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Effect of Modified Diets Pancreatic Oxidative Markers in Streptozotocin-Induced Diabetic Wistar rats Following the Administration of Alpha Lipoic Acid

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ABSTRACT: Despite the availability of various anti-diabetic drugs, diabetes mellitus (DM) remains one of the world's most prevalent chronic diseases leading to the generation of reactive oxygen species (ROS), triggering oxidative stress as well as numerous cellular and molecular modifications such as mitochondrial dysfunction affecting normal physiological functions in the body. This study aimed to investigate the ameliorative effect of modified diets on pancreatic oxidative markers in streptozotocin-induced diabetic Wistar rats following the administration of Alpha lipoic acid, using standard appropriate techniques. Result established that High protein diet (HPD), and with combined administration with alpha lipoic acid in this research resulted in an increased pancreatic superoxide dismutase (SOD) and catalase (CAT) and glutathione (GSH) levels respectively while decreasing pancreatic malondialdehyde (MDA) levels, hence is affirmed as a beneficial strategy in the management of oxidative stress in diabetes. The present study concludes that high protein diets alone and in combination with ALA decreases pancreatic oxidative stress damage caused by streptozotocin toxicity, hence, has the potential to manage diabetes by reducing Reactice oxygen species (ROS) mediated oxidative stress.

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The pancreas is an organ of the digestive system and endocrine system. It is a mixed or heterocrine gland, (Hall, 2016; Standring, 2016; Barrett, 2019; Kanth *et al.,* 2019). As an endocrine gland, it functions mostly to regulate blood sugar levels, secreting the hormones insulin, glucagon, somatostatin, and pancreatic polypeptide (Hall, 2016; Sadley, 2019), a role played by the islets of Langerhans Each islet contains beta, alpha, delta and Poly pancreatic cells that are responsible for the secretion of pancreatic hormones (Sembulingam and Sembulingam, 2019). whereas as a part of the digestive system, the pancreas functions as an exocrine gland secreting pancreatic juice into the duodenum through the pancreatic duct (Kanth *et al.,* 2019). This juice contains bicarbonate, which neutralizes acid entering the duodenum from the stomach; and digestive enzymes, which break down carbohydrates, proteins, and fats in food entering the duodenum from the stomach. Because of its role in the regulation of blood sugar, the pancreas is also a key organ in diabetes mellitus (Kanth *et al.,* 2019; Sadley, 2019). Oxidative stress is the excess formation and/or insufficient removal of highly reactive molecules such as reactive oxygen species (ROS) and reactive nitrogen species (RNS) (Turko *et al.,* 2001; Maritim *et al.,* 2003; Ojieh, 2020; Odeghe, 2023). Reactive

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Oxygen Spiecs include free radicals such as superoxide ($^{\bullet}O_2^-$), hydroxyl ($^{\bullet}OH$), peroxyl ($^{\bullet}RO_2$), hydroperoxyl ($^{\bullet}HRO_2^-$) as well as nonradical species such as hydrogen peroxide (H_2O_2) and hydrochlorous acid (HOCl) (Turko *et al.,* 2001; Evan *et al.,* 2002). Under normal conditions, $^{\bullet}O_2^-$ is quickly eliminated by antioxidant defense mechanisms. $^{\bullet}O_2^-$ is dismutated to H_2O_2 by manganese superoxide dismutase (Mn-SOD) in the mitochondria and by copper (Cu)-SOD in the cytosol (Evans *et al.,* 2003). H_2O_2 is converted to H_20 and O_2 by glutathione peroxidase (GSH-Px) or catalase in the mitochondria and lysosomes, respectively. H_2O_2 can also be converted to the highly reactive $^{\bullet}OH$ radical in the presence of transition elements like iron and copper (Nwogueze, 2023[a]).

While ROS are generated under physiological conditions and are involved to some extent as signaling molecules and defense mechanisms as seen in phagocytosis, neutrophil function, and shear-stress induced vasorelaxation, excess generation in oxidative stress has pathological consequences including damage to proteins, lipids and DNA (Nwogueze *et al.,* 2023[b]).

ROS can stimulate oxidation of low-density lipoprotein (LDL), and ox-LDL, which is not recognized by the LDL receptor, can be taken up by scavenger receptors in macrophages leading to foam cell formation and atherosclerotic plaques (Boullier *et al.,* 2001), $^{\bullet}O_2^-$ can activate several damaging pathways in diabetes including accelerated formation of advanced glycation end products (AGE), polyol pathway, hexosamine pathway and PKC, all of which have been proven to be involved in micro- and macrovascular complications. $^{\bullet}O_2^-$ and H_2O_2 stimulate stress-related signaling mechanisms such as NF-κB, p38-MAPK and STAT-JAK resulting in VSMC migration and proliferation. In endothelial cells, H_2O_2 mediates apoptosis and pathological angiogenesis (Taniyama and Griendling, 2003).

Diabetes mellitus is a collection of chronic metabolic illnesses defined by a persistently high blood sugar level (Seckold *et al.,* 2018; Ataihire, Nwangwa, and Igweh, 2019; Eke *et al.,* 2019; Kahn, 2020). According to the World Health Organization, Diabetes mellitus is a metabolic illness with numerous etiologies defined by persistent hyperglycemia and changes in carbohydrate, lipid, and protein metabolism caused by deficiencies in insulin production, insulin action, or both (Ojieh *et al.,* 2016; Krishnasamy and Abell, 2018; Fu *et al.,* 2019; Ojieh, 2020; Egboh *et al.,* 2022; Ossai *et al.,* 2024). It is caused by the body's inability to metabolize blood glucose, either due to a lack of insulin or an insufficient amount of insulin in the body (Saedi *et al.,* 2016). Direct evidence of oxidative stress in diabetes is based on studies that focused on the measurement of oxidative stress markers such as plasma and urinary F_2-isoprostane as well as plasma and tissue levels of nitrotyrosine and $^{\bullet}O_2^-$ (Ceriello *et al.,* 2003; Vega-López *et al.,* 2004; Oberg *et al.,* 2004). There are multiple sources of oxidative stress in diabetes including nonenzymatic, enzymatic and mitochondrial pathways. Nonenzymatic sources of oxidative stress originate from the oxidative biochemistry of glucose (Ojieh, 2020). Hyperglycemia can directly cause increased ROS generation (Ossai *et al.,* 2021). Glucose can undergo autoxidation and generate $^{\bullet}OH$ radicals (Turko *et al.,* 2001). In addition, glucose reacts with proteins in a nonenzymatic manner leading to the development of Amadori products followed by formation of AGEs. ROS is generated at multiple steps during this process. In hyperglycemia, there is enhanced metabolism of glucose through the polyol (sorbitol) pathway, which also results in enhanced production of $^{\bullet}O_2^-$.

Enzymatic sources of augmented generation of reactive species in diabetes include NOS, NAD(P)H oxidase and xanthine oxidase (Guzik *et al.,* 2000; Guzik *et al.,* 2002; Aliciguzel *et al.,* 2003; Ossai *et al.,* 2021). All isoforms of NOS require five cofactors/prosthetic groups such as flavin adenine dinucleotide (FAD), flavin mononucleotide (FMN), heme, BH_4 and Ca^{2+}-calmodulin. If NOS lacks its substrate L-arginine or one of its cofactors, NOS may produce $^{\bullet}O_2^-$ instead of $^{\bullet}NO$ and this is referred to as the uncoupled state of NOS (Guzik *et al.,* 2000; Guzik *et al.,* 2002; Aliciguzel *et al.,* 2003; Maritim *et al.*, 2003). NAD(P)H oxidase is a membrane associated enzyme that consists of five subunits and is a major source of $^{\bullet}O_2^-$ production (Guzik *et al.,* 2000; Guzik *et al.,* 2002; Kitada *et al.,* 2003; Etoh *et al.,* 2003). Guzik et al. investigated $^{\bullet}O_2^-$ levels in vascular specimens from diabetic patients and probed sources of $^{\bullet}O_2^-$ using inhibitors of NOS, NAD(P)H oxidase, xanthine oxidase and mitochondrial electron transport chain.

