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Phytochemical study and Biological Activity Study of *Cleome Rutidosperma*, Family-Capparidaceae.

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ABSTRACT

The present study was aimed to evaluate the antidiabetic activity of methaolic extract of the leaves of CR on streptozotocin (STZ)-induced diabetic rats. Experimental animal was induced by a single dose of intraperitoneal injection of STZ (65mg/kg). Adult male Wister albino rats were divided into five groups; normal control, diabetic control, diabetic glibenclamide (5mg/kg), diabetic CR M. extract (200mg/kg), and diabetic CR M. extract (400 mg/kg) for 14 days and analyzed for body weight (BW) and blood glucose. The STZ-treated diabetic control rats showed a significant increase in blood glucose with a concomitant decrease in BW. Oral administration of CR M. extract (200 and 400 mg/kg) and glibenclamide (5 mg/kg) for 14 days showed a significant reduction in blood glucose levels and elevation in the body weight studies as compared to control and glibenclamide-treated rats. The results of the present study showed that a potent antidiabetic activity was present in the aerial part of plant CR M. extract.

KEY WORDS: Diabetes mellitus, Methaolic extract, Glibenclamide, Blood glucose, Streptozotocin.

INTRODUCTION

Diabetes mellitus is the most prevalent metabolic disorder that causes changes in the metabolism of carbohydrates, fats, and proteins due to defects in insulin secretion or action, or both, and is characterized by loss of glucose homeostasis. It is one of the leading causes of morbidity and mortality worldwide[1]. By the year 2000, diabetes is expected to be one of the major, potentially fatal diseases, according to the World Health Organization [2]. The sixth most common cause of mortality worldwide is diabetes mellitus [3]. Diabetics have been reported to benefit from a wide variety of plants and plant extracts. Hypoglycemic properties have been suggested for the majority of these plants. Research is being done to find a new class of compounds that are necessary to combat the side effects of diabetes [4]. Only a few of the plants used in traditional herbal remedies for diabetes management have been scientifically proven to be effective [5]. Nowadays, the majority of diabetics taking biguanides or other conventional anti-diabetic medications experience side effects like headache, nausea, vomiting, abdominal pain, diarrhoea, and vomiting. It is crucial to create a new, safer, and effective herbal anti-diabetic formulation in order to counteract these side effects of medication [6-7].

Botanical name: *Cleome Rutidosperma.*

Kingdom: Plantae;	Phylum: Spermatophyte;	Subphylum: Angiosperm;
Class: Dicotyledonous;	Order: Brassicales;	Family: Cleomaceae;
Genus: Cleome;	Species: *Cleome Rutidosperma*	

Habitat: Invasive plant that thrives in dark, wet, and gloomy environments, such as rock walls and gaps. The plants may be found all across India, including disturbed soil, roadside ditches, abandoned gardens, and coastal woodlands.

Phytochemical Information: Phytochemical studies of *Cleome rutidosperma* showed the presence of tannins, lipids, amino acids, flavonoids, cardiac glycosides, alkaloids, steroids, saponins, terpenoids, poly- phenols, phlobatannins, pentose and reducing sugars, etc.[8]

Literature surveys: Researchers have discovered that this plant possesses powerful analgesic, antipyretic, anti-inflammatory, anti-convulsant, diuretic, anti-microbial, and wound-healing properties. Limited evidence from literature surveys exists regarding the pharmacological properties of managing diabetes. [9] The unique aspect of this study lies in the fact that no systematic research has been conducted on the aerial extracts of the *Cleome rutidosperma* plant to investigate it's in vivo anti-diabetic effects using an animal model. Therefore, the current study aims to unveil the anti-diabetic potential of CR M. (*Cleome rutidosperma* methanolic) extract both in vivo using a streptozotocin (STZ) model. Additionally, considering the plant's accessibility and feasibility, developing a new herbal formulation from the plant extract could lead to the creation of an innovative and cost-effective anti-diabetic remedy. [10]

METHODS

Plant material and authentication of plant:

