

Anti-diabetic and lipidemic effect of muskmelon fruits and seeds on streptozocin induced diabetic rat.

Abstract

Background: The increasing prevalence of diabetes mellitus Worldwide and its complex nature of predisposing one to different ailment like obesity, eye defect, cardiovascular diseases etc. calls for alternative measures in the management of the disease. Muskmelon (*Cucumis melo L.*) is an underutilized food with low glycemic index which are used traditionally in the management of different ailment like diabetes mellitus.

Objective: The aim of this study was to evaluate the anti-diabetic and anti-lipidemic effect of muskmelon fruits and seeds in streptozotocin induced-diabetic rats.

Method: Diabetes was induced using intravenous Streptozotocin at a dose of 42 mg/kg of body weight into the tail veins to group 2-5. Twenty-five male albino rats were divided into 5 groups, group 1 normal control, group 2- diabetic control, group 3- Glucophage treatment, group 4- 500mg/kg bw muskmelon fruits extract and group 5- 500mg/kg bw muskmelon seeds extract and were treated for 2 weeks. Fasting blood glucose, body weight, triglyceride, LDL, HDL and total cholesterol were evaluated.

Result: The result shows that the extract caused a significant increase in the body weight, HDL-cholesterol and a significant decrease in triglyceride, LDL-cholesterol, total cholesterol and fasting blood glucose.

Conclusion: The result proved the anti-diabetic and anti-lipidemic effect of muskmelon fruits and seeds which could be added to the pool of other food used in the management of diabetes mellitus which will lead to diet diversification.

Keywords: Muskmelon. Fruits, seeds, lipid profile, diabetes mellitus.

1. Introduction

Diabetes Mellitus is a metabolic disorder that affects human life both socially and economically. It is a complex and multifarious group of disorders characterized by hyperglycemia, and alterations in lipid and protein metabolism due to an absolute or relative lack of insulin. It is characterized by increased blood glucose levels resulting from the effects in insulin action [1]. It is a public health challenge that leads to serious consequences, causing damage to the body organs, tissues, heart, kidneys, eyes and nerves. Diabetes mellitus is considered as one of the most five leading causes of death Worldwide [2]. It is a syndrome of disordered metabolism, usually due to a combination of hereditary, environmental causes, change in lifestyle, which result in abnormal high blood sugar levels (hyperglycemia) [3]. The causes of diabetes mellitus and obesity are multifactorial and predispose an individual to different ailments that linger over a long period of time.

The World Health Organization opined that the prevention of diabetes and its complication is of great importance in the actualization of health for all [4,5]. Some plants have showed anti-diabetic activity during a trial study, these plants include *Panax species*, *Phyllanthus species*, *Acacia Arabica*, *Aloe vera*, *Trigonella fonum-graecum* etc [6]. Medicinal plants and its products continue to be an alternative therapeutic aid for alleviating the ailments of human kind [7,8]. The use of herbs and other dietary supplements as alternatives has been an aged long practice to mainstream western medical treatment.

There is high prevalence of Diabetes Mellitus Worldwide especially in developing countries such as Nigeria. The increasing cost of living in such countries, including high cost of medical services made most people to lack access to regular medical care. An insulin dependent patient on a minimum wage could spend 50% of the monthly income on insulin. There are various medicinal plants with bioactive compound such as muskmelon fruits and seeds which will serves as an alternative method in the control and treatment of diabetes.

Muskmelon (*Cucumis melo L.*) is a beautiful, juicy, and delicious fruit popular for its nutritive and medicinal properties [9]. It belongs to the family *Cucurbitaceae*, and consists of succulent stem with numerous seeds [10]. The *Curcurbitaceae* family includes squash, pumpkins, cucumbers, Musk melons, watermelons, and gourds. *Cucumis melo L* is one of the most important cultivated cucurbits, which is native to India and Africa [9]. It is a spreading, annually, more or less hairy vine. It grows well in all the tropical and subtropical areas of the world, but prefers a hot climate. Muskmelon is recommended for the treatment of cardiovascular disorders, as a diuretic, stomachic, antitussive and as a vermifuge. Muskmelon is enormously good for health as it is rich in ascorbic acid, carotene, folic acid, and potassium as well as a number of other human health bioactive compounds [11]. Its seeds are a rich natural source of polyunsaturated fatty acids, proteins, phytosterols, vitamins, trace elements (zinc) and antioxidant compounds [12]. This study was designed to determine the effect of muskmelon seed and fruits extract on the glucose and lipid profile of wistar rats.

