International Journal of Healthcare Sciences ISSN 2348-5728 (Online)
Vol. 3, Issue 1, pp: (356-360), Month: April 2015 - September 2015, Available at: www.researchpublish.com

The Effects of *Citrullus lanatus* Seed Extracts on Malondialdehyde and Serum Glucose in Streptozocin Induced Diabetic Rats

[1]Muhammad, Y., [2]Abubakar, N., [3]Musa, M.S., [4]Wali, U., [5]Yeldu, M.H., [6]Ahmed, A.Y., [7]Ngaski, A.A., [8]Ahmad, M.B., [9]Saidu, A.Y, [10]Gulumbe, N.S.

[1,2,3,4,5,6,7] Department of chemical Pathology, Faculty of Medical Laboratory Science, Usmanu Danfodiyo University, Sokoto;
[8]Department of Chemical Pathology, Bayero University, Kano and
[9]Dept. of Biological Sciences, Faculty of Sciences, Federal University Dutse, Jigawa, Nigeria
[10]Dept of Pure and Applied Chemistry, Kebbi State University of Science and Technology, Aliero

Abstract: **The use of natural products as a means of treatments for many physiological threats such as diabetes is currently gaining momentum in Nigeria. Diabetes is a metabolic syndrome that afflicts an estimate of 143 million people and that oxidative stress plays a vital role in development of diabetic and its attendant complications. Malondialdehyde (MDA) a product of lipid peroxidation is a convenient marker for the assessment of oxidative stress.**

Objective: The study was aimed at investigating the hypoglycaemic and antioxidants property of Watermelon (*Citrullus lanatus)* seed extracts on Streptozocin (STZ) induced diabetic rats.

Materials and Methods: A total of twenty five (25) rats were divided into five (5) groups of five (5) rats each; Non-Diabetic Control, Diabetic Control and the Diabetic treated with 50mg, 100mg and 200mg concentration of seed extracts for a period of four weeks. Diabetes was induced by a single intraperitonial injection of freshly prepared STZ (60mg/kg b.w). Fasting blood glucose was monitored on weekly basis using Fine test Auto coding glucometer. On the 29th day of treatment, the rats were sacrificed under Diethyl ether anaesthesia and the blood was collected directly from heart. Fasting blood sugar was measured weekly and after sacrifice in addition to MDA and were statistically analysed..

Results: The results showed that *Citrullus lanatus* seed extract at dose of 200mg can reduce serum glucose significantly (*P<0.05*) from 19.1±3.9 mmol/l (343.5 ± 69.7 mg/dl) to 13.1 ±1.2 mmol/l (235.9±22.4 mg/dl). Serum MDA of Diabetic control (3.3±0.6 µmol/l) was significantly (*P*<0.*05*) higher than that of Diabetes (xx µmol/l) treated with 200mg (2.1±0.4 nmol/dl) of seed extracts. *Citrullus lanatus* seed extract therefore prove to have hypoglycaemic and antioxidant properties.

Keywords: **Diabetes, *Citrullus lanatus*, Glucose, MDA, Oxidative Stress.**

1. INTRODUCTION

Herbal medicine represents one of the most important fields of traditional medicine globally; Different extracts from medicinal plants have been tested and believed to identify the source of therapeutic effects [6]. Diabetes mellitus is derived from Greek word (Diabetes means *siphon* and Mellitus refers to *Swee*t) [21]. Diabetes mellitus is a disease of carbohydrate metabolism characterized by hyperglycaemia as a result of defects in insulin secretion, insulin action or both [17]. The global burden of Diabetes is increasing and its one of the leading cause of morbidity and mortality worldwide [12]. The global prevalence of Diabetes in 2011 was 8% of the total population and the experts predicted by the year 2030 it will rise to 10% [10]. Streptozocin (STZ) is a diabetogen synthesized by streptomycetes achromogenes and is used for

