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A comparative study on the antidiabetic effects of *Trigonella foenum graecum* and *Caesalpinia bonducella* in alloxan induced diabetic rats

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ABSTRACT

Diabetes mellitus is a metabolic disease characterized by defects in insulin secretion. Allopathic medicines decrease the glucose level by acting on pancreatic beta cells. Indigenous medicines are used to treat diabetes mellitus because, the side effect are less, easy availability and less expensiveness. *Trigonella foenum graecum* seeds and *Caesalpinia bonducella* can increase glucose induced insulin release in human and rat pancreatic islets. The study was carried out to compare the antidiabetic potential of *Trigonella foenum graecum* seeds and *Caesalpinia bonducella* in alloxan induced diabetic rats. In our present study group I rats were treated with saline, group II rats with alloxan, group III rats with methanolic extract of *Caesalpinia bonducella* leaves along with alloxan and group IV rats with *Trigonella foenum graecum* seeds powder along with alloxan and group V rats with Glibenclamide along with alloxan for 21 days. The objective of the study was to analyze the diabetic profile, lipid profile, toxicity studies, changes in the levels of Homocysteine, (marker of cardiovascular disease), Folic acid (enhancer of endothelial function) and to compare the antidiabetic effects of *Trigonella foenum graecum* and *Caesalpinia bonducella* in alloxan induced diabetic rats.　© 2010 Trade Science Inc. - INDIA

INTRODUCTION

Diabetes is now regarded as a heterogenous group of diseases characterized by a state of chronic hyperglycemia, which causes a number of secondary complications like cardiovascular, renal, neurological and ocular diseases[10]. Oral hypoglycemic drugs are used only in the treatment of type 2 diabetes which is a disorder involving resistance to secreted insulin. The commonly used hypoglycemic drugs are Sulfonylureas - Glipizide,

Glyburide (Glibenclamide), Glimepiride, Metformin, Thiazolidinediones, Alpha-glucosidase inhibitors.

The uses of drugs, dietary supplements derived from plants have accelerated in recent years. Pharmacologists, microbiologists, botanists and chemists used natural products for the treatment of various diseases. In fact, according to the World Health Organization, approximately 25% of modern drugs used in the United States are been derived from plants[27].

Diabetes was induced in experimental animals by

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the administration of diabetogenic drugs like alloxan and streptozotozin. Alloxan, a ß -cytotoxic agent, rapidly and selectively accumulates in pancreatic ß -cells and causes ß-cell death and apoptosis by generation of reactive oxygen species (ROS), superoxide radicals and hydrogen peroxide[21]. ß cell death causes hyperglycemia due to insulin deficiency[31].

MATERIALS AND METHODS

Procurement of animals

Healthy young male albino rats (140gm- 160gm) were purchased from animal house, Madurai Kamaraj university, Madurai district, Tamil Nadu. The groups of rats were kept separately in individual stainless steel hoppers. The test animals were characterized by strain, source, sex, weight and age. The animals were kept individually for feeding in conventional laboratory diets with an unlimited supply of drinking water.

Procurement of diagnostic kits

Diagnostic kits used for the estimation of glucose, lipid profile, glycosylated haemoglobin were obtained from Agappe diagnostics, Thane, Maharashtra and other chemicals used are of analytical grade which were purchased from Southern India Scientific Company, Tiruchirappalli.

Diabetes induction

The alloxan monohydrate solution was made in normal water and administered with the single dose of 150 mg/kg body weight, i.m.[28].

Collection plant materials and standard drug

Caesalpinia bonducella was collected from Orathanadu, Tamilnadu and *Trigonella foenum graecum* (fenugreek) seeds were purchased from grocery shop. Glibenclamide was used as standard drug for the treatment of ulcer and was procured from Aventis Pharma Limited, Goa.

Preparation of herbal drugs and standard drug

Caesalpinia bonducella leaves were dried at 45°C for 48 hours, powdered using electric grinder and stored in a sterile container. This fine crude powder was used as herbal drug.

Measured amount of *Caesalpinia bonducella*

leaves powder were taken and added with 50 ml of 99.9% of hot methanol. The extraction was done with automatic soxhlet apparatus. The extract was administered at dosage of 200mg/kg body weight by oral gavages to the rats[7].

