Antioxidative and antidiabetic activities of watermelon (*Citrullus lanatus*) juice on oxidative stress in alloxan-induced diabetic male Wistar albino rats

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ABSTRACT

Background: The nutritional and medicinal importance of watermelon has been emphasized and its diseases preventive and curative power must be evaluated. Hence, this study was designed to evaluate the antioxidative and antidiabetic potentials of watermelon. Materials and Methods: The in vivo assay was carried out on 15 male albino rats which were divided into groups of three stages. In stage I, all animals received normal feeds and water for 1-week after, which five animals were selected and sacrificed for biochemical analyses which form the nondiabetic control, group. The remaining animals were fasted for 24 h before injected intra-peritoneally with a freshly prepared solution of alloxan at a dosage of 35 mg/kg body weight. Five out of the 10 rats were sacrificed as diabetic group while last five animals were fed with water melon juice for a week after, which they were sacrificed to form the treated group animals. In all the groups, body weights, fasting blood sugar, total protein level in the blood, and other biochemical parameters such as reduced glutathione (GSH), glutathione peroxidase (GPx), malondialdehyde (MDA) concentration; catalase, and superoxide dismutase (SOD) % inhibition activities were determined. Results: The results of the biochemical analyses showed a significant increase in the concentration of blood glucose level after treatment with alloxan, which indicates that diabetic was induced. Hence, watermelon juice caused increased in weight, hypoglycemia; and increases in GSH, GPx, catalase, and SOD % inhibition activities with reduced MDA concentration after treatments. Conclusion: The watermelon juice resulted in the restoration of impaired conditions of the rats.

Key words: Antidiabetic activity, antioxidant enzymes, oxidative stress, scavenging ability, therapeutic agent

INTRODUCTION

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Diabetes mellitus is a group of metabolic diseases characterized by abnormally high levels of plasma glucose or hyperglycemia in the fasting state or after administration of glucose during an oral glucose tolerance test.¹ More than 200 million people have type 2 diabetes; they continue to rise and have reached an epidemic proportion. The total number of people with diabetes is expected to reach 370 million worldwide in 2030.² Hyperglycemia induces glucose oxidation and initiates a nonenzymatic glycation

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of proteins, which in turn leads to enhanced production of reactive oxygen species.³ Herbal medicine provides a valuable therapeutic alternative. Traditional medicine has also been used in the treatment of diabetes in Iran for centuries.⁴ Many traditional plant treatments exist as a hidden wealth of potentially useful natural products for diabetes control.⁵ Despite recommendations by the World Health Organization in 1980, few traditional antidiabetic plants have received scientific or medical scrutiny. Currently, a number of natural products exist that demonstrate hypoglycemic activity. Indeed, depending upon the source that one might use, there are approximately 800-1200 plants that exhibit hypoglycemic activity.⁶

Watermelon (*Citrullus lanatus*, family Cucurbitaceae) is a vine-like (scrambler and trailer) flowering plant originally from Southern Africa. Its fruit, which is also called watermelon, is a special kind referred to by botanists as a pepo, a berry which has a thick rind (exocarp) and fleshy center (mesocarp and endocarp). Pepos are derived from an

inferior ovary and are characteristic of the Cucurbitaceae. The watermelon fruit, loosely considered a type of melon although not in the genus Cucumis, it has a smooth exterior rind (green, yellow, and sometimes white) and a juicy, sweet interior flesh (usually deep red to pink, but sometimes orange, yellow, and even green if not ripe.⁷ Watermelon is thought to have originated in Southern Africa, where it is found growing wild. It reaches maximum genetic diversity there, with sweet, bland, and bitter forms. In the 19th century, Candolle claimed that watermelon was indigenous to tropical Africa. Though Citrullus colocynthis is often considered to be a wild ancestor of watermelon and is now found native in North and West Africa, it has been suggested on the basis of chloroplast DNA investigations that the cultivated and wild watermelon diverged independently from a common ancestor, possibly Citrullus ecirrhosus from Namibia.8

MATERIALS AND METHODS

Sample collection and preparation

Fresh fruits of the watermelon were bought from Mojere market, Ado-Ekiti, Nigeria. The identification and authentication of both plants were carried out at the Department of Plant Science, Ekiti State University, Ado-Ekiti. The fruits were a squeeze and the extract was used in this work.