induction of both Type 1 and Type 2 Diabetes. STZ is taken up by pancreatic beta cells via GLUT 2 transporter and this result in changes of DNA in pancreatic B-cells comprising its fragmentation [22]. Action of STZ on B-cells is followed by characteristic alterations in blood insulin and glucose concentration and these changes reflect abnormalities in B-cells function [24]. Relative high cost and side effects associated with the conventional drugs remains one of the problem in the modern medicine and that the management of Diabetes without any side effects is still therefore a major concern [15]. Diabetes mellitus is a metabolic disease of increased free radicals generation [23], Free radicals are molecules of unpaired electrons, highly reactive, unstable, capable of damaging cells, proteins and DNA of biological system [19]. And when the abnormally excessive free radicals attacks lipids, Peroxidation of lipid initiates by abstracting a proton from fatty acid side chains and these results in the formation of degradative end products such as Alkanes, alkenes and aldehydes [4], [8]. Malondialdehyde (MDA) is a low molecular weight reactive aldehydic compound and spontaneous breakdown products of peroxides produced from free radicals action on Polyunsaturated Fatty acids (PUFA) [20]. MDA has been widely used as convenient biomarker of lipid Peroxidation because of its facile reaction with thiobarbituric acid [7]. Evidence is accumulating that oxidative damage to peripheral tissues resulting from the action of reactive oxygen species (ROS) leads to diabetic complications [3]. The current study was conducted to determine whether feeding the experimental rats with a dose of *Citrullus lanatus* (watermelon) seed extract (50, 100, 200mg/Kg) will have an impact on serum MDA and glucose in STZ-Induced diabetic rats.

2. MATERIALS AND METHODS

Experimental Animals:

Albino rats weighing 129-165g obtained from Department of Pharmacology, ABU Zaria were used in this study. The animals were housed in healthy condition at a constant environment with a 12-h light/dark cycle and nutritionally balanced pellets and water *ad libitum* and allowed to acclimatize for a period of two weeks before the commencement of the experiment.

Seed extract Collection and Preparation:

Water melon fruits were obtained from local market in Dutse, Jigawa state. It was dissected into halves, the flesh was removed and seeds collected were washed,dried under shed and milled into a fine powder. Using a weighing balance, powdered extract was weighed into a beaker, soaked in 250ml of absolute ethanol for 48hours. The solution was filtered using filter paper and concentrated at 50^0c with rotary evaporator, then freeze-dried prior to usage.

Induction of Diabetes:

Diabetes was induced by single intraperitonial injection of freshly prepared solution of STZ at a dose of 60mg/kg body weight in 0.1 M citrate buffer, pH 4.5. Diabetic was confirmed by assaying the concentration of 10-hr fasting blood glucose level taken from retro orbitalis plexus. Animal with blood glucose ≥ 15.6 mmol/l (280mgd/l) were considered diabetic and thus included in the study..

Study Design:

The experimental animals were randomly divided into five groups of five rats each (n=5) as follows:

Group A: Non Diabetic Control rats

Group B: Diabetic Control rats;

Group C: Diabetic rats treated with 50mg of seed extract

Group D: Diabetic rats treated with 100mg of seed extract

Group E: Diabetic rat treated with 200mg of seed extract.

The administration of extract was totally by enteral feeding for a period of four weeks. On the 29^{th} day of treatment and following an overnight fast, the animals were sacrificed under Diethyether anaesthesia and blood specimens were collected directly from heart.

Biochemical Analysis:

Serum MDA was estimated as a marker of lipid Peroxidation using the method of Nadigar *et al.* (1986) [14]. The method was based on the principle that acetic acid detaches the lipid and protein of the tissue. Thiobarbituric acid reacts with lipid

peroxide, hydroperoxide and oxygen labile double bond to form the colour adduct with absorption maxima at 530nm, and 0.5ml of serum was mixed with 3.6ml of 10% TCA. This mixture was centrifuged at 5000 rpm for 15minutes and the supernatant collected. Two milliliter (2.0ml) of supernatant was taken and mixed with 1.5ml of 0.67 % Thiobarbituric and 1.0ml of distilled water. This was kept in a boiling water bath for 10mins. After cooling, colour intensity was measured at 530nm wavelength spectrophotometrically. Glucose oxidase method was used for the assessment of serum glucose levels. This was based on the principle that oxygen reacts with 4-aminophenazone to produce a pink colour after conversion of glucose to hydrogen peroxide (H_20_2) and H_20_2 to oxygen by glucose oxidase and peroxidase respectively.

Statistical Analysis:

The data obtained was analysed using Microsoft Office Excel 2007 and Graphpad InStat® statistical soft ware Version 3.10, 32 Bit for windows (2009). Results are expressed as Mean ± SD. Group comparisons were made using one-way analysis of variance (ANOVA), paired comparisons were carried out using the Student's t-test, analysis and p-value of equal to or less than 0.05 ($P \leq 0.05$) was considered as significant.

3. RESULTS

Table 1.0: Serum Levels of Fasting Blood Glucose (Mg/dl) in Rats Prior and After Intraperitoneal Administration of Watermelon Seed Extracts.

