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ANTIHYPERGLYCEMIC AND HYPOLIPIDEMIC ACTIVITY OF LEAVES OF *GOSSYPIUM HERBACEUM* BY DEXAMETHASONE INDUCED DIABETIC RAT MODEL

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ABSTRACT

The hypoglycemic and hypolipidemic effect of ethyl ether and ethanolic fractions from leaves of *Gossypium herbaceum* (200mg/kg) (EEFGH and EFGH) was evaluated by dexamethasone-induced diabetic rats. Animals were induced for diabetes with dexamethasone (10 mg/kg of body weight- s.c.) and treated orally with different fraction of *Gossypium herbaceum.* Glibenclamide used as standard drug. The fractions showed significant ($p<0.01$) anti-hyperglycemic and hypolipidemic activity as compared to diabetic control. The fractions show beneficial effects on blood glucose like as standard. It also reduces the elevated biochemical parameters such as triglycerides (TGL), low density lipoprotein (LDL), very low density lipoprotein (VLDL), and total cholesterol (TC), increased the reduced level of high density lipoprotein (HDL) and maintains body weight. Thus both fractions could serve as good oral hypoglycemic agents and seems to be promising for the development of phytomedicines for diabetes mellitus.

KEYWORDS

Hypoglycemic, Hypolipidemic, *Gossypium herbaceum*, Dexamethasone and Glibenclamide.

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INTRODUCTION

According to WHO, the prevalence of diabetes is likely to increase by 35% by the year of 2025 currently there are over 150 million diabetics worldwide and this is likely to increase to 300 million or more. Statistical projection about India suggests that the number of diabetics will rise from 15 million in 1995 to 79.4 million by 2025, making it the country with the highest number of diabetics

in the world[1, 2]. Diabetes is a serious metabolic disorder with micro and macrovascular complication that results in significant morbidity and mortality[3]. Chronic hyperglycemia during diabetes causes glycation of body proteins that in turn leads to secondary complications affecting eyes, kidneys, nerves and arteries[4]. Modern medicines like Biguanides, Sulphonylureas and Thiozolidinediones are available for the treatment of diabetes. But they also have undesired effects associated with their uses[5]. Alternative medicines particularly herbal medicines are available for the treatment of diabetes. Common advantages of herbal medicines are effectiveness, safety, affordability and acceptability[6]. Medicinal plants and their products have been used in the Indian traditional system of medicine and have shown experimental or clinical anti-diabetic activity[7, 8]. Medicinal Plants are a rich source of natural products. Medicinal plants and their products have been widely used for treatment of diabetic populace all around the world with less known scientific basis of their functioning[9, 10]. Hence, natural products from medicinal plants need to be investigated by scientific methods for their anti-diabetic activity. The Survey of literature reveals that the medicinal plant of *Gossypium herbaceum* Linn. known as Karpasa and also called Karpasaaki, Samudranta, Tundikeri, Cavya, Picu belongs to the family Malvaceae is used in Ayurveda to treat various diseases. In Ayurveda the properties of Karpasa are Katu (Pungent), Kashaya (Astringent) in rasa; Laghu (Light), Tikshna (Penetrating) guna; Ushna (Hot) virya and Katu (Pungent) vipaka. It is used in Anartava (Amenorrhoea), Kashtartava (Dysmenorrhoea) and Prasutipashchatavikara (Purpueral disorders) [11, 12]. Roots are thermogenic, emollient, abortifacient, emmenogougue, diuretic, haematopurative[13] and root bark is anticancerous[14]. Gossypium herbaceum contains a Gossypol. Gossypol is a male contraceptive[12]. It also assists menstrual flow and effectively inhibits egg implantation[15, 16]. The literature showed GH used for number of ailments by traditionally and scientifically. The present study, we reported hypoglycemic and hypolipidemic potentials of *Gossypium herbaceum* in dexamethasone diabetic rat model.