All chemicals used were of the analytical grade while allglass distilled water used in the analysis. Alloxan used was obtained from Sigma/Aldrich.

Selection of animals and their care

Fifteen matured male Wistar albino rats weighing between 74.59 g and 87.29 g were used for this experiment. The animals were acclimatized for a period of 2 weeks to the laboratory conditions prior to the experiment at the animal house of Department of Biochemistry, Ekiti-State University. Rats were housed in a cage at room temperature with 12 h light and dark cycle with free access to drinking water and rat feed.

Experimental procedure

The animals were categorized into three as follows:

Group 1: Control group

All the animals received normal rat feed and water. After feeding them for a week, five rats were selected to determine their body weights. The animals were subsequently sacrificed to determine other parameters which included fasting blood sugar, blood total protein levels, reduced glutathione (GSH), glutathione peroxidase (GPx), malondialdehyde (MDA); catalase, and superoxide dismutase (SOD) activities from the plasma.

Induction of diabetes

After 1-week of control, the remaining rats were fasted for 24 h before injected intra-peritoneally with a freshly prepared solution of alloxan at a dosage of 35 mg/kg body weight. The animals were considered to be diabetic as the fasting blood sugar level was >115 mg/dL. This dose of alloxan produced type 1 diabetes. Five animals were selected to determine body weights. The animals were subsequently sacrificed to determine other parameters which included fasting blood sugar, blood total protein levels, GSH, glutathione peroxidase (GPx), MDA; catalase, and SOD activities from the plasma which forms the Group 2 diabetic animals.

At the expiration of 1-week of induction of diabetics, the animals were force-fed with the crude extracts of watermelon for a period of 1-week. The body weights of the last five rats were determined. The animals were subsequently sacrificed to determine other parameters which included fasting blood sugar, blood total protein levels, GSH, glutathione peroxidase (GPx), MDA; catalase, and SOD activities from the plasma which forms the Group 3 watermelon treated animals.

BIOCHEMICAL ASSAY

Determination of animals' weight

Individual animal weight was measured using Ohaus top-loading balance.

Determination of whole blood glucose

Glucose concentration in mg/dL was measured in the animal with the aid of On-Call Plus glucometer using compatible glucose test strips according to prescribed instructions.

Determination of plasma malondialdehyde

Lipid peroxidation was determined by measuring the formation of thiobarbituric acid reactive substances according to the method of Varshney and Kale.⁹ An aliquot of 0.4 mL of the plasma or other organ homogenates was mixed with 1.6 mL of Tris-KCl buffer to which 0.5 mL of 30% trichloroacetic acid was added. Then 0.5 mL of 0.75% TBA was added and placed in a water bath for 45 min at 80°C. This was then cooled on ice and centrifuged at 3000 × g. The clear supernatant was collected, and absorbance measured against a reference blank of distilled water at 532 nm. The MDA level was calculated according to the method of Adám-Vizi and Seregi¹⁰ lipid peroxidation in units/mg protein, or gram tissue was computed with a molar extinction coefficient of 1.56×10^5 /M/cm.

MDA (units / mg protein) = (

 $\frac{\text{Absorbance} \times \text{volume of mixture}}{\text{E}_{_{532nm}} \times \text{volume of sample} \times \text{mg protein}}$

Determination of reduced glutathione

The method of Jollow *et al.*¹¹ was followed in estimating the level of GSH 0.2 mL of sample was added to 1.8 mL of distilled water and 3 mL of the precipitating solution was mixed with the sample. The mixture was then allowed to stand for approximately 10 min and then centrifuged at 3000 × g for 5 min. 0.5 mL of the supernatant was added to 4 mL of 0.1 M phosphate buffer. Finally, 0.5 mL of the Ellman's reagent was added. The absorbance of the reaction mixture was read within 30 min of color development at 412 nm against a reagent blank.