This study demonstrated that there is enhanced production of $^{\bullet}O_2^-$ in diabetes and this is predominantly mediated by NAD(P)H oxidase. Furthermore, the NOS-mediated component is greater in patients with diabetes than in patients who do not have diabetes (Guzik *et al.,* 2002).The inhibition of intracellular free radical formation would therefore provide a causal therapy approach in the prevention of oxidative stress and related vascular complications in diabetes, Therefore, this study aimed to investigate the ameliorative effect of modified diets on pancreatic oxidative markers in streptozotocin-induced diabetic

Wistar rats following the administration of Alpha lipoic acid.

MATERIALS AND METHODS

Syringes And Needles (Lot: Ww-Ag-13024), Production Date-Oct 2013Expiring Date-Oct 2018, (NAFDAC No-03-0777), Manufactured For Agary Pharmaceutical Limited By Wuxi Yushou Medical Applainces Co.Ltd, Hand Gloves (Lot No 2116), Nafdac No-03-3206, Production Date-04 2015, Expiring Date-04 2018, Manufactured For Longer Life Health Care Ltd, No 5 Udi Street Onitsha Anambra State Nigeria, Incubactor (Model TT 9052), Company Name- Techmel And Techmel Usa., Glucometer Accu Checkwww.acu-check.com, Aucku Check active Strip (Lot No-24640133), Expiring Date-10 2025, Micropipette (Microlux)Vol.Range 0-1000ul, Stop Watch (Taksun: Ts-1809), Stop Timerq.C 2014-0907, Oven (Dhg-9023a), ELISA microplate (HIPO MPP-96, BIOSAN), Centrifuge Model 800 Made In China Zhengji, UV-VIS spectrophotometer (model 752N), Digital electronic weighing balance (Model JA 2003), Plastic cages, Animal cage and plates Sodium citrate (C_3H_4 (OH) $(COONa)_3 2H_2O$) (BDH chemicals LTD Pools England, Batch No. 5425/18/58), Streptozotocin (STZ): Sigma. Aldrich Co.3050 Spruce Street St. Louis Mo U.S.A, Nicotinamide (NAD) Santa Cruz biotechnology St. Dallas USA (Batch number 208096).

Drugs: Alpha lipoic acid (Batch number 539608101). Metformin Glucophage

Experimental Diets: Purchase of Animal Feeds

1. Soya beans were purchased from Dawanau market in Dawakin Tofa Local Government of Kano State, Nigeria.
2. Groundnut and Maize were purchased from the local market in Obiaruku, Ukwani Local Government Area, Delta State, Nigeria.
3. Standard commercial pelleted feed were purchased from Rainbow Top Feed Company, Amukpe roundabout, Sapele, Delta State, Nigeria

Identification: Soya bean, Groundnut and Maize were identified and authentication in the Department of Agronomy, Wildlife and Forestry, Faculty of Agriculture, Delta State University, Abraka, Nigeria.

Composition of Modified Special Diet (MSD): The composition of the various Nutrition Modified Diets (NMDs) formulated in the present study were based on AIN-93G rodent diet composition as recommended by the American Institute of Nutrition and with little modifications as adopted by Ossai *et al.* (2024), and care were taken to ensure that experimental diets have a similar nutrient to calorie ratio, since it has been reported that animals will mostly eat for calories and not weight of food.

The composition of different Nutrition Modify Diets (NMDs) used is illustrated in tables below. The modified diets were fed to the rats for 12 weeks *ad libitum*.

Preparation of Modified Animal Feeds: Soya beans, Groundnut and maize diets were prepared respectively according to the methods described by Aletor (2013). Soya beans, Groundnut and maize respectively were air-dried, milled and sieved to pass through a 0.5mm mesh and were properly stored to avoid contamination from pests and moulds.

Nutritional Composition of Modified Feeds: Eighty grams (80 g) of the milled feeds (Soya bean, Groundnut and Maize) were added respectively to 20 g of the normal commercial animal pellet to make a modified diets of each nutrients composition

Table 1: Composition of Modified Diets

Nutrient	Composition of Feed in 80 (g)			Composition of Normal Animal Meal 20 (g)			Composition of Modified Diets		
	Soya Ben	G. Nut	Maize	Soya Bean	G.Nut	Maize	Soya Bean	G.Nut	Maaie
Fat	19.90	40.10	3.40	4.50	17.00	4.50	24.40	57.10	7.90
Protein	36.50	25.30	9.00	17.00	4.50	11.30	53.50	29.80	20.30
CHO	30.20	19.00	74.50	11.30	11.30	17.0	41.50	30.30	91.50

Experimental Animal: One hundred and sixty (160) Adult male Wistar rats for this study were purchased from the animal house of the Faculty of Basic Medical Sciences, Delta State University, Abraka, Nigeria. They were acclimatized for seven days in a well aerated cages and conditioned in a breeding chamber with the natural controlled system (room temperature and a natural 12 h-12 h light-dark cycle).

Ethical Consideration: The protocol of the experiments in this study were obtained from the Research and Ethics Committee of the Faculty of

Basic Medical Sciences, Delta State University, Abraka, Nigeria (REC/FBMS/DELSU/21/134). This research were performed in accordance with the ethical standards on the care and use of animals as laid down (Helsinki, 1964).

Preparation of Sodium Citrate Buffer: Two grams (2g) of Sodium Citrate were dissolved in 100ml of water to yield 2% of citrate buffer

Preparation of Streptozotocin (STZ): Streptozotocin (STZ) of 0.6g were dissolved in 10ml of citrate buffer to yield 60mg/ml of stock solution (Diabetogenic agent).

Preparation of Nicotinamide solution: Nicotinamide (1000 g) were dissolved in 10 ml of water and were administered orally to the experimental rats in unit 2, 2 and 4 respectively fifteen (15) minutes before induction of Diabetes Mellitus (DM).

Preparation of Metformin Tablet Solution: Five hundred milligrams (500 mg) of metformin (Glucophage) were dissolved in 10ml of distilled water, to give 50mg/ml solution of anti-diabetic drugs.

Preparation of Alpha Lipoic Aicd (ALA) solution: Three hundred grams (300 mg) of alpha lipoic acid (ALA) were dissolved in 50 ml of distil water to yield 6mg/ml of alpha lipoic acid solution

Animal Grouping: The animals were randomly divided into four (4) units of four (4) groups in each unit, with ten (10) animals in each group. Only unit(s) 2, 3 and 4 were induced with diabetes while the unit 1 rats serve as the non-diabetic control unit. The animals were fasted for about 8 – 11 hrs with free access to water prior to the induction of diabetes.

Induction of Type II Diabetes: Rats in Unit(s) 2,3 and 4 received a single intraperitoneal injection of 100 mg/kg of Nicotinamide (Santa Cruz Biotechnology) 15 min before a single intraperitoneal (I.P) injection of freshly prepared solution of streptozotocin (Diabetic agent) were induced on them at a dose of 60 mg/kg according to their body weight.

Confirmation of Diabetes: The development of diabetes were checked with the aid of a glucometer (Accuk-check active, Germany) by measuring blood glucose level after 72hours of STZ injection. Diabetes Mellitus were confirmed by elevated fasting glucose over 200 mg/dL (Wilson *et al.,* 2021; Ossai *et al.*, 2021; Wilson *et al.*, 2022; Ossai and Ojieh, 2023; Ossai *et al.* 2024).

