



Modulatory effect of *Cassia auriculata* plant extraction on glucose metabolism in alloxan induced diabetic wistar rats

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Abstract

Cassia auriculata plant extraction has been reported to have anti oxidants, anti-diabetic, anti-lipidemic activities on diabetes induced rats. Therefore the present study objective is to study the effect of the *Cassia auriculata* plant extract on glucose metabolism in alloxan induced diabetic rats. Male Albino Wistar rats was used as a animal model. Diabetes was induced by giving alloxan. The diabetic rats were treated with extracts of *Cassia auriculata* with different doses. Glucose-6-phosphate, Lactate dehydrogenase, Hexokinase, and Glucose-6-Phosphate dehydrogenase were assayed. Glucose-6-Phosphatase liver enzyme was decreased to 14.42% when compared to the diabetic control group rats. LDH was decreased to 61.09% when compared to the diabetic control group rats. Hexokinase levels in Plant extract treated group rats was 1.45 U/mg, Glucose-6-Phosphate dehydrogenase levels in plant extract treated group rats was 2.12 U/mg. Thus the selected plant had showed significant level of glucose 6-phosphatase activity in our experimental animals.

Keywords: Diabetes, Ethnobotanical survey, Medicinal plants, Liver enzymes.

INTRODUCTION

Diabetes mellitus (DM) is a group of metabolic disorders with different etiologies (Desai MM.,2002). It is Characterized by derangements in Carbohydrate, Protein and fat metabolism, Caused by complete or relative insufficiency of insulin Secretion and or insulin action (Balkan *et al.*,2000). According to Food and agriculture organization by 35% by the year 2025 (Boyel *et al.*,2001) and approximately, 140 million people worldwide suffer from diabetes and it becomes a real problem of public health in the developing countries like India have today become the diabetic capital world with over 20 million diabetes and this number is set to increase to 57 million by 2025 which makes it a country with the highest number of diabetics in the world (king *et al.*,1998).

The control of blood glucose in diabetes patients is achieved mainly by the use of oral hypoglycemic/ anti hyper glycaemic agents and insulin. However, all these treatments have limited efficacy and have been reported to be associated with undesirable side effects (Harrower, 1994). In order to overcome the side effects associated with diabetes, interest has been shifted to the use of alternative medicine.

Medicinal plants and their bioactive constituents have been extensively used as alternative medicine for better control and management of diabetes mellitus. According to world ethnobotanical information reports ,almost 800 plants may possess anti diabetic potential (Alarcon-Aguilara *et al.*,1998), in the past decade , research has been focused on scientific evaluation of traditional drugs of plants origin and screening of more effective and safe hypoglycemic agents has continued to be an important area. In developing countries 80% of population are using traditional medicine in primary medical care (Grover and Yadav, 2004). However, a large number of herbs are now being used in the management of Diabetes mellitus. India has a wealth of medicinally important plants and ancient herbal treatment methods where traditional alternative medicines are popularly practiced among a large segments of its population .Although several medicinal plants have gained importance for the treatment of diabetes mellitus ,many

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remain to be scientifically investigated. Consumption of garlic (*Allium Sativum*, Liliaceae) and its components has been reported to have many medicinal properties including anti diabetic and anti lipidemic activities (McKenna *et al.*, 2002). *Cassia auriculata* plant extraction has been reported to have anti oxidants (Herrera-Mundo *et al.*, 2006), anti-diabetic (Lingaiah et al, 2014; Adisakwattana, S., 2011; Porik Raju, 2015), anti-lipidemic (Abdel-Sattar E., 2010) activities on diabetes induced rats. The objective of this study is to study the effect of the *Cassia auriculata* plant extract on glucose metabolism in alloxan induced diabetic rats.

MATERIALS AND METHODS

Animal:

Male Albino Wistar rats of body weight 150-250 gr were maintained on a standard pellet diet (AMRUT, Pune, India) and water at libitum. The protocol was approved by JLS, Hyd with CPCSEA /IAEC /JLS /005 /10 /16 /005.

Chemicals:

Alloxan monohydrate and Glybenclamide are procured from Sigma, Bangalore. Wellion LUNA duo Glucometer and Blood glucose strips procured from Med trust-Gluoworld, Kerala, India. The solvent and Chemicals used in the present study were of analytical grade and procured from Merk, India.

