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Research Article

Modulatory role of lauric acid supplement on lipid peroxidation and some antioxidant enzymes activity in high fat diet, streptozotocin-induced type 2 diabetic male wistar rats.

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Keywords:
Lipid peroxidation; antioxidant enzymes; lauric acid; metformin; type 2 diabetes

ABSTRACT

Background: Diabetes mellitus is a major health challenge in the world and is diagnosed by the presence of sustained hyperglycemia (high glucose levels in the blood). Oxidative stress is known to be actively involved in the onset and progression of diabetes and its complications. Antioxidants have important roles in biological systems by scavenging free radicals which may result in oxidative damage of biological molecules such as lipids, proteins and DNA. **Aim:** This study was designed to evaluate the effect of lauric acid on lipid peroxidation (serum malondialdehyde concentration) and some antioxidant enzymes (superoxide dismutase and Catalase) activities in high fat diet/streptozotocin-induced type 2 diabetic male wistar rats. **Study Design:** Thirty-five apparently healthy male wistar rats aged 6-8 weeks, weighing between 70-90 g were assigned into seven groups of five animals each (n=5) and administered graded doses of lauric acid supplement after validation of diabetes for a period of twenty-one (21) days. **Methodology:** Group 1: (Normal control), Group 2: (Diabetic control untreated), Groups 3: (Normoglycemic) received 125 mg/kg Lauric acid, Group 4, 5 and 6 were administered 125, 250 and 500 mg/kg body weight of lauric acid, Group 7: (Standard control) received metformin 100 mg/kg. At the end of twenty-one (21) days, rats were anaesthetized using ketamine and xylazine at 75 and 25 (mg/kg). Blood samples were taken from all treated groups for evaluation of serum MDA, SOD and CAT level. **Results:** Serum malondialdehyde (MDA) levels were significantly ($P < 0.05$) reduced (1.32±0.04, 1.40±0.04 and 1.42 ± 0.06 nmol/l) respectively when compared with the diabetic control (untreated) group with a value of (2.25±0.10 nmol/l), while there was up-regulated activities of serum endogenous antioxidant enzymes: SOD (1.97±0.08, 2.02±0.16, 1.98±0.12 IU/L), CAT (44.5±0.64, 43.2±0.85, 43.7±0.85 IU/L) relative to diabetic control (untreated) (1.35±0.02 and 34.0±0.91 IU/L) respectively. **Conclusion:** In conclusion, lauric acid decreases lipid peroxidation while increasing serum antioxidant activity in high fat diet/streptozotocin-induced type 2 diabetic male wistar rats after 21 days oral administration.

INTRODUCTION

Diabetes mellitus is a term that describes a metabolic syndrome of multiple etiology characterized by chronic hyperglycemia with disturbances of carbohydrates, fats and proteins metabolisms resulting from defects in insulin secretion, insulin action or both (WHO, 2016). The chronic hyperglycemia of diabetes is associated with long-term damage, dysfunction, and failure of different organs, especially the eyes, kidneys, nerves, heart and blood vessels (Cheungpasitporn *et al.*, 2016; Kalteniece *et al.*, 2018; Punthakee *et al.*, 2018). Diabetes represents one of the world's four major non

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communicable disease that are of great public health concern in the healthcare system. It accounts for about 1.6 million deaths each year globally (WHO, 2017). In 2015 in Nigeria alone, 1.56 million cases were reported including 105,091 deaths (IDF, 2015) and about 95% of all cases reported were attributed to Type-2 diabetes mellitus (T2DM), (IDF, 2015; WHO, 2016). In spite of advancements recorded to date on basic and clinical investigations into diabetes, a properly effective final remedy does not exist.

