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ANTI DIABETIC EFFECT OF THE AQUEOUS EXTRACT OF *CASSIA AURICULATA* FLOWER POWDER IN STEPTOZOTOCIN INDUCED DIABETIC RATS

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ABSTRACT

Cassia auriculata, a plant native to India and popularly known as 'avvarai' is distributed throughout central and south India. The roots of avvarai have been used in the treatment of skin diseases, leprosy, tumors, asthma and urethrorrhea. The leaves are recommended for leprosy, skin diseases, ulcers and diabetes mellitus for more than 2000 years. The flowers and bark of *Cassia auriculata* are used in diabetes, urethrorrhea, nocturnal emissions and pharyngopathy. The present study is to examine the antidiabetic effect of aqueous-extracted *Cassia auriculata* flower powder (CFP) on steptozotocin induced diabetic rats. The antidiabetic effect of CFP extract on steptozotocin induced diabetic rats was assessed by estimating the levels of blood glucose, liver glycogen, muscle glycogen, plasma insulin, fructosefructose amine and activities of carbohydrate metabolizing enzymes, namely glucose-6-phosphatase, fructose-1,6-bisphosphatase and glucose-6-phosphate dehydrogenase. The hypoglycemic activity of these herbal powders was compared with the allopathic drugs tolbutamide and metformin. The oral administration of CFP significantly lowered the blood glucose level and restored the normal liver and muscle glycogen levels in streptozotocin-induced diabetic rats. This effect is similar to that of tolbutamide and metformin.Plasma insulin levels of diabetic rats treated with CFP and those treated with tolbutamide were found to be similar. A significant reduction of fructosamine in diabetic rats indicates that the onset of secondary complications may be delayed by CFP. CFP decreased the activity of glucose-6-phosphatase and fructose-1,6-bisphosphatase and increased the activity of glucose-6-phosphate dehydrogenase. The study concludes that the flower powder of *Cassia auriculata* are hypoglycemic and antidiabetic. CFP contains components capable of enhancing insulin secretion and mimicking the effects of insulin on glucose metabolism.

KEY WORDS: Anti diabetic, *Cassia auriculata,* hypoglycemic, steptozotocin

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INTRODUCTION

Diabetes mellitus is characterized by hyperglycemia and disturbances of carbohydrate, fat and protein metabolism that are associated with insulin action and insulin secretion. It represents a heterogenous group of disorders having hyperglycemia, which is due to impaired carbohydrate (glucose) utilization resulting from a defective or deficient insulin secretory response or resistance or both. It is irreversible. Although patients can have a reasonably normal life, its late complications result in reduced life expectancy[1,19].Diabetes mellitus is rapidly becoming a major health problem and one of the challenges faced by modern medicine. It is the third leading cause of death (after heart disease and cancer) in many countries. It is a major cause of blindness, renal failure, amputation, heart attack and strokes. Synthetic hypoglycemic agents like glybenclamide, tolbutamide, acetohexamide and biguanides are prescribed for diabetes. But continuous intake of synthetic hypoglycemic agents results in serious side effects such as hematological, cardiovascular and gastrointestinal reactions, hypoglycemic coma and kidney and liver damage, which can endanger the life of the diabetic patients[21]. In recent years, one area receiving particular attention in the treatment of diabetes mellitus is the use of folk medicine.Plants have been used in the treatment of diabetes mellitus all over the world for centuries. Indigenous remedies have been in use for the treatment of diabetes mellitus since the time of Charaka and Sushruta (6th century BC, 400 BC). Papyrus Ebes of 1550 BC recommended a high fibre diet of wheat grains and ochre[26]. Today, more than 400 traditional plant treatments for diabetes have been described [23, 24].Many indigenous Indian medicinal plants have been successfully used to manage diabetes [18] and some of them have been tested and active principles isolated [15]. Among the classes of active chemical compounds isolated from plants with documented antidiabetic activity are alkaloids, glycosides, galactomannan, gum, peptidoglycan, glycopeptide, aminoacids and inorganic ions. To date metformin is the only ethical drug approved for treatment of non-insulin dependent diabetes mellitus derived from the medicinal plant *Galega officinalis* historically used to treat diabetes. There is every possibility of developing a few useful drugs from medicinal plants with a long history of human use [23]. *Cassia auriculata*, a plant native to India and popularly known as 'avvarai' is distributed throughout central and south India. The roots of avvarai have been used in the treatment of skin diseases, leprosy, tumors, asthma and urethrorrhea. The leaves are recommended for leprosy, skin diseases, ulcers and diabetes mellitus for more than 2000 years. The flowers and bark of *Cassia auriculata* are used in diabetes [3], urethrorrhea, nocturnal emissions and pharyngopathy.The flower powder of *Cassia auriculata* are being used as popular remedy for the treatment of diabetes mellitus in Ayurveda and Siddha medicine. However, no scientific data with regard to the effectiveness of this herb are available. *Cassia auriculata* has thus been chosen for the study to examine the antidiabetic effect on steptozotocin induced diabetic rats.

