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ANTIDIABETIC AND FREE RADICAL SCAVENGING ACTIVITIES OF METHANOLIC EXTRACT OF *CITRULLUS LANATUS* (FRUIT RIND)

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ABSTRACT

Free radicals are associated with Diabetes and its complications. The present study was designed to investigate DPPH scavenging in-vitro antioxidant activity and antidiabetic activity on alloxan induced diabetic mice. Mice treated with extract dose at 300mg/kg p.o, showed a remarkable decrease ($^{***}p<0.001$) of blood glucose concentration at 90min and 120min compared to diabetic control group and metformin was taken as a reference standard. In this study antioxidant activity of *Citrullus lanatus was* IC50 was 102.51 µg/ml in contrast with ascorbic acid IC50 was 36.34 µg/ml.

KEY WORDS: Antidiabetic, free radical, scavenging activity, fruit rind, *Citrullus lanatus.*

BACKGROUND

Herbal plants for their different medicinal values and their formulation has been found to be effective for the treatment of various diseases since ancient period. [1] Plant source traditional medicine plays an important role to manage diabetes mellitus. [2,3] Diabetes mellitus (DM) commonly known as diabetes, is a group of metabolic disorder with chronic high blood sugar.[4] Diabetes can cause acute and chronic complications. Acute complications include diabetic ketoacidosis and nonketotic hyperosmolar coma. Serious long-term complications include cardiovascular disease, stroke, kidney failure, foot ulcers and damage to the eyes.[5-6] Oxidative stress and hyperlipidemia associated lipid disorders develop premature atherosclerotic cardiovascular disease (CV). [7,8,9] Around 246 million people

worldwide suffering from diabetes and more than half them are in Asia and the Western Pacific.[10,11]

Oxidation reactions produce free radicals which damage or death the cell. Antioxidants end up free radical intermediates and inhibit other oxidation reactions. Antioxidants are often reducing agents such as thiols, ascorbic acid, or polyphenols.[12] Antioxidants are widely used in dietary supplements and have been investigated for the prevention of diseases such as cancer, coronary heart disease and even altitude sickness.[13] This undertaken study design to evaluate the hypoglycemic and anti-oxidant effect of methanolic extract of rind of *citrulus lanatus.*

MATERIALS AND METHODS

Plant materials' collection and extracts' preparation

Citrullus lanatus were collected from Market during the month of May 2014 and dried it rind and the plant authenticity was confirmed by the expert from the Bangladesh National Herbarium, Dhaka, Bangladesh.

The rinds of *Citrullus lanatus* were shade dried for fifteen days at room temperature (during this season Asian maximum temperature 38°C) to ensure the active constituents free from decomposition. The dried rinds were powdered in an electrical grinder after overnight drying in an oven below 50°C. The powder was extracted with 96% methanol at room temperature. The bottle were kept at room temperature and allowed to stand for 10 days with occasional shaking. When the solvent become concentrated, the liquid alcohol contents were filtered through cotton for several times and then through filter paper (Whatman Fitter Paper No. 1). After evaporation finally methanolic crude extract was obtained.

Drugs and Chemicals

The standard drug, Metformin hydrochloride was the generous gift samples from Beximco Pharmaceuticals Ltd of Bangladesh. Alloxan monohydrate was purchased from Loba chemie, India. Blood samples analyzed for blood glucose content by using Ok meter Match glucose test meter (Taiwan), DPPH from Sigma-aldorich, Germany and Ascorbic acid from Merck KGaA, Germany and All other chemicals and reagents used were of analytical grade.

Experimental Animals

Eight week-old Swice albino mice (30-35g) purchased from Jahangirnagar University animal lab, Dhaka, Bangladesh and were housed in animals cages under standard environmental conditions (22-25°C, humidity 60-70%, 12 hr light and dark cycle). The mice were feed with standard pellet diet taken from the mice supplied lab. The animals used in this study were cared in accordance with the guidelines on animal experimentation of our institute.

Method for Evaluation of Hypoglycemic Activity

Oral Glucose Tolerance Test (OGTT) in diabetic mice

After fasting 16hr, diabetes was induced into mice by in intra-peritoneal injection (i. p.) of alloxan monohydrade (90 mg/kg) dissolved in saline. After 48hrs, plasma glucose levels were measured by glucometer (Tyson, Taiwan) using a blood sample from tail-vein of mice. Mice with blood sugar higher than11.5 mmol/l were considered as diabetic.