Oxidative stress contributes significantly to the patho-physiology of several diseases which include diabetes. It is believed that in the onset and progression of late diabetic complication, free radicals play a major role due to their ability to cause alteration in enzymatic systems, lipid peroxidation, impaired glutathione metabolism and DNA damage (Ayepola, 2014; Ullah, *et al.,* 2016). Lauric acid (LA) or a dodecanoic acid is saturated fatty acids with a 12 carbon atom chain thus falling into the group of medium chain fatty acids (C6-C12) (Anzaku *et al.,* 2017). LA is the primary fatty acid of coconut oil which is present at approximately 45–53 % and its metabolic and physiological properties account for many of the medicinal and healing properties attributed to coconut oil. It is found in a wide variety of fruits, seeds (Silva *et al.,* 2015) and as a human milk component (Silberstein *et al.,* 2013).

Numerous methods for modeling diabetes have been developed. Among these techniques, the construction of a diabetic model with streptozotocin (STZ) combined with high-fat diet has been widely used. STZ, a monofunctional nitrosourea derivative, was first isolated from Streptomyces achromogene. It is also an alkylating agent and can lead to cell death. When STZ acts on β-cells, it may lead to beta cell death, insulin release decreases, and increase in blood glucose in the body. In addition, a high-fat diet can lead to obesity and insulin resistance. Therefore, diabetes induced by a high-fat diet combined with STZ can better mimic the pathogenesis of human type 2 diabetes and can be used widely in studies on hypoglycemic activity (Chen *et al.,* 2018).

This study was designed to evaluate the effect of oral administration of lauric acid on lipid peroxidation (using serum malondialdehyde concentration) and some antioxidant enzymes (superoxide dismutase and Catalase) activities in high fat diet/streptozotocin-induced type 2 diabetic male wistar rats.

MATERIALS AND METHODS

Experimental Site

This study was carried out in the Physiology Laboratory of the Department of Human Physiology, Faculty of Basic Medical Sciences, College of Health Sciences, Ahmadu Bello University Zaria, Kaduna state Nigeria. Zaria is located between latitudes 110 and 3′ N, and between 70 and 42′ E, at an altitude of 670 m above the sea level and 664 km away from the sea, in the Northern Guinea Savanna zone (Marthin, 2006)

Chemicals and Reagents

Lauric acid (white crystalline powder with CAS No-143-07-7, malaysia), Streptozotocin (Bristol sigma, Lagos), Metformin, Chloroform, (Zayo sigma company, Jos, Nigeria) Normal saline (0.9% w/v), Methylated spirit, Tween 80, Citrate buffer, fructose solution (Kem Light Laboratories PVT Ltd, India), Simas Margarine (PT Salim Ivomas Pratama Tbk, Indonesia), Groundnut meal, and ground nut oil were purchased in Samaru-Zaria market, Kaduna Nigeria. All chemicals were commercially obtained and were of analytical grade.

Experimental animals

Thirty-five (35) wistar rats aged 6-8weeks weighing 70-100 g were used for this study. The wistar rats were purchased from Faculty of Pharmaceutical Sciences, Ahmadu Bello University, Zaria, Nigeria. The animals were housed in plastic cages with bedding material (saw dust), in the animal house of department of Human Physiology, Faculty of Medicine, Ahmadu Bello University, Zaria, and were provided with food (pelletized growers mash) and water *ad libitum* for a period of two (2) weeks to acclimatize before the study began.

Preparation of High Fat Diet

Composition of the high fat diet was 25% margarine, 25% ground nut meal, 15% ground nut oil with 35% of the animal feed which was a modification of the composition described by Tanko *et al.,* (2016) and Okoduwa *et al.,* (2017).

Induction and Confirmation of Hyperglycaemia

Insulin resistance was induced by feeding the animals with high fat diet (25% margarine which is made up of (fat 99.9%, Emulsifiant E471, E322, E306, Beta Carotene C175130, Vitamin D 30,000 IU/kg), 25% ground nut meal, 15% ground nut oil with 35% of the animal feed (composed of crude protein 13%, fat 8%, crude fibre 15%, calcium 0.90%, available phosphorus 0.35%, methionine 0.37%, lysine 0.70%, metabolizable energy 2600kcal/kg) orally for a period of eight weeks along with 10% fructose solution as drinking water. On the 8th week, a single intraperitoneal dose (30mg/kg) of streptozotocin (STZ) dissolved in 0.1M fresh cold citrate buffer pH 4.5 was injected into overnight fasted

rats. The rats were provided 5% glucose solution as drinking water in the first 24 hrs after STZ induction (Srinivasan *et al*., 2005; Zhang *et al*., 2008; Wilson and Islam, 2012; Xiang *et al.*, 2018) as modified. After three (3) days, the blood glucose level of the rats were measured, and after two weeks, it was measured again and only animals with fasting blood glucose level greater than 11.1 mmol/L were considered diabetic (Xu *et al.*, 2017).