MATERIALS AND METHODS

2.1 INDUCTION OF DIABETES MELLITUS IN EXPERIMENTAL ANIMALS

Diabetes mellitus was induced in selected rats (bw 250-350g) by a single intraperitoneal injection of streptozotocin (Upjohn company, Kalamazoo, MI, USA) dissolved in 0.1M citrate buffer, pH 4.5 at a dose of 70 mg / kg body weight and after fifteen days diabetes was confirmed by the presence of high blood and urine glucose level. Control rats were given a vehicle injection at the same time when the diabetic condition was induced in experimental animals.

2.2 COLLECTION AND GROUPING OF EXPERIMENTAL ANIMALS (Common approval in Central Animal House, Raja Muthiah Medical College, not specific approval)

Healthy male adult albino rats of Wistar strain (bw 250-300g) were obtained from the Central Animal House, Raja Muthiah Medical College, Annamalai University, Chidambaram. The animals were housed in a clean and well ventilated animal house. They were fed with a standard pelleted diet (Gold Mohur, India) and drinking water *ad libitum*. The rats were divided into 6 groups of 10 rats each to determine the effective dose of flower powder of *Cassia auriculata*.

Experimental animal group

Group I :	Control rats given 1 ml of distilled water daily for 45 days
Group II :	Rats given *Cassia auriculata* flower powder (CFP) (250mg/kg bw) in distilled water (1 ml) daily for 45 days
Group III :	Diabetic control rats given 1 ml of distilled water daily for 45 days
Group IV :	Diabetic rats given CFP (250mg/kg bw) in distilled water (1 ml) daily for 45 days
Group V :	Diabetic rats given tolbutamide (100mg/kg bw) in distilled water (1 ml) daily for 45 days
Group VI :	Diabetic rats given metformin (10mg/kg bw) in distilled water (1 ml) daily for 45 days

2.3 PREPARATION OF PLANT MATERIALS TO BE ADMINISTERED

Fresh leaves and flowers of *Cassia auriculata* were collected and dried in shade. The dry flowers were finely powdered in a mill, sieved with a fine mesh and stored in an airtight container. The containers were kept in a deep freezer till the time of use.

2.4 TREATMENT OF ANIMALS

Rats were given oral dose of plant materials after 15 days of induction of diabetes. Base line body weight, fluid intake, and food consumption pattern were established and monitored during the following 45 days of oral treatment.

2.5 ESTIMATION OF BLOOD GLUCOSE

Blood glucose was estimated every 5 days in control as well as in experimental animals for 45 days by glucose oxidase method [13]. Added 0.1 ml of blood to 1 ml of 0.5 M NaOH. Then added 0.1 ml of 10% zincsulphate, mixed well and centrifuged. To 0.2 ml of the supernatant added 4 ml of the enzyme-dye reagent (125 mg glucose oxidase, 5 mg peroxidase and 0.5 ml of 1% o-dianizidine in 95% ethanol per 100 ml of phosphate (pH 7.0) solution). Standards (40-200µg glucose in 3% benzoic acid) were also treated in the same way as the test. For the blank 0.2 ml of distilled water and 4 ml of enzyme-dye reagent were taken. All the tubes were placed in the water bath at 37˚C for 45 minutes and read at 430 nm.