All the mice were divided into 4 groups, each group containing 5 mice. The divided groups are NC (normal control), DC (diabetic control), STD (diabetic mice receiving Metformin), ME (diabetic mice receiving methanolic extract). The mice were fasted over-night and next day blood samples were taken from all groups of animals to estimate fasting blood glucose level (0 min). All mice received 1gm /kg glucose. Without delay extract and were given per oral and three more blood samples were collected at 30, 90 and 120 minutes intervals and blood glucose level was estimated in all the experiments by using glucometer.[14]

DPPH radical-scavenging activity

Preparation of Control and Test Sample for Antioxidant Activity Measurement

Antioxidant activity of the extract of *Citrullus lanatus* was determined by DPPH free radical scavenging activity by the modified method of Gupta[15,16] Stock solutions (10 mg/ml) of the plant extracts were prepared in methanol from which serial dilutions were carried out to obtain concentrations of 1, 5, 10, 50, 100 and 500 μg/ml. Diluted extract solutions (2 ml) were added to 2 ml of a 0.004% ethanol solution of DPPH in test tube and in another test tube 2ml 0.004% DPPH & 2ml methanol is taken to prepare blank solution. Similar way by standard sample ascorbic acid was done. Both are mixed and allowed to stand for 30 min in a dark for proper reaction. The absorbance was determined at 517 nm using a double beam UV-visible spectrophotometer. Radical scavenging activity was expressed as the inhibition percentage (I %) and calculated as per the following equation:

%inhibition= [(Blank absorbance - Sample absorbance)/Blank absorbance] × 100

RESULT AND DISCUSION

Antidiabetic activity test

Table-1: Test materials used in the evaluation of hypoglycemic activity of crude extract of *Citrullus lanatus*

Group	Test Sample	Identification	Dose
NC	0.9% NaCl	Normal Control Group	10 ml/kg
DC	0.5% methyl cellulose	Diabetic Control Group	10 ml/kg
STD	Metformin	Standard Group	100mg/kg
ME	Methanolic Extract	Test Sample Group	300mg/kg

Table-2: Plasma level of glucose (mmol/L) of mice at different time.

Group	Blood glucose level (Mean ±SEM)			
	0 Minute	30 Minute	90 Minute	120 Minute
NC	5.65±0.38	5.65±0.38	5.86±0.52	5.82±0.22
DC	27.5±0.26	31.55±0.27	30.73±0.21	30±0.15
STD	27.2±0.20	19±0.25***	17.45±0.35***	16.85±0.33***
ME	27.08±0.24	24.98±0.12***	22.85±0.15***	19.73±0.10***

Values are expressed as Mean ±SEM (n=5). ***p<0.001 indicates significant changes compared with diabetic control.

Free radical scavenging screening

Table-3: DPPH scavenging assay

For Ascorbic Acid & Methanol Extract

Conc.	Absorbance			% inhibition of	
µg/ml	Blank	ASA	ME	ASA	ME
5	0.852	0.711± 0.007	0.565± 0.005	22.63	01.62
10		0.588± 0.002	0.515± 0.002	36.01	07.37
50		0.368± 0.002	0.304± 0.008	59.96	45.32
100		0.286± 0.003	0.194± 0.001	63.30	65.11
500		0.162± 0.003	0.152± 0.003	75.70	72.66
1000		0.102± 0.004	0.141± 0.004	88.90	74.64

Data are indicated as Mean ±SEM, ME= Methanol extract, ASA= Ascorbic acid

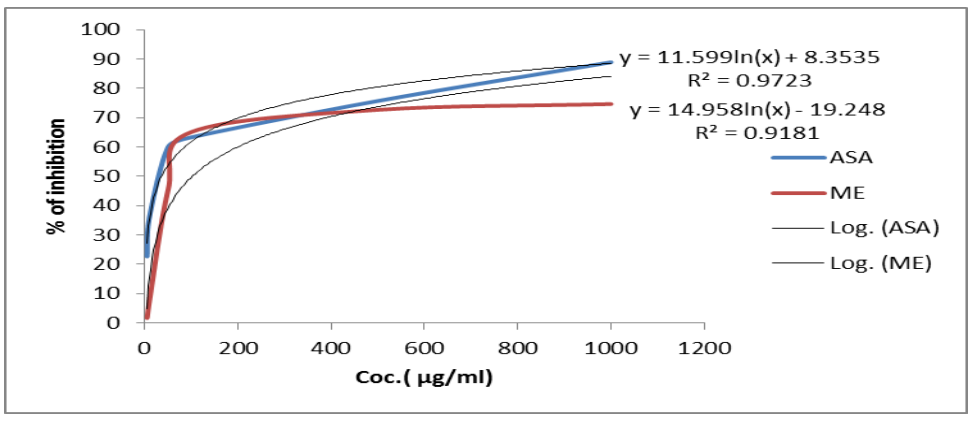

Figure-1: Anti-oxidant activity of ascorbic acid and *Citrullus lanatus.*

Table-4: IC_{50} of the extracts of *Citrullus lanatus* and standard.