Determination of catalase activity

This experiment was carried out using the method described by Sinha.12 0.2 mL of sample was mixed with 0.8 mL distilled H₂O to give 1 in 5 dilution of the sample. The assay mixture contained 2 mL of solution (800 mmol) and 2.5 mL of phosphate buffer in a 10 mL flat bottom flask. 0.5 mL of the properly diluted enzyme preparation was rapidly mixed with the reaction mixture by a gentle swirling motion. The reaction was run at room temperature. A 1.0 mL portion of the reaction mixture was withdrawn and blown into 1 mL dichromate/acetic acid reagent at 60 s intervals. The hydrogen peroxide content of the withdrawn sample was determined by the method described above. The mononuclear velocity constant, K, for the decomposition of H₂O₂ by catalase was determined by using the equation for a first-order reaction: $K = 1/t \log S_0/S$, where S_0 is the initial concentration of H₂O₂ and S is the concentration of the peroxide at t min. The values of the K were plotted against time in minutes and the velocity constant of catalase $K_{(0)}$ at 0 min determined by extrapolation. The catalase contents of the enzyme preparation were expressed in terms of Katalase feiahigkeit or "Kat.f" according to von Euler and Josephson.¹³

Kat. f =
$$\frac{K_{(0)}}{\text{mg protein} / \text{ml}}$$

Determination of glutathione peroxidase

GPx activity was measured using Paglia and Valentine's method. $^{\rm 14}$

The reaction mixture contained 2.6 mL of 100 mmol/L phosphate buffer (pH 7.0) with 3 mmol/L EDTA, 0.05 mL of 10 mg/mL GSH solution, 0.1 mL GR (10 mg/mL), 0.05 mL (10 mg/mL) NADPH-Na salt, 0.1 mL 90 mmol/L hydrogen peroxide solution, and 0.1 mL of sample. The GPx activity was monitored by the decrease in absorbance at 340 nm due to the consumption of NADPH.

Determination of superoxide dismutase

The level of SOD activity was determined by the method of Misra and Fridovich¹⁵ 1 mL of sample was diluted in 9 mL of distilled water to make a 1 in 10 dilution. An aliquot of the diluted sample was added to 2.5 ml of 0.05 M carbonate buffer (pH 10.2) to equilibrate in the spectrophotometer and the reaction started by the addition of 0.3 mL of freshly prepared 0.3 mM adrenaline to the mixture which

was quickly mixed by inversion. The reference cuvette contained 2.5 mL buffer, 0.3 mL of substrate (adrenaline), and 0.2 mL of water. The increase in absorbance at 480 nm was monitored every 30 s for 150 s.

Increase in absorbance per minute =
$$\frac{A_3 - A_0}{2.5}$$

where
$$A_0$$
 = Absorbance at 0 s
 A_3 = Absorbance 150 s

% inhibition
$$\frac{\text{Increase in absorbance for substrate}}{\text{Increase in absorbance of blank}} \times 100$$

One unit of SOD activity was given as the amount of SOD necessary to cause 50% inhibition of the oxidation of adrenaline to adrenochrome during 1 min.

Statistical analysis

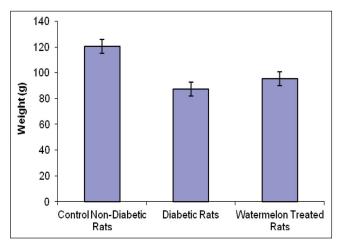
The results obtained were statistically analyzed and reported as mean \pm standard deviation of triplicate data in a bar chart.

RESULTS

The experimental results of the analyses were obtained in triplicates. Various formulae were used to calculate the individual parameter and enzyme activities. The means and standard deviations of the triplicates results were determined which were then used to construct the bar charts.

DISCUSSION

Figure 1 shows the effects of watermelon (*C. lanatus*) juice treatments on the weights of alloxan-induced diabetic rats. It was observed in this study that alloxan-induced diabetic rats had a marked loss in body weight. This would be expected as one of the effects of diabetes is body





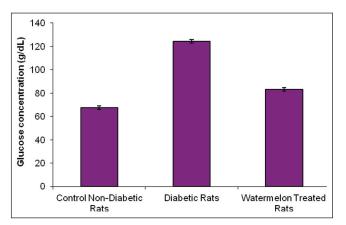
weight loss, an observation which was also seen in Eleazu *et al.*¹⁶ that reported weight loss in diabetic rat before the administration of unripe plantain. With the destruction of the pancreatic cells by alloxan, there is a deficiency of insulin leading to increased synthesis of ketone bodies which are excreted in urine.

