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ANTI-DIABETIC ACTIVITY OF METHANOLIC EXTRACT OF CHICORY ROOTS IN STREPTOZOCIN INDUCED DIABETIC RATS

Faheem Mubeen[1], Hardeep[2*] and Dhananjay Kumar pandey[3]

[1]Department of Pharmacology, Kasturba Medical College, Mangalore
[2]Department of Pharmacology, Punjab institute of medical sciences, Jalandhar
[3]Department of Pharmacology, Kasturba Medical College, Mangalore, India

***Corresponding author e-mail:** shergillhardeep21@gmail.com

ABSTRACT

To study the antidiabetic activity of Chicory roots methanolic extract (MEC) in streptozotocin (STZ) induced diabetic rats. MEC of root was subjected to preliminary qualitative phytochemical investigations by using standard procedures. The extract (400 mg/kg p.o.) was screened for antidiabetic activity in STZ-induced diabetic rats (30 mg/kg, i.p.). Acute oral toxicity study for the test extract of the plant root was carried out using OECD/OCED guideline 425. Phytochemical analysis of MEC of roots revealed the presence of inulin, sucrose, cellulose, protein, carbohydrates, lipids, alkaloids, glycosides and tannins compounds. In acute toxicity study, no toxic symptoms were observed for MEC up to dose 2000 mg/kg. Oral administration of MEC for 21 days exhibited highly significant ($P < 0.01$) hypoglycemic activity and also correction of altered biochemical parameters, namely cholesterol and triglycerides significantly ($P < 0.05$). Urine analysis on 1st day showed the presence of glucose and traces of ketone in the entire group except normal control group. However, on 21st day glucose and ketone traces were absent in MEC and glibenclamide-treated groups while they were present in diabetic control. The data were analyzed using analysis of variance followed by Dunnett's test. The observations confirm that methanolic extract of the root of the plant has antidiabetic activity and is also involved in correction of altered biological parameters. It also warrants further investigation to isolate and identify the hypoglycemic principles in this Chicory root so as to elucidate their mode of action.

Keywords: Antidiabetic activity, Chicory roots, hypoglycemic, streptozotocin

INTRODUCTION

Diabetes mellitus is a metabolic disorder characterized by disturbances in carbohydrate, protein and lipid metabolism and by complications like retinopathy, microangiopathy and nephropathy. [(1).] Currently available synthetic antidiabetic agents produce serious side effects like hypoglycemic coma[(2)] and hepatorenal disturbances[(3)]. Moreover they are not safe for use during pregnancy. [(4)] Hence, the search for safer and more effective hypoglycemic agents has continued. Following the WHO's recommendation for research on the beneficial uses of medicinal plants in treatment of diabetes mellitus [(5)] investigations on hypoglycemic agents derived from medicinal plants have also gained momentum. Several investigations have been conducted and many plants have shown a positive activity. [(6)] Although many drugs are available in modern medicine to treat diabetes mellitus, they produce various systemic side effects or exhibit tolerance upon chronic use. In Ayurveda, many plant products have been claimed to be free from side effects and less toxic than synthetic drugs [(7)].

Non-insulin- dependent diabetes mellitus (NIDDM) is a multifactorial disease, which is characterized by hyperglycemia and lipoprotein abnormalities [(8)]. These traits are hypothesized to damage cell membranes, which results in excess generation of reactive oxygen species. NIDDM has also been associated with an increased risk for developing

premature atherosclerosis due to an increase in triglycerides (TG) and low-density lipoproteins (LDL), and decrease in high-density lipoprotein levels (HDL) [9].

Chicory (Cichorium intybus L.) is widespread as a winter weed in the field of clover. It is edible fresh as well as lettuce. Chicory has a long history of herbal use and is especially of great value for its tonic effects upon the liver and digestive tract. The root and the leaves are appetizer, cholagogue, depurative, digestive, diuretic, hypoglycemic, laxative and tonic [10].

Bothayna (2000) noticed that feeding on chicory roots decreased the levels of plasma glucose, cholesterol, HDL-cholesterol and also reduced liver cholesterol, triglyceride and total lipids of streptozotocin induced diabetic rats[11]. Azorin et al (2009) found that chicory (inulin) effects on rats upon increase of HDL-C. This work was carried out to investigate the effect of chicory herb as hypoglycemic and hypolipidemic in diabetic rats [12].

MATERIALS AND METHODS

Experimental Animals: Male, Wistar albino rats weighing 200 to 250g (90 to 110 days old) bred in the central animal house of Kasturba Medical College, Mangalore, were used for the study. They were housed in clean, clear, polypropylene cages and maintained at 24.0±2ºC with 12 hrs light and dark cycles and have free access to food and water ad libitum. Animals were kept in experimental lab for seven days prior to experiment to acclimatize laboratory conditions. Each rat was used only once. Experiments were conducted between 9:00 to17:00 hrs. The norms for Good Laboratory Practice (GLP) were followed for care of laboratory animals. The study was approved by Institutional Animal Ethical Committee.