Fenugreek seeds were dried at 45°C for 48 hours powdered using electric grinder and stored in a container. This fine crude powder was administered with the dosage of 2g/kg b.w.[60]

Glibenclamide was administered with the dosage of 600µg/kg b.w[43].

EXPERIMENTAL

The rats were randomly divided into five groups of four animals each.

Group I   : Control rats treated with saline.

Group II : Alloxan induced rats.

Group III : Rats induced with alloxan and treated with methanolic extract of *Caesalpinia bonducella* leaves.

Group IV : Rats induced with alloxan and treated with *Trigonella foenum graecum* seed powder.

Group V : Rats induced with alloxan and treated with Glibenclamide.

All the animals were kept under treatment for 21 days.

Study protocol

The standard and test formulations were administered for 21 days, once in a day. Body weight was taken before and after experiment with the help of single pan balance. At the end of experiment, rats were sacrificed by cervical decapitation. Blood was collected and centrifuged for serum separation. For plasma, blood was collected with anticoagulant and centrifuged (2000 X g for 20min) to separate plasma. The tissues were dissected out, weighed and washed using ice cold saline solution and were homogenized (10% w/v) in Tris-Hcl buffer (0.1 M; pH 7.4) and centrifuged at 3000Xg for 20 minutes at 4°C. The resulting supernatant, plasma, serum were used for various assays.

Measurement of blood glucose levels

The body weight was measured at the beginning

and end of the experiment. Blood glucose levels was determined using a GOD - PAP method[58] Blood Glycosylated haemoglobin was determined by the method of[5,54].

Estimation of serum lipids

Serum Cholesterol levels was determined using the method of Allain CC *et al*.[3], HDL - Cholesterol was determined by Steele BW et al.[53], triglyceride was determined by Foster, C.S. and Dunn, O.[16]. LDL and VLDL were calculated using the following formulae:
LDL cholesterol = Total cholesterol – HDL + (TG/5) and
VLDL cholesterol was calculated using the formula (TG/5).

Estimation of lipid peroxidation

TBARS in pancreatic tissue was estimated by the method of Nichans and Samualson[35].

Estimation of enzymatic antioxidants

Superoxide dismutase (SOD) activity was determined by the modified method of Kakkar *et al*.[29]. Catalase (CAT) was assayed calorimetrically as described by Sinha[52]

Estimation of non-enzymatic antioxidants

Reduced glutathione (GSH) was determined by the method of Ellman[13]. Vitamin C (ascorbic acid) concentration was measured by Omaye *et al*.[37] method. Vitamin E (α-tocopherol) was estimated by the method of Desai[12].

Estimation of folic acid and homocysteine

Folic acid was determined by ELISA method[41]. Homocysteine was determined by ELISA method[64]

Statistical analysis

The data obtained in present investigation was subjected to statistical analysis. All results were expressed as Mean ± S.D. Student's T test was performed using SPSS software.

RESULTS

TABLE 1 shows the changes in the body weight, level of glucose and Glycosylated haemoglobin. The alloxan induced rats showed significant reduction in the body weight. On treatment with *Caesalpinia bonducella* and *Trigonella foenum graecum* the body weight was found to be increased when compared to alloxan induced group of rats. The results of *Caesalpinia bonducella* and *Trigonella foenum graecum* were comparable with glibenclamide treated group of rats.

The level of glucose (128.1%) and glycosylated haemoglobin (90.2%) was significantly elevated in alloxan-induced groups when compared to the control group of rats. On administration of herbal drugs viz *Caesalpinia bonducella*, *Trigonella foenum graecum* and Glibenclamide, the level of glucose (51.63%, 49.40% and 48.13%) and glycosylated haemoglobin (63.7%, 35.4% and 30.6%) was significantly reduced when compared to alloxan-induced group rats.