Experimental Design

Unit 1:

Group 1 (n = 6): Non-diabetic Rats feed with the normal chew.

Group 2 (n = 6): Non-diabetic Rats feed with the high fat diet (HFD)

Group 3 (n = 6): Non-diabetic Rats feed with the high protein diet (HPD)

Group 4 (n = 6): Non-diabetic Rats feed with the high carbohydrate diet (HFD)

Unit 2:

Group 1 (n = 6): Diabetic Rats feed with the normal chew.

Group 2 (n = 6): Diabetic Rats feed with the high fat diet (HFD)

Group 3 (n = 6): Diabetic Rats feed with the high protein diet (HPD)

Group 4 (n = 6): Diabetic Rats feed with the high carbohydrate diet (HFD)

Unit 3:

Group 1 (n = 6): Diabetic Rats feed with the normal chew and received 200 mg/kg alpha lipoic acid daily.

Group 2 (n = 6): Diabetic Rats feed with the high fat diet (HFD) and received 200 mg/kg alpha lipoic acid daily

Group 3 (n = 6): Diabetic Rats feed with the high protein diet (HPD) and received 200 mg/kg alpha lipoic acid daily

Group 4 (n = 6): Diabetic Rats feed with the high carbohydrate diet (HCD) and received 200 mg/kg alpha lipoic acid daily

Unit 4:

Group 1 (n = 6): Diabetic Rats feed with the normal chew received 200 mg/kg alpha lipoic acid and 50 mg/kg of metformin.

Group 2 (n = 6): Diabetic Rats feed with the high fat diet (HFD) received 200 mg/kg alpha lipoic acid and 50 mg/kg of metformin,

Group 3 (n = 6): Diabetic Rats feed with the high protein diet (HPD) received 200 mg/kg alpha lipoic acid and 50 mg/kg of metformin,

Group 4 (n = 6): Diabetic Rats feed with the high carbohydrate diet (HCD) received 200 mg/kg alpha lipoic acid and 50 mg/kg of metformin,

Sample Collection: Animals were euthanized by cervical dislocation after an overnight fast. Laparotomy were carried out on each animal to expose the internal organs; blood were collected via cardiac puncture, using 2ml syringes and 23G needle into plain blood sample containers.

Determination of Superoxide Dismutase (SOD): The activity of SOD in the tissue homogenates were estimated spectrophotometrically using the method adopted by Ossai and Ojieh (2023). The assay of SOD is an indirect method based on the inhibitory effect of SOD in the initial rate of epinephrine autooxidation. One unit of SOD was given as the amount of SOD necessary to cause 50% inhibition of the oxidation of epinephrine to adrenochrome during 60 seconds.

The homogenate supernatant (0.2ml) was added to 2.5ml of 0.05M carbonate buffer (pH 10.2) to equilibrate in the spectrophotometer and the reaction started by the addition of 0.3ml freshly prepared epinephrine (0.3mM) as the substrate to the buffer-supernatant mixture and quickly mixed by inversion. The reference cuvette contains 2.5ml of the buffer, 0.3ml of substrate and 0.2ml of deionized water. The increase absorbance at 480nm due to adrenochrome formed was monitored every 30 seconds for 120 seconds. The weight unit as calculated as in equation 1.

$$\text{wet tissue (unit/g)} = \frac{\%\, Inhibition\ x\ 1\ x\ 1000}{X^1\ \ x\ \ \ 50} \quad (1)$$

Where, X^1 = g of tissue in reaction mixture; 1/50 converts to 50% inhibition; 1000 converts to g of wet tissue and Where; 1 unit of SOD activity = Amount of SOD giving 50% inhibition

Determination of Catalase: The activity of catalase were determined in the tissue homogenates by the method adopted by Ossai and Ojieh (2023). In the assay, excess potassium permanganate is added and then residual unreacted permangantate is measured spectrophotometrically. It has been shown that the decomposition of hydrogen peroxide by catalase follows first order kinetics (Haber and Weiss, 1934).

This was carried out by pipetting 1.0ml phosphate buffer into a reference cuvette. Then, 2.0ml of sample was added into the reference cuvette and test cuvette respectively. Enzymatic reaction was initiated by adding 1.0ml of cold 10mM H_2O_2 into the test cuvette and mixing thoroughly. To stop the reaction, 7 ml of 0.1NKMnO4 was added within 30s and thoroughly mixed. The spectrophotometer standard was prepared by adding 7 ml of 0.1 N KMnO4 to a mixture of 5.5 ml of 0.05 N phosphate buffer, pH 7 and 1 ml of 6 N H2SO4. The reaction was carried out in an ice-water bath (0-2°C) and after exactly 3 minutes, the substrate concentration was measured at 240nm. The mathematical determination of the activity was done using the equation 2.

$$K = \frac{S_o}{S_3} x \frac{2.3}{t} \quad (2)$$

Where K = First order rate constant; t = time interval over which the reaction is measured (viz 3 mins); S_o = Substrate concentration at zero time; S_3 = Substrate concentration at 3 minutes

Glutathione (GSH): To estimate glutathione (GSH) activity, the harvested were lysed with stabilizing solution (1:10) and the measurements recorded were as prescribed by Paglia and Valentine (1967) with some modifications made by Flohé and Günzler (1984), which register NADPH + H+ oxidation at 340 nm.

Determination of Lipid Peroxidation: Into 2ml of glacial acetic acid was added 0.2ml of sample followed by 2ml 1% TBA in 0.05M NaOH. The loosely stopped tubes was immersed in boiling water bath for 15 minutes, allowed to cool and centrifuged at 800rpm for 15 minutes. The clear supernatant was carefully transferred into a cuvette and absorbance read at 532nm against a reagent blank. A molar extinction coefficient of 1.56 x 10^5 $m^{-1}cm^{-1}$ was used according to the expression of Adam Vizi and Seregi (1982). MDA was calculated as in equation 3.

$$\text{MDA (unit/g tissue)} = \frac{\Delta A\ x\ V_T}{E\ x\ V_3\ x\ X^1} \quad (3)$$

Where; MDA = Malondialdehyde, one of the final products in polyunsaturated fatty acid peroxidation in the cell; ΔA = Change in absorbance (nm); V_T = Total volume of reaction = 4.2ml; V_s = Volume of sample (0.2ml); X^1 = Weight of tissue in reaction mixture (g); E = Molar absorbance index = 1.56 x $10^5 m^{-1}cm^{-1}$

Statistical Analysis: Data were represented as mean ± Standard Error of Mean (SEM) and analyzed using GraphPad prism version 8.0 (GraphPad Software, San Diego, CA, USA). Comparison of mean differences between groups were performed using two-way analysis of variance (ANOVA), followed by Tukey post hoc test. *p value* ≤ 0.05 were considered statistically significant.

RESULTS AND DISCUSSION

Oxidative stress plays a pivotal role in cellular injury from hyperglycemia (Ossai and Ojieh, 2022; Ossai *et al,* 2023). Hyperglycaemia-induced oxidative stress has been singled out as one of the major links between diabetes and diabetic complications (Forrester *et al.,* 2018; Yuan *et al.,* 2019; Bhatti *et al.,* 2022).