2.6 ESTIMATION OF GLYCOGEN

Glycogen was estimated in experimental rats by the procedure based on Good *et al.* [9]. The liver was taken out rapidly from the animal and the excess blood removed by blotting between folds of filter paper and weighed quickly to the nearest 0.1 g. Minced the liver and a portion of it was immediately put into a weighed stoppered test tube containing 30% KOH and weighed again. The difference between this weight and the original weight of the tube plus the KOH solution gives the weight of the liver sample used. It was digested in a boiling water bath for 1½ hour. Cooled in ice-cold water. Two volumes of 95% ethanol were then added and the mixture heated just to boiling. Spurting was avoided. It was left to stand overnight in the cold, then homogenized and centrifuged; the precipitate was dissolved in 5-10ml warm water. The glycogen was reprecipitated with 2 volumes of 95% ethanol. It was centrifuged and washed several times with 60% ethanol. 2ml of 2N H_2SO_4 per gram of liver was added and hydrolysed in a boiling water bath for 3-4 hours. The solution was neutralised with NaOH using phenol red as indicator, then made to a known volume and filtered. Glucose was determined in an aliquot. The factor 0.93 was used to convert glucose to glycogen.

2.7 QUANTITATIVE DETERMINATION OF PLASMA INSULIN

The plasma insulin was assayed by ELISA method using Boehringer Mannheim kit (Boehringer analyser ES 300). 0.1 ml of plasma was injected into the plastic tubes coated with monoclonal anti-insulin antibodies. 40mM phosphate buffer (pH 7.0) and anti-insulin POD conjugate were added to form anti-insulin antibody-POD conjugate. Substrate [phosphate / citrate 10 mM, pH 4.4 / H_2O_2 (sodium perborate) 3.2 mM] and chromogen [di-ammonium 2,2-azino-bis (3-ethyl benzothiazoline-6-sulphonate)] solutions were then added to form indicator reaction. A set of standards (insulin in bovine serum matrix) was also treated in a similar manner. After the development of colour, the absorbance was read at 420nm. The values were expressed as µU / ml of plasma.

2.8 ESTIMATION OF FRUCTOSAMINE

The term fructosamine refers to glycosylated albumin and other proteins. Haemoglobin is not the only protein, which reacts with glucose to form a carbohydrate protein derivative. Serum albumin and a wide variety of other proteins in blood and tissues also undergo this reaction. Glycosylated haemoglobin measurements provide an indication of glucose control over the last 3 months or so, measurements of fructosamines gives a clear picture of more short-term glucose levels [5].Fructosamine was estimated in serum of control and experimental animals by the procedure described by Armbruster [2]. The reaction mixture contained 50mM potassium phosphate buffer (pH 7.0), 100 µM nitro blue tetrazolium and 0.1 ml serum in a final volume of 3 ml. Incubated at 25°C for 30 minutes. The standards (fructose 40 µg – 200 µg) were also treated as above and read the colour developed at 530nm.

2.9 ASSAY OF HEXOKINASE (ATP:D-HEXOSE-6-B-PHOSPHOTRANSFERASE; E.C.2.7.1.1)

Hexokinase is a key glycolytic enzyme. It phosphorylates glucose to glucose-6-phosphate with the help of ATP. Hexokinase has low K_m value like 50 mM for glucose, and high V_{max}. Hexokinse is allosterically inhibited by glucose-6-phosphate. Increased glycolysis and β-oxidation in diabetes enhances the concentration of ATP and raises the ATP/ADP ratio. These inhibit phosphofructokinase; fructose-6-phosphate and glucose-6-phosphate accumulate and inhibit the hexokinase, stopping further glycolysis. Hexokinase activity was assayed [4]. The reaction mixture in a total volume of 5 ml contained the following viz., 1 ml of 5mM glucose solution, 0.5 ml of 72 mM ATP solution, 0.1 ml of 50 mM magnesium chloride solution, 0.4 ml of 12.5 mM potassium dihydrogen phosphate, 0.4 ml of 0.1 M potassium chloride, 0.4 ml of 0.5 M sodium fluoride and 2.2 ml of 0.01 M Tris-HCl buffer (pH 8.0). The mixture was pre-incubated at 37°C for 5 min. The reaction was initiated by the addition of 2 ml of tissue homogenate. 1 ml of the reaction mixture was immediately removed to the tubes containing 1 ml of 10% TCA which was considered as zero time. A second aliquot was removed after 30 min incubation at 37°C. The protein precipitate was removed by centrifugation and residual glucose in the supernatant was estimated by the O-toluidine method. The enzyme activity is expressed as µmoles of glucose phosphorylated / min / mg protein.