Collection and authentication Plant Material: Fresh plant of chicory roots is collected in winter (January and February) from the agriculture fields of Medchal Village, Hyderabad. The botanical identity was confirmed by a taxonomist Prof. Kamal, Department of Botany; Gorakhpur University, Gorakhpur where voucher specimen (No. GU0309186) has been deposited.

Preparation of Chicory root extract: The Chicory roots were washed, shade dried and powdered. The powdered material was defatted with petroleum ether (60–80°C) and then extracted with methanol in Soxhlet apparatus (40 cycles). The extract was concentrated for further studies at reduced pressure and temperature in a rotary evaporator. MEC was tested for the presence of secondary metabolites by various phytochemical tests

Drugs and chemicals used: Glibenclamide, streptozotocin (STZ), and sodium citrate buffer were used in this study. Other chemicals used for extraction purpose and phytochemical tests were of laboratory grade.

Phytochemical screening: The plant may be considered as biosynthetic laboratory for the chemical compounds such as inulin, sucrose, cellulose, protein, carbohydrates, lipids, alkaloids, glycosides, tannins. The compounds that are responsible for therapeutic effect are usually the secondary metabolites. A systematic study of a crude drug embraces thorough consideration of both primary and secondary metabolites derived as result of plant metabolism. The plant material may be subjected to preliminary phytochemical screening for detection of various plant constituents.

Standard screening test of the extract was carried out for various plant constituents. The crude extract was screened various plant constituents. The crude extract was screened for the presence or absence of secondary metabolites such as alkaloids, carbohydrate, phenolic compounds, inulin, saponins, steroids, tannins, etc. by using standard procedures [13, 14].

Acute toxicity test: Acute oral toxicity study for the test extract of the root was carried out using OECD/OCED guideline 425. The test procedure minimizes the number of animals required to estimate the oral acute toxicity. The test also allows the observation of signs of toxicity and can also be used to identify chemicals that are likely to have low toxicity. Healthy, young adult albino Wistar rats (200–250 g) were used for this study. Animals were fasted prior to dosing. The fasted body weight of each animal was determined, and the dose was calculated according to the body weight. The drug was administered in the dose of 2000 mg/kg body weight orally to one animal. This first test animal survived. Then, four other animals were dosed sequentially; therefore, a total of five animals were tested. Animals were observed individually at least once during the first 30 min after dosing, periodically during the first 24 h (with special attention given during the first 4 h), and daily thereafter, for a total of 14 days. No animal died. Therefore, the LD_{50} is greater than 2000 mg/ kg [15].

An investigation with 1/20th, 1/10th and 1/5th of 2000 mg/ kg, i.e. 100, 200, and 400 mg was done in pre-screening. Only 400 mg/kg was found to be effective against diabetes, hence this dose was used in final screening.

Induction of non-insulin dependent diabetes mellitus (NIDDM): [16-18] After fasting, diabetes mellitus (DM) was induced by intraperitoneal injection of STZ dissolved in 0.1 M cold sodium citrate buffer (pH 4.4) at a dose of 30 mg/kg b.w. The animals were allowed to drink 5% glucose solution overnight to overcome the drug-induced hypoglycemia. After 72 h, STZ-treated animals were considered as diabetic when the fasting plasma levels were observed above 200 mg/dl with glucosuria. The experiments were conducted on animal groups to see the effect of MEC on diabetic rats.

Five rats were used in each of the four groups which were as follows:
Group I: Normal control (vehicle).
Group II: Diabetic control (vehicle).
Group III: Diabetic rats treated with MEC (400 mg/kg p.o.).
Group IV: Diabetic rats treated with glibenclamide (5 mg/ kg p.o.).

Vehicle, MEC, and glibenclamide were administered once daily for 21 days from the day of induction. Blood was drawn from tip of the tail, and blood glucose level was estimated on 0, 7th, 14th and 21st day of experiment with the help of glucometer (one touch ultra, Johnson and Johnson Ltd.) using strip method. On 21st day, blood sample was taken by orbital sinus bleeding method for measuring serum cholesterol and TG level using an auto-analyzer (Semi auto chemistry analyzer, CHEM 400). Fresh urine was collected, and glucose and ketone in urine were checked using keto-diastix strips on 0 and 21st day of the experiment.

Statistical analysis: All results were expressed as Mean ± SE. The data were analyzed using analysis of variance (ANOVA) and then group were compared by Dunnett's test. Values were considered statistically significant with $P < 0.05$. Graph Pad Instat was used for the analysis of data.

