WORLD JOURNAL OF PHARMACEUTICAL RESEARCH
SJIF Impact Factor 5.045
Volume 3, Issue 8, 497-504. Research Article ISSN 2277 – 7105

SYNERGETIC ACTIVITY OF METHANOLIC EXTRACT OF *SYZYGIUM CUMINI* (L) SKEELS (SEEDS), *COSTUS IGNEUS* (NAK) (LEAVES), AND *MUCUNA PRURIENS* (L.) DC (SEEDS) FOR ANTIDIABETIC ACTIVITY.

Arjun .G*, Kiran.B, Yogisha.S

[1]PG Department of Biochemistry, CMR Institute of Management Studies (Autonomous) C.A. #2, 3rd 'C' Cross, 6th 'A' Main, HRBR layout, 2nd Block, Kalyana Nagar, Bangalore-560043, Karnataka State, India.

[2]Department of Botany, Sikkim Central University, 6th Mile, Tadong, Gangtok Sikkim - 737102, India.

[3]Skanda Life Sciences Pvt Ltd, Sri Shaila Bramara Complex, Srigandha Kaval, Nagarbhavi - Sunkadakatte link road, Bangalore - 560 091, Karnataka, India.

Article Received on
28 July 2014,

Revised on 21 August 2014,
Accepted on 16 Sept 2014

***Correspondence for Author**
Dr. Arjun .G
PG Department of Biochemistry CMR Institute of Management Studies (Autonomous) C.A. #2, 3rd 'C' Cross, 6th 'A' Main, HRBR layout, 2nd Block Kalyana Nagar, Bangalore Karnataka State, India.

ABSTRACT

In vitro evaluation of three plants viz., *S. cumini* (Seeds) *C. igneus* (Leaves) and *M. pruriens* (Seeds) showed a promising result in inhibition of α- Glucosidase tested at 0.0, 2.5, 5.0, 10.0, 20.0, 40.0, 80.0 and 160 µg/ml concentration. Moderate activity was recorded in 2.5, 5.0, 10.0 µg/ml concentration. 22.11 percent of inhibition was recorded in 20.0 µg/ml, 38.22 percent in 40.0 µg/ml, 42.11 percent in 80.0 µg/ml and 49.13 percent in 160.0 µg/ml. all the result was compared to standard acarbose. In aldose reductase inhibition assay tested at 5 to 320 µg/ml concentration showed significant activity and recorded 8.90 percent in 20.0 µg/ml, 12.33 percent in 40.0 µg/ml concentration. Highly significant activity was observed in 80, 160 and 320 µg/ml concentration and recorded 20.11, 24.55 and 34.55 percent respectively.

KEYWORDS: *S. cumini, C. igneus, M. pruriens,* Anitdiabetic, α-Glucosidase, aldose reductase.

INTRODUCTION

Diabetes mellitus (DM) is a chronic disease characterized by a deficiency in insulin production and its action or both. That leads to prolonged hyperglycemia with disturbances in most metabolic processes inside the human body [1].Diabetes mellitus (DM) is a disease of civilization and the prevalence of this disease has risen worldwide in large parts because of an increase in obesity and sedentary life styles. About 90% DM patients are of type II DM within insulin resistance which plays an important role in the development of the disease. It was estimated that about 200 million people worldwide suffered from DM in 2010, and it is expected to reach 300 million by 2025[2]. The progression of type II DM begins with an impairment of glucose tolerance which is often associated with a state of insulin resistance. Both insulin resistance and decreased insulin secretion delivers the patho physiology of T_2DM. In T_2DM the increased circulating glucose concentration is associated with abnormalities in carbohydrate, protein, and lipid metabolism and a variety of micro vascular, macro vascular, neurological, and infectious complications. According to recent estimates, approximately 285 million people worldwide (6.6%) in the 20–79 year age group will have diabetes in 2013. Diabetes mellitus is a widespread disease, If left untreated, diabetes can cause many complications such as cardiovascular disease, hypertension, dental disease, retinopathy, cataracts, renal disease, neuropathies, Because of their insulin-insensitivity, organs such as the lens, retina, nerves and kidney are target organs for these diabetic complications[3]. Glucose from the digested food circulates in the blood as a ready energy source for any cells that need it. Insulin is a hormone or chemical produced by cells in the pancreas, an organ located behind the stomach. Insulin bonds to a receptor site on the outside of cell and through which glucose can enter into the cells. Some of the glucose can be converted to concentrated energy sources like glycogen or fatty acids and saved for later use. When there is not enough insulin produced, glucose stays in the blood rather entering the cells. At the same time that the body is trying to get rid of glucose from the blood, the cells a restarving for glucose and sending signals to the body to eat more food, thus making patients extremely hungry. To provide energy for the starving cells, the body also tries to convert fats and proteins to glucose[3,4]. *S. cumini* is also known as jambu**,** *S. cumini* has been widely used for the treatment of various diseases in traditional and folk medicine. The fruits and seeds are used to treat diabetes, pharyngitis, spleenopathy, urethrorrhea. *C. igneus* commonly known as 'Insulin plant' was introduced from Mexico to India (Kerala) very recently. Plant grows very quickly, and the propagation is by stem cutting. It needs sunshine but it also grows in slightly shady areas. *M. pruriens* also nown as velvet bean and cowitch, is an herb used as a minor