2.10 ASSAY OF LIVER FRUCTOSE 1,6 BISPHOSPHATASE (FRUCTOSE1,6-BISPHOSPHATEPHOSPHOHYDROLASE; EC 3.1.3.11)

Fructose 1,6 bisphosphatase, the key gluconeogenic enzyme hydrolyses, fructose-1,6-bisphosphate to fructose-6- phosphate and inorganic phosphate. This enzyme occurs in the hepatic and renal cytosol. It is strongly and allosterically inhibited by AMP, but is activated by citrate. Insulin represses the enzyme and reduces gluconeogenesis. Decreased insulin concentration, increased glucagon in diabetes enhances gluconeogenesis by inducing the activity of fructose-1,6-bisphosphatase [3]. Fructose-1,6-bisphosphatase was assayed by the method of Gancedo and Gancedo[8]. The assay mixture in a final volume of 2 ml contained 1.2 ml of 0.1M Tris HCl buffer, pH 7.0, 0.1 ml of substrate (0.05M fructose-1,6-bisphosphate), 0.25 ml of 0.1M magnesium chloride, 0.1 ml of 0.1M potassium chloride solution, 0.25 ml of 0.001M EDTA solution and 0.1 ml of enzyme homogenate. The incubation was carried out at

37°C for 15 min. The reaction was terminated by the addition of 10% TCA. The suspension was centrifuged and the supernatant was used for phosphorus estimation by the method of Fiske and Subbarow [7]. The supernatant was made upto a known volume. To this 1 ml of ammonium molybdate (2.5g of ammonium molybdate in 100 ml 3N H_2SO_4) was added followed by 0.4ml of ANSA (0.02 g of ANSA + 0.12 g sodium bisulphite + 0.12 g sodium sulphite dissolved in 10 ml water). The blue colour developed after 20min was read at 660 nm. Enzyme activity was expressed as µmoles of inorganic phosphorus liberated / min / mg protein.

2.11 ASSAY OF LIVER GLUCOSE-6-PHOSPHATASE (GLUCOSE-6-PHOSPHATE PHOSPHO HYDROLASE; E.C.3.1.3.9)

Glucose-6-phosphatase occurs in hepatic microsomal membrane, hydrolyses glucose-6-phosphate to release glucose in the endoplasmic reticulum. It is induced by glucagon, adrenaline, and glucocorticoid. It enhances gluconeogenesis. Insulin represses the enzyme. The activity of the glucose-6-phosphatase is enhanced during diabetes. Glucose-6-phosphatase was assayed [6]. The incubation mixture contained 0.7ml of 0.1M citrate buffer pH 6.4, 0.5 ml of substrate (0.01M glucose-6-phosphate) and 0.3 ml of tissue homogenate. The reaction mixture was incubated at 37°C for 1hr. Addition of 1 ml of 10% TCA to the reaction tubes terminated the reaction of the enzyme. The suspension was centrifuged and the phosphorus content of the supernatant was estimated by the method of Fiske and Subbarow [7]. Enzyme activity was expressed as µmoles of inorganic phosphorus liberated/min/mg protein.

2.12 ASSAY OF LIVER GLUCOSE-6-PHOSPHATE DEHYDROGENASE (GLUCOSE-6-PHOSPHATE: $NADP^+$ OXIDO REDUCTASE, EC 1.1.1.49)

Glucose-6-phosphate dehydrogenase was assayed by the method of Kornberg and Horecker,[17]. Measured into a test tube 2.0 ml of 0.05 M triethanolamine buffer pH 7.6, 0.1 ml 0.01 M NADP and 1 ml of liver homogenate. Mixed and allowed to stand for 5 min at 25°C. Added 0.05 ml of 0.031M glucose-6-phosphate and after about 2min read the extinction at 340nm every minute for 5 minutes. Used a blank with tissue homogenate plus buffer but without NADP and glucose-6-phosphate. The glucose-6-phosphate dehydrogenase activity was measured by the initial rate of reduction of $NADP^+$ at 25°C by following the increase in absorption at 340nm.

RESULTS AND DISCUSSION

3.1 EFFECT OF CFP ON BLOOD GLUCOSE, LIVER AND MUSCLE GLYCOGEN

Effect of CFP on blood glucose, liver and muscle glycogen levels is presented in Table 1.