Hyperglycaemia leads to generation of free radicals due to autoxidation of glucose and glycosylation of proteins (Harding *et al.,* 2019; Natrajan, 2021; Arif *et al.,* 2022). The persistent increase in reactive oxygen species (ROS) and reactive nitrogen species (RNS) accompanied by a decrease in antioxidant activity leads to the occurrence of oxidative stress which can cause endothelial dysfunction, insulin resistance, and alterations in number and functions of pancreatic cells and eventually leads to diabetic micro-vascular and macrovascular complications (Einarson *et al.,* 2018; Dal Canto *et al.,* 2019; Forman and Zhang, 2021; Herb *et al.,* 2021; Wang *et al.,* 2021; Tan, Cheong and Cheang, 2022). Once ROS is produced in excess, they cause the structural deterioration of macromolecules (carbohydrates, proteins, lipids, and DNA) leading to their instability and consequently loss of function (Sharifi-Rad *et al.,* 2020; Herb *et al.,* 2021; Bano *et al.,* 2022). ROS have also been reported to induce several cellular signaling cascades that ultimately lead to the transcription of stress-related genes which promote the development of diabetic complications (Martemucci *et al.,* 2022). In recent years, the attention to diet as an essential source of exogenous antioxidants has increased (Najafi *et al.,* 2022). Medicinal plants and food are commonly highly recommended as sources of natural antioxidants to reduce the risk of diabetic complications especially cardiovascular diseases, and their efficacy has been proven in clinical trials (Zhang *et al.,* 2018; Ren *et al.,* 2019; Park *et al.,* 2021; Tan, Cheong and Cheang, 2022). Compared with artificial antioxidants, the antioxidant ingredients in natural plants and food are not a single compound but might work together to achieve antioxidant activity (Tan, Cheong and Cheang, 2022). The demand for natural antioxidants has reduced the use of synthetic antioxidants due to their toxicity, carcinogenicity or hepatotoxicity in the human body (Ojieh, 2020; Martemucci *et al.,* 2022). The dietary antioxidants naturally present in food have aroused considerable interest because of their safety and potential nutritional and therapeutic effects on health (Anachuna *et al.,* 2020; Martemucci *et al.,* 2022). Natural antioxidants can be found in all parts of plants such as fruit, vegetables, nuts, seeds, leaves, roots and bark (Martemucci *et al.,* 2022; Najafi *et al.,* 2022), and people receive antioxidant supplements directly from fresh fruit and vegetables. If antioxidant supplements are consumed in large doses, they can act as pro-oxidants (Martemucci *et al.,* 2022). Compared to supplements, the dietary intake of antioxidants from natural fruit and vegetables may be a safe way to avoid overdosing (Martemucci *et al.,* 2022). Foods rich in micronutrients such as α-tocopherol (vitamin E) and minerals have been considered useful to alleviate ROS-related damage. For example, selenium and zinc interact with GPx and SOD, respectively, to combat oxidative stress (Martemucci *et al.,* 2022). The combination of selenium and vitamin E has shown protective effects against oxidative damage in the colon of rats with ulcerative colitis (Martemucci *et al.,* 2022). Although dietary antioxidants are essential in supplying endogenous antioxidants for the neutralization of oxidative stress, inappropriate use may be detrimental for the physiological scavenging of ROS (Martemucci *et al.,* 2022). In fact, malnutrition and antioxidant deficiency have been related to diseases such as chronic obstructive pulmonary disease (COPD) and Crohn's disease (Martemucci *et al.,* 2022). The lack of antioxidants induced by malnutrition can increase the risk of disease and negative treatment outcomes (Martemucci *et al.,* 2022). Decreased intake or availability of dietary antioxidants such as vitamins C and E, carotenoids and polyphenols can reduce the efficiency of the antioxidant system and aggravate disease progression (Martemucci *et al.,* 2022).

The outcome of this study in figure 1 and 2 showed that in the HFD and HCD of the non-diabetic rats as well as the diabetic rats, pancreatic MDA were raised markedly while SOD, CAT and GSH levels were significantly decreased, however, intervention with HPD, lowered the MDA level while increasing the SOD, CAT and GSH level respectively in the pancreatic tissues.

A significant increase were observed in MDA levels in non-diabetic rats fed with HFD and HCD respectively when compared to the non-diabetic control rats fed with rat chaw, however, non-diabetic rays fed with protein diet showed a significant decrease in MDA activity when compared to the non-diabetic control rats fed with rat chaw. Significant decrease in SOD, CAT and GSH were also observed in non-diabetic rats fed with HFD and HCD respectively when compared to the non-diabetic control rats fed with rat chaw, however, non-diabetic rats fed with HPD showed a significant increase in SOD, CAT as well as GSH levels when compared to the non-diabetic control rats fed with rat chaw.

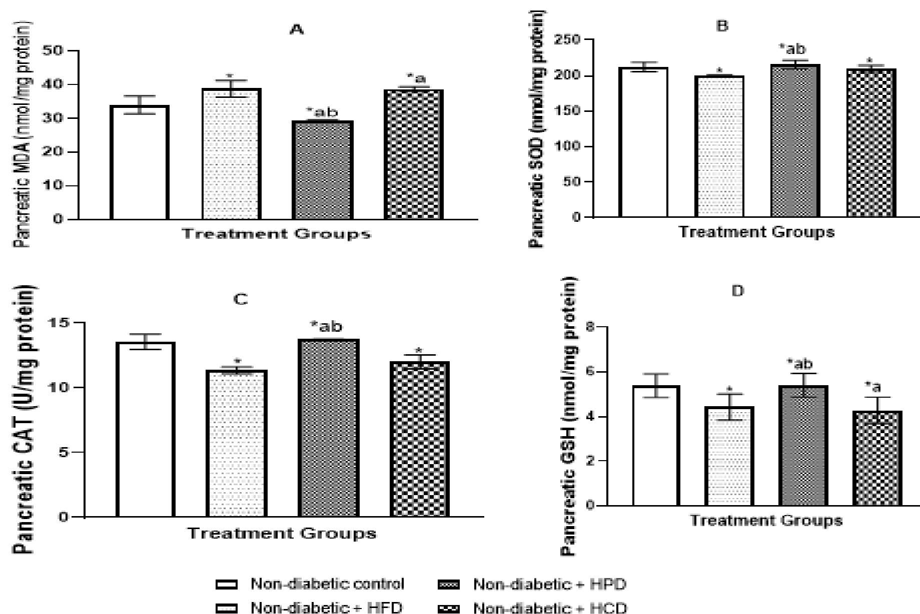

Fig 1: Effect of high fat (HFD), high protein (HPD), and high carbohydrate diet (HCD) on pancreatic (a) MDA, (b) SOD, (c) CAT and (d) GSH levels in non-diabetic male Wistar rats.
Bars represent Mean ± S.E.M. (n = 6) (One-way ANOVA followed by Bonferroni *post hoc* test). $^{*}p < 0.05$, relative to controls; $^{a}p < 0.05$ relative to HFD group $^{b}p < 0.05$, relative to HCD group.