TABLE 1 : Changes in the body weight, level of glucose and glycosylated haemoglobin

Parameters	Control group	Alloxan induced group	*Caesalpinia bonducella* treated group	*Trigonella foenum graecum* seed treated group	Glibenclamide treated group
Body Weight(g)Gain/ Loss	+40 ± 14.1	- 30 ± 3.55	+ 20 ± 6.16	+30 ± 9.39	+ 20 ± 6.97
Glucose (mg/dl)	137.75 ± 2.62	314.25 ± 0.24* 128.1%	152 ± 1.63** 51.63%	159 ± 5.22*** 49.40%	163 ± 8.52**** 48.13%
Glycosylated Haemoglobin (mg/dl)	6.52 ± 0.38	12.4 ± 0.25* 90.2%	7.5 ± 0.42** 63.7%	8 ± 0.31*** 35.4%	8.6 ± 0.16**** 30.6%

Values are expressed as Mean ± SD., n=4 : * - group II compared with group I, ** - group III compared with group II, *** - group IV compared with group II, **** - group V compared with group II.

TABLE 2 represents the lipid profile of experimental animals. The level of lipid profile viz TC (83%), TGL (121.1%), LDL (152%) and VLDL (121.1%) was significantly elevated whereas HDL Cholesterol (29.5%) was significantly reduced in alloxan induced group when compared to the control group of rats. On administration with herbal drugs viz *Caesalpinia bonducella*, *Trigonella foenum graecum* and Glibenclamide, the

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level of lipid profile viz TC (34.1%, 38.5% & 28%), TG (44.3%, 50.3% & 39.7%), LDL (40.9%, 48.3% & 39.7%) and VLDL (44.3%, 48.2% & 39.7%) were significantly reduced whereas HDL cholesterol (12.9%, 25.8% & 9.7%) was significantly elevated, when compared to alloxan induced rats.

TABLE 2 : Changes in the lipid profile

Parameters	Control group	Alloxan induced group	*Caesalpinia bonducella* treated group	*Trigonella foenum graecum* seed treated group	Glibenclamide treated group
Total Cholesterol (mg/dl)	124 ± 1.82	227 ± 1.82* 83%	149.5 ± 4.43** 34.1%	139.5 ± 4.04*** 38.5%	163.5 ± 6.19**** 28%
Triglyceride (mg/dl)	90 ± 1.82	199 ± 4.24* 121.1%	110 .75 ± 4.64** 44.3%	99 ± 3.16*** 50.3%	120 ± 3.36**** 39.7%
HDL (mg/dl)	44 ± 3.36	31 ± 1.82* 29.5%	35 ± 0.81** 12.9%	39 ± 0.81*** 25.8%	34 ± 2.16**** 9.7%
LDL (mg/dl)	62 ± 4.08	156.25 ± 2.62* 152%	92.25 ± 4.27** 40.9%	80.75 ± 3.09*** 48.3%	105.5 ± 7**** 39.7%
VLDL (mg/dl)	18 ± 0.36	39.8 ± 0.84* 121.1%	22.15 ± 0.92** 44.3%	19.8 ± 0.63*** 48.2%	24 ± 0.67**** 39.7%

Values are expressed as Mean ± SD., n= 4: * - group II compared with group I, ** - group III compared with group II, * - group IV compared with group II, **** - group V compared with group II.**

TABLE 3 shows the levels of TBARS in pancreas of experimental animals. There was a significant elevation in the levels of TBARS (46.3%) in alloxan-induced group compared to normal. On treatment with anti diabetic drugs viz, *Caesalpinia bonducella*, *Trigonella foenum graecum* and Glibenclamide, there was a significant decrease in the levels of TBARS (26.7%, 23.3% and 22%) when compared to alloxan-induced group of rats.

TABLE 3 : Changes in the levels of TBARS in pancreatic tissue

Parameters	Control group	Alloxan induced group	*Caesalpinia bonducella* treated group	*Trigonella foenum graecum* seed treated group	Glibenclamide treated group
TBARS (mM/mg in tissue)	1.025 ± 0.15	1.5 ± 0.21* 46.3%	1.1 ± 0.40** 26.7%	1.15 ± 0.1*** 23.3%	1.17 ± 0.25**** 22%

Values are expressed as Mean ± SD., n= 4: * - group II compared with group I, ** - group III compared with group II, * - group IV compared with group II, **** - group V compared with group II.**

