



SJMLS-2(4)-2017-008

Effects of Crushed Watermelon Seed on Blood Glucose and Electrolytes Levels in Laboratory Induced Hyperglycaemia

Shingdang, J.*¹, Okunlola, S.¹, Bot, Y.¹, Ekwempu, A.¹

Department of Medical Lab. Science, Faculty of Medical Sciences, University of Jos, Nigeria ¹.

Author for Correspondence: johnshindang@gmail.com +234-810-810-7686.

Abstract

Diabetes mellitus is a non-communicable disease with a high prevalence globally. It is associated with several complications arising from high blood glucose level (Khaki et al., 2013). This study was carried out to investigate the effects of crushed watermelon seed (*Citrullus Lanatus*) on blood glucose and electrolytes profile of alloxan induced hyperglycaemic Wistar rats. The study design was experimental in nature comprising sixteen (16) male Wistar rats grouped into four groups: Group 1, negative control (n=4); group 2, positive control (n=4); group 3, test group (n=4) and group 4, test group (n=4). Animals in group one were administered normal rat feed (vital feed) and tap water. Groups 2, 3 and 4 were made hyperglycaemic by a single dose of intraperitoneal injection of Alloxan monohydrate 180mg/kg body weight. Crushed watermelon seed (5g per 100g body weight) was added to their normal rat feed and administered orally as treatment after 72-hour post-Alloxan injection. Fasting blood glucose and electrolyte parameters were determined weekly for a period of four weeks. After the 4th week of treatment, the rats were sacrificed and blood collected via cardiac puncture into lithium heparin tube and plain tubes for electrolyte and blood glucose estimation. The results obtained for basal fasting blood glucose was 3.2mmol/L for group 1 (negative control), 3.5mmol/L for group 2 (positive control), 3.0mmol/L for group 3 and 4.0mmol/L for group 4 (test groups). After the fourth week of administration of crushed watermelon seeds, these values decreased to 3.0 mmol/L and 3.5mmol/L for group 3 and group 4 respectively. Our work suggests that the glucose concentration decreased

significantly ($p < 0.05$) at week 3 and 4 with no significant decrease in electrolyte parameters. Crushed watermelon seed may be considered as an intervention for hyperglycemic control.

Key Words: Crushed Watermelon Seed, Wistar Rats, Blood Glucose, Electrolytes.

Introduction

The use of natural products for the prevention and treatment of different pathologies is continuously expanding throughout the world (Cragg and Newman, 2013). The use of various herbs in medicine has been reported to be on the increase in many developing and industrialized countries (Ernst and White, 2000). World health Organization, reported that between 65% and 80% of the world's population use herbs as their primary form of healthcare due to their nutritional components (Johnson, 2012).

In line with this, WHO in 1985 directed that developing countries should supplement their health programmes with traditional herbal preparations, provided they are proven to be non-toxic (WHO, 1985). In Nigeria, commonly used medicinal plants include: *Moringa lucida* otherwise known as Oruwo root in Yoruba, *Azadirachta indica* or Dogon yaro leaf in Hausa and *Astonia boonei* or Ahum bark in Igbo, to

which users have testified to their analgesic, anti-inflammatory and antipyretic properties (Olajide *et al.*, 2000). Various researchers have documented the role of herbal medicines in management of both minor and major illness (Gardiner and Riley, 2007). In order to achieve a more effective healthcare programme, the National Agency for Food, Drugs, Administration and Control (NAFDAC) in Nigeria set out in 2012 to document ethno-medicinal preparations (herbal medicine) in the Nigerian market for onward utilization (Awodele *et al.*, 2012).

Indigenous cultures around the world recognize the use of plants as a therapeutic tool in the management of high blood glucose (Ezugwu and Eze, 1998). Several researchers have reported more than four hundred plants used for lowering high blood glucose in Southern Western Nigeria. Some of the plants include: *Cola acumina* stem bark, *Ficus exasperate* leaf and watermelon rind (Ogunleye *et al.*, 2003) to mention a few. With so much attention drawn towards the medicinal benefits of water melon fruit, this work is therefore aimed at investigating the effects of crushed water melon seeds on blood glucose and electrolytes in induced hyperglycemic male Wistar rats.

Materials and Methods

Study Area

This was an experimental quantitative cohort study and included male Wistar rats fed with crushed water melon seeds to determine its effects on blood electrolytes and glucose. This work was carried out in the University of Jos, Plateau State, Nigeria. The water melon fruit (*Citrullus Lanatus*) was obtained from fruit and vegetable market in Farin Gada, Jos, Plateau State, Nigeria.

