citrate buffer (pH 4.5), after an overnight fast (Sankar and Pari, 2011). The rats were kept for 24 hours on five (5%) percent glucose solution (4 hours post STZ induction) as described by Stanley *et al.,* 2001. Diabetes was confirmed by the determination of fasting blood glucose concentration by tail prick using glucometer (Roche one touch glucometer, Germany) Seventy two (72) hours post streptozotocin injection and rats that had blood glucose level greater than 250 mg/dL were selected for this experiment (Zulfiker *et al*, 2010).

Experimental Design

Diabetic and normal rats were randomly assigned into four groups of six rats each and treated for four weeks as follows: Group 1: Normal controls and received water only; Group 2: Diabetic controls received water only; Group 3: Normal rats treated with 200 mg/kg MB; Group 4: Diabetic rats treated with MB (200 mg/kg).

Preparation of Sample and Biochemical Analysis

At the end of the treatment period, the rats were fasted overnight and sacrificed by cervical decapitation. The liver was dissected out and rinsed in ice-cold phosphate buffered saline (PBS) (0.01mol/L, pH 7.0-7.2) to remove excess blood before weighing. The liver tissue (50mg/ml buffer) was cut into small pieces and homogenized in PBS. The resulting mixture was centrifuged for 10 minutes at 10,000 rpm and the supernatant was used for hepatic oxidative stress biomarkers determination. Superoxide dismutase (SOD) and Catalase (CAT) (R & D Systems, China), reduced glutathione (GSH) and Glutathione-s-transferase (GST) (Uscn Life Sciences Inc, USA) were determined by ELISA methods as described by Tietz (1999) using commercial kits. Glutathione peroxidase (GPx) (Randox Laboratories Limited, UK) was determined by the UV method as described by Paglia and Vanlentine (1967). Thiobarbituric acid reactive substance (TBARS) was assayed by the method described by Nichans and Samuelson (1968).

Statistical Analysis

Values were expressed as mean ± standard deviation. Statistical analysis was carried out using one way analysis of variance (ANOVA) followed by Tukey's post hoc test. Values of $p<0.05$ were considered significant.

Results and Discussion

The effect of the aqueous leaf extract of *M. balsamina* on hepatic oxidative stress status was evaluated in STZ-induced diabetic rats using some selected oxidative stress biomarkers. As shown in Table 1, antioxidant enzymes; SOD, CAT and GPx and GSH levels was significantly decreased ($p<0.05$) in the untreated diabetic rats compared to the normal controls. In addition, lipid peroxidation was significantly increased ($p<0.05$) as evidenced by an increased level of TBARS when compared to the normal controls. However, treatment with 200 mg/kg of MB for 28 days resulted in a significantly elevated ($p<0.05$) levels of antioxidant enzymes and GSH as compared to the diabetic untreated rats (Table 1). Also, MB significantly reduced ($p<0.05$) the degree of hepatic lipid peroxidation as compared to untreated diabetic rats (Table 1).

With respect to the normoglycemic rats treated with 200 mg/kg of MB, an insignificant increase ($p>0.05$) was observed in the antioxidant enzymes; SOD, CAT and GST levels when compared to the normal controls. In addition, an insignificant difference ($p>0.05$) was observed in the level of TBARS. However, treatment of the normoglycemic rats with MB, resulted a significantly elevated level of GSH and significantly decreased level of GPx as compared to the normal controls.

Table 1: Effect of Aqueous Leaf Extract of *M. balsamina* on Hepatic Oxidative Stress in STZ- induced Diabetic rats

Biomarkers	Normal Control	Diabetic Control	Normal + MB	Diabetic + MB
SOD (U/mg protein)	15.44 ± 1.54^{d}	4.20 ± 1.12^{a}	10.95 ± 1.83^{d}	8.17 ± 0.67^{b}

CAT (U/mg protein)	59.75±4.44[c]	25.80±2.89[a]	61.46±4.33[c]	42.69±2.42[b]
GST (U/mg protein)	9.78 ± 0.38[d]	6.87± 0.51[a]	9.48 ± 0.60[d]	7.98± 0.21[b]
GSH (μg/g tissue)	47.68±6.50[d]	27.05±1.50[a]	52.63± 5.87[e]	35.17±3.13[b]
GPx (U/mg protein)	21.71±2.09[e]	7.50±0.63[a]	16.45± 4.33[d]	10.74±2.44[b]
TBARS (nmol/g tissue)	0.98 ± 0.08[a]	2.86 ± 0.14[d]	1.02± 0.25[a]	1.89 ±0.16[c]