Induction of Diabetes:

Diabetes was induced (Kaleemet, et al., 2006) in overnight fasted adult Wistar rats weighing 150-250gr by a single intraperitoneal injection of 120mg/kg/bw alloxan monohydrate. Alloxan monohydrate 120mg/kg was dissolved in 0.1M citrate buffer (PH405). Hypoglycemia was confirmed by the elevated glucose levels (>180mg/dl) in plasma determined at 72h and after injection.

Experimental Design:

After induction of Diabetes, the rats were divided in to Six groups each group comprising a minimum 6 rats. These are Group-I control rats, Group-II Diabetic control group, Group-III Diabetic group rats administered

with Glybenclamide Drug (10mg kg/bw), Group-IV Diabetic rats administered with *Acalypa indica* plant extraction (100mg/kg/bw), Group-V Diabetic rats administered with *Cassia auriculata* plant extraction (200mg/kg/bw) and group-VI Diabetic rats administered with *Cassia auriculata* plant extraction (400mg/kg/bw), with orally treatment. Body weight and blood glucose levels measurements were conducted periodically. At the end of the experiment rats were anaesthetized and scarified by cervical decapitation. The liver was collected and, homogenized.

Biochemical Determination:

At the end of the experiment rats were anaesthetized and scarified and dissect the liver. The liver were immediately dissected and washed in ice-cold saline to remove the blood and homogenized in 0.1M Tris HCL buffer PH 7.4. The supernatant was used for enzyme activity assay. Glucose-6-phosphate, Lactate dehydrogenase, Hexokinase, and Glucose-6-Phosphate dehydrogenase were assayed by the method of Oliver Bevel B (1986), Mukherjee et al (1981).

Statistical analysis:

All the grouped data were statically evaluated with SPSS software. Hypothesis testing methods included one way analysis of variance (ANOVA) followed by least significant different test. P Value of less than 0.05 were considered to indicate statistical significance. All the results were expressed as the mean $\pm$ S.D for Six animals in each group.

RESULTS

Effect of *Cassia auriculata* Flower extract on blood glucose levels of Wistar rats:

In Table-1, the blood glucose levels after treating wistar rats with *Cassia auriculata* were given. The normal Control group rats had a blood glucose levels was 52.5 mg/dl, Diabetic control group rats had 281.2 mg/dl, Standard Control group rats Blood Glucose levels was 170.3 mg/dl and *Cassia auriculata* flower extract treated group rats blood glucose levels was 155.4 mg/dl. The blood glucose levels significantly decreased up to

Table-1. Effect of *Cassia auriculata* Flower extract on Liver enzymes of Wistar rats.

	Glucose-6-phosphatase (U/mg)	Lactate dehydrogenase (U/mg)	Hexokinase (U/mg)	Glucose-6-phosphate dehydrogenase (U/mg)
Group-I (NC)	10.1	3.46	1.84	3.62
Group-II (DC)	21.5	8.3	0.9	2.45
Group-III (SC)	13.36	4.26	1.57	3.1
Group-IV <i>Cassia auriculata</i> (7mg/bw)	18.4	3.23	1.45	2.12

44.74% in *Cassia auriculata* experimental rats when compared to the Diabetic control group rats.

The Effect of *Cassia auriculata* flower extract on liver enzymes of Wistar rats (Table-1 and Figure-1). Glucose-6-Phosphatase liver enzyme was decreased to 14.42% when compared to the diabetic control group rats. LDH was decreased to 61.09% when compared to the diabetic control group rats. The Hexokinase liver enzyme was increased to 61.11% when compared to the diabetic control group rats and the Glucose-6-Phosphate dehydrogenase was increased to 13.47% when compare to the diabetic control group rats.

Comparative Studies of Blood Glucose levels and Liver Enzymes of Wistar rats which is Treated by *Cassia auriculata* Flower extract:

The effect of *Cassia auriculata* flower extract on blood Glucose levels and Liver enzymes of Wistar rats were given in Table No.21 and Graph 18. Blood glucose levels in normal control group rats was 52.5 mg/dl, and liver enzymes like Glucose-6-Phosphatase in normal control group rats was 10.1 U/mg, Lactate dehydrogenase in normal control group rats was 3.46 U/mg, Hexokinase in normal control group rats was 1.84 U/mg and Glucose-6-Phosphate dehydrogenase in normal control group rats was 3.64 U/mg. Blood glucose levels in diabetic control group rats was 281.2 mg/dl and liver enzymes Glucose-6-Phosphatase levels in diabetic control group rats was 21.5 U/mg, Lactate Dehydrogenase levels in diabetic control group rats was 8.3 U/mg, Hexokinase levels in diabetic control group rats was 0.9 U/mg, Glucose-6-Phosphate dehydrogenase levels in diabetic control group rats was 2.45 U/mg. Blood glucose levels in standard control group (Diabetic+ treated with Glybenclamide drug) rats was 170.3 mg/dl and liver enzymes Glucose-6-Phosphatase levels in standard control group rats was 13.36 U/mg, Lactate dehydrogenase levels in standard control group rats was 4.26 U/mg, Hexokinase levels in standard control group rats was 1.57 U/mg, Glucose-6-Phosphate dehydrogenase levels in standard control group rats was 3.1 U/mg. Blood glucose levels in plant extract

treated group (Diabetic +Treated with *Cassia auriculata* flower extract) rats was 155.4 mg/dl and liver enzymes Glucose-6-Phosphatase levels in plant extract treated group rats was 18.4 U/mg, Lactate dehydrogenase in levels plant extract treated group rats was 3.23 U/mg, Hexokinase levels in Plant extract treated group rats was 1.45 U/mg, Glucose-6-Phosphate dehydrogenase levels in plant extract treated group rats was 2.12 U/mg.

DISCUSSION

The liver enzymes like Hexokinase, Glucose-6-phosphatase, Lactate Dehydrogenase and Glucose-6-phosphate dehydrogenase were studied to understand the impact of the selected plant extracts on carbohydrate metabolism to further confirm their role in diabetes management. These enzymes were selected by earlier researchers to study carbohydrate metabolism (Monica Nannipieri et.al 2005. Anna Ludovica Fracanzani et.al., 2008). Glucose-6-phosphatase generally catalyses an exchange reaction between glucose and Glucose-6-phosphate and this enzyme forms a phosphor enzyme intermediate (Hass, and Byrne, 1960). It is a mechanism that is also consistent with the multiple phosphate transferase activities (Van Schaftingen and Isabelle Gerin, 2002). Administration of *Boerhavia diffusa* plant extracts decreased the glucose-6-phosphatase activity and increased plasma insulin level and also acted as an antioxidant in the test animals. (Pari and Amarnath Satheesh, 2004). It was also reported earlier that *Encostema littorale* plant extracts also decreased glucose 6-phosphatase activity (Maroo, Vasu, and Gupta. 2000. Vijayvargia, Kumar, and Gupta, 2003). The Glucose-6-Phosphatase in normal control Wistar rats group was 10.1 U/mg, in diabetic control group 21.5 U/mg, in standard control group 13.36 U/mg it was observed that when plant extracts were administered to wistar rats there is variations in the activity of Glucose-6-Phosphatase. In *Cassia auriculata* plant extract treated group the Glucose-6-Phosphatase 18.4 U/mg. Thus the selected plant had showed significant level of glucose 6-phosphatase activity in our experimental animals.

Table-2. Comparative Studies of Blood Glucose levels and Liver Enzymes of Wistar rats which is Treated by *Cassia auriculata* Flower extract.

	Blood Glucose levels (mg/dl)	Glucose-6-phosphatase (u/mg)	Lactate dehydrogenase (u/mg)	Hexokinase (u/mg)	Glucose 6-phosphate dehydrogenase (u/mg)
Group I (NC)	52.5	10.1	3.46	1.84	3.62
Group II (DC)	281.2	21.5	8.3	0.9	2.45
Group III (SC)	170.3	13.36	4.26	1.57	3.1
Group IV <i>Cassia auriculata</i> (7mg/bw)	155.4	18.4	3.23	1.45	2.12

CORRIGENDUM

The authors wish to clarify that the ethical statement in the “**Animals**” section of the published article has been corrected. The corrected statement is as follows:

“The protocol was approved by JLS, Hyderabad, under CPCSEA/IAEC/JLS/005/10/16/005.”

This correction is limited to the ethical statement in the “**Animals**” section. No other scientific content, data, results, figures, tables, conclusions, or interpretations of the published article have been changed. This correction does not affect the scientific validity or conclusions of the original article.

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Competing interests

The authors have declared that no competing interests exist.

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