food crop and medical bean. The pods of the Mucuna have hair-like needles covering the outside. These hairs contain mucunian and setotonin and can cause itching , plisteres and dermatitis. In the present study , three medicinal plants viz., *S. cumini* , *C.igneus* and *M. pruriens* were evaluated for inhibition of α-Glucosidase and aldose reductase to test the antidiabetic activity.

MATERIALS AND METHODS

Plant Materials

Healthy plant parts of three medicinal plants viz., seeds of *S. cumini* (L.)Skeels belongs to family Myrtaceae, leaves of *C. igneus* (Nak). belongs to family Costaceae and seeds of *M. pruriens* (L.) DC belongs to family Fabaceae were collected in and around Bangalore, Karnataka state. The plant material were Washed thoroughly 2-3 times with running tap water and once with sterile distilled water, air dried at room temperature on a sterile blotter and used for preparation of extract [5].

Solvent Extraction: All the three plants viz., *S. cumini* (Seed), *C. igneus* (Leaves), *M. pruriens* (Seed) were powdered and subjected to methanol solvent extraction using Soxhlet apparatus[6]. The solvent extract was collected and stored in small brown bottle at 5^0 C until further use. One gram of each of solvent extract was dissolved in 10ml of methanol, which served as the mother solvent extracts[7].

Reagents And Enzymes Used: RPMI-1640 and Dulbecco's Modified Eagle Medium (DMEM) were optained from Gibco Life Technologies, DL Glyceraldehyde and NADPH were obtained from Sigma Aldrich Chemicals Pvt Ltd. Acarbose and Sucrose were obtained from Himedia Laboratory, Mumbai

Collection of Rats: The rats were supplied by Achrya Pharmacy College, Bangalore. Permission was taken from ethical committee to conduct experiment with its reference number CPCSEA/CH/org/2000/241.

Preparation of Enzyme

Extraction of A-Glycosidase: Rats are sacrificed, intestine is removed and chilled with ice cold 80 mM phosphate buffer (pH 7.0). The intestine is then cut open, the mucosa is scraped off with a piece of glass rod and homogenized in homogenizer with four parts (v/w) of cold 80 mM buffer (pH 7.0). The tube is chilled with crushed ice during homogenization.

Nuclei and large cell debris are removed by centrifugation at 2000 to 4000 rpm for 10 minutes and supernatant is stored at – 20°C[8]. The protein content was estimated following the procedure of Lowry [9].

Extraction of Aldose Reductase: Crude aldose reductase(AR) was prepared from rat lens. Eyeballs were removed from 9 week old Wistar /NIN (WNIN) male rat . Lenses were dissected by posterior approach and homogenized in 10 volumes of 100 mM potassium phosphate buffer pH 6.2. The homogenate was centrifuged at 15,000x g for 30 min at 4^0C. The supernatant was used as the source of AR and stored at – 20°C [10].

Standard (Inhibitor): Dissolve 50 mg of acarbose in 50 ml of phosphate buffer and dilute appropriately to get concentration of 5 µg/ml using phosphate buffer pH 7.0.

ENZYME ASSAY

Inhibition of A-Glucosidase: 50 µl of enzyme was taken and added to 250 µl of buffer or test sample and incubate at 37°C for 30 minutes. After incubation, 500 µl of sucrose solution was added and incubate at 37°C for 20 minutes and heated on boiling water bath for 2 minutes to arrest the reaction and subjected for cooling. The optained solution was taken and subjected to measure glucose concentration by Glucose Oxidase method [11].