Experimental Design

Animal grouping

Thirty-five (35) wistar rats were divided into seven (7) groups containing 5 wistar rats each (n=5) as follows:

Group 1: Normoglycemic rats administered 1 ml/kg Distilled water via intragastric intubation once daily for three weeks

Group 2: Diabetic rats administered 1 ml/kg Tween 80 via intragastric intubation once daily for three weeks

Group 3: Normoglycemic rats administered 125 mg/kg of Lauric Acid via intragastric intubation once daily for three weeks

Group 4: Diabetic rats administered 125 mg/kg of Lauric Acid via intragastric intubation once daily for three weeks

Group 5: Diabetic rats administered 250 mg/kg of Lauric Acid via intragastric intubation once daily for three weeks

Group 6: Diabetic rats administered 500 mg/kg of Lauric Acid via intragastric intubation once daily for three weeks

Group 7: Diabetic rats administered 100 mg/kg Metformin via intragastric intubation once daily for three weeks (Tikoo *et al.*, 2016)

Estimation of Biomarkers of Oxidative Stress and Lipid Peroxidation

Catalase (CAT) activity:

Catalase (CAT) activity was measured using the method described by Aebi (1984). Exactly 10µl of serum was added to a test tube containing 2.80ml of 50mM potassium phosphate buffer (pH 7.0). The reaction was initiated by adding 0.1ml of freshly prepared 30mM H_2O_2 and the decomposition rate of H_2O_2 was measured at 240nm for 5 minutes on a spectrophotometer. A molar extinction coefficient (E) of $0.041mM^{-1}\text{-}cm^{-1}$ was used to calculate the Catalase activity. Catalase Conc.= Absorbance/E. Catalase Activity = Catalase Con./Protein Conc. (mg/ml)

Superoxide dismutase (SOD) activity:

Superoxide dismutase (SOD) activity was determined by the method described by Fridovich (1989).

Principle:

The ability of superoxide dismutase (SOD) to inhibit auto-oxidation of adrenaline at pH 10.2 forms the basis of this assay.

Procedure:

An aliquant mixture of 0.2ml of the diluted micro some was added to 2.5ml of 0.05M carbonate buffer. The reaction was started with the addition of 0.3ml of 0.3mM Adrenaline. The reference mixture contained 2.5ml of 0.05M carbonate buffer, 0.3ml of freshly prepared 0.3mM Adrenaline (0.01g of adrenaline dissolved in 17ml of distilled water) and The reference mixture contained 2.5ml of 0.05M carbonate buffer, 0.3ml of 0.3mM Adrenaline and 0.2ml of distilled water. The Absorbance was measured over 30 seconds up to 150 seconds at 480nm

Calculations:

Increase in absorbance per minute = $(A_2 - A_1)/2.5$

% Inhibition = 100 - { (Incr. in absorbance for sample/Incr. in absorbance of blank) x 100}

1 unit of SOD activity is the quantity of SOD necessary to elicit 50% inhibition of the oxidation of Adrenaline to adenochrome in 1 minute.

Estimation of lipid peroxidation (malondialdehyde):

Lipid peroxidation was estimated spectrophotometrically as thiobarbituric acid reactive substances (TBARS). A principal component of TBARS is malondialdehyde (MDA), a product of lipid peroxidation. The assay for malondialdehyde was estimated colorimetrically by measuring malondialdehyde (MDA) by the method of Albro *et al.* (1986) and Das *et al.* (1990). In brief, 0.1 ml of plasma was treated with 2 ml of (1:1:1 ratio) TBA–TCA–HCL reagent (TBA 0.37%, 0.25N HCL and 15% TCA) and placed in water bath for 15 min, cooled and centrifuged and then clear supernatant was measured at 535nm against reference blank.