Table I

LEVELS OF BLOOD GLUCOSE, LIVER AND MUSCLE GLYCOGEN IN DIFFERENT GROUPS OF RATS

Groups	Blood glucose (mg/dl)	Glycogen (mg/g tissue)			
		Liver	% increase (+) or % decrease (-)	Muscle	% increase (+) or % decrease (-)
I	79.4 ± 4.1 [a]	60.0 ± 1.2 [a]	NS	8.3 ± 0.4 [a]	NS
II	77.8 ± 8.6 [a]	60.0 ± 1.5 [a]	NS	8.2 ± 0.3 [a b]	NS
III	264.2 ± 23. 8 [c]	35.5 ± 3.5 [c]	-41	5.2 ± 0.8 [c]	-38
IV	79.1 ± 6.1 [a]	59.9 ± 1.9 [a]	+68	8.2 ± 0.4 [a b]	+58
V	87.7 ± 12.1 [b]	55.9 ± 2.9 [b]	+57	8.2 ± 0.4 [a b]	+58
VI	85.8 ± 6.8 [a]	57.9 ± 2.4 [a b]	+63	7.9 ± 0.4 [a b]	+52
CD (0.05)	8.02	2.3		0.33	

Values are mean ± SD of ten rats.NS – Not significant, Values with different superscripts differ significantly (p<0.05)
I - Control II - C + CFP (250mg) III - Diabetic control (D)
IV – D + CFP (250mg) V - D + Tolbutamide (100mg) VI - D + metformin (10mg)
Percent decrease (-) and increase (+) in glycogen are calculated over control for group (II) an diabetic control for groups (IV –VI).

a) BLOOD GLUCOSE

In control rats, oral administration of CFP did not alter the blood glucose level from the normal levels. It indicates that the CFP do not have hypoglycemic effect on normoglycemic rats. It has been reported that oral antidiabetic drugs produce hypoglycemia in normal rats by stimulating the pancreatic β-cells to produce more insulin and by increasing glycogen deposition in the liver. Also the exogenous administration of insulin is well known to produce hypoglycemia in both normal and diabetic subjects [11]. It is noteworthy that the blood glucose level of the normal rats treated with CFP was not significantly different from that of the controls. It did not produce hypoglycemia in normal rats. Hypoglycemic coma is one of the serious side effects with some of the antidiabetic drugs. This means that there is no risk of hypoglycemia, when *Cassia auriculata* is used in the treatment of diabetes. Oral administration of CFP produced a significant ($p<0.05$) lowering of blood glucose in streptozotocin-induced diabetic rats. The elevated blood sugar level in the streptozotocin-diabetic rats was reduced to a level on par with those of the controls. This effect was found to be similar to that of tolbutamide (100mg/kg bw) and metformin (10mg/kg

bw), the known oral hypoglycemic agents used for comparison.