The increased in the synthesis of ketone bodies coupled with increased lipolysis leads to a severe body weight loss. However, the diabetic rats treated differently with watermelon (*C. lanatus*) juice had a remarkable gain in body weight.

Figure 2 revealed the effects of watermelon (*C. lanatus*) juice treatments on the blood glucose concentration of alloxan-induced diabetic rats. The concentration of fasting blood glucose was an increase in the alloxan-induced diabetic rats in this study which was in correlation with Edoga *et al.*¹⁷ who also reported increased glucose concentration at diabetics. Alloxan is known to destroy the cells of the pancreas that function in the regulation of insulin secretion and thus leads to an increase in the concentration of blood glucose.

However, the glucose concentration significantly reduced in the diabetic rats after treatment with the juice of watermelon. This is in agreement with earlier works done by Edoga, *et al.*, Jaiswal *et al.*, and Mohammed *et al.*¹⁷⁻¹⁹ who reported hypoglycemic actions in their various works when treated diabetic animals with some plants extracts. Somani and Singhai²⁰ had also reported the hypoglycemic activity of *Myristica fragrans* extract in alloxan-induced diabetic rats.

Figure 3 shows the effects of watermelon juice treatment on the MDA concentration of alloxan-induced diabetic rats. The concentration of plasma malonadildehyde was shown to be significantly increased in diabetic rats when compared with the control, but grossly reduced on treatments with the juice; this is in accordance with Ochuko *et al.*²¹ who reported decreased MDA in brain tissues of diabetic rats by certain plant fiber.





It has also been reported by Ceriello *et al.*²² that diabetic patients show during the postprandial period, an increased plasma MDA levels. However, the diabetic rats, when placed on the watermelon juice had a remarkable decrease in plasma MDA level when compared with the control, thus indicating the free radical scavenging activity of watermelon (*C. lanatus*) juice on oxidative stress in diabetics.

The effects of watermelon (*C. lanatus*) juice treatments on the GSH concentration of alloxan-induced diabetic rat showed a significant reduction in the GSH levels of the diabetic rats when compared with the control as shown in Figure 4. This depletion in blood glutathione concentration is attributing primarily to the alloxan injected in the rats, which act as a xenobiotic and an inducer of diabetes. Both xenobiotics and normal metabolism are known to deplete antioxidants as they are consumed in the course of scavenging reactive species generated.

The reduction in glutathione concentration to the level that was observed, could lead to a devastating decrease in the total antioxidant status of the animals because glutathione helps in recycling cellular antioxidants, inhibits free radical damage, and plays a key role in the detoxification of harmful compounds.²³ The observation in this work, however, agrees with earlier works carried out by Eleazu *et al.*, Domínguez *et al.*, and Polidori *et al.*,^{16,24,25} who reported reduced total plasma antioxidant capacity in uncontrolled diabetes. Watermelon (*C. lanatus*) juice intake by the diabetic rats thus increased their glutathione status to near the control level and this is remarkable as this implies that the diet could have an ameliorating effect on the altered antioxidant status of a diabetic rat.

Figure 5 explained the effects of juice of watermelon (*C. lanatus*) treatment on the glutathione peroxidase (GPx) activity of alloxan-induced diabetic rat. GPx activity of the alloxan-induced diabetic rat showed a decrease when

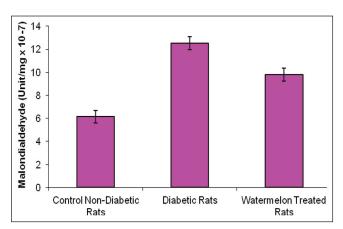
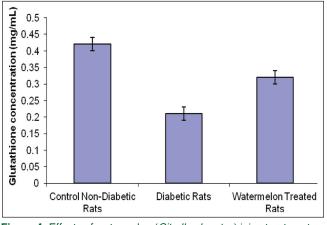