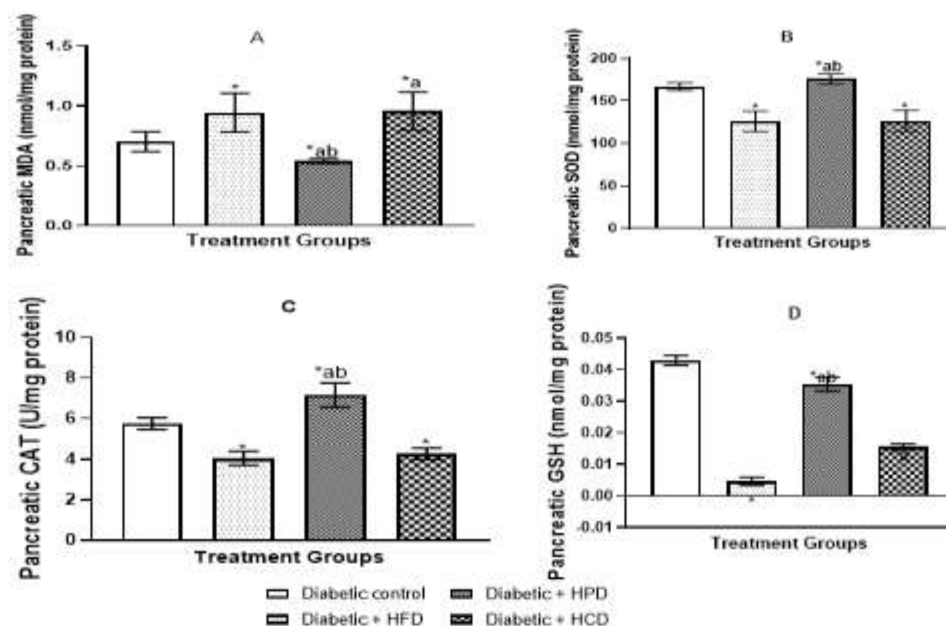

Fig 2: Effect of high fat (HFD), high protein (HPD), and high carbohydrate diet (HCD) on pancreatic (a) MDA, (b) SOD, (c) CAT and (d) GSH levels in streptozotocin-induced diabetes male Wistar rats
Bars represent Mean ± S.E.M. (n = 6) (One-way ANOVA followed by Bonferroni *post hoc* test). $^{*}p < 0.05$, relative to controls; $^{a}p < 0.05$ relative to HFD group $^{b}p < 0.05$, relative to HCD group.

In diabetic rats fed with HFD and HCD respectively, MDA level were significantly increased while SOD, CAT and GSH levels were significantly reduced when compared to the diabetic control rats fed with rat chaw. However, intervention with HPD to the diabetic rats significantly decreases the MDA level while increasing SOD, CAT and GSH level respectively when compared to the diabetic rats fed with rat chaw. However, in figure 3 which is the unit 3 protocol, pancreatic MDA levels were significantly increased whereas, pancreas SOD, CAT and GSH levels were significantly decreased in the diabetic rats fed with HFD + ALA as well as diabetic rats fed with HCD + ALA when compared to diabetic rats fed with rat chaw

+ ALA. Intervention with HPD + ALA significantly decreased pancreatic MDA level while increasing pancreas SOD, CAT and GSH levels in diabetic rats when compared to the diabetic rat fed with HFD + ALA as well as diabetic rats fed with HCD + ALA respectively.

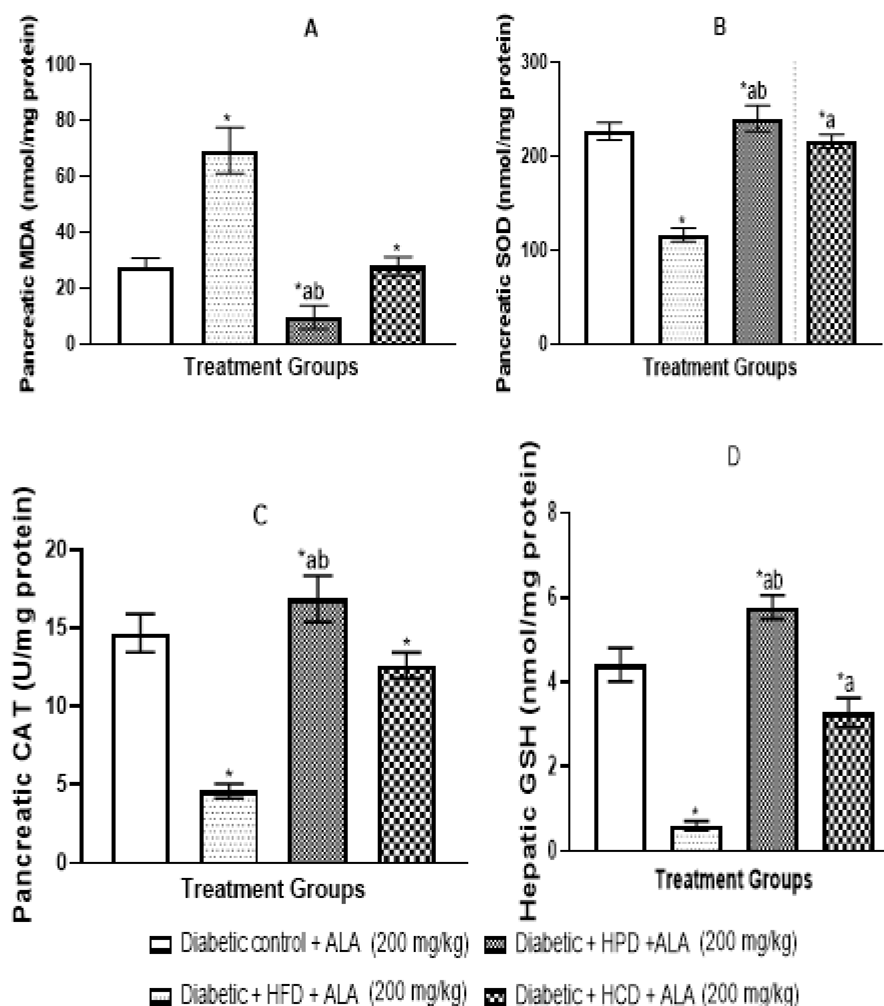

Fig 3: Effect of high fat (HFD), high protein (HPD), and high carbohydrate diet (HCD) and co-treated with alpha lipoic acid (ALA) on pancreatic (a) MDA, (b) SOD, (c) CAT and (d) GSH levels in streptozotocin-induced diabetic male Wistar rats

Bars represent Mean ± S.E.M. (n = 6) (One-way ANOVA followed by Bonferroni *post hoc* test). $^{*}p < 0.05$, relative to controls; $^{a}p < 0.05$ relative to HFD group $^{b}p < 0.05$, relative to HCD group.

In diabetic rats fed with HFD + ALA, MDA level were significantly increased when compared to diabetic rats fed with rat chaw and ALA, however, MDA level in diabetic rats fed with HPD + ALA were significantly decreased when compared to that of the diabetic rats fed with rat chaw and ALA as well as diabetic rats fed with HCD + ALA respectively. But no significant difference in MDA level were observed in diabetic rats fed with HCD + ALA when compared to diabetic rats fed with rat chaw and ALA. Statistically significant increase in SOD, CAT and GSH levels were also observed in diabetic rats fed with HPD + ALA when compared to diabetic rats fed with HFD + ALA, and HCD + ALA respectively. In figure 4 which is the unit four treatment group, Statistically significant increased pancreatic MDA level with decreased pancreatic SOD, CAT and GSH levels were observed in diabetic rats fed with HFD + ALA + MFM as well as HCD + ALA + MFM respectively when compared to diabetic control rats fed with rat chaw, ALA and metformin. Intervention with HPD + ALA + MFM significantly increases the pancreatic SOD, CAT and GSH levels while decreasing pancreatic MDA level in diabetic rats when compared with diabetic rats fed with HFD + ALA + MFM as well as in HCD + ALA + MFM respectively