b) LIVER AND MUSCLE GLYCOGEN

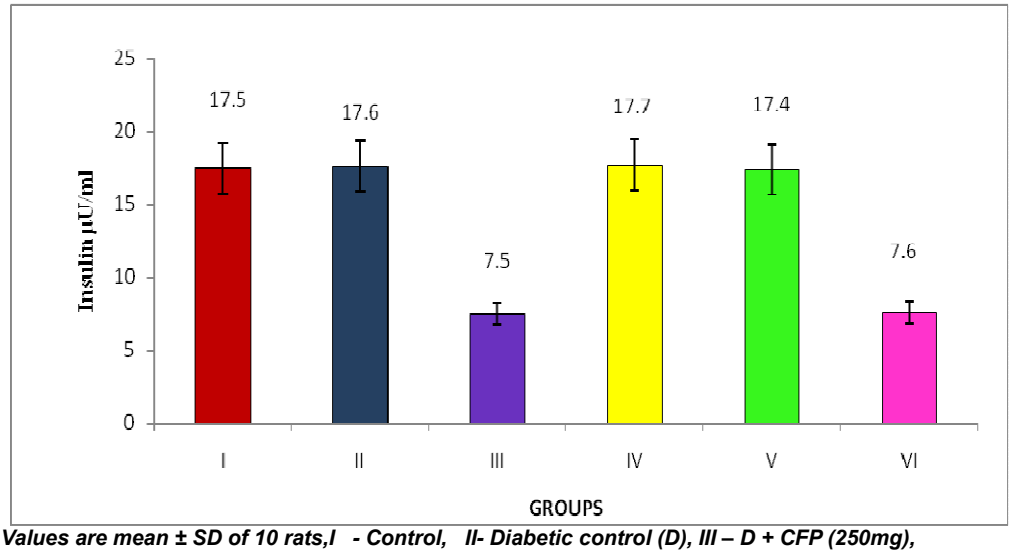
Administration of CFP did not produce any change in liver and muscle glycogen levels in control rats. The values were within normal range, liver glycogen: 5.23–6.17g/100 g liver, muscle glycogen: 0.8 – 1.05g/100 g muscle. Liver and muscle glycogen levels were significantly ($p<0.05$) reduced in streptozotocin-induced diabetic rats. The decrease in hepatic and muscle glycogen content in diabetics has been observed earlier [20]. The change observed is probably due to the lack of insulin in the diabetic state, which results in the inactivation of glycogen synthase system. Treatment with CFP restored the hepatic and muscle glycogen to near normal levels ($p<0.05$). The percent increase in hepatic glycogen on administration with CFP, compared to that of the streptozotocin-diabetic rats was 68 per cent, whereas the same for muscle glycogen was 58. This shows that in streptozotocin diabetes CFP restored the liver and muscle glycogen levels to normal and this effect is similar to that caused by tolbutamide and metformin.

3.2 EFFECT OF CFP ON PLASMA INSULIN CONCENTRATION

Figure-1 illustrates the effect of CFP on the plasma insulin levels of control and diabetic rats.

Figure 1

Effect of CFP on plasma insulin concentration



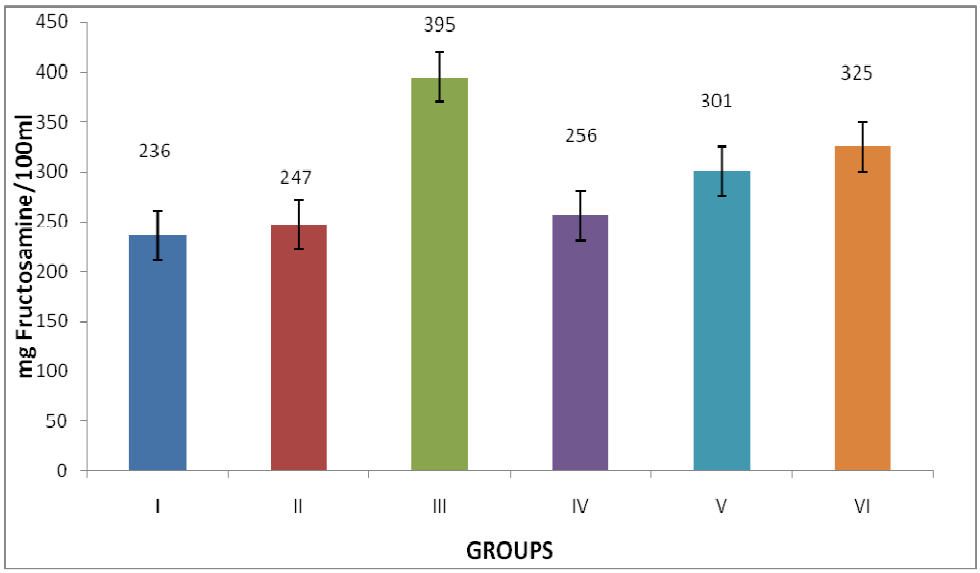
Values are mean ± SD of 10 rats,I - Control, II- Diabetic control (D), III – D + CFP (250mg), IV - D + Tolbutamide (100mg), VI - D + metformin (10mg)