Lauric Acid Preparation and Administration

Lauric acid, due to its low solubility in water, was suspended in 1ml/kg of tween 80, and administered orally.

Ethical Approval

The rats were handled in accordance with the principles guiding the use and handling of experimental animals Ahmadu Bello University Zaria, Nigeria.

Statistical Analysis

Data collected was analyzed by one-way analysis of variance, (ANOVA), followed by *Tukey's post-hoc* test

which was used to compare the level of significance between the controls and treatment groups. Results were expressed as mean ± SEM. Statistical package for social science (SPSS) version 22.0 was used for the analysis and Values of $p < 0.05$ was considered significant.

RESULTS

Effect of Three Weeks Oral Administration of Lauric Acid on Serum Malondialdehyde (MDA) level in HFD/STZ-Induced Type 2 Diabetes Mellitus in Male Wistar Rats.

Fig. 1 Shows, a significant increase (P < 0.05) in malondialdehyde (MDA) levels in the diabetic control (untreated) group (2.25±0.10 nmol/l) when compared with that of normal control (normal saline) (1.37±0.06 nmol/l) and a significant decrease (P < 0.05) in malondialdehyde level in groups treated with lauric acid at doses of 125 mg/kg (1.32±0.04 nmol/l), 250 mg/kg (1.40±0.04 nmol/l) and 500 mg/kg (1.42 ± 0.06 nmol/l) respectively when compared with the diabetic control (untreated) group with a value of (2.25±0.10 nmol/l)

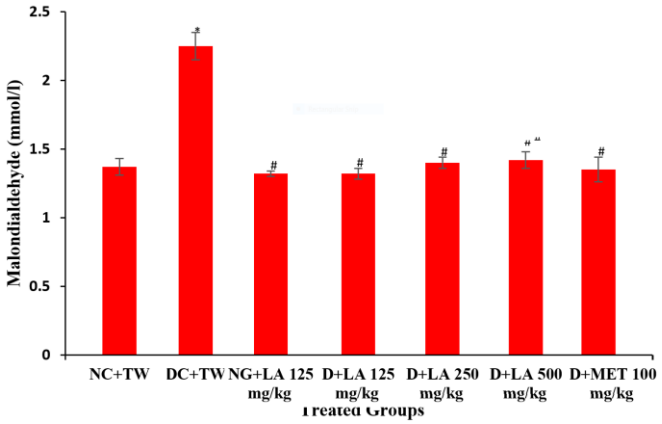

Fig 1. Se.rum Malondialdehyde (MDA) level in HFD/STZ-Induced Type 2 Diabetes mellitus in Male Wistar Rats Treated with Different Doses of Lauric acid for 21days. *= Means with this superscript are significant p< 0.05 compared to normal control #= Means with this superscript are significant p< 0.05 compared to diabetic control (untreated). DC= Diabetic control, NC= Normal (Non-diabetic) control, NG= Normoglycemic, D = Diabetic, LA = Lauric acid, MET = Metformin, TW= Tween 8.

Effect of Three Weeks Oral Administration of Lauric Acid on Serum Superoxide Dismutase (SOD) Activity in HFD/STZ-Induced Type 2 Diabetes Mellitus in Male Wistar Rats.

The results (Fig. 2) show a significant (P < 0.05) increase in serum superoxide dismutase (SOD) activity in groups treated with lauric acid 125 mg/kg (1.97±0.08 IU/L), 250 mg/kg (2.02±0.16 IU/L) and 500 mg/kg (1.98±0.12 IU/L) as compared with diabetic control (untreated) (1.35±0.02). The result also shows significant increase (P < 0.05) in serum superoxide dismutase (SOD) activity in groups treated with lauric acid 125 mg/kg (1.97±0.08 IU/L), 250 mg/kg (2.02±0.16 IU/L) and 500 mg/kg (1.98±0.12 IU/L) as compared with standard control (metformin treated group) (1.80±0.07). However, only group treated with lauric acid 250 mg/kg (2.02±0.16 IU/L) shows significant increase (P < 0.05) when compared with normal control (1.97±0.08 IU/L)