TABLE 4 shows the changes in the levels of enzymatic antioxidants in pancreatic tissue. The activities of SOD (70.8%) and CAT (50.52%) were decreased significantly in alloxan-induced group compared to normal. *Caesalpinia bonducella*, *Trigonella foenum graecum* and Glibenclamide treatment significantly in-

TABLE 4 : Changes in the activities of antioxidants in pancreatic tissue

Parameters	Control group	Alloxan induced group	Caesalpinia bonducella treated group	*Trigonella foenum graecum* seed treated group	Glibenclamide treated group
SOD (Units/mg protein)	48 ± 2.16	14 ± 0.81* 70.8%	31.25 ± 2.62** 123.2%	28.28± 4.5*** 102%	21 ± 4.32**** 50%
CAT (µM of H_2O_2 consumed /min/mg protein)	95 ± 2.16	47 ± 4.96* 50.52%	86 ± 2.58** 82.97 %	76 ± 2.44*** 61.7 %	72.25 ± 2.5**** 53.72 %
GSH (mg / 100 g of tissue)	65 ± 2.16	36.5 ± 2.38* 43.84 %	62 ± 1.58** 69.86 %	55.5 ± 2.08*** 52.05 %	50 ± 1.41**** 36.98 %
Vitamin C (mg/dl)	3.9 ± 0.16	2.4 ± 0.21* 38.5%	2.7 ± 0.29** 25%	3.1 ± 0.16*** 29.2%	2.9 ± 0.21**** 20.8%
Vitamin E (mg/dl)	1.225 ± 0.18	0.85± 0.05* 30.61%	1.2 ± 0.08** 41.17%	1.12 ± 0.15*** 31.76%	1.025 ± 0.12**** 20.58%

Values are expressed as Mean ± SD., n= 4: * - group II compared with group I, ** - group III compared with group II, * - group IV compared with group II, **** - group V compared with group II.**

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creased the activities of SOD (123.2%, 102% and 50%) and CAT (82.97%, 61.7% and 53.72%) in pancreatic tissue compared to alloxan-induced group.

There was a significant decrease in the levels of non enzymatic antioxidants such as GSH (43.84%), vitamin C (38.5%) and vitamin E (30.61%) in pancreatic tissue of alloxan induced rats when compared to normal. Treatment with *Caesalpinia bonducella*, *Trigonella foenum graecum* and Glibenclamide showed a significant increase in the levels of GSH (69.86%, 52.05% 36.98%), vitamin C (25%, 29.2%, 20.8%) and vitamin E (41.17%, 31.76%, 20.58%) when compared to alloxan induced group of rats.

TABLE 5 shows the levels of homocysteine & folic acid in plasma. There was a significant elevation in the levels of homocysteine (15.26%) in alloxan-induced group compared to normal. On treatment with *Caesalpinia bonducella*, *Trigonella foenum graecum* and Glibenclamide, there was a significant decrease in the levels of homocysteine (12.16%, 10.27% and 9.81%) when compared to alloxan-induced group. The activities of folic acid was decreased (-7.36%) significantly in alloxan-induced group compared to normal. *Caesalpinia bonducella*, *Trigonella foenum graecum* and Glibenclamide treatment significantly increased the activities of folic acid (4.25%, 3.32% & 2.38%) in

TABLE 5 : Changes in the levels of homocysteine & folic acid in plasma

Parameters	Control group	Alloxan induced group	*Caesalpinia bonducella* treated group	*Trigonella foenum graecum* seed treated group	Glibenclamide treated group
Homocysteine (nmoles/ml)	9.63 ± 0.06	11.1 ± 0.27* 15.26%	9.75 ± 0.17** 12.16%	9.96 ± 1.11*** 10.27%	10.01 ± 0.81**** 9.81%
Folic acid (ng/mL)	280.65 ± 0.20	261.75 ± 17.17* 7.36%	288.5 ± 3.10** 4.25%	291.30 ± 2.12*** 3.32%	294.12 ± 3.00**** 2.38%

Values are expressed as Mean ± SD., n= 4: * - group II compared with group I, ** - group III compared with group II, *** - group IV compared with group II, **** - group V compared with group II.

plasma compared to alloxan-induced group of rats.

