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Anti-diabetic activity of *Cassia auriculata* flowers in α- amylase inhibition and glucose uptake by isolated rat hemi-diaphragm

Muthukumar A.[1]*, S. Mohan[2], R. Sundharaganapathy[3] and G. Nagaraja Perumal[4]

[1,4]Department of Pharmacology, [2]Department of Pharmaceutics, Karpagam College of Pharmacy, Coimbatore-641032, Tamil Nadu India
[3]Faculty of Pharmacy, Karpagam University, Coimbatore-641021, Tamil Nadu India

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ABSTRACT

Diabetes mellitus (DM) has a profound adverse effect on quality of life in terms of social, psychological well-being as well as physical health. Diabetic complications are mainly mediated through oxidative stress such as increased production of Reactive Oxygen Species (ROS) or impaired antioxidant defence system. Enhancement of lipid peroxidation, alteration in antioxidant enzymes and impaired glutathione metabolism are the main factors involved in the development of diabetes. Production of free radicals is also involved in the pathogenesis of various type of disease including diabetes mellitus. Increased formation and accumulation of advanced glycation products elimination (AGEs) is also involved in the diabetic complications, such as retinopathy, neuropathy, nephropathy and renal dysfunction through a series of pathological changes. the aim present study was evaluate In vitro Anti-diabetic activity of Cassia Auriculata flowers and its phytochemical studies done by In vitro α- Amylase Inhibition Assay and In vitro Glucose Uptake by Isolated Rat Hemi-Diaphragm.

Key words: Diabetes mellitus, *Cassia Auriculata* and Anti-diabetic activity.

INTRODUCTION

The incidence of diabetes is growing rapidly both in the United States and worldwide. For example, it is estimated that more than 180 million peoples are affected with diabetes, and also prevalence will be expected to more than double by the year of 2030. In the United States, approximately 21 million peoples are estimated to suffer from diabetes, and it is a major cause of morbidity and mortality. Diabetes is heterogeneous group of syndrome characterized by an elevation of blood glucose caused by a relative or absolute deficiency of insulin[1-4].In recent times, focus on plant research has increased all over the world and a large body of evidence has collected to show immense potential of medicinal plants used in various traditional systems. Over the last few years, researchers have aimed at identifying and validating plant derived substances for the treatment of various diseases. Similarly it has been already proved that various parts of plants such as Leafs, fruits, seeds etc. provide health and nutrition promoting compounds in human diet[5-6]. The *Cassia auriculata Linn* is another Indian plant, which has enormous traditional uses against various diseases. The present review aims to compile medicinal values of *Cassia auriculata Linn* generated through the research activity using modern scientific approaches and innovative scientific tools. *Cassia auriculata* is one of the most traditionally using hypoglycemic agents among tribes in India and it is not scientifically validated. Based on the above mentioned reasons, one new research is required to develop one new drug with more anti diabetic activity, which have fewer side effects, which will be work multifactorial anti diabetic mechanism[7-8]. The present study is attempted to develop a novel plant based antidiabetic drug, which will be evaluated by using *In vitro* methods.

MATERIALS AND METHODS

Plant collection

The flower part of plant was collected from less rain fall irrigation hills and which authenticated by recognized botanical survey of India (BSI) Coimbatore.

Extraction procedure by Cold maceration

the preparation of extract using cold maceration process.1000g of coarse powder of plants, mixed with 2000ml of solvent in round bottom flask, which kept for 15days and shaken regularly 2 times per day and decanted. The extracts (methanol & petroleum ether) are dried under reduced pressure and stored in a desiccators[9-10].

Anti Diabetic Activity

***In vitro* α- Amylase Inhibition Assay**

A total of 500 µl of test samples and standard drug (100-1000µg/ml) were added to 500 µl of 0.20 mM phosphate buffer (pH 6.9) containing α-amylase (0.5mg/ml) solution and were incubated at 25°C for 10 min. After these, 500 µl of a 1% starch solution in 0.02 M sodium phosphate buffer (pH 6.9) was added to each tube. The reaction mixtures were then incubated at 25°C for 10 min. The reaction was stopped with 1.0 ml of 3, 5 dinitro salicylic acid colour reagent. The test tubes were then incubated in a boiling water bath for 5 min, cooled to room temperature. The reaction mixture was then diluted after adding 10 ml distilled water and absorbance was measured at 540nm. Calculation of 50% inhibitory concentration (IC_{50}).The concentration of the plant extracts required to scavenge 50% of the radicals (IC_{50}) was calculated by using the percentage scavenging activities at five different concentrations of the extract[11-13].