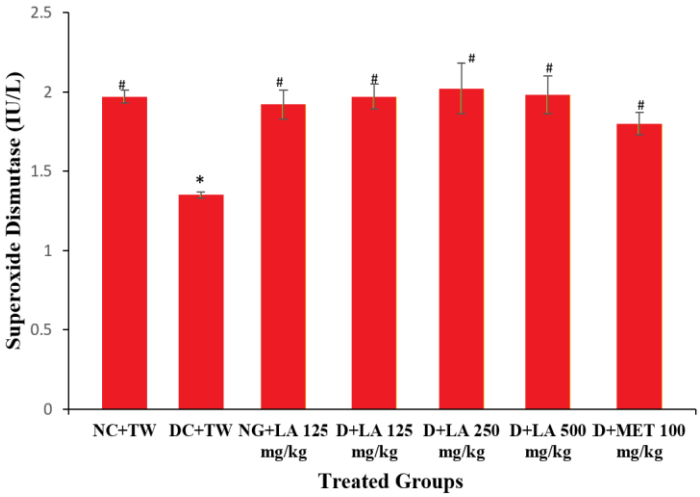

Fig. 2. Serum Superoxide Dismutase (SOD) Activity in HFD/STZ-Induced Type 2 Diabetes Mellitus in Male Wistar Rats Treated with Lauric acid and Metformin for 21days. *= Means with this superscript are significant p< 0.05 compared to normal control; #= Means with this superscript are significant p< 0.05 compared to diabetic control (untreated); DC= Diabetic control, NC= Normal (Non-diabetic) control, NG= Normoglycemic, D = Diabetic, LA = Lauric acid, MET = Metformin, TW= Tween 80

Effect of Three Weeks Oral Administration of Lauric Acid on Serum Catalase (CAT) Activity in HFD/STZ-Induced Type 2 Diabetes Mellitus in Male Wistar Rat

The results in Fig. 3 show a significant (P < 0.05) increase in serum catalase (CAT) activity in groups treated with lauric acid 125 mg/kg (44.5±0.64 IU/L), 250 mg/kg (43.2±0.85 IU/L) and 500 mg/kg (43.7±0.85 IU/L) as compared with diabetic control (untreated) (34.0±0.91). The result also shows significant increase (P < 0.05) in serum catalase (CAT) activity in groups treated with lauric acid 125 mg/kg (44.5±0.64 IU/L), 250 mg/kg (43.2±0.85 IU/L) and 500 mg/kg (43.7±0.85 IU/L) as compared with standard control (metformin treated group) (40.7±0.47 IU/L).

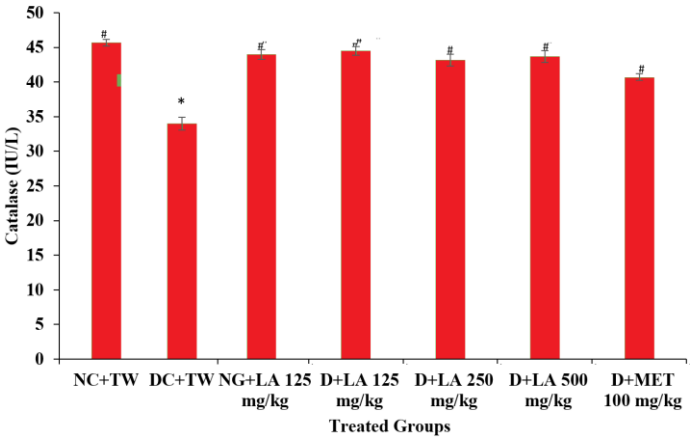

Fig. 3. Showing Serum Catalase (CAT) Activity in HFD/STZ-Induced Type 2 Diabetes Mellitus in Male Wistar Rats Treated with Lauric acid and Metformin for 21days. ; *= Means with this superscript are significant p< 0.05 compared to normal control; #= Means with this superscript are significant p< 0.05 compared to diabetic control (untreated); DC= Diabetic control, NC= Normal (Non-diabetic) control, NG= Normoglycemic, D = Diabetic, LA = Lauric acid, MET = Metformin, TW= Tween 80