Parameter	GROUPS				
	A (n=5)	**B** (n=5)	**C** (n=5)	**D** (n=5)	**E** (n=5)
Baseline	72.6±2.3	72.4±1.7	71.1±2.1	72.4±2.0	72.9±2.6
Induction		353.1±51.0	367.2±40.0	341.9±64.4	343.5±69.7
Week 1	72.4±1.7	372.9±42.2	348.2±47.3	345.7±49.4	314±30.5
Week 2	71.2±1.7	390.6±69.3	382.5±22.8	338.0±30.4	301.4±7.5
Week 3	72.3±2.3	418.0±40.2	389.1±9.8	357.3±56.6	283.5±15.5
Week 4	73.1±1.3	391.1±7.9	384.1±11.6	300.1±3.5	235.9±22.4

Results are Mean ±SD; n=number of rats; Group A=non-Diabetic control; Group B=Diabetic control; Group C= Diabetes+50mg of extract; Group D= Diabetes +100mg of extract and Group E= Diabetes +200mg of extract.

Table 2.0: Serum Malondialdehyde Concentration in Streptozocin Induced Diabetic Rats and Control after 28days of Treatment with watermelon seed extracts

GROUPS	MALONDIALDEHYDE (nmol/dl)
A (Non Diabetic Control)	1.8±0.2
B (Diabetic Control)	3.3±0.6
C (Diabetes+50mg of seed extracts)	3.2±0.4
D (Diabetes+100mg of seed extracts)	2.8±0.8
E (Diabetes+200mg of seed extracts)	2.1±0.4

Results are Mean ±SD; n=number of rats; Group A=non-Diabetic control; Group B=Diabetic control; Group C= Diabetes+50mg of extract; Group D= Diabetes +100mg of extract and Group E= Diabetes +200mg of extract.

4. DISCUSSION

The hypothesis that Diabetes is a degenerative disease of increased oxidative stress leads to the premise that higher intake of antioxidant could help in neutralizing the effects of radicalized molecules which precipitated the diabetic complications [19], [23]. The current paper aimed to reports the hypoglycaemic and antioxidative property of Citrullus lanatus seed extract.

Reduction of serum glucose is the classical and clinical target of any forms of Diabetes and the results of current study clearly indicate that administration of 50mg and 100mg of *C. lanatus* seed extract to diabetic rats doesn't statistically reduce the serum glucose rather a significant reduction ($p<0.05$) was found in Diabetic group treated with 200mg watermelon seed extract at week 2, 3 and 4 (Table 1) when compared with Diabetic control. The results are in agreement with Omigie, 2014 Nassiri *et al.*, (2009) [13], also suggest that the presence of tannins, Saponins and soluble fibre in watermelon may be the contributing factors to this hypoglycaemic effect. The study shows that hyperglycaemia was interrelated to the higher lipid Peroxidation and the lower total antioxidant capacity, this verify the direct relationship between Diabetes and Oxidative stress condition.

Significantly ($p<0.05$) increased of MDA concentrations observed in Diabetic control compared to Non Diabetic control were as a result of oxidative stress. The result of the current study however revealed a statistical increase in serum total antioxidant capability and decrease in MDA levels following an oral administration of 200mg *C lanatus* seed extract for 28days.

Presence of Saponins, Flavanoids, Terpenoids, some basic cardiac glycosides and lycopenes in watermelon has been reported by (Oseni and Okoye, 2013[16] and Ambreen et al., 2014) [1]. Flavanoids are potent super antioxidants and free radical scavengers which mimic the oxidative cell damage [9]. It is further proposed by Mahesh and Menon (2004) [11] that flavanoids causes proliferation of pancreatic β-cells and consequent secretion of more insulin which reduces hyperglycaemia in Diabetic rats treated with *C. lanatus* seed extracts. A vibrant tetrapenic carotenoid Lycopene in watermelon has the potential of preventing oxidative cell damage in diabetes for its singlet oxygen scavenging ability [18].

5. CONCLUSION

In conclusion, administration of 200mg/kg of *Citrullus lanatus* seed extract has Antihyperglycaemic and antioxidative properties on STZ-Induced diabetic rat which can make it an attractive candidate for prophylactic treatment of diabetes, although further investigation is needed to determine toxicity, exact dose and duration of supplements.