***In vitro* Glucose Uptake by Isolated Rat Hemi-Diaphragm**

Wistar albino rats (150-200gms) of both sexes were procured from College of Veterinary and Animal Science, Mannuthy, Thrissur- Kerala, India. Prior to the experiment the rats were housed in a clean polypropylene cages (6rats/cages) for a period of 7days under standard temperature (25-30^{o}C), relative humidity (45-55%), dark/light cycle (12/12hrs). The studies were performed with the approval of institutional animal ethics committee (IAEC) (KU/IAEC/B. Pharm/148). The animals were put in overnight fasting were deprived of food for 16hrs but allowed free access of water. Glucose uptake by rat hemi-diaphragm was estimated by the methods described elsewhere (Walaas and Walaas, 1952; Chattopadhyay et al., 1992) with some modifications. Four sets containing six numbers of graduated test tubes (n=6) for each extract are taken. Group I serve as a control which contained 2 mL of Tyrode solution with 2% glucose, Group II contained 2 mL Tyrode solution with 2%glucose and regular insulin (Nova Nardisk) 0.62 mL of 0.4 units per mL solution. Group III contained 2 mL Tyrode solution with 2% glucose and 1.38 mL of Pet. Ether extract of *Cassia Auriculata* and the Group IV contained 2 mL Tyrode solution with 2% glucose and regular insulin0.62 mL of 0.4 units per ml solution and 1.38 mL Methanolic extract of *Cassia Auriculata* fraction and glucose estimated by UV Spectrophotometer[14-15].

RESULTS AND DISCUSSION

***Invitro* antidiabetic study (α- Amylase Inhibition Assay)**

The α-Amylase Inhibition results were indicated (Table-1to 3 & Fig- 2 to 4), standard drug have more α-Amylase Inhibition property (IC_{50}= 3µg/ml) very less concentration 50 % inhibition was more when compared to each other extracts. Methanolic Extract of *Cassia Auriculata* have more α-Amylase Inhibition property (IC_{50}= 5µg/ml) very less concentration 50 % inhibition was more when compared with Petroleum Ether Extract of *Cassia Auriculata*.

