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Research Article

Anti-Diabetic activity of Gossypium Herbaceum by Alloxan Induced Model in Rats

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ABSTRACT

The hypoglycemic and hypolipidemic effect of ethyl ether and ethanol extracts of Gossypium Herbaceum (200mg/kg) leaves was evaluated with measurements including, blood glucose level and biochemical parameters. The extracts of the leaves was tested for its efficacy in alloxan-induced diabetic rats. Animals were induced for diabetes with Alloxan (150 mg/kg of body weight- i.p.). The extracts of Gossypium Herbaceum administered daily. The extracts were also evaluated for acute oral toxicity studies and its effect on different biochemical parameters. The extracts showed significant ($p<0.01$) antihyperglycemic and hypolipidemic activity as compared to diabetic control. The extracts show beneficial effects on blood glucose level in alloxan model. It also reduces the elevated biochemical parameters such as triglycerides (TGL), low density lipoprotein (LDL), very low density lipoprotein (VLDL), Total Cholesterol (TC) and increased the reduced level of high density lipoprotein (HDL), which might be due to presence of flavanoids, tannins, alkaloids and phenolic compound present in that extract. Thus extracts of Gossypium Herbaceum could serve as good oral hypoglycemic agents and seems to be promising for the development of phytomedicines for diabetes mellitus associated cardiac disorders.

Keywords: Hypoglycemic, Hypolipidemic, Gossypium herbaceum, Alloxan

INTRODUCTION

Diabetes mellitus is a chronic metabolic disorder characterized by a high blood glucose concentration-hyperglycaemia (fasting plasma glucose > 7.0 mmol/l or plasma glucose > 11.1 mmol/l2 hours after a meal) – caused by insulin deficiency, often combined with insulin resistance. Hyperglycemia occurs because of uncontrolled hepatic glucose output and reduced uptake of glucose spills over into the urine (glycosuria) and causes an osmotic diuresis (polyuria), which in turn results in dehydration, thirst and increased drinking (polydipsia). The IDF (International Diabetes federation) has subsequently released estimates of the numbers of people with diabetes for 2003 and forecasts for 2025 of 194 million and 334 million, respectively.[1] International Diabetes Federation, 2006 published the number of people with diabetes in India currently is around 40.9 million and is expected to rise to 69.9 million by 2025 unless urgent preventive steps are taken. [2]

In recent years, there has been renewed interest in the treatment against different diseases using herbal drugs as they are

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generally non-toxic and WHO has also recommended the evaluation of the effectiveness of plants in condition where we lack safe modern drugs. In the Indigenous system of medicine like Ayurveda, many herbal medicines have been less-toxic recommended for the treatment of diabetes or madhumeha and some of them experimentally evaluated [3]. Although diet and exercise are the first steps toward achieving treatment goals of diabetics, 90% of patients with diabetes cannot maintain long term glycemic control with diet and exercise alone. Thus, anti hyperglycemic drugs are necessary for the treatment of diabetes.

Diabetes complications are divided into microvascular and macrovascular. Microvascular complications include damage to eyes (retinopathy) leading to blindness, to kidneys (nephropathy) leading to renal failure and to nerves (neuropathy) leading to impotence and diabetic foot disorders (which include severe infections leading to amputation). Macrovascular complications include cardiovascular diseases such as heart attack, stroke and insufficiency in blood flow to legs [4].

Gossypium Herbaceum L. belongs to Malveceae and commonly called as cotton plant [5, 6]. It is oldest Indian herbal drug, which is included in our present study is widely used by tribal people. Ayurvedic system has already noticed the importance of this plant and prepared the herbal drug formulation "Diabecon." This is used for diabetes [7]. It has several experimentally proven pharmacological activities, which includes Antitumor[8], Antimutagenic[9], Anticonvulsant[10] antibacterial, antihelmenthic [11] and antifungal activities [12]. The cotton seed has already proved anti-diabetic so based on the literature review the present study was carried out anti-diabetic and hypolipidemic activity of leaves of Gossypium Herbaceum.