MATERIAL AND METHODS

Preparation of extracts

The collected leaves were shade dried completely. The dried leaf was then coarsely powdered and was sieved (sieve # 60) to get uniform powdered. The powdered materials was loaded in Soxhlet's extractor and defatted with N-hexane. The marc was dried and extracted with solvent ethyl ether and 80% ethanol in a Soxhlet's apparatus. Final compound was concentrated by vacuum drying. The traces of the solvents were removed by keeping the dried extracts in to desiccators.

Preliminary phytochemical screening

The fractions of leaves of *Gossypium herbaceum* was screened for the presence of various phytoconstituents like alkaloids, flavonoids, saponin, tannin and glycosides[17] etc.

Experimental Animals

All the experiments were carried out using Swiss Albino mice (25-30 g) and Wister rats (150-200 g). The animals were placed at random and allocated to treatment groups in polypropylene cages with paddy husk as bedding. Animals were housed at a temperature of 24 ± 2^0C and relative humidity of 30-70%. A 12:12 light: day cycle was followed. All animals were allowed free access to water and fed. Ethical clearance was obtained from Institutional Animal Ethical Committee (IAEC) of Sri K.V College of Pharmacy, Chickballapur, Karnataka. No: SKVCP/IAEC/PGCOL/10-11/01.

Acute oral toxicity studies

The acute toxicity study was carried out with fractions of *gossypium herbaceum* as per OECD 425 Guidelines. Mortality in each group within 24 h was recorded. The animals were observed for a further 14 days for any signs for delayed toxicity.

Dexamethasone induced diabetic model[18]

In the experiment a total of 30 overnight fasted rats were used. The 24 rats were rendered diabetic by the aqueous solution of Dexamethasone (10mg/kg, s.c.). The animals divided into seven groups of six rats

each. Group I normal control received 1% v/v tween 80, Group II served as Diabetic control, Group III served as standard treated with 5 mg/kg of Glibenclamide, Group IV and V treated with 200 mg/kg of ethyl ether and ethanolic fractions of *gossypium herbaceum* respectively. Treatment was continued for 11th consecutive days along with dexamethasone except normal control. The blood samples were collected from the retro orbital of each rat under mild ether anesthesia on 11th day and serum separated by centrifugation of blood at 4000 rpm for 10mins. Blood and serum were subjected to glucose measurement. The biochemical parameter such as TGL, HDL, LDL, VLDL and TC was estimated by a semi auto analyzer in serum. The percentage reduction of blood glucose level in serum and blood was calculated by following formula.

$$\frac{\text{Control-Test}}{\text{Control}} \times 100$$

Statistical Analysis

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

RESULTS

Preliminary phytochemical screening studies

The preliminary phytochemical analysis of fractions of *Gossypium herbaceum* shows presence of steroids, alkaloids, flavonoids, glycosides, tannin and carbohydrate (Table No.1).

Acute toxicity

The ethyl ether and ethanol fractions 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 fractions of *Gossypium herbaceum* was considered as 2000mg/kg. Studies were carried out with 1/10 of the LD50 as effective dose 200mg/kg.

Body weight

The Table No.2 shows the body weight of the normal and treated groups significantly differ from diabetic control on 11th day. The treated groups animal body weight maintained throughout the experiment compare to diabetic control.

Blood and serum glucose level

The standard (Glibenclamide (5mg/kg) and ethyl ether and ethanol fractions (200 mg/kg) treated groups, the peak values of sugar level significantly decreased in serum and blood [(77.53%, 71.82%, 69.48%) and (73.42%, 72.65%, 70.49%)] simultaneously on the 11th day (Figure No.1, Table No. 3 and 4). Thus, the fractions was found to be more significant ($p<0.01$) as standard drug in lowering blood glucose level compare to diabetic control and equal to normal control.

Biochemical parameters

Table No.5 shows GH have significantly reversed the diabetes-induced hyperlipidemia Compared to diabetic control. A significant percentage 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.

