European Journal of Medicinal Plants

26(4): 1-6, 2018; Article no.EJMP.45794
ISSN: 2231-0894, NLM ID: 101583475

The Effect of Ethanolic Seed Extract of *Citrullus lanatus* (Watermelon) on Blood Glucose Level and Lipid Profile of Diabetic Wistar Rats

Deborah Francis[1], Celestine Ani[2*], Choice Nworgu[1], Okorie Pamela[1], Igwe Uzoma[3], Jide Uzoigwe[1], Princewill Ugwu[1] Pamela Anyaeji[1] and Nwachukwu Daniel[1]

[1]Department of Physiology, College of Medicine, University of Nigeria, Enugu Campus, Nigeria.
[2]Department of Human Physiology, College of Medicine, Enugu State University of Science & Technology, Parklane, Enugu, Nigeria.
[3]Department of Physiology, Faculty of Basic Medical Sciences, Alex Ekwueme Federal University, Ndufu-Alike, Ebonyi State, Nigeria.

Authors' contributions

This work was carried out in collaboration between all authors. All authors read and approved the final manuscript.

Article Information

Editor(s):
(1) Dr. K. Himakar Reddy, Department of Advanced Research Center, Narayana Medical College &Hospital, India.
(2) Prof. Marcello Iriti, Professor of Plant Biology and Pathology Department of Agricultural and Environmental Sciences Milan State University, Italy.
Reviewers:
(1) Dr. Nahida Tabassum, University of Kashmir, Srinagar, India.
(2) Dr. Dennis, Amaechi, Veritas University, Abuja, Nigeria.
(3) E. Siva Rami Reddy, Tantia University, India.
Complete Peer review History: http://www.sdiarticle3.com/review-history/45794

Original Research Article

Received 21 September 2018
Accepted 08 December 2018
Published 09 March 2019

ABSTRACT

Aims: This study investigated the effect of ethanolic seed extract of *Citrullus lanatus* (ESECL) on blood glucose level and lipid profile in Alloxan-induced diabetic male Wistar rats.
Study Design and Methodology: 30 male adult wistar rats were grouped randomly into six experimental groups of five rats each. Diabetes was induced by intraperitoneal injection of 100 mg/kg of alloxan monohydrate, dissolved in normal saline, while the normal control group (group 1) was given the vehicle only. Three days after induction of diabetes, were treated further for four weeks with ESECL at doses of 100, 200 and 400 mg/kg for groups 4, 5, and 6 respectively.

**Corresponding author: E-mail: anicelestine2006@gmail.com;*

Animals in Group 3 were treated with Glibenclamide (2.5 mg/kg), while group 2 served as the negative control group.
Results: Administration of ESECL caused significant decrease in blood glucose levels in groups 4, 5 and 6 compared to rats of group 2 (p=0.00125). There was also significant decrease in the levels of cholesterol (TC) , triglycerides (TG), low density lipoprotein (LDL), with increase in high density lipoprotein (HDL) in Groups 4, 5 and 6, compared to Group 2 rats (p=0.00125). The results obtained from the rats of the group receiving extract were similar to that obtained from Groups 3.
Conclusion: Thus, this study suggests that this extract could possibly normalize abnormalities in blood glucose levels and lipid profiles in diabetic conditions in a dose dependent manner.

Keywords: Diabetes; Citrullus lanatus; fasting sugar level; hypolipidaemic and phytochemical.

1. INTRODUCTION

Drugs made from natural herbs, rinds and seeds from fruits, and stems, have been recognized to be less toxic and free from adverse effects than synthetic types in the management of diabetes and this has become necessary to search for more economic and effective treatment of diabetes by Noor et al. [1]. Fruits have also been recognized as natural source of various bioactive compounds [2] which could be attributed to their phytochemical constituents [3]. One of such fruit is *C. lanatus*. Studies have shown that *C. lanatus* contains various amounts of vitamins, minerals, and also serves as good source of antioxidants that help combat formation of free radicals [4]. There is paucity of information on the effect of the seed extract of *C. lanatus* on blood glucose level and lipid profile in diabetic conditions in our environment. The seed of *C. lanatus* is often discarded as waste but proximate analysis shows that it possesses phytochemical such as phenols, flavonoids, and alkaloids [5]. The need to search for non pharmacological antidiabetic agents that are cheap and affordable with little or no side effects necessitated this study. to investigate the effect of seed extract of *C. lanatus* on blood glucose level (bgl), total cholesterol (tc), triglycerides (tg), high density lipoprotein (hdl), and low density lipoprotein (ldl).