2. METHODS

2.1 Procurement of raw materials

The muskmelon fruits (*cucumis melo L.*) that was used for the study was purchased from Ogbete main market, Holy Ghost, Enugu, Enugu State. Streptozotocin and other chemicals that was used for the study was purchased from a local chemical store at Enugu. Glucophage tablets was purchased from a local pharmacy store at Enugu.

2.2 Processing of raw materials

The fruits were cleaned well with water, sliced open, removed the seeds, the fruits were sliced into smaller pieces, de-hulled the seeds and oven dried at 40°C. After complete drying, the fruits and the dried seeds were blended using attrition mill. The muskmelon fruits and seeds flour were stored differently at 4°C in the freezer for further use.

2.3 Preparation of musk melon extract

Methanolic extraction was done using a modified method of Bhandari and Kawabata [13]. Five hundred grams (500g) of muskmelon fruits and seeds flour were soaked in 200 ml of methanol and kept overnight. The suspension was filtered through Whatman No.1 filter paper, and the filtrate was diluted to make up to 100ml with methanol. Sample solutions were stored at 4°C in amber bottles and served as the stock solution for subsequent analysis.

2.4 Design of Study

The diabetic studies was carried out using the Completely Randomized Design (CRD). Rats was randomly assigned to the treatments based on their weights. There were five treatments each replicated five times. The rats were the replicates while the different diets were the treatments.

2.5 Animal experiment

2.5.1 Animal housing

Twenty-five adult male Albino rats were purchased from the same colony, which weighed between 151-153g at the Faculty of Veterinary Medicine, University of Nigeria Nsukka, Nigeria. The rats were housed individually in metabolic cages built to separate faeces and urine. The rats had exactly 12 h of light and 12 h of darkness in a day. The experiment was performed in compliance with the National Research council guidelines on the care and use of laboratory animals [14]. The Animal Experimentation Ethics Committee of University of Nigeria Nsukka, approved the study.

2.5.2 Induction of diabetes

The rats were grouped into 5. They were fed with standard rat chow for 7 days. After a 7-day acclimatization period, the rats were weighed prior to grouping such that each group will be $\leq 5g$ more than each other [15]. Animals were fasted for 12 h and diabetes was induced using intravenous Streptozotocin at a dose of 42 mg/kg of body weight into the tail veins to group 2-5. Group one were fed rat chow alone throughout the experiment without inducing diabetes. Group two received rat chow alone, group three received rat chow and Glucophage tablets, group four received rat chow and 500mg/kg body weight of muskmelon fruits extract and group 5 received 500mg/kg body weight of muskmelon seeds extract. The diets were formulated using AIN-93G (American Institute of Nutrition) method [16]. Only animals that showed clinical signs of severe diabetes and fasting glucose ≥ 126 mg/dL in two successive determinations were used for the study. The body weight, water intake, food intake, urine output, blood glucose, hemoglobin, total cholesterol, triglycerides, and high and low density lipoproteins were analyzed. Some parameters were obtained using individual metabolic cages while blood glucose tests were performed using evolve glucometer. Analytical tests were done on day 7, 14, 21 and 28.

Table 1: Diet Composition

Groups	Group 1	Group 2	Group 3	Group 4	Group 5
Diets/Treatment	Normal control	Diabetic control.	Treated with Glucophage tablet.	Treated with 500mg muskmelon fruits extract	Treated with 500mg muskmelon seeds extract.