Estimation of Glucose by Glucose Oxidase method: 100 µl of sample was taken and mixed with 500 µ of glucose reagent (Glucose reagent kit) and incubated at room temperature for 10 minutes. After incubation , the absorbance was measured at 510 nm. The percentage inhibition of alfa glucosidase is calculated as follows: % inhibition= Absorbance (control) – Absorbance test)/ Absorbance (control) x100[12].

Inhibition of Aldose Reductase: The assay mixture in 1 ml contains 50 µM potassium phosphate buffer (pH 6.2), 0.4 mM lithium sulfate, 5 µM 2-mercaptoethanol, 10 µM DL-glyceraldehyde, 0.1 µM NADPH, and enzyme preparation (rat lens or recombinant enzyme). Appropriate blanks were employed for corrections. The assay mixture was incubated at 37 ^{0}C and initiated by the addition of NADPH at 37 ^{0}C. The change in the absorbance at 340 nm due to NADPH oxidation was followed in a Cary Bio 100 spectrophotometer. AR activity was assayed following standard procedure[10].

Statistical Analysis: All data are expressed as the mean ± SEM, n=3. The IC50 value was determined by nonlinear regression curve fit using Graph pad prism5. The data were subjected to Tukey's HSD analysis. Data on percentages were transformed to arcsine and analysis of variance (Anova) was carried out with transformed values. The means were compared for significance using Tukey's HSD (P=0.05).

RESULT

A-Glucosidase Inhibition Assay: All the three plants showed a significant activity in α-Glucosidase inhibition tested at different concentration. At 2.5 µg/ml, the percentage of inhibition was 3.05, at 5.0 µg/ml, it was recorded 5.25, 12.99 percent in 10.0 µg/ml, 22.11 percent in 20.0 µg/ml, 38.22 percent in 40.0 µg/ml, 42.11 percent in 80.0 µg/ml and 49.13 percent in 160.0 µg/ml. Compared to standard, Acarbose, it was recorded (Table 1).

Table 1: Absorbance values of Methanol extract of three Medicinal plants for α-Glucosidase inhibition assay

Plant Materials	Concentration in µg/ml	% Inhibition
S. cumini (Seed) *C. igneus* (Leaves) *M. pruriens* (Seed)	0.0	0.0^{a} ±0.0
	2.5	3.05^{b} ±0.1
	5.0	5.25^{c} ±0.1
	10.0	12.99^{d} ±0.1
	20.0	22.11^{e} ±0.0
	40.0	38.22^{f} ±0.0
	80.0	42.11^{g} ±0.1
	160.0	49.13^{h} ±0.0

1. Values are means of three replicates, ±standard error
2. Analysis of Variance (ANOVA) , $P < 0.001$
3. a to h means with different letters are significantly different from each other.

Aldose Reductase Inhibition Assay: The aldose reductase inhibition assay tested at 5 to 320 µg/ml concentration showed significant activity and recorded 0.99 percentage inhibition at 5.0 µg/ml, 4.50 percent in 10 µg/ml, 8.90 percent in 20.0 µg/ml, 12.33 percent in 40.0

µg/ml concentration. The inhibition percentage goes on increasing with increasing concentration. At 80.0 µg/ml concentration, the inhibition percentage was 20.11 and at 160 µg/ml concentration, it was recorded 24.55 percent of inhibition and at 320 µg/ml concentration, the inhibition percentage was 34.55 respectively. Compared to standard, it was recorded (Table 2).

Table 2 : Absorbance values of Methanol extracts of three Medicinal plants for for aldose reductase inhibition assay

Plant Materials	Concentration in µg/ml	% Inhibition
S. cumini (Seed) *C. igneus* (Leaves) *M. pruriens* (Seed)	0.0	0.0^{a} ±0.0
	5.0	0.99^{b} ±0.1
	10.0	4.50^{c} ±0.0
	20.0	8.90^{d} ±0.1
	40.0	12.33^{e} ±0.0
	80.0	20.11^{f} ±0.0
	160.0	24.55^{g} ±0.0
	320.0	34.55^{h} ±0.1

- Values are means of three replicates, □□standard error
- Analysis of Variance (ANOVA) , $P < 0.001$
- a to h means with different letters are significantly different from each other.