2. MATERIALS AND METHODS

2.1 Plant Collection, Identification and Authentification

Fresh fruits of *C. lanatus* were obtained from Ogbete main market in Enugu State, Nigeria. Sample of the seeds from the fruit was identified and authenticated at the herbarium section of the Department of Plant Science and Biotechnology, University of Nigeria Nsukka, Nigeria. A voucher specimen was deposited in the herbarium for future reference with no (UNH 851b).

2.2 Preparation of Aqueous Extract of *C. lanatus* Seed

$$\text{Yield (\%)} = (g)\ \frac{\text{Weight of extract}}{\textit{weight of the plant material}} \times \frac{100}{1}$$

The method of extraction employed was according to Trease and Evans [6] The fruits of *C. lanatus* were cut into halves and the seeds removed from the pulp, washed and dried under room temperature, then pulverized into coarse powder using a Herbal Medicine Pulverizer (Model 220v UPS), then stored at -2˚C until needed. 1114 g of the dried powder was macerated with 600 ml of 98% ethanol (analytical grade) for 48 hours and vortex vigorously every 3 hrs. The mixture was filtered through Whatman No 1 filter paper and concentrated using a vacuum rotary evaporator to obtain 65.559 g weight of extract. The extractive yield was calculated using the relation to obtain 5.8%. 4 g of the extract was later reconstituted in 20ml of distilled water to obtain stock solution of 200 mg/ml.

2.3 Phytochemical Analysis

Analysis for the presence of phytochemical was determined using standard methods as described by many researcher [7,8]

2.4 Acute Toxicity Studies

This was determined using Lorke's method [9]. A total of 21 mice of 3 in each group were used. They were fasted overnight (8-12 hrs) then orally fed with the extract in increasing dose of 500 mg/kg, 1000 mg/kg, 2000 mg/kg, and 3000 mg/kg body weight to four groups of three mice each. The general behavior and signs of toxicity of the animals were observed continuously for 24 hours thereafter 48 hours following extract administration. No sign of toxicity was recorded even at the dose of 3000mg/kg. Doses of 100, 200 and 400 mg/kg of extract were selected for

the study. The general behavior and signs of toxicity of the animals were observed continuously for 24 hours thereafter, for 48 hours following extract administration. No signs of toxicity were recorded even at the dose of 3000 mg/kg. Doses of 100 mg/kg, 200 mg/kg and 400 mg/kg of extract were selected for the study.

2.5 Experimental Procedure

A total of 45 male Wistar rats weighing 100-200g were obtained from the Animal House Unit, Faculty of Basic Medical Sciences, University of Nigeria, Enugu campus. The rats were housed in standard cages with 12 hrs dark/light cycle allowed to acclimatized for 14 days during which they were provided with normal rat chow (Vital feeds ltd Jos, Plateau State) and drinking water *ad libitum*.

2.6 Induction of Diabetes

After an overnight fast, diabetes was induced in all the rats by intraperitoneal injection of 500mg of alloxan monohydrate dissolved in 5ml of normal saline at a dose of 100 mg/kg of body weight to get a concentration of 100 mg/ml [10]). After 72 hours of induction, the blood glucose level of the rats were monitored using glucose strips with Accu-Answer ® ZH-G01 glucometer Guilin Zhonghui Technology Co Ltd China and the rats with a blood sugar level >200 mg/dl were classified as diabetic and used for the study [10]. While non- diabetic ones were selected at random to form the control group. Blood glucose level were checked weekly during 28 days of experimental period.