DISCUSSION

Glucocorticoids are widely used therapeutic tools, particularly in treatment for anti-inflammatory and immunomodulatory purposes. Side effects of glucocorticoid treatment include steroid diabetes[19, 20]. Glucocorticoid induced hyperglycemia is partially due to increased hepatic glucose production and insulin resistance of peripheral tissues. Moreover, glucocorticoids are known to inhibit insulin secretion[21, 22]. The underlying mechanism involves increased α_2- adrenoceptor signaling[23], increased Potassium channel activity[24] and impaired glucose metabolism[25, 26]. Although reduced insulin secretion during glucocorticoid treatment can be overcome by blocking adrenoceptor signaling or by inhibition of potassium channel, compelling evidence suggests that the proper functioning of β-cells also depends on cell survival[27]. Accordingly, a reduction of β-cell mass in long-standing glucocorticoid therapy may contribute to the consecutive development of steroid diabetes. 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, *Gossypium herbaceum* Fractions showed significant reduction in TC, TG, LDL, VLDL levels and increased level of HDL in diabetic model rats. However, the increased HDL (cardioprotective lipid) Therefore, *Gossypium herbaceum* has potential role to prevent formation of atherosclerosis and coronary heart disease. The results declared number of possible mechanism of GH inhibit the dexamethasone induced diabetic by decrease insulin resistant, promote glycogenesis in tissues and increase the glucose metabolism in cell and interfere with α_2 adreno-receptor this may be influenced on the level of insulin to maintain the normal glucose level. Several authors reported those secondary metabolites, such as saponins, flavonoids, phenolic compounds, and triterpenoids have anti-hyperglycemic and hypolipidemic activity[28-30]. Hence, the activity of *Gossypium herbaceum* may be due to different types of active secondary metabolites, each with a single or diverse range of biological activities.

Table No.1: Phytochemical screening of different fractions of *Gossypium herbaceum*

S.No	Fractions	Steroids	Alkaloids	Glycosides	Saponin	Flavonoid	Tannin	Carbohydrates
1	Ethyl ether	-	-	-	-	+	+	-
2	Ethanol	-	+	+	-	++	+	-

Table No.2: Effect of Effect of EFGH and EEFGH leaves of *gossypium herbaceum* on body weight by Dexamethasone induced rats

S.No	Groups	Treatment	Body weight in gm	
			0^{th} day	11^{th} day
1	I	Normal control	163.33±4.10	216.66±2.10*
2	II	Diabetic control	164.16±3.96	115.83±4.54
3	III	Standard treated glibenclamide (5mg/kg)	157.15±3.81	185.83±1.53*
4	IV	EEFGH 200 mg/kg +Dexamethasone	161.66±4.21	172.5±2.14*
5	V	EFGH 200 mg/kg+ Dexamethasone	160.83±3.00	167.5±3.81*

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

Table No.3: Effect of EFGH and EEFGH leaves of *gossypium herbaceum* on serum glucose level on Dexamethasone induced rats after 11 days

S.No	Groups	Groups	Serum glucose concentration (mg/dl)
1	I	Normal control	82.43±2.24*
2	II	Diabetic control	416±9.25
3	III	Standard treated glibenclamide (5mg/kg)	93.45±2.36*
4	IV	EEFGH 200 mg/kg +Dexamethasone	117.22±8.42*
5	V	EFGH 200 mg/kg+ Dexamethasone	122.96±10.10*

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

Table No.4: Effect of EFGH and EEFGH leaves of *gossypium herbaceum* on blood glucose level on Dexamethasone induced rats after 11 days