Test Samples	Regression line	R^2	IC_{50} µg/ml
ASA	y = 11.59ln(x) + 8.353	R^2 = 0.972	36.342
ME	y = 14.95ln(x) - 19.24	R^2 = 0.918	102.514

IC_{50} of Ascorbic acid=36.342 µg/ml and Methanolic extract=102.514 µg/ml

CONCLUSION

The present design study showed that the blood glucose levels were significantly higher in diabetic mice after oral administration of glucose and experimental groups of mice shown in Table-2. In undertaken study blood glucose concentration was observed after 30 min, 90 min and 120 min. Experimental Mice treated with extract in 300mg/kg, showed a significant decrease in blood glucose concentration at 90min and 120min compared to diabetic control mice. In this study hypoglycemic effect was significant ($p<0.05$) reducing blood glucose level that from 27.08mM to 19.73mM ± SEM ,where standard reduces 27.2 to 16.85 mM ± SEM. An evidential scavenging activity was observed i.e IC_{50} of Methanolic extract of *Citrullus lanatus* (102.514 µg/ml) compared to Ascorbic acid (36.342 µg/ml).

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REFERENCES

1. Senthilkumar R, Ahmedjohn S, Archunan G, Manoharan N. Antioxidant activity of *Wedelia chinensis* in alloxan induced diabetic rats. *Pharmacologyonline*, 2008; 640-651.
2. Ahemed I, Adeghate E, Cummings E, Sharma AK, Singh J. Beneficial effects and mechanism of action of Momordica juice in the treatment of Streptozotocin- induced diabetes mellitus in rats. Molecular Cell Biochemistry, 2004; 26: 63-70.
3. Karunanayake EH, Tennekon KH. Search of novel hypoglycemic agents from medicinal plants. In: Sharma, A.K., (Eds). Diabetes mellitus and its complication, An Update. Edn 1, Macmillan India Ltd, New Delhi, 1993; 435- 469.
4. Jump up "About diabetes". World Health Organization. Retrieved 4 April 2014.
5. Jump up to "Diabetes Fact sheet N°312". *WHO*. October 2013. Retrieved25 March 2014.
6. Kitabchi, AE; Umpierrez, GE; Miles, JM; Fisher, JN (Jul). "Hyperglycemic crises in adult patients with diabetes.". *Diabetes Care*, 2009; 32(7): 1335–43.

7. Anonymous, Diabetes now a global threat gets own day. Sunday Times of India, 2006; 24: 11.
8. http://www.bbc.com/news/world-asia-24740288
9. El-Hilaly J, Tahraoui A, Israili Z H and Lyoussi B, Hypolipidemic effects of acute and sub-chronic administration of an aqueous extract of *Ajuga iva* L. whole plant in normal and diabetic rats, *J Ethnopharmacol*, 2006; 105(3): 441-448.
10. Saravanan R, Prasad N R and Pugalandi K V, Effect of *Piper betle* leaf extract on alcoholic toxicity in the rat brain, *J Med Food*, 2003; 6(3): 261-265.
11. Yokozawa T, Ishida A, Cho E J and Nakagawa T, The effects of *Coptidis rhizome* extract on a hypercholesterolemic animal model, *Phytomedicine*, 2003; 10(1): 17-22.
12. Sies, Helmut "Oxidative stress: Oxidants and antioxidants".*Experimental physiology*, 1997; 82(2): 291-95.
13. Baillie, J.K.; Thompson, A.A.R.; Irving, J.B.; Bates, M.G.D.; Sutherland, A.I.; MacNee, W.; Maxwell, S.R.J.; Webb, D.J. Oral antioxidant supplementation does not prevent acute mountain sickness: double blind, randomized placebo-controlled trial, 2009; 102 (5): 341–8.
14. Hossain MS, Asadujjaman M, Khan MRI, Ahmed M, and Islam A, Antidiabetic and glycogenesis effects of different fractions of methanolic extract of Momordica charantia (Linn.) in alloxan induced diabetic rats, International Journal of Pharmaceutical Sciences and Research, 2011; 2(2): 404-412.
15. Gupta M, Mazumdar UK, Sivahkumar T, Vamis MLM, Karki S, Sambathkumar R, Manikandan L. Antioxidant and anti-inflammatory activities of *Acalypha fruticosa*. *Nig. J. Nat. Prod. Med*, 2003; **7**: 25-29.
16. Brand-Williams, W., Cuvelier, M. and Berset, C. Use of a free radical method to evaluate antioxidant activity. Lebensmittel-Wissens-chaft-und-Technologie, 1995; 28: 25-30.