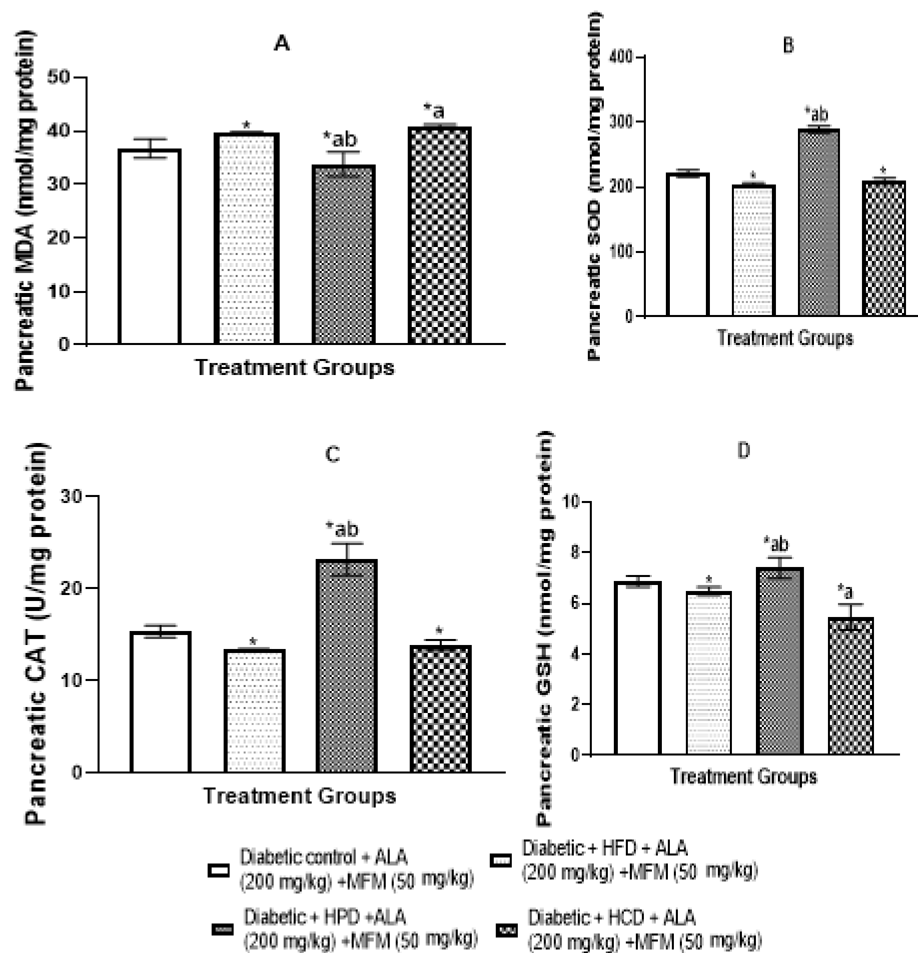

Fig 4: Effect of high fat (HFD), high protein (HPD), and high carbohydrate diet (HCD) and co-treated with alpha lipoic acid (ALA) and Metformin (MFM) on pancreatic (a) MDA, (b) SOD, (c) CAT and (d) GSH levels in streptozotocin-induced diabetic male Wistar rats Bars represent Mean ± S.E.M. (n = 6) (One-way ANOVA followed by Bonferroni *post hoc* test). $^{*}p < 0.05$, relative to Non diabetic ; $^{a}p < 0.001$ relative to Diabetic + HCD group $^{b}p < 0.05$, relative to Diabetic + HCD + ALA group.

Statistically significant increase in MDA level were observed in diabetic rats fed with HFD + ALA + MFM as well as HCD + ALA + MFM respectively when compared to diabetic control rats fed with rat chaw, ALA and metformin. However, a significant decrease in MDA level were observed in diabetic rats fed with HPD + ALA + MFM when compared to diabetic control rats fed with rat chaw, ALA and metformin. Intervention with HPD + ALA + MFM significantly increases the SOD, CAT and GSH levels in diabetic rats, which were significantly decreased in diabetic rats fed with HFD + ALA + MFM as well as in HCD + ALA + MFM respectively when compared to the diabetic rats fed with rat chaw, ALA and metformin. Alpha-lipoic acid (ALA) is thought to be the most successful antioxidant in clinical trials (Bobe *et al.,* 2020; Altunina, Lizogub and Bondarchuk, 2020; Viana *et al.,* 2022; Najafi *et al.,* 2022; Al Mosawi, 2023). It is a natural compound with diverse biochemical functions, had been reported to acts as a metal chelator, regenerates endogenous antioxidants such as vitamins C and E, oxidizes glutathione within the cell (Maciejczyk *et al.,* 2022), and is a modulator of the signalling transduction of several pathways (Capece *et al.,* 2022; Capece *et al.,* 2023). It is the only antioxidant capable of dissolving in both water and fats (Solmonson and DeBerardinis, 2018; Nguyen and Gupta, 2021; Trapali and Fotia, 2022; Viana *et al.,* 2022; Ossai *et al.*, 2024). ALA can be biosynthesized in plants and animals where it is metabolized to dihydrolipoic acid (DHLA) upon uptake into cells (Carli *et al.,* 2021; Al Mosawi, 2023). ALA is also a cofactor for a number of mitochondrial enzymes (Maciejczyk *et al.,* 2022). Lipoic acid are has also been reported to be involved in the termination of the lipid peroxidation process (Maciejczyk *et al.,* 2022), a process which is necessary in the amelioration of diabetic complication as reported also by Kaviyani, Keshavarz, and Abbasi (2020). Many studies have indicated its potential role in the regulation of glucose

metabolism, highlighting its effects on insulin sensitivity, insulin secretion (Capece *et al.,* 2022), the reduction of circulating lipid levels (Ghelani *et al.,* 2017; Capece *et al.,* 2022) and the increase of nitric oxygen (Capece *et al.,* 2022). Further, ALA also seems to play a role in improving peripheral diabetic polyneuropathy (Capece *et al.,* 2022). It is, therefore, widely prescribed in both type 1 and type 2 diabetes (T1D; T2D) (Mezza *et al.,* 2019; Mezza *et al.,* 2020; Capece *et al.,* 2022) diabetic neuropathy and in other insulin resistance conditions such as metabolic syndrome (MS), polycystic ovary syndrome (PCOS) and obesity (Genazzani *et al.,* 2018; Moffa *et al.,* 2019; Capece *et al.,* 2022).

In figure 5, a statistically significant increased pancreatic MDA level with decreased pancreatic SOD, CAT and GSH levels respectively were observed in diabetic rats fed with HPD when compared to the non-diabetic rats fed with HPD, however, intervention with HPD + ALA significantly decreased pancreatic MDA level while increasing pancreatic SOD, CAT and GSH levels. Pancreatic MDA level were further decreased while increasing SOD, CAT and GSH levels in diabetic rats fed with HPD + ALA + MFM when compared to diabetic rats fed with HPD + ALA.