2.5.3 Blood collection

Blood was collected from the ocular vein and used for the laboratory analysis. Total cholesterol assay was performed following a colorimetric enzymatic method described by Trinder using Dialab kit [17]. The HDL-c assay was determined using the method described by Wiebe *et al.* [18] with Inmesco kit. The triglycerides level was determined by enzymatic colorimetric method described by Cole *et al.* [19] using Dialab kit. The LDL-c level was deduced from the other lipids previously obtained using the formula described by Richmond [20].

3.5.4 Determination of Blood Glucose of Rat Sample

The blood glucose was determined using EVOLVE[®] Diabetes Monitoring kit. Blood glucose level by inserting the code key of the glucometer into the code key opening and a test strip inserted to make sure that the code on the glucometer matches the code on the test strip. The thumb finger is puncture with the kit needle, a drop of blood placed on the test strip, it was allowed to stand for 2 minutes and the reading was taken in g/dl.

Statistical Analysis

The data generated was subjected to Analysis of Variance (ANOVA) using Statistical Package for Social Science (SPSS version 20) to detect significant differences among the sample ($p < 0.05$). Significance means was separated using Turkey's Least Significant Difference (TLSD) test.

3.0 RESULTS

Table 1: Effect of muskmelon fruits extract on the body weight of Rats (g)

Day	GP1	GP2	GP3	GP4	GP5
7	151.30 ^a ± 1.34	151.90 ^a ±0.34	152.80 ^a ±0.34	154.08 ^a ±0.76	153.28 ^a ±0.43
14	151.75 ^a ± 0.89	120.64 ^b ±0.48	122.43 ^b ±1.45	122.11 ^b ±0.83	121.01 ^b ±0.50
21	152.30 ^a ±0.93	120.20 ^e ±1.03	145.27 ^b ±0.44	140.13 ^c ±0.42	134.22 ^d ±0.10
28	152.48 ^a ± 0.56	121.45 ^d ± 0.91	150.62 ^a ±0.33	141.45 ^b ±0.62	136.17 ^c ±0.29

Table 2: Effect of muskmelon fruits extract on the fasting blood glucose of Rats (mg/dl)

Day	GP1	GP2	GP3	GP4	GP5
14	108.72 ^b ±0.54	183.01 ^a ±0.30	184.60 ^a ±0.56	183.70 ^a ±0.85	182.50 ^a ±0.52
21	109.24 ^e ±0.93	189.83 ^a ± 0.51	126.20 ^d ±0.13	139.10 ^c ±0.67	157.13 ^b ±0.33
28	108.10 ^e ±0.35	188.36 ^a ±0.20	122.13 ^d ±0.84	132.98 ^c ±0.27	146.25 ^b ±0.12

Table 3: Effect of muskmelon fruits extract on the total cholesterol of Rats (mg/dl)

Day	GP1	GP2	GP3	GP4	GP 5
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14	32.53 ^b ±0.09	44.56 ^a ±0.21	45.08 ^a ±0.84	43.90 ^a ±0.70	44.96 ^a ±0.20
21	30.27 ^c ±0.72	44.68 ^a ±0.30	34.48 ^d ±0.92	37.46 ^c ±0.14	40.12 ^b ±0.10
28	31.90 ^e ±0.83	44.23 ^a ±0.89	33.02 ^d ±0.40	35.89 ^c ±0.26	38.43 ^b ±0.12

Table 4: Effect of muskmelon fruits extract on the triglyceride of Rats(mg/dl)

Day	GP1	GP2	GP3	GP4	GP 5
14	21.60 ^b ±0.27	34.40 ^a ±0.86	34.94 ^a ±0.93	34.62 ^a ±0.07	34.62 ^a ±0.12
21	22.14 ^c ±0.68	34.80 ^a ±0.51	25.16 ^d ±0.16	27.80 ^c ±0.42	30.46 ^b ±0.08
28	23.08 ^c ±0.43	35.98 ^a ±0.23	22.28 ^d ±0.90	24.30 ^c ±0.0.25	25.28 ^b ±0.11

Table 5: Effect of muskmelon fruits extract on the HDL of Rats (mg/dl)