Table No-1 α-Amylase Inhibition of Standard Drug

CONCENTRATION((µg/ml)	% OF INHIBITION (%)
0	0
2.5	46.8±0.2
5	77.5±0.2
7.5	80±0.1
10	90.1±0.2

Fig No:2,Alfa-Amylase Inhibition of standard drug

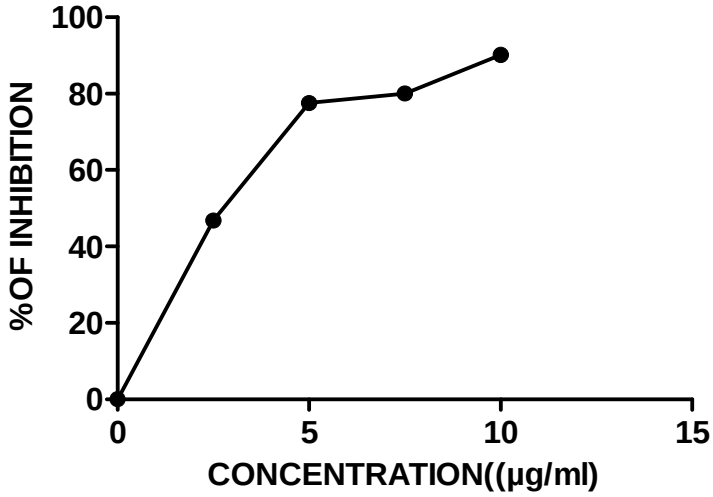


Table-2, α-Amylase Inhibition of Methanolic Extract of *Cassia Auriculata*

CONCENTRATION((µg/ml)	% OF INHIBITION (%)
0	0
2.5	38.3±0.2
5	51.25±0.1
7.5	75.5±0.3
10	81.3±0.4

***In vitro* Glucose Uptake by Isolated Rat Hemi-Diaphragm**

Fig No:3, Alfa-Amylase Inhibition of Methanolic Extract of *Cassia Auriculata*

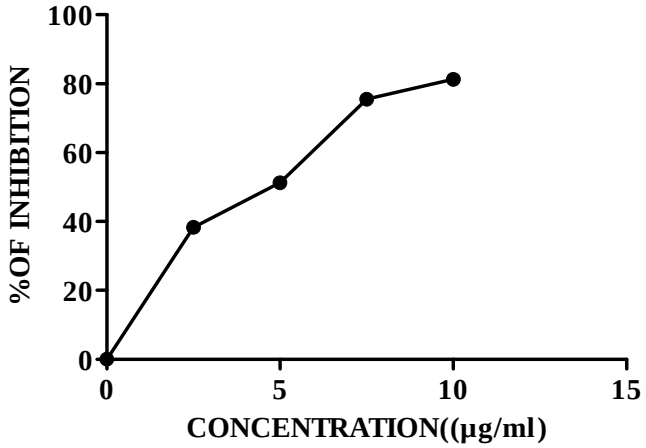
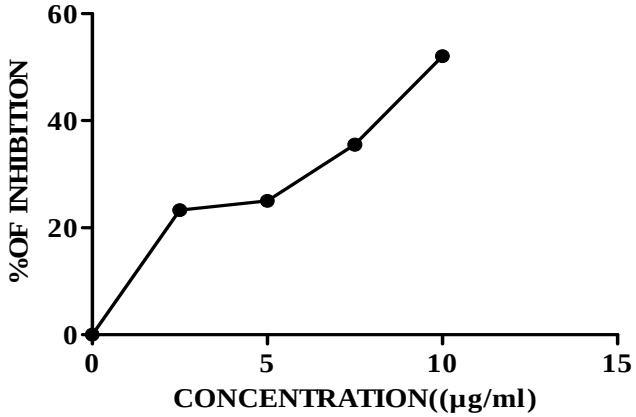


Table-3, α-Amylase Inhibition of Petroleum Ether Extract of *Cassia Auriculata*

CONCENTRATION((µg/ml)	% OF INHIBITION (%)
0	0
2.5	23.3±0.2
5	25±0.3
7.5	35.5±0.4
10	52±0.3

IC_{50}= 9.8µg/ml

Fig No:4, Alfa-Amylase Inhibition of Petroleum Ether Extract of *Cassia Auriculata*



***In vitro* Glucose Uptake by Isolated Rat Hemi-Diaphragm**

Glucose uptake using rat hemi-diaphragm study results revealed that, glucose uptake has been indicated by Methanolic Extract of *Cassia Auriculata* incubated groups (8.1 ± 0.04**) utilized more (*P<0.05, **P<0.01, ***P<0.001) glucose when compared to control and Petroleum Ether Extract of *Cassia Auriculata* (4.5±0.06*), but positive control have more glucose uptake (***P<0.001) when compared with each other groups (8.2±0.1***).

GROUPS	TISSUE UTILIZED GLUCOSE LEVEL (µg/ 30 ml)
Control (2 ml of Tyrode solution with 4% Glucose).	3.5±0.4
Positive Control (2 ml Tyrode solution with 4% Glucose and regular insulin (Nova Nardisk) 0.62 ml of 0.4 units per ml).	8.2±0.1***
Petroleum Ether Extract (2 ml Tyrode solution with 4% Glucose and 1.38 ml of Petroleum Ether extract of *Cassia Auriculata)*	4.5±0.06*
Methanolic Extract (2 ml Tyrode solution with 2% Glucose and 1.38 ml of Methanolic Extract of *Cassia Auriculata)*	8.1±0.04**

The glucose uptake were analyzed by using rat hemi-diaphragm and all values are expressed as Mean±SEM (*n*=6), All groups were compared to control and standard, (*P<0.05, **P<0.01, ***P<0.001) evaluated by one way, ANOVA followed by Dunnet 't' test.

DISCUSSION

Invitro study is on the principle of Inhibition of α-amylase, enzyme that plays a role in digestion of starch and glycogen are considered a strategy for the treatment of disorders in carbohydrate uptake, such as diabetes[16]. Pancreatic α-amylase is a key enzyme in the digestive system and catalyses the initial step in hydrolysis of starch mixture to smaller particles. Each extracts were tested for α-amylase inhibition to get the extraction with minimum IC_{50} value[17]. As per the above mechanism all the extract have concentration dependent affinity towards the inhibition of α-amylase enzyme. Skeletal muscle is a major tissue for blood glucose utilization and a primary target tissue for insulin action. Insulin increases glucose uptake in skeletal muscle by increasing functional glucose transport molecules by the way of GLUT-4 in the plasma membrane[18]. Glucose transport in skeletal muscle can also be stimulated by contractile activity. Drugs like glibenclamide come under the class of sulfonylureas that act by stimulating pancreas to trigger the GLUT-4 receptors[19]. Rosiglitazone come under the class of Peroxisome poliferator Gamma agonist, which acts by stimulating pancreas to trigger the GLUT-4 receptor. Above two concepts indicated our extracts acts on pancreas has activate the GLUT-4 receptor to reduce blood glucose level[20].

CONCLUSION

The two *invitro* antidiabetic methods have been performed and found to be α-amylase inhibition and glucose uptake of different extracts. To identify the active constituents. Further studies are required to purify the active principle and study the molecular mechanism of the exact pathway. This information's will be useful for the development of alternative method rather than insulin and hypoglycemic agents for the treatment of diabetes mellitus.

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