RESULTS

Preliminary phytochemical screening: Phytochemical screening was done using color forming and precipitating chemical reagents to generate preliminary data on the constituents of the root extract. The chemical tests revealed the presence of major secondary metabolites such as alkaloids, carbohydrate, phenolic compounds, inulin, saponins, steroids, tannins, etc. in the extract of the root of Chicory. The results indicated the presence of inulin, sucrose, cellulose, protein, carbohydrates, lipids, alkaloids, glycosides, and tannins compounds in methanolic extract of Chicory.

Acute toxicity studies: A preliminary toxicity study was designed to demonstrate the appropriate safe dose range that could be used for subsequent experiments rather than to provide complete toxicity data on the extract. Acute toxicity studies conducted revealed that the administration of methanolic extract (up to a dose of 2000 mg/kg) of Chicory root did not produce significant changes in behavior of the animals. No death was observed up to the dose of 2000 mg/kg b.w. The rats were physically active. These effects were observed during the experimental period (14 days). The results showed that in single dose the plant extract had no adverse effect, indicating that the median lethal dose (LD_{50}) could be greater than 2000 mg/kg body weight in rats. In acute toxicity study, no toxic symptoms were observed for MEC up to dose of 2 g/kg body weight. All animals behaved normally. No neurological or behavioral effects could be noted. No mortality was found up to 14 days study.

Blood glucose level: In STZ -induced diabetic rats, the blood glucose levels were in the range of 279–281 mg/dl, which were considered as severe diabetes. In the glibenclamide (5 mg/kg) and methanolic extract (400 mg/kg) treated groups, the peak values of blood sugar significantly decreased from 281.2 mg/ dl to 114.6 mg/dl and from 280.6 mg/dl to 119.2 mg/dl on the 21st day, respectively [Table 1]. Hence, in this study observations showed that the MEC reduced the blood glucose level in diabetic rats but values did not return to those of normal controls. Therefore, MEC possesses significant ($P < 0.01$) antidiabetic activity, when compared with diabetic control. There was a marked reduction in blood glucose level (in 21 days) in STZ -diabetic animals. This effect of the MEC (400 mg/kg) is nearly equal to, if not better than, that of glibenclamide (5 mg/kg) [Table 1].

Serum lipid profile: The effect of the MEC and glibenclamide, in the untreated diabetic rats, serum levels of cholesterol and TG were significantly increased [Table 2]. These complications of diabetes were attenuated with the administration of the methanolic extract. The effects of the standard drug (glibenclamide) on serum TG and cholesterol in the diabetic rats were comparable to those of the herbal

extract. Total cholesterol and TG were significantly elevated in diabetic group in comparison to control group. Administration of MEC for 21 days significantly reduced the serum levels of cholesterol and TG in comparison to diabetic control rats [Table 2].

Urine glucose and ketone: Urine analysis on day 0 showed the presence of glucose (+++) and ketone (trace) in the entire group, except normal control. However, on 21st day glucose and ketone traces were absent in MEC and glibenclamide treated groups while they were present in diabetic control group [Table 3].

DISCUSSION

The various numbers of plants have been traditionally used to treat diabetes, and some have been proven to have hypoglycemic effects. These studies have identified that compounds such as polysaccharides [(19)], inulin [(12)], terpenoids and tannins [(20)], and alkaloids [(21)] are responsible for antidiabetic effect. MEC also contains inulin, sucrose, cellulose, protein, carbohydrates, lipids, alkaloids, glycosides, tannins compounds. The observed hypoglycemic effects of this plant could have resulted from the combined activity of these compounds present in the extract.
Administration of STZ caused rapid destruction of pancreatic β-cells in rats, which led to impaired glucose stimulated insulin release and insulin resistance, both of which are marked feature of type II diabetes [(22)]. Oral hypoglycemic agents and insulin are currently available for treating DM. There is, however, a growing interest in herbal remedies due to the side effects associated with the existing drugs [(23)]. The present investigation indicates the hypoglycemic and also protective effects of MEC on serum lipid profile of STZ-diabetic rats. We have observed a significant ($P < 0.01$) decrease in blood glucose in MEC-treated diabetic rats, when compared with diabetic control rats. The possible mechanism of MEC on hypoglycemic action may be through potentiating pancreatic secretion of insulin from β-cells of islets and/or due to enhanced transport of blood glucose to the peripheral tissue or by other mechanisms such as stimulation of glucose uptake by peripheral tissue, inhibition of endogenous glucose production or activation of gluconeogenesis in liver and muscles [(12)].