Day	GP1	GP2	GP3	GP4	GP5
14	12.65 ^a ±0.96	10.22 ^b ±0.22	9.93 ^b ±0.22	10.36 ^b ±0.45	10.42 ^b ±0.13
21	13.07 ^a ±0.67	10.17 ^c ±0.64	11.69 ^b ±0.64	10.90 ^c ±0.19	10.26 ^d ±0.36
28	12.70 ^a ±0.19	9.16 ^c ±0.33	12.20 ^b ±0.33	11.93 ^c ±0.55	10.58 ^d ±0.22

Table 6: Effect of muskmelon fruits extract on the LDL of Rats (mg/dl)

Day	GP1	GP2	GP3	GP4	GP5
14	16.44 ^b ±0.58	26.74 ^a ±0.22	26.93 ^a ±0.37	27.02 ^a ±0.12	26.92 ^a ±0.46
21	15.96 ^c ±0.13	27.30 ^a ±0.11	18.20 ^d ±0.20	21.41 ^c ±0.69	24.10 ^b ±0.38
28	16.37 ^c ±0.95	26.94 ^a ±0.07	16.71 ^d ±0.10	18.10 ^c ±0.34	22.74 ^b ±0.15

RESULTS

BODY WEGHT: Table 1 shows the mean body weight of the rats. On day 7 which was the day after acclimatization, the mean body weight of rats ranged between 151.03-154.80g. The mean body weight of rats on day 14 which was the day diabetics was confirmed were in the range of 120.64-151.75g. The mean body weight of rat on day 21 which was the day for the first test of recovery were in the range of 120.20-152.30g. On day 28 which was the day for the last test of

recovery the mean body weight of the rats was between 121.45-152.48g. The results show that there is a decrease in the body weight of the rats from 151.03-154.80g to 120.64-151.75g on day 14 when diabetics was confirmed. There was an increase in the mean body weight of rats in group 3 to 5 as was observed from the results of day 14 and day 21. The mean body weight increased from 121.01-122.43 to 134.22-145.27.

FASTING BLOOD GLUCOSE: Table 2 shows the mean fasting blood glucose of the rats. The mean fasting blood glucose of rats in group 1 which were the positive control without diabetics ranged between 108.10-109.24mg/dl. The mean blood glucose of the rats on day 14 which was the day diabetics was confirmed were in the range of 182.50-184.60mg/dl for group 2 to group 5. The mean blood glucose of rat on day 21 which was the day for the first test of recovery were in the range of 126.20-157.13mg/dl for group 3 to group 5. On day 28 which was the day for the last test of recovery the mean fasting blood glucose of the rats was between 122.13-146.25mg/dl for group 3 to group 5. The results show that there is a decrease in the fasting blood glucose of the rats from 182.50-184.60mg/dl on day 14 when diabetics was confirmed to 122.13-146.25mg/dl on day 21 after the first test of recovery for group 3 to group 5. There was also decrease in the mean fasting blood glucose of the rats from 182.50-184.60mg/dl on day 14 when diabetics was confirmed to 33.02- 38.43122.13-146.25mg/dl on day 21 after the last test of recovery for group 3 to group 5. The mean fasting blood glucose of rats in group 2 which was the diabetic rats with no treatment was between 183.01-189.83mg/dl.

Total cholesterol: Table 3 shows the mean Total cholesterol of the rats. The mean Total cholesterol of rats in group 1 which were the positive control without diabetics ranged between 30.27- 32.53mg/dl. The mean blood glucose of the rats on day 14 which was the day diabetics was confirmed were in the range of 43.90-45.08mg/dl for group 2 to group 5. The mean blood glucose of rat on day 21 which was the day for the first test of recovery were in the range of 34.48-40.12mg/dl for group 3 to group 5. On day 28 which was the day for the last test of recovery the mean Total cholesterol of the rats was between 33.02-38.43mg/dl for group 3 to group 5. The results show that there is a decrease in the fasting Total cholesterol of the rats from 43.90-45.08mg/dl on day 14 when diabetics was confirmed to 34.48-40.12mg/dl on day 21 after the first test of recovery for group 3 to group 5. There was also decrease in the mean Total cholesterol of the rats from 43.90-45.08mg/dl on day 2 when diabetics was confirmed to 33.02-

38.43mg/dl on day 28 after the last test of recovery for group 3 to group 5. The mean Total cholesterol of rats in group 2 which was the diabetic rats with no treatment was between 44.23-44.68mg/dl.