MATERIAL AND METHODS

Collection and authentication of the plant material

The leaves of *Gossypium herbaceum* had been collected from the field of tirumala forest, Chittoor District, Andhra pradesh, India. The plant was identified and authenticated by the Botanist Dr. K. Madhava Chetty, Assistant Professor, Department of botany, Sri Venkateswara University, Tirupathi.

Preparation of extracts

The fresh leaves of *Gossypium herbaceum* were collected and dried under shade and ground into powder with mechanical grinder. The powder was passed through sieve no.30 and stored in a container. The dried powder of leaves of *Gossypium herbaceum* was defatted with petroleum ether. The defatted powder material (marc) thus obtained was successively extracted with Ethyl ether and ethanol by maceration. The solvent was removed by distillation under reduces pressure and evaporation. The resulting semisolid mass was vacuum dried by using rotary flash evaporator.

Data showing the extractive values of dried leaves powder of Gossypium *herbaceum* L.

Plant name	Part Used	Method of extraction	% yield	
			Ethyl ether	Ethanol
Gossypium herbaceum L.	Leaves	Maceration	14.16%	12.16%

Preliminary phytochemical screening

The extracts obtained by solvent extraction method are subjected to qualitative phytochemical analysis in order to identify the nature of constituents present in the leaves of *Gossypium herbaceum.* [13]

Experimental Animals

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Swiss albino mice (20-25 g) and Sprague drawly rats (150-200 g) of either sex and of approximate same age used in the present studies were procured from listed suppliers of Sri Venkateswara Enterprises, Bangalore, India. The animals were fed with standard pellet diet (Hindustan lever Ltd. Bangalore) and water ad libitum. All the animals were housed in polypropylene cages. The animals were kept under alternate cycle of 12 hours of darkness and light. The animals were acclimatized to the laboratory conditions for 1 week before starting the experiment. The animals were fasted for at least 12 hours before the onset of each activity. The experimental protocols were approved by Institutional Animal Ethics Committee SKCP/IAEC/PGCOL/11-12/03 after scrutinization.

Acute Toxicity Studies

Swiss albino mice with weight ranging (20-25 gm female) were taken for the experiment. The animals were made into a group of 3 each, dose of ethyl ether and ethanol extracts were given according to the body weight (mg/kg), starting dose of 5 mg /kg was given to the first individual animal, no death was occurred, higher doses were given to next group of animals.

Acute toxicity study of Ethyl ether and ethanolic extracts of leaves *Gossypium herbaceum* L. Based on OECD 423 guidelines

S. No	Number of animals	Dose in mg/kg	Report
1	3	5mg/kg	No death
2	3	50mg/kg	No death
3	3	300mg/kg	No death
4	3	2000mg/kg	No death

From the observation the ethyl ether and ethanol extracts of leaves of *Gossypium herbaceum* were screened for acute toxicity study by OECD guidelines 423 for determining the $LD_{50.}$ The results showed that LD50 was found to be 2000mg/kg. Therefore its ED50 was found to be 200mg/kg [14]

PHARMACOLOGICAL STUDIES

Anti-diabetic activity [15, 16]

Induction of Diabetes

Diabetes was induced for all groups except normal control group rats. Animals were allowed to fast 24 hrs prior to injection with freshly prepared saline solution of alloxan (150 mg/kg, i.p.). After 48 Hrs, rats with marked hyperglycemia (fasting blood glucose >200mg/ dl) were selected to determine the efficacy of extracts of the plant.

Alloxan induced diabetic model in rats

To investigate the hypoglycemic effect of the Ethylether and ethanol extracts, the fasted rats were divided into 5 groups of 6 each.

Group I (Normal control) - Rats were received 1% v/v tween 80

Group II (Diabetic Control) - Rats were administered alloxan (150 mg/kg, i.p)

Group III (Glibenclamide treated Group) - Rats were received (5 mg/kg, p.o.) for 14 days.

Group IV (Gossypium herbaceum ethyl ether extract) - Rats were received (200 mg/kg, p.o.) for 14 days.

Group V (Gossypium herbaceum ethanolic extract) - Rats were received (200 mg/kg, p.o.) for 14 days.