S.No	Groups	Treatment	Blood glucose level in mg/dl				
			0th day	3st day	6th day	9th day	11th day
1	I	Normal control	81.05±31.833	83.8±11.32	76.63±15.28	79.83±21.39	85.6±15.79
2	II	Diabetic control	314.5±23.1	382.16±10.9	424.33±19.35	451.66±15.38	489.16±19.51
3	III	Standard Glibenclamide (5mg/kg)	310.50±6.01	271.88±3.32*	235.0±9.02*	171.7. ±3.88*	130.0±13.39*
4	IV	EEFGH 200mg/kg +Dexamethasone	310.166±3.86	274.83±6.17*	241.44±12.50*	175.0±5.87*	133.75±10.48*
5	V	EFGH 200mg/kg+ Dexamethasone	301.0±10.36	276.66±5.88*	246.16±9.52*	215.30±9.77*	144.33±13.87*

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

Table No.5: Effect of EFGH and EEFGH leaves of *gossypium herbaceum* on biochemical parameters after 11 days treatment by dexamethasone induced rats

S.No	Groups	Groups	TG (mg/dl)	TC (mg/dl)	HDL (mg/dl)	LDL (mg/dl)	VLDL (mg/dl)
1	I	Normal control	116.74±2.67	96.36±4.96	37.83±0.72	41.00±2.80	11.96±0.72
2	II	Diabetic control	278.41±10.16	169.81±10.11	21.5±1.4	93.50±2.38	30.33±1.22
3	III	Standard glibenclamide (5mg/kg)	136.88±24.93*	118.86±11.42*	38.33±6.72*	52.67±1.82*	13.98±1.12*
5	IV	EEFGH 200 mg/k + Dexamethasone	159.00±8.33*	142.56±10.33*	36.67±1.26*	58.67±1.52*	14.10±1.42*
6	V	EFGH 200 mg/kg+ Dexamethasone	200.41±11.05*	154.87±10.51*	34.83±0.94*	62.33±1.44*	14.55±1.55*

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

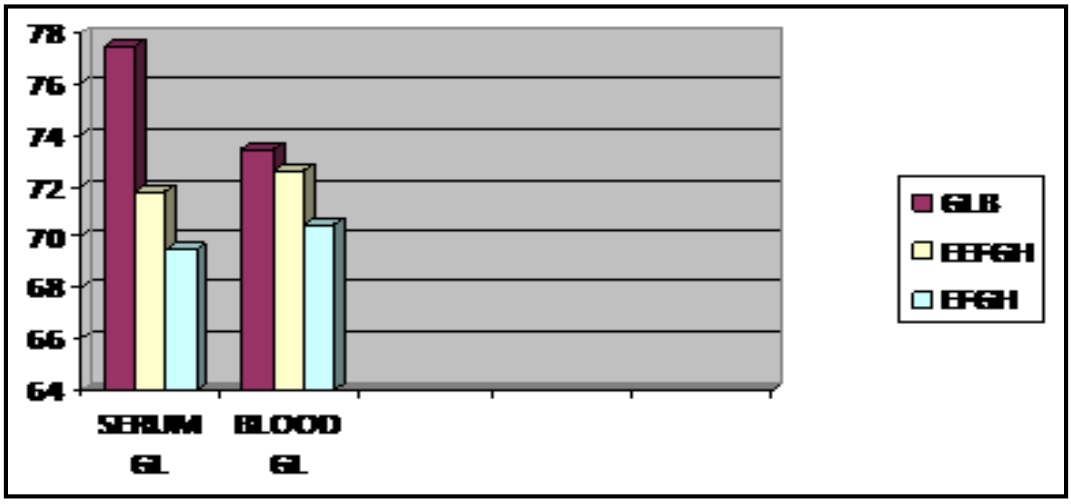

Figure No.1: Percentage reduction of serum and blood glucose level by Glibenclamide and *Gossypium herbaceum* on 11th day

CONCLUSION

The present study demonstrated that both extracts of *Gossypium herbaceum* could be useful in management of diabetes associated with abnormalities in lipid profiles. Further study need to be isolate, identify the active compounds and find out the possible mechanism of actions.

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CONFLICT OF INTEREST

We declare that we have no conflict of interest.

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