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RESEARCH ARTICLE

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ANTIDIABETIC ACTIVITY OF METHANOLIC EXTRACT OF HIBISCUS CANNABINUS IN STREPTOZOTOCIN INDUCED DIABETIC RATS

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ABSTRACT

The aim of present study was to evaluate antidiabetic activity of methanolic extract of Hibiscus cannabinus; family Malvaceae leaves in streptozotocin induced diabetic rats. The alcoholic extract of Hibiscus cannabinus was studied for antidiabetic activity in streptozotocin induced diabetic rats by oral administration of extract 400mg/kg body weight for 15 days. The effect was compared with oral dose of 0.5mg/kg Glibenclamide. The determination of blood glucose level by GOD-POD kit method. The result shows the alcoholic extract of Hibiscus cannabinus leaves significantly lowered the blood glucose of hyperglycemic rats. From the toxicity study it was observed that methanolic extract of Hibiscus cannabinus was nontoxic up to 5g/kg body weight and phytochemical study showed the presence of phytosterols, flavonoids and glycosides. It is concluded that Hibiscus cannabinus leaf extract has significant antidiabetic activity, which lowered the fasting blood glucose level in Streptozotocin induced diabetic rats.

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KEY WORDS

Anti diabetic activity, Hibiscus cannabinus, streptozotocin, GOD-POD.

INTRODUCTION

Diabetes mellitus is a serious complex chronic condition that is a major source of ill health worldwide. This metabolic disorder is characterized by hyperglycemia and disturbances of carbohydrate, protein, and fat metabolisms, secondary to an absolute or relative lack of the hormone insulin. Besides hyperglycemia, several other factors including dislipidemia or hyperlipidemia are involved in the development of micro and macrovascular complications of diabetes that are the major causes of morbidity and death[1].According to WHO projections, the prevalence of diabetes is likely to increase by 35%. Currently, there are over 150 million diabetic patients worldwide and this is likely to increase to 300 million or more by the year 2025. Statistical projection about India suggests that the number of diabetics will rise from 15 million in 1995 to 57 million in the year 2025, the highest number of diabetics in the world[2]. Reasons for this rise include increase in sedentary lifestyle, consumption of energy-rich diet, obesity, higher life span, etc. Other regions with greatest number of diabetics are Asia and Africa, where diabetes mellitus rates could rise to twofold to threefold than the present rates[3]. Evaluation of plant products to treat diabetes mellitus is of growing interest as they contain many bioactive substances with therapeutic potential. In recent years, several authors evaluated and identified the antidiabetic potential of traditionally used Indian medicinal plants using experimental animals. Previous studies confirmed the efficacy of several medicinal plants in diabetes mellitus. Although a large number of medicinal plants have been already tested for their antidiabetic effects, these effects remain to be investigated in several other Indian medicinal plants[4].

Hibiscus cannabinus (Malvaceae) is an annual or perennial herbaceous bush and has several forms with varying colors of flowers. It is native to China and grown widely as an ornamental plant throughout India. The flowers are considered emollient, and an infusion of the petals is used as a demulcent. Its decotion is given in bronchial catarrh in India. Previous studies show that the plant possesses anti-complimentary, antidiarrhetic and antiphologistic activities[5]. The leaves and flowers have been found to be effective in the treatment of heart disorders. No reports are available on the antidiabetic activity of Hibiscus cannabinus leaves. Hence, the present study focuses on the scientific investigation of antidiabetic activity of Hibiscus cannabinus leaves[6, 7].

MATERIALS AND METHODS

(i) Plant material

Fresh leaves were collected from tropical area in Yercaud and authentified by G.V.S Moorthy, Joint director, Botanical survey of India; Coimbatore was submitted to department of pharmacology for further reference.

(ii) Extraction

The leaves, shade dried, Powdered in a grinder mixture to obtain coarse powder and then passed through 60 mesh sieve. The powdered leaves were extracted using continuous hot extraction method by gradient extraction technique. The extracts were evaporated to dryness and phytochemical screenings were performed[8]

(iii) Animals

Swiss albino mice of female sex weighing 20-25gms were employed for toxicity study. Albino wistar rats of male sex weighing 200-250 gms were employed for antidiabetic study. They were housed in standard environment condition and fed with standard rodent diet with water and ad libitum. Ethical clearance for the animal study was obtained from Institutional Animal Ethical Committee (09MP03AUG2009) of CPCSEA (887/ac/CPCSEA).

(iv) Toxicity Study

An acute oral toxicity study was performed as per OECD guidelines 423. By Acute toxic class method Swiss albino mice of female sex weighing 20-25gms were used for the study. Acute toxic class method is a stepwise procedure with use of three animals of a single sex per step. Depending on mortality or morbidity status of the animals. Average 2-4 steps may be necessary to allow judgement on the acute toxicity of the substance. Three animals were used for each step. The animal were placed individually and observed for any sign of toxicity, morbidity or mortality during the first 24hrs, with special given attention during the first 4 hours and daily thereafter for a total of 14 days.[9]

(v) Induction of diabetes

All the rats were fasted overnight before the administration of Streptozotocin. Diabetes was induced in rats by intra peritoneal injection of streptozotocin dissolved in 0.1M sodium citrate buffer pH4.5 at the dose of 50mg/kg body weight. After the injection they had free access to food and water. The animals were allowed to drink 5% glucose solution overnight to overcome hypoglycaemic shock. The development of diabetes was confirmed after 48hrs of Streptozotocin injection. The animals having fasting blood glucose level more than 200mg/dl were considered as diabetic rats and used for the experimentation[10]. Diabetic animals were grouped five days after induction of diabetes Effect of Methanolic Extract of Hibiscus cannabinus in streptozotocin induced diabetes in rats.