Diabetes is associated with hyperlipidemia. It is well known that insulin activates enzyme lipoprotein lipase, which hydrolyzes triglyceride under normal conditions. Destruction of β-cells leads to depletion of plasma insulin, which results in hyperlipidemia. The significant control of plasma lipid levels suggests that the MEC may produce its action by improving insulin secretion [(11)].

Diabetogenic agents significantly increase the cholesterol and TG levels. The abnormally high concentration of serum lipids in DM is mainly due to an increase in the mobilization of free fatty acids from the peripheral fat depots, since insulin inhibits the hormone-sensitive lipase. The marked hyperlipidemia that characterizes the diabetic state may, therefore, be regarded as a consequence of the uninhibited actions of lipolytic hormones on the fat depots. Excess of fatty acids in plasma produced by STZ promotes the liver conversion of some fatty acids to phospholipids and cholesterol. These two substances, along with excess of TG formed in the liver, may be discharged into lipoproteins in the blood. As a result, serum phospholipids are elevated [(24)]. Administration of MEC to diabetic rats improved the cholesterol and TG [Table 2]. This effect may be due to low activity of cholesterol biosynthesis enzymes and/or due to decrease in lipolysis which are under the control of insulin [(25)]. Defects in carbohydrate metabolizing machinery and consistent efforts of the physiological system to correct the imbalance in carbohydrate metabolism place an overexertion on the endocrine system, which leads to the deterioration of endocrine control. Continuing deterioration of endocrine control exacerbates the metabolic disturbances and leads primarily to hyperglycemia [(18)]. The most significant findings of this study is that the MEC has shown beneficial effect not only on blood glucose, but also on glucose and ketone levels of urine in STZ -induced diabetic rats. Urine analysis on 0 day showed the presence of glucose and traces of ketone in the entire group except normal control. However, on 21st day glucose and ketone traces were absent in MEC and glibenclamide-treated groups while they were present in diabetic control [Table 3]. Therefore, results obtained from this study are quite promising and comparable with glibenclamide, a standard drug used to treat DM.

The observations confirm that methanolic extract of the leaf and stem of the plant has antidiabetic activity and is also involved in correction of altered biological parameters mainly Cholesterol metabolism. It also warrants further investigation to isolate and identify the hypoglycemic principles in this plant so as to elucidate their mode of action.

Table 1: **Effect of MEC root on plasma glucose STZ induced in diabetic rats.**

Groups	Blood glucose (mg/dl) in day			
	Zero time	7 days	7 days	7 days
Normal control (–)	94.2 ± 3.65	95 ± 2.7	95.8 ± 3.06	95.2 ± 2.37
Diabetic control (–)	279 ± 6.21	280.4 ± 6.01	282.6 ± 5.82	284.4 ± 5.25
Diabetic rats + MEC (400 mg/kg)	280.6 ± 5.16^{ns}	256.6 ± 4.95**	190.4 ± 5.25**	119.2 ± 2.27**
Diabetic rats + glibenclamide (5 mg/kg)	281.2 ± 5.89^{ns}	247.8 ± 5.76**	179.4 ± 6.48**	114.6 ± 3.17**

*Values are mean ± SEM; n = 5 in each group except in diabetic control group where n = 4 because one animal died on the 8th day. Ns P > 0.05 (non-significant); **P < 0.01 (highly significant) when compared to diabetic control rats; MEC or glibenclamide was administered daily for 21 days. For glucose estimation, blood was collected just before the drug administration on the 0 day and 1 h after the drug administration on the 7th, 14th day and 21st day.*

Table 2: **Effect of MEC root on serum lipid profile in STZ-induced diabetic rats.**

Groups	Cholesterol (mg/dl)	Triglycerides (mg/dl)
Normal control (–)	81.8 ± 3.69	74.6 ±5.19
Diabetic control (–)	130 ± 8.47	120.2 ± 5.24
Diabetic rats + MEC (400 mg/kg)	105.6 ± 6.06*	96.2 ± 4.38*
Diabetic rats + glibenclamide (5 mg/kg)	87.2 ± 3.14**	84.4 ± 7.52**

*Values are mean ± SEM; n = 5 in each group except in diabetic control group where n = 4 because one animal died on the 8th day. *P < 0.05 (significant); **P < 0.01 (highly significant) when compared to diabetic control rats.*

Table 3: **Effect of MEC root on urine glucose and ketone in STZ-induced diabetic rats**

Groups	0 day		21 day	
	Glucose	Ketone	Glucose	Ketone
Normal control (–)	-	-	-	-
Diabetic control (–)	+++	Trace		Trace
Diabetic rats + MEFJ (400 mg/kg)	+++	Trace	-	-
Diabetic rats + glibenclamide (5 mg/kg)	+++	Trace	-	-

Glucose - = absence of glucose, +++ = 1 g/dl; Ketone - = absence of ketone, Trace = 5 mg/dl.

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