DISCUSSION

Medicinal plants constitute an important natural wealth of India and man plant species possess diverse medicinal properties .These medicinal plants play a vital role in providing primary health care services to rural people. Medicinal plants play a pivotal role in the health care of ancient and modern cultures. Ayurveda, the Indian system of medicine mainly uses plant based drugs or formulations to treat various human ailments because they contain the components of therapeutic value. In addition, plant based drugs remain an important source of therapeutic agents because of the availability, relatively cheaper cost and non-toxic nature when compared to modern medicine. Many herbs contain antioxidant compounds which protects the cells against the damaging effects of reactive oxygen species. The methanol

extract of three plant parts viz., *S. cumini* (Seeds) , *C. Igneus* (Leaves), and *M. pruriens* showed significant activity in inhibiting the alpha-glycosidase ,aldos reductase, enzyme with IC 50 value 24.11 and 22.64 .The biological compounds like flavanoids ,phenols, alkaloids, polyphenols, , and anthocyanins present in the herbal formulation promote the 3T3L1 adipocyte cells to differentiate into many cells by transport of glucose across the membrane through Glut 4 mechanism. The retardation of membrane bound α-glucosidase inhibitory reaction or inhibition of passive glucose transport would successfully flatten the postprandial blood glucose excursions or reduce hyperglycemia. The present observation showed moderate synergistic activity in α-glucosidase avtivity and Aldose reductase inhibition . A further investigation is necessary to isolate and identify the bioactive compound responsible for anitdiabetic activity.

CONCLUSION

This study is significantly covers various pathways and metabolic aspects for progression of diabetes. These plant extracts have definitely shown antidiabetic activity, whether all the components act independently or in a synergetic manner because active principles or biomolecules are responsible for their antidiabetogenic effect. Plants selected for formulations exhibited antidiabetic agent's carbohydrate blockers and Aldose reductase that hold the hope for development of new alternative therapies to control hypoglycaemia.

ACKNOWLEDGEMENT

The authors are thankful to the CMR Institute of Management Studies (Autonomous), PG Department of Biosciences, Bhuvanagiri, OMBR layout, Bangalore, and Skanda Life Sciences Private Limited, Sri Shaila Bramara Complex, Srigandakaval Nagarbhavi-Sunkadakatte link road, Bangalore for providing facilities.

REFERENCES

1. Singab AN, Youssef FS, Ashour ML. Medicinal Plants with Potential Antidiabetic Activity and their Assessment. Med Aromat, 2014; 3(1): 151.
2. Amos AF, McCarty DJ, Zimmet P. The rising global burden of diabetes and its complications: estimates and projections to the year 2010. Diabetic medicine. 1997; 14: 85-87
3. Salim B. *Review,* Diabetes mellitus and its treatment. Int J Diabetes & Metabolism. 2005; 13:111-134.

4. Ramachandran A, Das AK, Joshi SR, Yajnik CS, Shah S, Prasanna Kumar KM. Current Status of Diabetes in India and Need for Novel Therapeutic Agents. 2010; 58:7-9
5. Satish S, Raveesha KA, Janardhana GR. Antibacterial activity of plant extracts on Phytopathogenic *Xanthomonas campestris* pathovars. Letters in Applied Microbiology. 1999; 28 : 145-147.
6. Nostro A, Germano MP, Angelo VD, Marino A, Cannatelli MA. Extraction methods and bioautography for evaluation of medicinal plant antimicrobial activity. Planta Medica. 2000; 25: 20-24.
7. Pinto CMF, Maffia LA, Casali VWD, Cardoso AA. *In vitro* effect of plant leaf extracts on mycelial growth and sclerotial germination of *Sclerotium cepivorum.* J. phytopathology, 1998; 146: 421-425.
8. Ranjana , Yamini BT. Insulin secreting and α-glucosidase inhibitory activity of hexane extract of Annona squamosa Linn. in streptozotocin (STZ) induced diabetic rats . Indian Journal of Experimental Biology. 2014; 52: 623-629.
9. Lowry OH, Rosebrough NT, Farr AL, Randall RJ. Protein estimation by Lowrey's method. J.Biol.Chem. 1951; 193: 265.
10. Suryanarayana P, Anil KP, Megha S,Mark PJ, Bhanuprakash GR. Inhibition of aldose reductase by tannoid principles of *Emblica officinalis*: Implications for the prevention of sugar cataract. *Molecular Vision*, 2004; 10:148-54.
11. Lee SY, Mediani A, Ashikin N, Azliana AH, Abas F. Antioxidant and α-glucosidase inhibitory activities of the leaf and stem of selected traditional medicinal plants. International Food Research Journal, 2014; 21(1): 165-172
12. Ketan H, Annapurna A. The Effect of Quercetin on Blood Glucose Levels of Normal and Streptozotocin Induced Diabetic (Type I & T Ype Ii) Rats. International Journal of Pharmaceutical, Chemical and Biological Sciences. 2014; 3: 613-619.