2.7 Experimental Design

The experimental animals were divided into 6 groups of five rats (n =5) as follows:

Group 1: Served as the normal control and received only feed and water *ad libitium*.
Group 2: Diabetic untreated (Negative control)
Group 3: Diabetic + 2.5mg/kg Glibenclamide.
Group 4: Diabetic + 100 mg/kg ESECL.
Group 5: Diabetic + 200 mg/kg ESECL
Group 6: Diabetic + 400 mg/kg ESECL

At the end of the experimental period (28 DAYS), fasted rats were under chloroform anaesthesia. Blood samples were carefully obtained via retro-orbital puncture using a plain capillary tube.Glucose levels were checked using Accu - Answer ® ZH-G01 glucometer Guilin Zhonghui Technology Co Ltd China.

2.8 Method of Lipid Profile Analysis

The lipid profiles (TC, TG, HDL) of the rats were analyzed using the method of Siyem et al. [11] where a handheld 3-in-1 Combo Test Device for a complete lipid panel (Mission cholesterol meter) Model CCM-111 produced by ACON laboratories (Hanghzhou) Co. Ltd China was used .The blood samples were allowed to stand for 15 minutes to clot and then centrifuged at 6000 rev/min for 5 minutes. 35ul of Serum was collected with Pasteur pipette and introduced into the cholesterol test strips after inserting the MEMO clip and allowed for 60 seconds for the reading to be displayed. The LDL-C was calculated from the values obtained from the meter using the Fredwald's equation.

2.9 Statistical Analysis

All results were expressed as the Mean±SEM. The results were analyzed for statistical significance using statistical package for social science (SPSS) version 21.One-way analysis of variance (ANOVA) was used to compare differences between means of different groups. Value of $P < 0.05$ was considered significant.

3. RESULTS

3.1 Phytochemical Analysis of *Citrullus lanatus* Seed Extract

Different doses of the extract were administered, the extract was found to significantly reduce the blood glucose level of Wistar rats in Groups 4, 5, and 6 ($P= 0.01$) when compared with group 1 and group 2. The standard drug, glibenclamide administered to animals of group 3 was found to have the same potency with the extract at 400mg/kg. The effect of the ESECL on lipid profile is shown in Table 3. Different doses of the extract were administered caused a significant reduction in the values of total cholesterol, triglyceride and low density lipoprotein in Groups 4, 5 and 6 as compared to Group 2 and 1. At the end of drug treatment, the extract also caused a significant elevation in the values of high density lipoprotein in Groups 4, 5 and 6, an effect similar to that of glibenclamide seen in Group 3.

Table 1. Qualitative and quantitative analysis of seed extract shows the following constituents

Constituents	Qualitative	Quantitative (mg/100 g)
Citrulline	+++	7.22
Flavonoids	++	3.03
Phenols	+	3.20
Saponin	++	2.41
Tannin	+	2.10
Alkaloid	+++	4.22

+= Trace, ++ = moderate, +++ high concentration

Table 2. Effect of the ethanolic seed extract of *Citrullus lanatus* on blood glucose levels during four week study

Groups	Diabetic stage	Week 1	Week 2	Week 3	Week 4
1		104.00±2.74	112.25±4.32	118.00±7.07	113.50±5.80
2	223.60±14.20	224.00±6.32$^{\alpha}$	226.20±42.60$^{\alpha}$	227.60±43.78$^{\alpha}$	229.00±30.89$^{\alpha}$
3	227.60±90.31	220.20±122$^{\alpha}$	214.20±73.96$^{\alpha}$	161.00±62.22$^{\alpha\beta}$	129.80±55.57$^{\beta}$
4	225.40±66.90	222.80±55.54$^{\alpha\beta}$	217.40±41.44$^{\alpha}$	214.40±47.62$^{\alpha}$	173.00±32.99$^{\alpha}$
5	224.60±88.64$^{\beta}$	218.40±66.34$^{\alpha\beta}$	206.80±46.61$^{\alpha}$	183.80±4.76$^{\alpha}$	154.80±73.21$^{\beta}$
6	226.00±105$^{\beta}$	202.75±152$^{\alpha\beta}$	198.75±119$^{\alpha}$	175.00±75.95$^{\alpha}$	125.00±8.45$^{\beta}$

Results are expressed as Mean±SEM; (P<0.05). Results are expressed as Mean±SEM; $^{\alpha}$ P<0.05 compared with group 1; $^{\beta}$ P<0.05 Compared to group 2