Research Design

Sixteen male albino Wistar rats of average weight 264.5g were obtained from the animal house of the University of Jos, Plateau state, Nigeria. The animals were maintained under standard laboratory conditions of twelve-hour light-dark cycle (Shindang *et al.*, 2011) for one week to acclimatize, and were fed with food (vital feed) and water. The rats were then divided into 4 groups thus; Group 1 – Negative (non - Hyperglycemic) control(n=4); Group 2 – Positive (Hyperglycemic) control(n=4), Group 3 – Hyperglycemic plus water melon seed(n=4), Group 4 – Hyperglycemic plus water melon seed(n=4).

The water melon fruit (*Citrullus Lanatus*) seed was obtained and treated according to the method previously described by Omiege *et al.*, (2014). After drying, the seeds were crushed into coarse powder using Kenwood blender and kept in a well labeled plastic container.

Prior to the induction, basal glucose and electrolyte levels for all the rats in the groups were determined. The rats were also fed once daily on vital feed and water. Hyperglycaemia was then induced by a single intraperitoneal injection of 180mg per kg body weight of Alloxan monohydrate (Ochuko *et al.*, 2012). Animals were observed for 72 hours. After a 12 hour fast, blood samples were obtained for glucose and electrolyte determination. Consequently, 5g per 100g body weight (Shindang *et al.*, 2011) of crushed seed of water melon was mixed with their meal and fed to animals in group 3 and 4 daily. Animals in group 1 and 2 were fed with normal vital feed. Fasting blood glucose and electrolyte was estimated weekly for three weeks. At the end of the feeding and treatment, rats were fasted over-night and sacrificed. Blood was collected by cardiac puncture into lithium heparin tube



for blood glucose and electrolytes estimation (Na^+ , K^+ , Cl^- and HCO_3^-) at Jos University Teaching Hospital, Nigeria. Glucose and electrolytes were estimated on COBAS C311 automated clinical chemistry analyser.

Statistical Analysis

Data obtained were analysed by SPSS software and presented as means \pm SD and significance determined at 5% level of probability.

Results

The 16 male Wistar albino rats used for the experiment were healthy and active, with an average basal weight and temperature of 239.5g and 32.7°C respectively. The mean basal fasting glucose (mmol/L) for test group 3 was 3.1 ± 0.28 and 4.1 ± 0.53 for test group 4 (Table 1). After hyperglycaemic induction with alloxan, there was significant increase in fasting blood glucose levels in the positive control and test groups with Group 3 having the highest mean of 11.0mmol/L compared to the positive group (group 2) with 10.5mmol/L as shown in

Table 1. One week after treatment with crushed water melon seeds, there was a significant fall in the fasting glucose values to 3.9mmol/L and 3.8mmol/L for rats in group 3 and 4 respectively. This trend continued up to the 4th week (Table1).

The mean basal electrolytes values (Meq/L) was Na^+ (141.1 ± 0.2), K^+ (4.11 ± 0.08), HCO_3^- (98.3 ± 0.42) and Cl^- (98.3 ± 0.42) for test group 3. The values for test group 4 and the two control groups were all within normal limits (Table 2). However, the mean electrolytes values after induction with alloxan were (144.4 ± 0.2) Na^+ , (4.8 ± 0.01) K^+ , (100 ± 0.61) Cl^- and (27.3 ± 1.30) HCO_3^- in the positive control group and a non-significant increase in the test groups at $p > 0.05$ (Table 2). After treatment with crushed watermelon in the test groups, there was a fall in the serum electrolytes levels in the 4th week in comparison with the control groups. However, there was sustained slight increase in the HCO_3^- level from the induction phase to the treatment level. Details are in Table 2.

Table 1. Mean and Standard Deviation of fasting blood glucose for Baseline, Induction and 4 weeks Treatment (mmol/L)

Period	Group1 (Negative Control)	Group2 (Positive Control)	Group 3 (Test group)	Group 4 (Test group)
Baseline	3.2 ± 0.27	3.2 ± 0.23	3.1 ± 0.28	4.1 ± 0.53
Induction	*	10.5 ± 0.3**	11.0 ± 1.34	10.0 ± 7.2
Week 1			3.9 ± 0.68	3.8 ± 0.57
Week 2			3.4 ± 0.64	3.7 ± 0.48
Week 3			3.3 ± 0.68	3.7 ± 0.44
Week 4			3.0 ± 0.72	3.5 ± 0.71

Key:

* Not induced

**Induced

Table 2. Mean ± Standard Deviation for Baseline, Induction and Treatment Values for Serum Electrolytes (Meq/L).