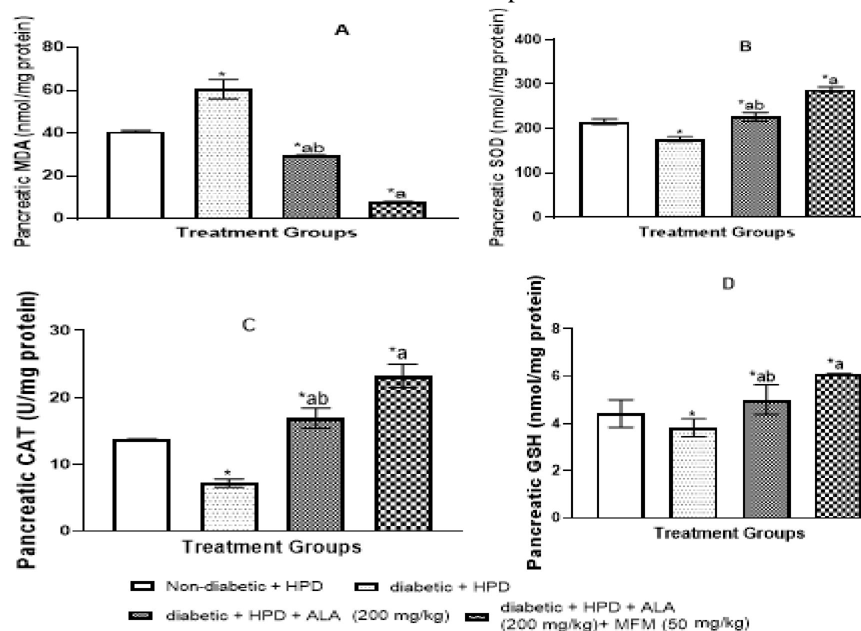

Fig 5: Effect of alpha lipoic acid (ALA) and Metformin (MFM) on pancreatic (a) MDA, (b) SOD, (c) CAT and (d) GSH levels in non-diabetic and streptozotocin-induced diabetic male Wistar rat fed with high protein diet (HPD)
Bars represent Mean ± S.E.M. (n = 6) (Two-way ANOVA followed by Bonferroni *post hoc* test). $^{*}p < 0.05$, relative to Non diabetic ; $^{a}p < 0.001$ relative to Diabetic + HPD group $^{b}p < 0.05$, relative to Diabetic + HPD + ALA group.

A statistically significant increase in MDA were observed in diabetic rats fed with HPD when compared to the non-diabetic rats fed with HPD, however, a significant decrease in MDA levels were observed in diabetic rats fed with HPD + ALA when compared to diabetic rats fed with HPD only. Further decrease in MDA were observed in diabetic rats that received HPD + ALA + MFM when compared to diabetic rats that received HPD + ALA. A significant decrease in pancreatic SOD, CAT and GSH levels were observed in diabetic rats fed with HPD when compared to the non-diabetic rats fed with HPD, however, in diabetic rats fed with HPD + ALA, significant increase in pancreatic SOD, CAT and GSH levels respectively were observed when compared to diabetic rats fed with HPD only. Intervention with HPD + ALA + MFM further increases the activities of SOD, CAT and GSH levels in diabetic rats when compared to the diabetic rats fed with HPD + ALA. Observed result in figure 6 showed that in diabetic rats fed with HFD, a significant increased pancreatic MDA level with decreasing SOD, CAT and GSH levels where observed when compared to non-diabetic rats fed with HFD, however, decreased pancreatic MDA level with increased SOD, CAT and GSH levels were observed in diabetic rats fed with HFD + ALA as well as in diabetic rats that received HFD + ALA + MFM when compared to diabetic rats fed with HFD only.

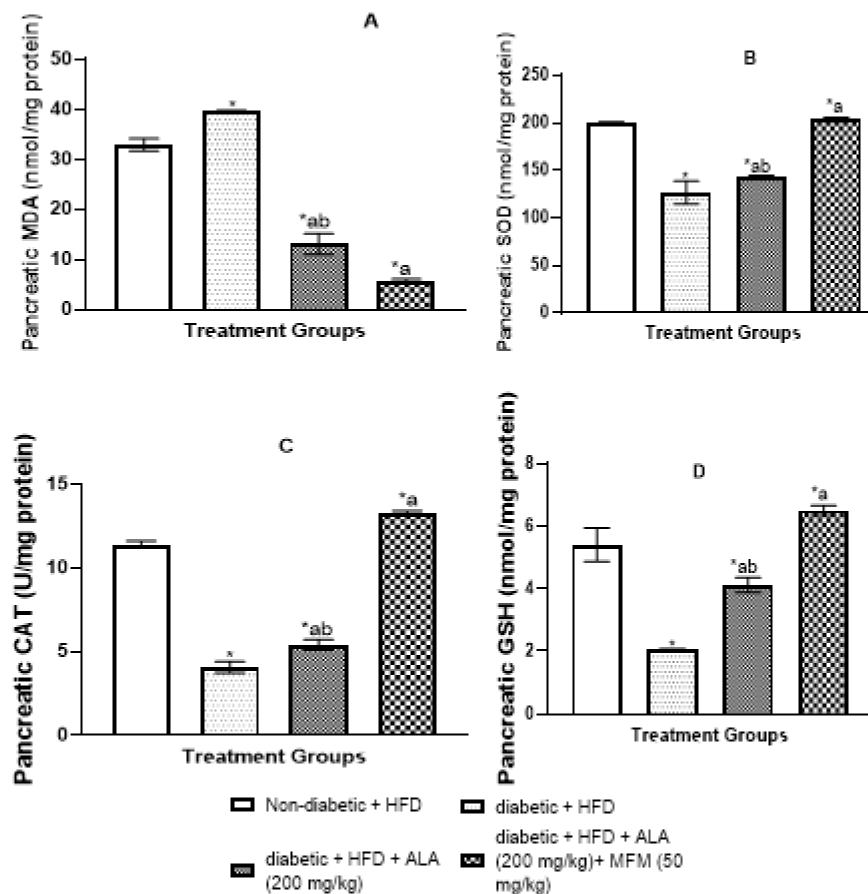

Fig 6: Effect of alpha lipoic acid (ALA) and Metformin (MFM) on pancreatic (a) MDA, (b) SOD, (c) CAT and (d) GSH levels in non-diabetic and streptozotocin-induced diabetic male Wistar rat fed with high fat diet (HFD)
Bars represent Mean ± S.E.M. (n = 6) (Two-way ANOVA followed by Bonferroni *post hoc* test). $^{*}p < 0.05$, relative to Non diabetic ; $^{a}p < 0.001$ relative to Diabetic + HFD group $^{b}p < 0.05$, relative to Diabetic + HFD + ALA group.

In diabetic rats fed with HFD, a significant increase in MDA level were observed when compared to non-diabetic rats fed with HFD, however, MDA level were significantly decreased in diabetic rats fed with HFD + ALA as well as in diabetic rats that received HFD + ALA + MFM when compared to diabetic rats fed with HFD only. Pancreatic SOD, CAT and GSH levels were significantly decreased in diabetic rats fed with HFD only when compared to the non-diabetic rats fed with HFD, however, intervention with HFD + ALA to diabetic rats significantly increases the activities of SOD, CAT and GSH respectively, while HFD + ALA + MFM further cause a significant increase in SOD, CAT and GSH level when compared to diabetic rats fed with HFD + ALA only Also, in figure 7, a statistically significant increase pancreatic MDA level with a decreased SOD, CAT and GSH levels were observed in diabetic group fed with HCD when compared to the non-diabetic group fed with HCD, however, treatment with ALA significantly decreases pancreatic MDA level while increasing SOD, CAT and GSH levels in diabetic rats fed with HCD when compared to diabetic rats fed with HCD only. Further decrease in pancreatic MDA and increased SOD, CAT and GSH levels were observed in diabetic rats fed with fed with HCD + ALA + MFM when compared to diabetic rats fed with HCD + ALA. This result is in agreement with Jimenez-Pulido *et al.* (2022) on the impact of Protein Content on the Antioxidants, Anti-Inflammatory Properties and Glycemic Index of Wheat and Wheat Bran. A report on the role of dietary proteins and amino acids in the pathogenesis of insulin resistance published in Annu Rev Nutr by Tremblay and co. also suggested that modulating dietary proteins and the flux of circulating amino acids generated by their consumption and digestion might underlie powerful new approaches to treat various metabolic diseases such as obesity and diabetes (Ke *et al.,* 2018).