EXPERIMENTAL DESIGN

In the experiment rats were divided into the following groups with six animals each

Group I **:** Normal control received 1% w/v gum acacia 1ml/kg for 15 days orally.
Group II **:** Diabetic control received 1% w/v gum acacia 1ml/kg for 15 days orally.
Group III **:** Diabetic rats received methanolic extract of Hibiscus cannabinus leaf 400mg/kg body weight once a day orally for 15 days.
Group IV **:** Diabetic rats treated with Glibenclamide 0.5mg/kg orally once a day for 15 days.

Rats were fasted overnight and the blood was withdrawn from the orbital sinus of the eye on the 5th day, 15th day and 20th day post induction to determine blood glucose by GOD-POD kit method. The change body weight was observed throughout treatment period in experimental animals.

STASTISTICAL ANALYSIS

All values were expressed as Mean ± S.D. The differences between control and treatment groups were tested for significance using ANOVA followed by Dunnet's t test. $P<0.05$ were considered significant.

RESULTS

The preliminary phytochemical studies indicate the presence of phytosterols, Flavonoids and glycosides in methanolic extract Hibiscus cannabinus leaf. In acute toxicity study the methanolic extract of Hibiscus

cannabinus did not produce lethality up to the dose level of 2000mg/kg.

Table- 1
Effect of Hibiscus cannabinus leaf extract on body weight in Streptozotocin induced diabetic rates

Groups	Body weight in gms(Mean±SEM)		
	Post induction days		
	5th day	15th day	20th day
Control	167.2±3.25	173±3.54	181±3.34
Diabetic control	163.8±3.34	136.8/±2.10*	125.3±2.39*
Diabetic rats+ Control	164.3±1.98	170.3±1.764*	175.8±1.47*
Diabetic rats+ glibenclamide	165.1±2.77	170.8±82.62*	178.5±2.37

Values are expressed as Mean ± S.E. n=6.
P<0.05 Experimental groups were compared with diabetic control.*
P<0.05 Diabetic groups were compared with control group.*

In the antidiabetic activity, the effects of Hibiscus cannabinus leaf extract on body weight is measured on 5th, 15th and 20th day of post induction and were compared with normal and diabetic control groups. The values are shown in Table No-1. Streptozotocin induced diabetic rats showed a significant decrease (P<0.05) in body weight compared to normal rats. Oral administration of leaf extract at the dose of 400mg/kg showed a significant) increase (P<0.05) in body weight on 15th and 20th day of post induction when compared to untreated diabetic rats.

Table- 2
Effect of Hibiscus cannabinus leaf extract on blood sugar level in streptozotocin induced diabetic rats.

Groups	Blood glucose level in mg/dl (Mean±SEM)		
	Post induction days		
	5th day	15th day	20th day
Control	62.2±1.22	61.05±1.11	60.47±1.16
Diabetic control	273.46±14.7	260.2±1.34*	269.8±11.88*
Diabetic rats+ Control	273.10±17.04	135.4±13.99*	70.61±2.24*
Diabetic rats+ glibenclamide	263.20±3.59	127.06±8.07*	69.06±1.28*

Values are expressed as Mean ± S.E. n=6.
P<0.05 Experimental groups were compared with diabetic control.*
P<0.05 Diabetic groups were compared with control.*

The effect Hibiscus cannabinus leaf extract on fasting blood glucose level is measured on 5th, 15th and 20th day of post induction and compared with normal and diabetic control groups. The values are shown in table No-2. Streptozotocin induced rats showed a significant increase (P<0.05) in fasting blood glucose level compared to normal rats. Oral administration of leaf extract at the dose of 400mg/kg body weight showed a significant

decrease (P<0.05) in blood glucose level in 10 and 15 days of treatment. The fasting blood glucose level on 15th day of post induction (10 days of treatment) was 135.4±13.99 mg/dl compared to fasting blood glucose of diabetic control animal 260.2±1.34mg/dl. The group treated with Glibenclamide 0.5 mg/kg showed fasting blood glucose level of 127.06±8.07 mg/dl. On 20th day of post induction (15days of treatment), the leaf extract treated group showed a fasting blood glucose level of 70.61 ± 2.24 mg/dl, compared to untreated diabetic animal which showed a fasting blood glucose level of 269.8 ± 11.88 mg/dl. The group treated with Glibenclamide 0.5 mg/kg orally showed fasting blood glucose level of 69.06± 1.28 mg/dl.

DISCUSSION

In the present study the hypoglycemic activity of methanolic extract of Hibiscus cannabinus leaves was evaluated in Streptozotocin induced diabetic rats. The continuous treatment of leaf extract for a period of 15 days produced a significant decrease in blood glucose level in diabetic rats which is comparable to that of standard drug Glibenclamide which is used in treatment of type II diabetes mellitus. The standard drug Glibenclamide stimulates insulin secretion from beta cells of islets of langerhans. From the study, it is suggested that the possible mechanism by which the plant extract decreases the blood glucose level may be by potentiation of insulin effect either by increase in pancreatic secretion of insulin from beta cells of islets of langerhans or by increase in peripheral glucose uptake.

CONCLUSION

The methanolic extract of Hibiscus cannabinus leaf exhibited significant hypoglycemic activity in streptozotocin induced diabetic rats. From the phytochemical analysis it was found that the major chemical constituents of the leaf extract were flavonoids and glycosides. On the basis of above evidence it is possible that the presence of flavonoids may be responsible for the observed antidiabetic activity. Further pharmacological and biochemical investigations are underway to find out the active constituents responsible for antidiabetic activity and to elucidate its mechanism of action.

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