Plasma insulin concentrations were not significantly different in control rats treated with CFP comparison with the untreated control rats. It has been reported that sulphonylurea compounds stimulate the pancreatic β-cells to produce more insulin and lead to hypoglycemia. It is an adverse effect of the drug sulfonylurea used in the treatment of diabetes. Administration of CFP did not produce any significant increase in plasma insulin level of normoglycemic rats. This indicates that there is no risk of increased secretion of insulin and hypoglycemia while treating diabetes with CFP.A significant ($p<0.05$) decrease in the plasma insulin level in streptozotocin-induced diabetic rats has been reported [27]. Streptozotocin administration has also been reported to cause the destruction of β-cells resulting in hypoinsulinemia and severe hyperglycemia in experimental animals [12]. A significant ($p<0.05$) increase in plasma insulin level was observed in streptozotocin diabetic rats treated with CFP. Sugihara *et al*. [25] have also reported similar plasma insulin levels of diabetic animals treated with various medicinal plants. The insulin levels of diabetic rats treated with CFP and those treated with tolbutamide (a sulfonylurea) were found to be similar. The sulfonylureas act directly by stimulating β-cells of islets of Langerhans to release more insulin[14]. This indicates that CFP also stimulate β-cells of islets of Langerhans to release insulin, but does not lead to hypoglycemia as the blood glucose level was within the normal range in these groups. Another well-known antidiabetic drug metformin (a biguanide) failed to improve the plasma insulin level in streptozotocin diabetic rats. Sulfonylureas and Biguanides are different in their mechanism of hypoglycemic activity. Biguanides lower blood glucose through suppression of gluconeogenesis and by enhancing the anaerobic glycolysis [28].The potency of the effect of CFP was approximately equal to that of tolbutamide. CFP enhances insulin secretion from intact pancreatic β-cells of islets of Langerhans.

3.3 INFLUENCE OF CFP ON SERUM FRUCTOSAMINE LEVEL

Serum fructosamine concentration in control and experimental groups of rats is presented in Figure-2.

Figure 2
Influence of CFP on serum fructosamine level



Values are mean ± SD of 10 rats , I - Control, II - Diabetic control (D), III – D + CFP (250mg), IV - D + Tolbutamide (100mg), VI - D + metformin (10mg)

Serum fructosamine concentrations were not significantly different in control rats treated with CFP. There was a significant ($p<0.05$) increase in the plasma fructosamine level in streptozotocin-induced diabetic rats. Glycoprotein, glycosylated proteins and glycosylated haemoglobin are elevated in uncontrolled diabetes and can be used as an excellent marker of overall glycaemic control. Glycosylation of proteins depends on the glucose level in the cell. A significant ($p<0.05$) reduction of fructosamine (glycosylated plasma proteins) in diabetic rats treated with CFP, tolbutamide and metformin treatment confirms the overall blood glucose control. The hypoglycemic effect produced by CFP in this respect was significantly more ($p<0.05$) than the effect produced by the well-known oral hypoglycemic agents tolbutamide and metformin. It suggests that the onset of secondary complications (microangiopathy or diabetic kidney disease and retinopathy) may be delayed by the herbal therapy [22].

3.4 EFFECT OF CFP ON HEPATIC KEY ENZYMES OF CARBOHYDRATE METABOLISM

Liver is the candidate organ for glucose metabolism. Glycolysis and gluconeogenesis are the two prime complementary events balancing the glucose load in our body. Effect of CFP on hepatic key enzymes of carbohydrate metabolism hexokinase, glucose-6-phosphate dehydrogenase, glucose-6-phosphatase and fructose-1,6-bisphosphatase is shown in Table 2.

Table 2
ACTIVITIES OF HEXOKINASE, GLUCOSE-6-PHOSPHATE DEHYDROGENASE,FRUCTOSE-1, 6-PHOSPHATASE AND GLUCOSE-6-PHOSPHATASE

Groups	Hexokinase (U/mg protein)	Glucose-6-phosphate dehydrogenasese (U/mg protein)	Fructose-1,6 bisphospatase (U/mg protein)	Glucose-6-phosphatase (U/mg protein)
I	58.3 ± 2.9 [a]	155.9 ± 8.8 [a]	284.9 ± 18.5 [a]	571.0 ± 16.1 [a]
II	57.6 ± 4.6 [a]	154.9 ± 4.3 [a]	285.4 ± 15.4 [a]	558.4 ± 16.9 [a]
III	23.3 ± 4.1 [c]	83.0 ± 4.4 [b]	780.7 ± 18.2 [c]	1254.2 ± 22.6 [b]
IV	58.8 ± 7.3 [a]	151.4 ± 6.4 [a]	285.5 ± 16.4 [a]	573.4 ± 17.8 [a]
V	54.4 ± 4.2 [b]	154.4 ± 8.8 [a]	275.2 ± 24.7 [a b]	560.9 ± 30.4 [a]
VI	53.6 ± 4.2 [b]	156.4 ± 4.3 [a]	263.8 ± 26.8 [b]	557.1 ± 26.0 [a]
CD $_{(0.05)}$	3.89	5.80	16.97	27.74