Triglyceride: Table 4 shows the mean triglyceride of the rats. The mean triglyceride of rats in group 1 which were the positive control without diabetics ranged between 21.60-23.08mg/dl. The mean triglyceride of the rats on day 14 which was the day diabetics was confirmed were in the range of 34.40-34.94mg/dl for group 2 to group 5. The mean triglyceride of rat on day 21 which was the day for the first test of recovery were in the range of 25.16-30.46mg/dl for group 3 to group 5. On day 28 which was the day for the last test of recovery the mean triglyceride of the rats was between 22.28-25.28mg/dl for group 3 to group 5. The results show that there is a decrease in the triglycerides of the rats from 34.40-34.94mg/dl on day 14 when diabetics was confirmed to 25.16-30.46mg/dl on day 21 after the first test of recovery for group 3 to group 5. There was also decrease in the mean triglyceride of the rats from 34.40-34.94mg/dl on day 14 when diabetics was confirmed to 22.28-25.28mg/dl on day 28 after the last test of recovery for group 3 to group 5. The mean triglyceride of rats in group 2 which was the diabetic rats with no treatment was between 34.40-35.98mg/dl.

HDL: Table 5 shows the mean HDL of the rats. The mean HDL of rats in group 1 which were the positive control without diabetics ranged between 12.65-13.07mg/dl. The mean HDL of the rats on day 14 which was the day diabetics was confirmed were in the range of 9.93-10.42mg/dl for group 2 to group 5. The mean HDL of rat on day 21 which was the day for the first test of recovery were in the range of 10.26-11.69mg/dl for group 3 to group 5. On day 28 which was the day for the last test of recovery the mean HDL of the rats was between 10.58-12.20mg/dl for group 3 to group 5. The results show that there is an increase in the HDL of the rats from 9.93-10.42mg/dl on day 14 when diabetics was confirmed to 10.26-11.69mg/dl on day 21 after the first test of recovery for group 3 to group 5. There was also an increase in the mean HDL of the rats from 9.93-10.42mg/dl on day 14 when diabetics was confirmed to 10.58-12.20mg/dl on day 28 after the last test of recovery for group 3 to group 5. The mean HDL of rats in group 2 which was the diabetic rats with no treatment was between 9.16-10.22mg/dl.

LDL: Table 6 shows the mean LDL of the rats. The mean LDL of rats in group 1 which were the positive control without diabetics ranged between 15.96-16.44mg/dl. The mean LDL of the rats on day 14 which was the day diabetics was confirmed were in the range of 26.74-27.02mg/dl for group 2 to group 5. The mean LDL of rat on day 21 which was the day for the first test of recovery were in the range of 18.20-24.10mg/dl for group 3 to group 5. On day 28 which was the day for the last test of recovery the mean LDL of the rats was between 16.71-22.74mg/dl for group 3 to group 5. The results show that there is a decrease in the triglycerides of the rats from 26.74-27.02mg/dl on day 14 when diabetics was confirmed to 18.20-24.10mg/dl on day 21 after the first test of recovery for group 3 to group 5. There was also decrease in the mean LDL of the rats from 18.20-24.10mg/dl on day 14 when diabetics was confirmed to 22.28-25.28mg/dl on day 28 after the last test of recovery for group 3 to group 5. The mean triglyceride of rats in group 2 which was the diabetic rats with no treatment was between 16.71-22.74mg/dl.

DISCUSSION

In the recent time, there has been a high prevalence of diabetes mellitus all over the World but the situation is more worrisome in developing Nation like Nigeria where there is little or no quality health care services. Insulin has been the most reliable therapy but often time are not accessible due to economic status of the people and this calls for an alternative that can be accessible and affordable by the users. Most plants foods contain some bioactive substance with antidiabetic effect. This study was conducted to investigate the antilipidemic and antidiabetic effect of muskmelon fruits and seed extract (500mg/kg) on *Streptozotocin*-induced rats.