Parameter	Group 1	Group 2	Group 3	Group 4	p-value
Baseline					
Sodium	143.5 ± 0.19	141.0 ± 0.89	141.1 ± 0.00	141.3 ± 0.2	p>0.05
Potassium	4.4 ± 0.48	4.2 ± 0.11	4.11 ± 0.08	4.1 ± 0.06	
Bicarbonate	23.8 ± 0.85	23.8 ± 1.31	20.6 ± 0.48	23.5 ± 1.0	
Chloride	99.1 ± 0.39	96.5 ± 1.54	98.3 ± 0.42	97.0 ± 0.47	
Induction					
Sodium		144.4 ± 0.72	144.5 ± 2.29	144.6 ± 2.04	0.0505
Potassium		4.8 ± 0.01	4.8 ± 0.01	4.8 ± 0.28	0.0093
Bicarbonate		27.3 ± 1.30	26.8 ± 0.50	30.1 ± 0.079	0.0001
Chloride		100.1 ± 0.69	99.8 ± 0.60	100.0 ± 0.47	0.0330
Treatment					
Sodium			142.8 ± 0.31	141.3 ± 0.50	0.5262
Potassium			4.2 ± 0.58	4.3 ± 0.17	0.8206
Bicarbonate			29.7 ± 1.20	26.3 ± 0.88	0.0002
Chloride			98.3 ± 3.14	98.3 ± 0.55	0.7168

Discussion

Male Wistar rats were used for this experiment because of their physiological and biochemical similarity to humans (Wang *et al.*,2012). Consequently, they may have lower insulin resistance than females (Murphy *et al.*, 2004;

Hoffman *et al.*, 2000). There was successful induction of hyperglycaemia after alloxan administration at the dose of 5g/100g body weight of rats in the control and test groups: 10.5 ± 0.3, 11.0 ±1.34 and 10.0±0.72 mmol/L respectively. Omigie *et al.* (2014) and Ezeigbo



et al. (2016) reported a similar success at induction of hyperglycaemia in Wistar rats. After administration of crushed water melon seed at 5g/100g body weight in the normal rat feed in the treatment group (3 and 4) there was dramatic fall in the glucose value from week 1 to week 4 (Table 1). This finding was consistent with that reported by Omigie *et al.* (2014) who administered methanolic extract of water melon seeds in female Wistar rats.

In addition, this finding also revealed that the active ingredient, which are flavonoids, are present in the crushed water melon seed as well, even though not as high as would be in a concentrated extract. These, along with the carbohydrates in the crushed water melon seed, could have been responsible for inducing the release of insulin from the pancreas of the rats leading to lowering of the blood glucose (Nasiri, *et al.*, 2009). The mean glucose concentration at induction (Mmol/L) in group 2, 3, and 4 was 10.5 ± 0.3 , 11.0 ± 1.34 and 10.0 ± 0.72 respectively. This finding was at variance with that reported by Chirinos *et al.* (2013) who after alloxan induction reported an unusual rise in basal glucose level of 29.6 ± 22 mmol/L. This could be associated with differences in the strength of the alloxan used.

The basal electrolyte values were all within normal reference limits (Anonymous, 1982). This report showed that the animals were healthy. However, after induction, there was a non-significant increase in the K^+ , Na^+ and Cl^- ions when compared to the baseline values. In addition, while there was a non-significant decrease in the Na^+ , K^+ and Cl^- ions, there was an initial increase in the HCO_3^- at induction which dropped after treatment in group 4 but was increased in group 3. This difference has not been reported by other researchers who all reported that the electrolyte levels were un

affected by hyperglycaemia. Our finding is therefore significant as the HCO_3^- level could be due to the generation of CO_2 in the liver from metabolic activity (Robert and Joseph, 1999). This finding will require further work to create a better understanding regarding the interaction between crushed water melon seeds and serum bicarbonate concentrations. Szkudelski, (2001), reported Alloxan response in three phases: initial hyperglycaemia lasting about 2 hours, probably due to hepatic glycogenolysis; transient hyperglycaemia, at approximately 6 hours due to the output of insulin from damaged cells and finally hyperglycaemia permanent, noticeable at 12 hours. This process may have been responsible for an increase in CO_2 and thus carbonic acid which is unstable leading to the formation of H^+ ions and bicarbonate. However, after completing 4 weeks of administration of crushed water melon seed, it was observed that the effect of this seed was more intense at the 1st week and 4th week of treatment than in the 2nd and 3rd week. The reason for this variation may be that crushed *Citrullus lanatus* seeds induce hypoglycaemia by triggering insulin release at the onset of hyperglycaemia as seen in the first week (Omigie *et al.*, 2014).

Our work suggests that the effect on the electrolytes could have been caused by the crushed water melon seed.

In conclusion, this study suggests that crushed water melon seed may be considered for use as an agent for regulating blood glucose and electrolyte levels. However, more work might be needed to ascertain the role of electrolytes in regulating hyperglycaemia.

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Citation: Shindang, J., Okunlola, S., Bot, Y., Ekwempu, A. *Effects of Crushed Watermelon Seed on Blood Glucose and Electrolytes Levels in Laboratory Induced Hyperglycaemia. Sokoto Journal of Medical Laboratory Science*;2(4): 63 – 69.

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