DISCUSSION

Diabetes mellitus is the most common hereditary disease associated with carbohydrate metabolism, affecting about 200 million people worldwide. It is a metabolic disorder resulting in elevated blood sugar. Diabetes mellitus is also considered to be a serious endocrine syndrome[42].

Conventionally diabetes is managed through a combination of insulin administration, lifestyle and dietary modification and oral hypoglycemic agents. However, conventional treatment is both expensive and oral hypoglycemic agents have potentially undesirably side effects if consumed over a prolonged period of time. In recent years, cases of insulin resistance have also been encountered with some diabetic patients. This leads to an increased demand for natural products with antidiabetic activity with fewer side effects[30].

Many herbs and plant products have been shown to have hypoglycemic action[22]. In the light of above concept we have selected *Trigonella foenum graecum*

and *Caesalpinia bonducella,* as natural products to evaluate their hypoglycemic effects and to compare them with standard drug Glibenclamide for their efficacy[14].

There was significant reduction in the body weight in alloxan-induced diabetic rats (p<0.05). Loss of body weight is one of the symptoms of diabetes[17]. This loss of body weight in diabetes is due to increased lipolysis and increased muscle wasting and loss of tissue proteins caused by insulin deficiency[59]. Protein synthesis is decreased in all tissues due to absolute or relative deficiency of insulin (an anabolic hormone) in alloxan-induced diabetic rats[55]. There was significant increase in the body weight in *Caesalpinia bonducella* and *Trigonella foenum graecum* seed extract treatment on alloxan-induced diabetic rats (p<0.05). The ability of the *Caesalpinia bonducella* and *Trigonella foenum graecum* to protect from maximum body weight loss seems to be due to its ability to reduce hyperglycemia. The results of extract of *Caesalpinia bonducella* and *Trigonella foenum graecum* on bodyweight of are comparable with standard drug Glibenclamide.

Blood glucose level was increased as expected in

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alloxan-injected animals, since alloxan causes a massive reduction in insulin release, by the destruction of the beta-cells of the islets of Langerhans and inducing hyperglycemia[20]. Oral administration of *Caesalpinia bonducella*, *Trigonella foenum graecum* and standard drug glibenclamide resulted in a significant reduction in the blood glucose. The glycosylated hemoglobin gives an idea about glucose levels[39]. In our study, the glycosylated hemoglobin level was significantly elevated in alloxan-induced group showing that the diabetic animals had high blood glucose level, where as the level of glycosylated hemoglobin was significantly decreased in *Caesalpinia bonducella* and *Trigonella foenum graecum* administered animals showing the hypoglycemic effect of *Caesalpinia bonducella* and *Trigonella foenum graecum.*

The hypoglycemic effects of *Trigonella foenum graecum* have been attributed to several mechanisms. Sauvaire *et al.*[48] demonstrated *in vitro* the amino acid 4-hydroxyisoleucine in *Trigonella foenum graecum* seeds increased glucose-induced insulin release in human and rat pancreatic islet cells. This amino acid appeared to act only on pancreatic beta cells, since the levels of somatostatin and glucagon were not altered. *Trigonella foenum graecum* seeds exert hypoglycemic effects by stimulating glucose-dependent insulin secretion from pancreatic beta cells[2], as well as by inhibiting the activities of alpha-amylase and sucrase[4], two intestinal enzymes involved in carbohydrate metabolism.

In addition to the amino acid in the *Trigonella foenum graecum*, a high-fibre content is also associated with the improved ability to handle blood glucose. In the presence of a high fibre diet, the cells are more sensitive to insulin and an increase in the number of insulin receptor sites occurs or alternatively, there is a stimulation of the cell's ability to burn glucose. Certain dietary fibres reduce the rate of food passage through the intestine and into the bloodstream, thereby helping to control the increase in postprandial blood sugar levels. High-fiber diets are associated with less glycosuria, lower fasting blood sugar levels, and lower insulin requirements. Water-retaining fibres, especially the mucilaginous compounds, such as the gel fibre present in *Trigonella foenum graecum* seeds, reduce the rate of glucose absorption and may also delay gastric emptying thereby preventing the rise in blood sugar levels following a meal[19].