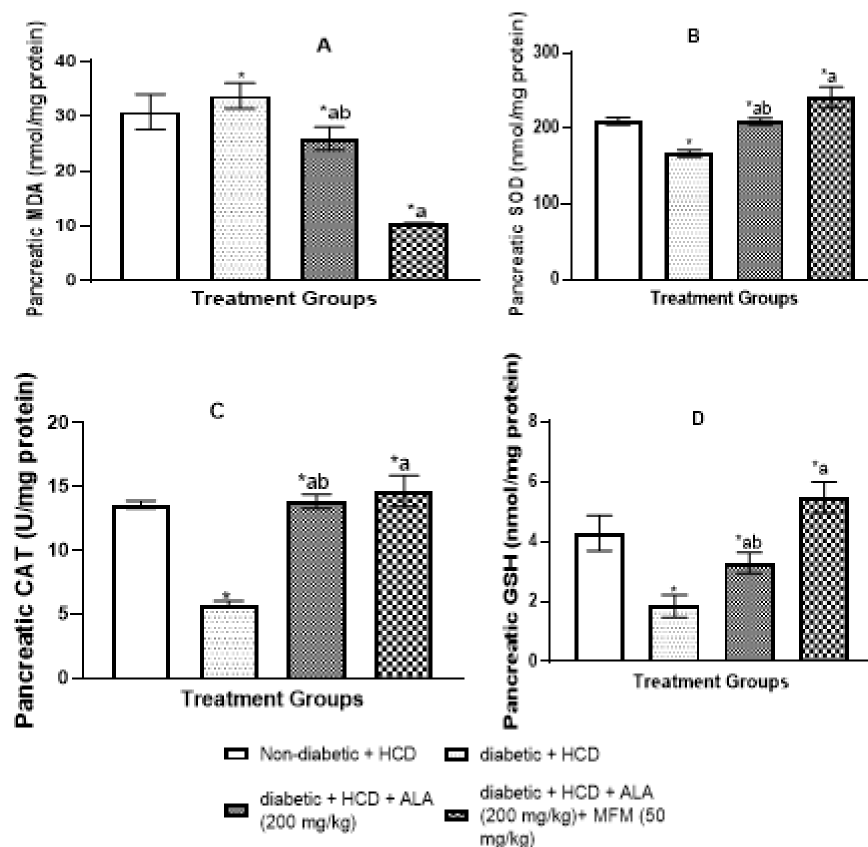

Fig 7: Effect of alpha lipoic acid (ALA) and Metformin (MFM) on pancreatic (a) MDA, (b) SOD, (c) CAT and (d) GSH levels in non-diabetic and streptozotocin-induced diabetic male Wistar rat fed with high carbohydrate diet (HCD)
Bars represent Mean ± S.E.M. (n = 6) (Two-way ANOVA followed by Bonferroni *post hoc* test). $^{*}p < 0.05$, relative to Non diabetic ; $^{a}p < 0.001$ relative to Diabetic + HCD group $^{b}p < 0.05$, relative to Diabetic + HCD + ALA group.

A statistically significant increase in MDA level were observed in diabetic group fed with HCD when compared to the non-diabetic group fed with HCD, however, a significant decrease in MDA level were observed in diabetic rats fed with HCD + ALA when compared to diabetic rats fed with HCD only. Further decrease in MDA were observed in diabetic rats fed with fed with HCD + ALA + MFM when compared to diabetic rats fed with HCD + ALA. The SOD, CAT and GSH level were also significantly decreased in diabetic rats fed with HCD when compared to non-diabetic rats fed with HCD, however, significant increase in SOD, CAT and GSH levels were observed in diabetic rats fed with HCD + ALA when compared to diabetic rats fed with HCD. Further increase in SOD, CAT and GSH levels were observed in diabetic rats fed with HCD + ALA + MFM when compared to diabetic rats fed with HCD + ALA. Several reports claimed that some specialized proteins also function as antioxidants such as peroxiredoxins, thioredoxins, and glutaredoxins (Vanacker *et al.,* 2018; Fernández-Trijueque, Serrato and Sahrawy, 2019; Jacquot and Zaffagnini, 2019; Yu *et al.,* 2022). However, Ke *et al.* (2018) concluded in a similar findings that the association between protein intake and T2DM varies by dietary pattern, hence dietary pattern may be considered into the recommendation of protein intake for diabetes prevention. Dietary proteins and amino acids have been reported as important modulators of glucose homeostasis by promoting insulin resistance and increasing gluconeogenesis (Ke *et al.,* 2018; Ossai *et al.,* 2024). Although high-protein diet has shown beneficial effects on glucose homeostasis in short-term trials (Ke *et al.,* 2018), emerging evidence suggest that protein actions on Type 2 diabetes mellitus incidence may vary by the amino acid types and food sources (Ke *et al.,* 2018). Previous findings from a few long-term epidemiologic studies evaluating food sources of protein reported the conflicting associations of animal and plant protein with risk of T2DM. High total and animal protein intake were associated with a modest elevated risk of T2DM in a large cohort of European adults, but plant protein intake was not associated with T2DM (Ke *et al.,* 2018). Higher intake of animal protein such as red and processed meat has been positively associated

with risk of T2DM, while intake of plant-based sources of protein, such as nuts legumes and soy food, has been associated with a significantly lower risk of T2DM (Ke *et al.,* 2018, Ojieh *et al.,* 2020).

Lipid oxidation in food systems had been reported to lead to undesirable flavors and the formation of toxic compounds (Zhu *et al.,* 2022). In biological systems, however, a clear link has also been established between lipid oxidation products and the etiology of many diseases such as atherosclerosis, Alzheimer's disease, diabetes, and cancer (Zhu *et al.,* 2022). Lipid peroxidation reactions can result from direct oxidation by reactive free radicals or can be triggered by the mediation of redox-active metals (Zhu *et al.,* 2022). Transition metals such as Fe2+ and Cu2+ are pro-oxidants that catalyze the breakdown of hydro-peroxides into free radicals, thus indirectly initiating the oxidative degradation of lipids (Zhu *et al.,* 2022). However, antioxidant can act in two established ways, namely by protecting target lipids from oxidative initiators or by impeding the propagation of chain lipid peroxidation. In the first case, the active peptide inhibits the production of ROS or scavenges the active species that cause oxidative initiation such as O2•- (Nwogueze *et al.,* 2021). In the second case, the antioxidant peptide molecule can intercept lipid peroxyl radicals (LOO•) generated by lipid autoxidation by providing a hydrogen atom, forming a less reactive hydro-peroxide, thus interrupting the chain reaction of lipid radicals (Olatunde and Benjakul, 2018; Nwogueze *et al.,* 2020; Zhu *et al.,* 2022) In addition, the strong emulsifying properties of these antioxidants may give them a unique inhibitory capacity, since this allows them to adsorb well on the surface of the lipid molecules, blocking their contact with oxygen. In experimental models, ALA was reported to decrease lipid peroxidation, reduce oxidative stress, and improve nerve blood flow and distal, sensory, and motor nerve conduction in diabetic animals (Zhou *et al.,* 2022; Capece *et al.,* 2023; Al Mosawi, 2023). The role of ALA in ameliorating the symptoms of DN has been demonstrated in several clinical trials (Zhou *et al.,* 2022; Capece *et al.,* 2023; Al Mosawi, 2023). ALA is known to reduce oxidative stress by inhibiting hexosamine and AGEs pathways (Zhou *et al.,* 2022). In a recent report, ALA 600 SOD (an oral formulation of ALA and superoxide dismutase) improved symptoms and electroneurographic parameters among subjects with DN (Zhou *et al.,* 2022; Capece *et al.,* 2023; Al Mosawi, 2023).

Conclusion: The results of the present study concludes that high protein diets alone and in combination with ALA decreases pancreatic oxidative stress damage caused by streptozotocin toxicity, hence, has the potential to manage diabetes by reducing Reactive oxygen speices (ROS) mediated oxidative stress.

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