The blood samples were collected from the retro orbital of each rat under mild ether anesthesia on 0^{th}, 3^{rd}, 6^{th}, 9^{th} and 14^{th} day and serum separated by centrifugation of blood at 4000 rpm for 10mins. Blood Samples were subjected to glucose measurement by glucometer and separated serum was used for

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the estimation biochemical parameters of TGL, HDL, LDL, VLDL and TC by a semi auto analyzer.

Statistical Analysis

One-way analysis of variance (ANOVA) followed by Dunnett's method of multiple comparisons was employed using Graphpad Instat 3.0 software. $p<0.01$ was considered to be statistically significant.

RESULTS

Preliminary phytochemical screening

The preliminary phytochemical analysis of fractions of *Gossypium herbaceum* shows presence of steroids, alkaloids, flavonoids, glycosides, tannin and carbohydrate. (Table1)

Table 1: Phytochemical screening of different fractions of *Gossypium herbaceum*

Extracts	Steroids	Alkaloids	Glycosides	Flavonoid	Tannin	Carbohydrates	Phenolic compound
Ethyl ether	+	+	+	+	+	+	+
Ethanol	+	+	+	+	+	+	+

Acute toxicity

The ethyl ether and ethanol extracts of *Gossypium herbaceum* had good margin of safety and did not shown any lethal effects on the animals up to the doses of 2000mg/kg. Hence the LD50 of both extracts of *Gossypium herbaceum* was considered as 2000mg/kg. Studies were carried out with 1/10 of the LD50 as effective dose 200mg/kg.

ALLOXAN INDUCED DIABETIC MODEL IN RATS

Body weight

The table 2 shows the body weight of the normal and treated groups significantly differ from diabetic control on 14th day. The treated groups animal body weight maintained throughout the experiment compare to diabetic control.

Table 2: Effect of ethyl ether and ethanolic extracts of leaves of Gossypium herbaceum L. on body weight by alloxan induced rats.

Groups	Treatment	Body weight in gm	
		0^{th} day	11^{th} day
I	Normal control	160.25±3.52	183.33±5.42**
II	Diabetic control	154.65±2.98	129.27±4.56
III	Glibenclamide	160±4.64	171.59±5.27**
IV	EEEGH	160.82±4.31	167.5±5.94**
V	EEGH	151.66±6.20	158.45±3.64**

The values are mean±SEM, n=6 when compared with diabetic control **p<0.01

Blood glucose level

The standard (glibenclamide 5mg/kg) and ethyl ether and ethanol extract (200 mg/kg) treated groups, the peak values of blood sugar significantly decreased to 130, 145.56, and 133.75 mg/dl simultaneously on the 14th day (Table 10& Figure 6). Thus, the result was found to be more significant ($p<0.01$) in

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lowering blood glucose level compare to diabetic control. There was no significant variation between treated groups during the 14 days study.

Table 3: Effect of Ethyl ether and ethanolic extracts of dried leaves of Gossypium herbaceum L. on blood glucose level by alloxan induced rats.

Groups	Treatment	Blood glucose level in mg/dl				
		0th day	3st day	6th day	9th day	14th day
I	Normal control	81.05±31.833**	83.8±11.32**	76.63±15.28**	79.83±21.39**	85.6±15.79**
II	Diabetic control	314.5±23.1	382.16±10.9	424.33±19.35	451.66±15.38	489.16±19.51
III	Glibenclamide	310.50±6.01	271.88±3.32**	235.0±9.02**	171.7±3.88**	130.0±13.39**
IV	EEEGH	308.66±4.26	281.58±6.82**	243.0.±40.93**	195.0±11.68**	145.56±7.09**
V	EEGH	310.166±3.86	274.83±6.17**	241.44±12.50**	175.0±5.87**	133.75±10.48**

The values are mean±SEM, n=6 when compared with diabetic control **p<0.01

Biochemical parameters

Table 4 shows extract has significantly reversed the diabetes-induced hyperlipidemia Compared to diabetic control. A significant reduction of total cholesterol level, LDL, TGL and VLDL in extracts treated was significant to diabetic group. However HDL level increased with extracts and GLB group respectively when compare to diabetic control.