The antidiabetic effect of *Caesalpinia bonducella* extract could be linked to more than one mechanism. Leaf extract of *Caesalpinia bonducella* caused an increase in glycogen concentration of the liver probably by stimulating the enzymes glycogen synthase and hexokinase, both of which contribute to increase glycogen synthesis[19]. Diminished phosphatidylinositol 3-kinase (PI-3K) activation in diabetes as a result of insulin deficiency has been reported to be associated with impaired skeletal muscle glycogen synthase enzyme[36]. Leaf extract of *Caesalpinia bonducella* increased PI-3K activation leading to stimulation of muscle glycogen synthase. The increase concentration of glycogen in skeletal and cardiac muscle also might be due to increased expression and translocation of GLUT-4 glucose transporters as a result of increased PI-3K activation, leading to increased peripheral uptake of glucose[11].

Sharma et al.[51], have reported the hypoglycaemic properties of the *Caselpinia bonducella*, in normal as well as alloxan induced diabetic rats. Leaves of *Caselpinia bonducella* have been traditionally used in diabetes. The drug has potential to act as antidiabetic agent[32].

The antidiabetic activity of *Caesalpinia bonducella* might be attributed to the presence of flavonoids, known to be natural antioxidants[15], which protect the existing ß-cells (which escaped alloxanization) from dying by their free radical scavenging action[31]. Tiwari AK *et al.*[57], reported that Saponins inhibited glucose transport across the intestine by inhibiting sodium glucose co-transporter-1 (S-GLUT-1).

Hypercholesterolemia and hypertriglyceridemia have been reported to occur in diabetics[46,50,56]. A significant increase in cholesterol and triglycerides, LDL, VLDL and a decrease in the level of HDL- Cholesterol was observed in our experiment in alloxan induced group in accordance to these studies.

The level of TG and TC of *Trigonella foenum graecum* treated diabetic animals were lower than those of untreated diabetic animals ($p<0.001$). These results are consistent with the previous findings[26]. Several studies have shown that an increase in HDL-C is associated with a decrease in coronary risk. Yet most of the drugs that decrease TC also decrease HDL-C[40]. In the present study, *Trigonella foenum graecum*, while

lowering TG, LDL, VLDL and TC, significantly increased the concentration of HDL-C ($p<0.001$) also. These effects may be due to sapogenins, which increase biliary cholesterol excretion, in turn leading to lowered serum cholesterol levels[9,45,48].

The lipid-lowering effect of *Trigonella foenum graecum* might also be attributed to its estrogenic constituent, indirectly increasing thyroid hormone T_4. Another mechanism by which *Trigonella foenum graecum* exerts the hypolipidemic action could be as the result of retardation of carbohydrate and fat absorption due to the presence of bioactive fiber[26].

Administration of *Caesalpinia bonducella* extract to diabetic animals normalizes triglyceride levels and cholesterol levels in alloxan induced diabetic rats. Studies by Ram *et al.*[44] and Sharma *et al.*[51] have reported a similar lipidemic-lowering activity of some medicinal plants. D.M. Kannur *et al.*[32] reported that the drug (*Caesalpinia bonducella extract*) has the potential to act as antidiabetic as well as antihyperlipidemic. Bouquet A.[8] observed that the ethanolic and aqueous extract of *Caesalpinia bonducella* also has blood pressure lowering capacity in animals.

The level of lipid peroxidation in alloxan-induced animals was significantly increased (p <0.001) when compared to normal. *Caesalpinia bonducella* and *Trigonella foenum graecum* significantly reduced (p < 0.001) the level of lipid peroxidation and increased the glutathione in diabetic animals, which might be due to its antioxidant and the free radical quenching property of the phytoconstituents of *Caesalpinia bonducella* and *Trigonella foenum graecum*.

Anti-oxidants commonly present in plants, such as phenolic compounds and saponins, are known to reduce ROS in diabetes[47]. Preliminary phytochemical screening by Rajasekaran S *et al.*[43] revealed the presence of phenolic compounds and saponins in the gel extract of *Trigonella foenum graecum*. These saponins might have lowered the level of TBARS and increase the level of SOD, CAT GSH, Vitamin C and E.