DISCUSSION

Oxidative stress is a product of an imbalance between reactive oxygen species (ROS) such as superoxide anion (O_2^-) and the antioxidant defense systems such as superoxide dismutase (SOD). Antioxidants enzymes involved in the elimination of ROS include SOD, CAT and GSH, respectively. Antioxidant enzymes are a critical part of cellular protection against reactive oxygen species and ultimately oxidative stress. They form the first line of the antioxidant defense system against ROS generated *in vivo* during oxidative stress and act cooperatively at different sites in the metabolic pathway of free radicals (Cheng and Kong, 2011).

The present study showed a significant decrease in the activity of measured antioxidants enzymes (SOD and CAT) in diabetic control (untreated) rats when compared with normal control while there was a significant increase in the level of malondialdehyde (MDA) when compared with the normal control. There was also a significant increase in the activity of measured antioxidants enzymes (SOD and CAT) in diabetic rats treated with graded doses of lauric acid (125, 250, 500 mg/kg respectively) when compared with diabetic control untreated while there was a significant decrease in the level of malondialdehyde (MDA) when compared with diabetic control untreated. There was a significant increase in activity of antioxidant enzymes (SOD and CAT) and a significant decrease in MDA levels in diabetic rats treated with graded doses of lauric acid (125, 250, 500 mg/kg respectively) when compared with standard control (metformin) however only group that received 250 mg/kg lauric acid has a significant increase in SOD activity when compared with normal control. There was no significant difference between normoglycemic group that received 125 mg/kg lauric acid when compared with normal control (1 ml/kg distilled water).

This finding is consistent with the reports of various researchers (Cheng and Kong, 2011; Singh *et al.*, 2012; Cheng *et al.*, 2013 Iranloye *et al.*, 2013; Akinnuga *et al.*, 2016). This indicates a decrease in the antioxidant defense system and an increase in lipid peroxidation. Hyperglycemia is a main cause of increase production of ROS by generation of free radicals due to auto-oxidation of glucose and glycosylation of proteins (Al-Faris *et al.*, 2010) which could lead to increased lipid peroxidation and altered antioxidant defense and further impair glucose metabolism in biological system. The SOD, a superoxide radical scavenging enzyme is considered the first line of defense against the deleterious effect of oxygen radicals in the cells and it scavenges reactive oxygen radical species by catalyzing the dismutation of O_2^- radical to H_2O_2 and O_2^- thereby reducing the likelihood of superoxide anion interacting with nitric oxide to form reactive peroxynitrite. As a result, reduction in SOD activity in diabetic animals observed in the present study may be as a results of an increased formation of O_2^- radical which overwhelms the enzymes ability to counteract its effect and hence may reflects the cause of tissue injury (Singh *et al.*, 2012).

Catalase (CAT) is an enzymatic antioxidant widely distributed in all animal tissues. CAT decomposes hydrogen peroxide formed from the dismutation of superoxide anion by SOD to water (H_2O) and oxygen (O_2) which protects the tissues from highly reactive hydroxyl radicals (Onyeka *et al.*, 2012); however, treatment with graded doses (125, 250 and 500 mg/kg) of LA significantly increased the activities of the antioxidant enzymes (SOD and CAT) and also significantly reduced lipid peroxidation as seen in the increased levels of MDA in the treatment groups when compared with the diabetic control (untreated) group. SOD and CAT must have been up regulated by LA and this may indicate a pronounced antioxidant effect of the treatment given to the group. Since oxidative stress contributes significantly to the pathophysiology of diabetes and its complications substances that suppress oxidative stress might be therapeutically beneficial.

CONCLUSION

Lauric acid possesses potent antioxidant activity and also decrease lipid peroxidation by decreasing serum malondialdehyde concentration in HFD/STZ-induced type 2 diabetes mellitus in male wistar rat treated for twentyone days.

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