Table 4: Effect of Ethyl ether and ethanolic extracts of dried leaves of Gossypium herbaceum L. on biochemical parameters by alloxan induced rats.

Groups	Treatment	TC mg/dl	TGL mg/dl	HDL mg/dl	LDL mg/dl	VLDL mg/dl
I	Normal control	124.66±.12.02**	152.6±5.9**	63.66±2.51**	81.5±4.63**	24.83±1.034**
II	Diabetic control	276.16±3.52	226.83±6.01	25.3±3.2	170±8.58	48.66±3.56
III	Glibenclamide	128.16±10.60**	156.83±7.51**	68.6±1.15**	74.83±4.16**	22.83±2.66**
IV	EEEGH	132±3.53**	164.16±8.21**	52.83±4.8**	73.83±6.11**	27±6.5**
V	EEGH	134.66±7.78**	162.33±6.504**	60.66±4.58**	71.66±2.51**	24.16±4.87**

The values are mean±SEM, n=6 when compared with diabetic control **p<0.01

DISCUSSION

The present study was undertaken to investigate the antidiabetic and hypolipidemic effects of *Gossypium herbaceum* by alloxan induced diabetic model in rats. Alloxan produce highly reactive hydroxyl radicals the actions of reactive oxygen species with a simultaneous massive increase in cytosolic calcium concentration cause rapid destruction of β-cells and thus increase the blood sugar [17, 18]. Lipid

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abnormalities accompanying with atherosclerosis is the major cause of cardiovascular disease in diabetes. Therefore ideal treatment of diabetes, in addition to glycemic control, should have a favorable effect on lipid profiles. High level of TC and LDL are major coronary risk factors [19].

In the present study, diabetic rats had lower body weight, high blood sugar levels as compared to normal rats. However, orally administered ethyl ether and ethanol extracts of *Gossypium herbaceum* significantly decreased the blood glucose level. This could be due to potentiation of the insulin effect of plasma by increasing the pancreatic secretion of insulin from existing β-cells of islets of Langerhans or its release from bound insulin. The extract producing its hypoglycemic activity by a mechanism independent from the insulin secretion, it may be by inhibition of endogenous glucose production or by the inhibition of intestinal glucose absorption or the consistent antidiabetic effect of ethyl ether and ethanol extracts of *Gossypium herbaceum* in alloxan induced rats may also be due to enhanced glucose utilization by peripheral tissues. Lipid abnormalities accompanying with atherosclerosis is the major cause of cardiovascular disease in diabetes. Therefore ideal treatment of diabetes, in addition to glycemic control, should have a favorable effect on lipid profiles. High level of TC and LDL are major coronary risk factors. Hence, measurements of biochemical parameters are necessary to prevent cardiac complications in diabetes condition. In this study reveals *Gossypium herbaceum is* not only lowered TC, TG, LDL, VLDL levels but also increased level of cardioprotective lipid HDL Therefore, *Gossypium herbaceum* has potential role to prevent formation of atherosclerosis and coronary heart disease. Several authors reported those secondary metabolites, such as saponins, flavonoids, phenolic compounds, and triterpenoids have anti-hyperglycemic and hypolipidemic activity [20-22]. The phytochemical screening of *Gossypium herbaceum* revealed the presence of flavonoids, steroids, alkaloids, carbohydrates, tannins and phenolic compounds. Hence, the antidiabetic and hypolipidemic activity of ethyl ether and ethanol extracts is probably due to the presence of several bioactive anti-diabetic principles and their synergistic properties.

CONCLUSION

The present study demonstrated that both extracts of *Gossypium herbaceum* could be useful in management of diabetes associated with abnormalities in lipid profiles. The activity of gossypium herbaceum L. may be due to natural constituent like flavanoids, phenolic compounds, tannins and alkaloids. Each phytoconstituents is responsible number of activities, for example earlier studies on flavanoids proved many pharmacological activities like anti-diabetic, antioxidant, CNS disorder, ulcer, anti-inflammatory, anti arthritis, memory learning and anti-depression and not only flavanoids, alkaloids, glycosides, tannins, saponins. So further study need to be identified, isolate the active compounds, formulation and possible mechanism of actions.

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