REFERENCES

[1] Ambreen, N, Masood, SB, Muhammad T,S, Mir, MNQ, Rani, SN (2014). Watermelon Lycopene and allied health claims.EXCLI Journal (13): 650-666

[2] Arash khaki, Elaleh Ouladsahebmadarek, AfshinZahedi Benham Ahmadnezhad. (2013): Effects of watermelon seeds extracts (C. vulgaris) on sperms in diabetic rats Indonesia J of Pharm.14:2

[3] Baynes JW and Thorpe SR (1999). Role of Oxidative stress in diabetic Complications. Diabetes 48:1-9)

[4] Benedetti, A., Casini, AF, Ferrari, M, Comporti M (1979): Effects of diffusible products of Peroxidation of rats liver microsomal lipids, Biochem J 180(2) 303-312

[5] Benzie, I. (2003): "Evolution dietary antioxidants" comparative Biochemistry and physiology 136(1): 113-126

[6] Cragg GM, Newman DJ, Snader KM (1997). Natural products in drug discovery and development .J. Nat. Prod., 60:52-60)

[7] Esterbauer, H and Cheeseman, K.H (1990); Determination of aldehydic lipid Peroxidation products: Malondialdehyde and 4-hydroxynonenal, Methods in Enzymology,vol 186, pp 407-421)

[8] Esterbauer, H (1982): Free radicals, lipid Peroxidation and cancer, McBrien DCH, Slater, TF (eds) Academic press, London, 101-128

[9] Hour, SS., Ahmed EM and Carter RD. (1980): Concentration of Watermelon juice. J. Food Sci. (45) 718-719

[10] International Diabetes Federation (IDF) Country estimates table 2011. IDF Diabetes atlas. 6th edition 2012. Available from http://www.idf.org/sites/defaull/files/EN_6E_Atlas accessed 7 june 2013

[11] Mahesh T and Menon VP (2004).Quercetin alleviates oxidative stress in Streptozocin-induced diabetic rats. Phytother Res. 18:123-127

[12] Murray CJ, VOST, Lozano R, Naghavi, M, Flaxman AD, Michaud, C,(2012): Disability-adjusted life years` (DALYs) for 291 diseases and injuries in 21 regions, 1990-2010: A systematic analysis for the global burden of disease study 2010. Lancet 380:2197-2223

[13] Nassiri, M., Khaki, A., Gharachurlu, S., Ashteani, A., Rastegarnea, K.,Rezazadeh, S (2009.)Effects of ginger on spermatogenesis in Streptozocin induced Diabetic Rat, Iran. J. Med. Plants, 8(31):118-125

[14] Nadigar MA, Chandrakala MV. (1986). Malondialdehyde levels in different organs of rats subjected to acute alcohol toxicity. Indian jour Clin Biochem 1:133

[15] Nweze EI (2009). Justification for the use of Ocimum gratissimum in herbal medicine and its alteration with disc antibiotics. www.biomedicine.com

[16] Oseni, OA and Okoye, VI (2013): Studies and Antioxidant Properties of the Fruit Watermelon (Citrullus lanatus), J Pharm Biomed Sci (14) 508-414

[17] Orr, DP. (2000). Contemporary management of adolescents with Diabetes mellitus. Part 1: Type1 Diabetes. Adolescents health update 12(2) p3

[18] Sesso HD, Buring JE, Norkus P, Gaziano, JM.(2005). Plasma lycopene, other carotenoids and retinal and risk of cardiovascular diseases in men. Am J Nutr 79:47-53

[19] Sies, H (1997). Oxidative Stress: Oxidants and antioxidants. Exp. Physiol; 822:291-295

[20] Slatter, DA, Bolton, CH, Beiley AJ (2000): The importance of lipid derived Malondialdehyde in Diabetes mellitus. Diabetologia 43:550-557

[21] Vasudevan, DM and SreeKumari,S. (2010). Textbook of Biochemistry for Medical Students. "Regulation of blood sugar; Insulin and Diabetes mellitus." 6th edition.Jaypee brothers Medical publishers, New Delhi.

[22] Yamamato, H, Uchigata, Y, Okamoto, H (1986) Streptozocin and alloxan induced DNA strands breaks poly (ADP-ribose) synthetase in pancreatic islets. Nature 294:284-286

[23] Wali, U, Jogana, MU, Zarummai AL and Saidu, Y. (2011). Antioxidant Vitamins and Trace elements Status of Diabetics in Sokoto, Nigeria. Nig J of Pure and Applied Sci. 19(1): 130-134.

[24] West, E, Simon, OR and Morrison, EY (1996): Streptozocin alters pancreatic beta cells responsiveness to glucose within six hours of injection into rats. West Indian Med J. 45 (60-62)