Values are mean ± SD of 10 rats Values with different superscripts differ significantly ($p<0.05$)
Hexokinase : Units : µmoles of glucose phosphorylated/hr
Glucose-6-phosphate dehydrogenase : Units: 50% reduction in NADP
Fructose-1,6 bisphosphatase: Units: µmoles of pi liberated/hr
Glucose-6-phosphatase: Units: µmoles of pi liberated/hr

Oral administration of CFP (250mg/kg bw) to control rats did not change the activities of glycolytic enzyme, hexokinase, pentose phosphate pathway enzyme, glucose-6-phosphate dehydrogenase and gluconeogenic enzymes, glucose-6-phosphatase and fructose-1, 6-bisphosphatase. In streptozotocin-induced diabetic rats a significant decrease in the activity of hexokinase and glucose-6-phosphate dehydrogenase was observed. The gluconeogenic enzymes glucose-6-phosphatase and fructose-1,6-bisphosphatase activities ($p<0.05$) were significantly increased in diabetic rats.The decreased activity of the key glycolytic enzyme, hexokinase and pentose phosphate pathway enzyme glucose-6-phosphate

dehydrogenase during diabetes has reduced the disposal of glucose as glucose-6-phosphate. On the contrary, the activities of the gluconeogenic enzymes, viz. glucose-6-phosphatase and fructose-1,6-bisphosphatase were enhanced during diabetes. Gupta *et al*. [10] have also made a similar observation in diabetic rats. Changes in the activities of these enzymes have impaired glucose homeostasis leading to hyperglycemia.CFP decreased the activity of gluconeogenic enzyme glucose-6-phosphatase and fructose-1,6-bisphosphatase and enhanced the glucose oxidation by the pentose phosphate shunt through activation of its principle enzyme glucose-6-phosphate dehydrogenase in streptozotocin-induced diabetic rats and increased the glycogen synthesis by activating its key enzyme hexokinase. Treatment with increased hexokinase and glucose-6-phosphate dehydrogenase activity and suppressed hepatic gluconeogenesis by reducing the activities of fructose-1,6-bisphosphatase and glucose-6-phosphatase significantly ($p<0.05$). The effects of CFP treatment on enzyme parameters are very similar to those caused by the reference drugs tolbutamide and metformin. The enhanced activities of the enzymes in CFP treated groups may be due to increased utilization of glucose and increased level of serum insulin. Insulin integrates hepatic carbohydrate metabolism by increasing the biosynthesis of enzymes of glycolysis, glycogenesis, and pentose oxidative pathway and by inhibiting gluconeogenesis [6].

CONCLUSION

Oral administration of cassia auriculata flower powder significantly lowered the blood glucose level in streptozotocin-induced diabetic rats. It showed that the hypoglycemic activity of these herbal powders is on par with that of the allopathic drugs tolbutamide and metformin. Administration of CFP restored the normal liver and muscle glycogen levels. This effect is similar to that of tolbutamide and metformin. Plasma insulin levels of diabetic rats treated with CFP and those treated with tolbutamide were found to be similar. This indicated that these herbal powders also stimulate the cells of islets of Langerhans to release insulin and maintain normoglycemia. A significant reduction of fructosamine in diabetic rats indicates that the onset of secondary complications may be delayed by CFP. CFP decreased the activity of gluconeogenic enzymes glucose-6-phosphatase and fructose-1,6-bisphosphatase and enhanced the glucose oxidation by the pentose phosphate shunt through activation of its principle enzyme glucose-6-phosphate dehydrogenase in streptozotocin-induced diabetic rats and increased the glycogen synthesis by activating its key enzyme hexokinase. The enhanced activities of the enzymes in CFP treated groups may be due to increased utilization of glucose and increased level of plasma insulin. Insulin integrates hepatic carbohydrate metabolism by increasing the biosynthesis of enzymes of glycolysis, glycogenesis, and pentose oxidative pathway and by inhibiting gluconeogenesis.

CONFLICT OF INTEREST

Conflicts of interest are declared none.

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