Data are mean ±SD, n= 6 and different letters within a row indicate significant difference at $P<0.05$, (Tukey's multiple comparisons test)
SOD Superoxide dismutase; CAT Catalase; GST Glutathione-S-Transferase GSH Reduced Glutathione; GPx Glutathione Peroxidase; TBARS Thiobarbituric acid reactive substances

Oxidative stress (OS) in diabetes co-exists with diminished antioxidant capacity, which increases the deleterious effects of free radicals (Martim *et al.,* 2003). Oxidative stress result from an imbalance between radical generating and antioxidant defense system in favour of free radical generating system which damages complex cellular molecules such as lipids, proteins and DNA (Atangwho *et al.,* 2010). STZ generates free radicals which selectively causes damage to pancreatic beta cell thus inducing diabetes. Diminished levels of antioxidant enzymes, reduced glutathione levels and increased peroxidation of membrane lipids of liver tissues in STZ-induced diabetic rats observed in the present study correlates with reports of Ayoub *et al*. (2013) and Stanley *et al*. (2001). This finding indicates oxidative stress in the liver of the STZ-induced diabetic rats. Lipid peroxidation; measured as thiobabituric acid reactive

substance (TBARS), is considered as a reliable marker of oxidative stress in biological system. Malondialdehyde is a secondary product of lipid peroxidation and is known to cause cross-linkage of membrane components containing amino group which makes the membrane fragile resulting in altered cellular functions (Srivasatava, 1998). The elevated TBARS observed in the STZ induced diabetic rats may also be attributed to the increased oxidation of glucose, free radical formation due to increased polyol pathway flux, glycation of hemoglobin and nitric oxide donor property of STZ (Srivasatava, 1998).
Cooperative defense systems that protect the body from free radical damage which includes the antioxidant nutrients and enzymes are the first line of defense against reactive oxygen species (ROS) induced oxidative damage (Sellamuthu *et al.,* 2013). The ability of the aqueous leaf extract of *M. balsamina* to improve the oxidative stress status in the present study agrees with reports of Ayoub *et al.* (2013), Cheng *et al.* (2013) and Hassan *et al.* (2015). Increased level of the enzyme SOD may protect CAT against enzyme inactivation by superoxide radical. Thus, the increase in level of SOD may indirectly play an important protective role in preserving the activity of CAT (Ayoub *et al*., 2013). Glutathione constitutes the major non-enzymatic antioxidant capacity of the cytoplasm as such protects the cellular system against toxic effects of lipid peroxidation. Diminished levels of GSH, SOD and CAT in the liver, pancreas and kidney during diabetes is indicative of its increased utilization due to oxidative stress as observed in the untreated diabetic rats in the present study (Almeida *et al.,* 2012). GPx; a selenium containing enzyme and GST collaborately work together with glutathione to play a central role in the decomposition and detoxification of endogeneous metabolic peroxides and organic hydroperoxides to non-toxic products (Ayoub *et al*., 2013). GST catalyzes the nucleophilic attack of glutathione to electrophilic

substrates, resulting in addition or substitution reactions (Armstrong, 1997; Josephy and Mannervik, 2006). Observed reduction of GPx after STZ induction could be the result of decreased level of GSH, because GSH is an essential co-substrate for GPx. Additionally, The observed decreased level of GPx and GST in the present study could be attributed to radical-induced inactivation and/or inactivation of the enzyme by glycation (Ayoub *et al.,* 2013; Almeida *et al.,* 2012).

NADPH is required for regeneration of glutathione via the glutathione redox cycle. Improved antioxidant status in diabetic rats treated with the aqueous leaf extract of MB could be accompanied with a consequent increase in the NADPH/NADP+ ratio resulting in an increased GSH reductase activity and decreased aldose reductase activity. Consequently, this elevates the level of GSH which in turn reduces the deleterious effects of reactive oxygen species generated during STZ-induced diabetes. Elevated GSH, SOD, CAT, GPx and GST levels could therefore confer protection to cellular macromolecules against oxidation and also detoxify and protect pancreatic beta cells from reactive oxygen species generation induced by exposure to streptozotocin (Naito *et al.,* 2010).

Conclusion

Findings of the present study demonstrated that the aqueous leaf extract of *M. balsamina* possess antioxidant potential in STZ-induced diabetic rats as evidenced by its ameliorative effect on hepatic oxidative stress biomarkers. Therefore, it has a potential to confer protection against oxidative stress-induced liver damage in diabetes. Further studies on identification and characterization of bioactive antioxidants in *M. balsamina* with the utilization of appropriate tools are recommended.

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