There was a decrease in the body weight of the rats after injecting the rats with *Streptozotocin*. This was as a result of the destruction of the pancreatic islet β -cell by the antibiotic *Streptozotocin* which led to hyperglycemia in the rats. These also resulted in the decrease in endogenous insulin release, which affect the utilization of glucose by the tissues. The finding is in line with some previous works which observed that Sulfonylureas drugs are known to increase the blood glucose level by stimulating β cells to release insulin [21;22; 23;24]. Farida and Shouky [25] noted that the main characteristics of type 1 diabetes is severe loss of body weight, which could be attributed to muscle atrophy. The result of group 2 which was fed with rat chow and water alone after inducing diabetics tends to be decreasing and not capable of recovering

from the weight loss thereby prove that the gain in weight as seen in group 4 and 5 was as a result of the muskmelon fruits and seed extract.

There was an increase in the mean body weight of rats in group 4 and 5 as was observed from the results of day 2 and day 9. The mean body weight increased from 121.01-122.43 to 134.22-145.27 which could be attributed to bioactive substances present in the extract. Oliveria et al. [26]; Miaffo [24] observed that chemical compounds like glycosides, saponins, phenols, alkaloids and flavonoids are responsible for the hypoglycemic, lipid-lowering effects observed in their work.

Although there was significant difference between group 3 and group 4 and 5 at $p < 0.05$ but the increase observed in group 4 and 5 which were fed with muskmelon fruits and seeds extract shows that the extract had the potential to replace loss weight as it can be deduce from the results.

The extract of muskmelon fruits and seeds significantly decreased the blood glucose level. The result is in line with previous study that stated that some plant extract has the capacity to mimic the action of insulin and stimulate its secretion by the β -cells of the islets of Langerhans [27, 28]. This could also be attributed to the stimulation of glucose uptake by peripheral tissues, the inhibition of endogenous glucose production and the activation of gluconeogenesis in the liver and muscles [29].

The extract of muskmelon fruits and seeds had a significant decrease in some lipid profiles like glyceride, low density protein, and total cholesterol level in the rats and at the same time increased the high density lipoprotein cholesterol level. This could be as a result of increased utilization of glucose which led to the inhibition of lipid peroxidation and control of lipolytic hormones. Studies shows that some plants have anti-hyperlipidemic effects [30]. Studies had also shown a strong association between hyperlipidemia and diabetes mellitus. Akpan et al. [31] stated that hyperlipidemia occur in diabetes- induced rat as a result of excess mobilization of fat from the adipose tissue due to the underutilization of glucose. Rajaei et al [32] observed that when an animal is in diabetic state some hormones sensitive lipase has an increased ability to break down stored triglycerides into fatty acids resulting in a larger quantity being discharged into circulation. This subsequently promotes the conversion of excess fatty acids into phospholipids and cholesterol in the liver. The phospholipids and cholesterol coupled with the

excess triglyceride formed in the liver may be discharged into the blood in the form of lipoproteins. There is inactivation of lipoprotein lipase enzyme that are responsible for the hydrolysis of triglycerides [33]. Murali et al. [34] opined that hypercholesterolemia develops in the diabetic state because insulin has an inhibitory action on β -hydroxy- β -methylglutaryl-Coenzyme-A (HMG-CoA) reductase, an enzyme responsible for the metabolism of cholesterol-rich LDL particles.

Conclusion

The present study revealed that muskmelon fruits and seeds extract possess hypoglycemic, and hypolipidemic properties. This was demonstrated in their ability to decrease the blood glucose, total cholesterol, low density lipoprotein cholesterol and increased the high density lipoprotein cholesterol in streptozotocin induced- diabetic rats hence they are recommended for people with diabetes mellitus challenge.

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Plate 1 and 2: Musk melon seed and fruit



Plate 3: De-hulled muskmelon seed.

UNDER PEER REVIEW