Phytochemical analysis of *Caesalpinia bonducella* by Gaur *et al.*[18], Gupta et al.[23] has revealed the presence of alkaloids, flavonoids, glycosides, saponins, tannins and triterpenoids. Many flavonoids may help to provide protection against ROS, by scavenging these radicals along with antioxidant vitamins and enzymes. Phytochemi-

cal studies of Katbamna et al.[33] also indicated the presence of predominant active constituent - the flavonoid in *Caselpinia bonducella*. The leaf of *Caesalpinia bonducella* also contain phenolic compound of Brazilin and bonducin[19]. The leaf extract showed markedly high antioxidant activity and free radical scavenging activity. Gupta et al.[24], reported hepatoprotective and antioxidant role of *Caesalpinia bonducella*.

In alloxan induced diabetic control rats the level of GSH, vitamin C and E was found to be decreased where as *Caesalpinia bonducella* and *Trigonella foenum graecum* seed extract treatment showed a significant increased in the level of GSH, vitamin C and E.

Vitamin C is an excellent hydrophilic antioxidant in plasma, because it disappears faster than other antioxidants when plasma is exposed to reactive oxygen species. As an antioxidant, vitamin C's primary role is to neutralize free radicals. Since ascorbic acid is water soluble, it can work both inside and outside the cells to combat free radical damage[1].

One of the best-characterized antioxidant is vitamin E, a fat-soluble vitamin that helps prevent damage to lipids by oxygen free radicals[38]. When highly reactive species attack lipids within membranes or lipoproteins, they set off the chain reaction of lipid peroxidation[25]. Vitamin E halts this chain reaction by breaking the chain of lipid peroxidation[38].

In our present study, there was an increase in the level of homocysteine ($p<0.001$) and decrease in the level of folic acid ($p<0.001$) in alloxan induced group of rats when compared to normal. Ethanolic extract of *Caesalpinia bonducella*, aqueous extract of *Trigonella foenum graecum* seed significantly decreased the level of homocysteine ($p<0.001$) and the level of folic acid was significantly elevated ($p<0.05$) when compared to diabetic control.

Homocysteine, an atherogenic amino acid, has emerged as a novel independent marker of risk for the development of cardiovascular disease, metabolic syndrome and diabetes mellitus over the past three decades. The water-soluble B vitamins (especially folate and cobalamin-vitamin B_{12}) have been shown to lower homocysteine[6].

Insulin acutely increases homocysteine transmethylation, transsulfuration and clearance in control rats, thus stimulating homocysteine removal from plasma. But a

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reverse mechanism is seen in alloxan induced diabetic rats, a remarkable increase in homocysteine was noticed in diabetic control rats due to the observed lack of insulin[62].

The ameliorative effects of folic acid on endothelial function have been traditionally ascribed to its homocysteine lowering effects[34]. It is well established that homocysteine acutely and chronically impairs endothelial function by inhibiting the synthesis and release of nitric oxide and enhancing the production of superoxide[34,63]. Increase in the concentration of the folic acid might lower the homocysteine levels, which enhances the endothelial function[61]. Our results also correlated with above findings.

A comparative study of the antihyperglycemic activity, hypolipidaemic activity and antioxidant activity of *Caesalpinia bonducella, Trigonella foenum graecum* and Glibenclamide was well displayed in our present study. The results of treatment with *Caesalpinia bonducella*, *Trigonella foenum graecum* was quite comparable with Glibenclamide and *Caesalpinia bonducella* is better remedy when compared to *Trigonella foenum graecum* and the standard drug.

CONCLUSION

In conclusion, we suggest that *Caesalpinia bonducella* and *Trigonella foenum graecum* have antidiabetic, antilipidaemic, antioxidant properties in animal model. Although the exact chemical compound(s) responsible for the antidiabetic activity of *Caesalpinia bonducella* and *Trigonella foenum graecum* extract still remain speculative. More detailed studies on *Caesalpinia bonducella* and *Trigonella foenum graecum* using different doses and covering longer period of observation are needed before reaching a clearcut conclusion. Further research may be needed to refine the extraction procedure of *Caesalpinia bonducella* and *Trigonella foenum graecum* could lead to improved pharmaceutical products.

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