4. DISCUSSION

The present study determined the effect of ESECL on blood glucose level and serum lipid profile of alloxan- induced diabetic male Wistar rats. Alloxan has been found to be selectively toxic to pancreatic beta cells as it preferentially accumulates in the beta cells as glucose analogues leading to glucose oxidation and reduction in insulin release by the destruction of β-cells of islets of Langerhans [11] The presence of plant secondary metabolites like flavonoids and phenols, reported to be present in *C. lanatus* extract are known for their antioxidant and possible hypoglycemic activities [12]. Furthermore, *C. lanatus* is a rich source of a precursor (i.e. citrulline) for arginine synthesis and dietary arginine supplementation has been shown to decrease plasma glucose concentration in diabetic rats [13].This result is in agreement with the previous work carried out by Omigie and Agoreyo, [14] which suggests that the presence of Saponins, flavonoids and phenols in watermelon may be the contributing factors to this hypoglycemic effect. Increased levels of lipid profiles have been associated with diabetic mellitus [15].The abnormally high concentration of serum lipids in diabetics is mainly due to increase in the mobilization of free fatty acids from the peripheral fat depots [16].The ESECL was found to significantly reduce TC, TG and LDL concentration and significantly increase the HDL concentration in the diabetic rats. This result is in agreement with the previous work done by [17], which stated that treatment with methanolic seed extract of *C. lanatus* seeds restored all these parameters with an increase in HDL. The mechanism of the action of the extract of the seed of *C. lanatus* on fat metabolism is uncertain. However, studies have shown that nitric oxide synthesized from arginine plays an important role in regulating the oxidation of fatty acids and glucose [18] and *C. lanatus* is a bio-available source of a precursor (i.e. citrulline) for arginine synthesis.

Table 3. Effect of the ethanolic seed extract of *C. lanatus* on lipid profile level (mg/dl) during four week study

Groups	Total cholesterol	Triacylglycerol	HDL	LDL
1	28.76±10.26	36.17±1.53	49.48±1.37	21.61±13.60
2	46.84±2.35$^{\alpha}$	75.88±15.14$^{\alpha}$	11.08±0.60$^{\alpha}$	30.63±2.46
3	34.76±3.64$^{\beta}$	53.93±7.30$^{\alpha\beta}$	36.72±4.89 $^{\alpha}$	20.04±4.88
4	42.80±2.11$^{\alpha}$	64.43±12.38 $^{\alpha}$	34.72±4.89 $^{\alpha\beta}$	27.62±2.88
5	31.90±1.85$^{\beta}$	56.52±1.80$^{\alpha\beta}$	34.61±2.17$^{\alpha\beta}$	18.81±2.71$^{\beta}$
6	29.14±1.95$^{\beta}$	54.79±0.72$^{\alpha\beta}$	38.20±2.60$^{\alpha\beta}$	15.13±1.73$^{\beta}$

Results are expressed as Mean±SEM; $^{\alpha}$P<0.05 compared with group 1; $^{\beta}$P<0.05 Compared to group 2

5. CONCLUSION

The present study has shown that *C. lanatus* significantly caused a *significantly* reduction in blood glucose level in diabetic rats. It also significantly reduced total cholesterol, triglyceride, low density lipoprotein and increased high density lipoprotein concentrations in diabetic rats. This reduction was found to be dose dependent. The seed extract was found to be equally effective as that of the standard drug (glibenclamide). Thus, these results suggest that the seed of *C. lanatus* may be useful in the treatment of diabetes mellitus.

CONSENT

It is not applicable.

ETHICAL APPROVAL

The ethical clearance for this study was obtained from the research and ethics committee of the college of medicine, University of Nigeria, Enugu Campus. The protocol number is: 038/02/2018.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES

1. Noor A, Gunasekara S, Manickam AS,.Vijayalakshmi MA. Antidiabetic activity of *Aloe veranad*. Histology of organs in streptozotocin induced diabetic rats. Current Science. 2008;94(8):1070-1075. Elujoba AA, Odeleye OM. Ogunyemi. Traditional medical development for medical and dental primary healthcare delivery system in Afr.J. Trad, Compl and. Alt. Med. 2005;2(1):46-61.
2. Pennington JA, Fisher RA. Food component profiles for fruit and vegetable subgroups. J. Food Comp and Analysis. 2010;23(3):411-418.
3. Gonzalez-Aguiller GA, Celis J, Sotelo-Mundo RR, Dela LA. Physiological and biochemical changes of different fresh-cut mango cultivars stored at 5°C. Int. J. Food Sci. Technol. 2008;43(1):91-101
4. Ajayi LA, Ajibade O, Oderinde RA. Preliminary phytochemical analysis of some plant seeds. Res J. Chem Sci. 2011;1:58-62. Varghese S, Gomathic D, kalaisehi K, Devaki K. Phytochemical screening and HPTLC finger printing analysis of *Citrullus lanatus* (Thunb). Seed. J. Acute Dis. 2013;122-126.
5. Adesanya AO, Olaseinde OO, Oguntayo OD, Otulana JO, Adefule AK. Effects of Methanolic Extract of *Citrullus lanatus* seed on experimentally induced prostatic hyperplasia. EJMP. 2011;1(4): 171-179.
6. Trease GE, Evans WC. Pharmacognosy, 11th edition. Brailliar Tinidel Can. Macmillian Publishers; 2005.
7. Harbone JB. Phytochemical methods. A guide to modern technique of plant analysis, Chapman and Hall, London. 1984;134-60.
8. Lorke D. A new approach to practical acute toxicity testing, Arch Toxical. 1983;54:275-87.
9. Akpotu AE, Ani CO, Nworgu C, Okorie PO, Igwe U, Uzoigwe J, et al. Antidiabetic and anti-hyperlipidemic effects of ethanolic extract of *Dryopteris dilatata* leaves. J. Diabetes. Endrinol. 2018;9(3):20-2.
10. Ani CO, Agu UF, Nworgu CC; Uzoigwe, UJ, Okorie PO, Adugba AO, et al. Investigation of antihypertensive effect of Nigerian varieteies of solanium Lycopersicon on rats. Afri. J. Pharm. Pharmacol. 2017;1(34):419-425.
11. Siyem D, Syngai G, Khup PZ, Khongwir BS, Kayang H. Hypoglyceamic effects of *Potentilla fulgens* L. in normal and alloxan diabetic mice. J. Ethnophamacol. 2012; 83:55-61.
12. Mowla A, Alauddin M., Rahman A. Ahmed R. Antihyperglycemic effect of *Trigonellafoenum-grace* (fenugreek) seed extract in alloxan-induced diabetic rats and its use in diabetes mellitus: A brief qualitative phytochemical and acute toxicity test on the extract. Afri. J. Compl. Alt. Med 2009;6 (3):255-261.
13. Kohli R, Meininger CJ, Haynes TE, Yan J, Self T, Wu, G. Dietary L-arginine supplementation enhances endothelial nitric oxide synthesis in streptozotocin induced diabetic rats. J. Nutri. 2004;134(3): 60-68.
14. Omigie IO, Agoreyo FO. Effects of Watermelon (*Citrullus lanatus*) seed on blood glucose and electrolyte parameters in diabetic Wistar rats. J. Appl Sci. Env. Man. 2014;18(12):231-233
15. Nikkila EA. Plasma lipid and lipoprotein abnormalities in diabetes. In: Diabetes and heart diseases. Jarret RF, (Ed.). Elsevier

Science Publishers B.V. Amsterdam, The Netherlands. 1984;134-167
16. Bopama KN, Kanna J, Sushma G, Balaraman R, Rathod SP. Antidiabetic and antihyperlipidemic effects of neem seed kernel powder on alloxan diabetic rabbits. Indian J. Pharmacol. 1997;29:162-167.
17. Chinmay D, Deshmukh H, Anurekha J. Antidiabetic and antihyperlipidemic effects of methanolic extract of Citrullus lanatus seeds in rats. IJPPS. 2015;7. [ISSN-0975-1491]
18. Jobgen WS, Fried SK, Fu WJ, Meininger CJ, Wu G. Regulatory role for the arginine-nitricoxide pathway in metabolism of energy substrates. J. Nutr. Biochem. 2006;17:571–88.

Peer-review history:
The peer review history for this paper can be accessed here:
http://www.sdiarticle3.com/review-history/45794