Figure 3: Effects watermelon (*Citrullus lanatus*) juice treatment on the malondialdehyde concentration (malondialdehyde units/mg protein $\times 10^{-7}$) of alloxan-induced diabetic rat

compare with the control animals in the watermelon (*C. lanatus*) juice treated rats. However, the watermelon treated diabetic rats showed an increase in the activity of Gpx status which was almost close to the control level and this is remarkable as this implies that the juice could have an ameliorating effect on the altered antioxidant status of a diabetic.

Figure 6 revealed the effects of watermelon (*C. lanatus*) juice treatment on the catalase activity of alloxan-induced diabetic rat. The reduction in catalase activity after injection with alloxan is another significant finding in this study. The decreased concentration of plasma catalase is attributable in part to the reduced synthesis of this antioxidant enzyme (which functions in the detoxification of hydrogen perioxide) whose concentrations would have fallen with the alloxan that was injected into the animals. Though some studies have reported no alterations in the activity of red cell catalase in diabetics.²⁶ However, this study is in agreement with earlier reports by Ochuko *et al.*, Udoh *et al.*, and Tagami *et al.*^{21,27,28} who reported a





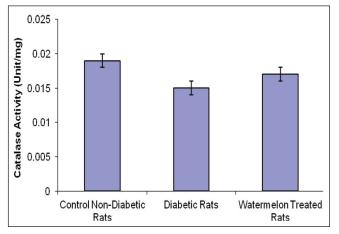


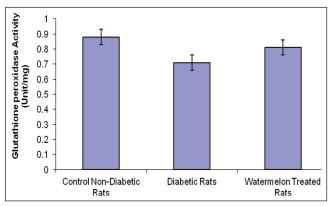
Figure 6: Effects of watermelon (*Citrullus lanatus*) juice treatment on the catalase activity of alloxan-induced diabetic rat

decreased in red blood cell catalase activity in a diabetic. While treatment with watermelon (C. *lanatus*) juice caused a slight increase in catalase activity in their respective groups.

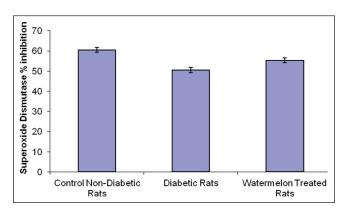
Figure 7 emphasized the effects of watermelon (*C. lanatus*) juice treatments on the SOD % inhibition in alloxaninduced diabetic rats. Similarly, as observed for catalase, there was a slight reduction in the alloxan-induced diabetic rats compared to control stage, but treatment with the juice caused an increase in the SOD percentage inhibition activity.

The ability of any compound to inhibit the formation of superoxide radicals which are toxic species can be viewed as a good index for measuring antioxidant activities.

The observation in this study, however, agrees with earlier work of Oseni and Idowu²⁹ in the study of inhibitory activity of aqueous extracts of *Moringa oleifera* and *M. fragrans* on oxidative stress in alloxan-induced diabetic male Wistar albino rats where it was evidenced that the two extracts played a key role in inhibiting superoxide radicals.









CONCLUSION AND RECOMMENDATION

Alloxan is a potent diabetic agent that induces diabetes by destroying the pancreas cells, with a significant increase in blood glucose level. Direct consumption of alloxan or any foods that contain alloxan may result in glucose metabolism disorder and as such should be avoided. This preliminary study has been able to demonstrate the diabetic effect of alloxan and hypoglycemic potentials of watermelon juice in alloxan-induced diabetic rats. It further shows the benefits of watermelon juice as they significantly reduced the extent of antioxidant loss and restoration of diabetes caused by alloxan in the rat. All the biochemical analysis showed watermelon effectively protected pancreatic cells death. These results suggest that watermelon has a beneficial effect on diabetes. The hypoglycemic potential shown by watermelon might be due to the presence of some bioactive compounds in the plant juice.

Further investigation should be conducted to identify these bioactive compounds present in watermelon juice.

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