The herbs of *Cleome rutidosperma* (CR) were obtained in quantity during the month of October 2018 from local regions of Khordha district, Odisha. For usage, the leaves were freshly separated. The plant specimens were identified and certified by Dr. P. C. Panda, Principal Scientist, RPRC, Bhubaneswar, Odisha. A voucher specimen (V No. UIH-22548)

was placed at the herbarium of Dept. of Pharmacognosy, Hi-Tech College of Pharmacy, Bhubaneswar, for future reference. (Fig 1 & 2)

Preparation of plant extract:

The coarse powder (500 g) was heated using a Soxhlet apparatus and subjected to extraction using a solvent system constituting Petroleum Ether (40 - 60 ^{O}C) (PE), Chloroform (CL), Methanol (ME) and Purified water (AQ) for a duration of 24 hours each. The collected samples were dried at room temperature and the resulting product was kept in a sealed container. [11]

Drugs and chemicals:

Sodium chloride, D-glucose, methanol, sodium citrate tribasic dehydrate, citric acid monohydrate, STZ, and glibenclamide were all purchased from M/s. Scientificocity, Bhubaneswar.

Experimental animals and sampling:

Male and female Wister albino rats weighing between 150-200 g were utilized in the study. The rats were kept in a well-ventilated animal facility that maintained a consistent temperature and relative humidity of 55-60%, following a 12-hour light and dark cycle. They were housed in spacious polypropylene cages with paddy husk serving as bedding material. The animals had access to food and water *ad libitum*, except during fasting periods. Bedding material was changed twice weekly, and all cages were properly labelled for identification purposes. Each rat was marked on the head and/or tail with picric acid for individual identification within the cage.

EXPERIMENTAL PROCEDURE

Phytochemical screening

The methaolic extract was analyzed using qualitative tests to identify various components including tannins, alkaloids, saponins, glycosides, terpenes, phenolics, flavonoids, carbohydrates, proteins, and steroids. These constituents were identified using established and straightforward qualitative methods as outlined by Trease and Evans (Table-1) [12-14]

Toxicity studies:

Acute toxicity testing was conducted according to the OECD guidelines (423) [13]. Adult albino mice in good health, weighing from 20 g to 25 g, were chosen for the research. Food was not given to the three animals overnight before dosing, but water was still provided. [15] Being a conventional form of remedy derived from plants, it was improbable for the mortality to occur at the most elevated initial dosage amount (2000 mg/kg body weight [BW]). Therefore, all animals underwent a limit test at a single dose level of 2000 mg/kg BW. The animals were monitored individually following administration for the first 30 minutes, regularly for the first 24 hours, with a focus on the initial 4 hours, and then over the next 72 hours to track any signs of death. The subsequent clinical examinations were conducted and documented. [16]

In vivo anti-diabetic effect:

C.R was studied for the antidiabetic activity in diabetic Wistar rats. Diabetes was induced by intraperitoneal injection of STZ 50 mg/kg BW. The antidiabetic effect of plant extract was compared with standard drug glibenclamide. [17-22]

Induction of diabetes in rats:

Following a week of acclimatization, the rats underwent overnight fasting. Diabetes was induced by injecting STZ intraperitoneally, which was freshly dissolved in citrate buffer pH 4.5 (a mixture of two parts 0.1 M sodium citrate and three parts 0.1 M citric acid) [23]. To counteract the drug-induced hypoglycaemia resulting from the significant release of insulin from β-cells, the animals were allowed to consume a 5% glucose solution overnight. [24] After 3 days, blood glucose levels were measured, and animals with a blood concentration exceeding 250 mg/dl were considered diabetic and included in the experiment. The administration of the plant extract began on the 4th day following STZ injection, marking the start of the treatment period, which lasted for 14 days [25]

Experimental design

At the commencement of the study, the initial fasting glucose levels and body weights of all animals were documented. Blood glucose levels were monitored using the Accucheck glucometer consistently during the duration of the study. The 30 rats utilized in the experiments were segregated into groups consisting of six rats each.

Gr 1: Normal control rats received distilled water.

Gr 2: STZ-induced diabetic rats received (from 1st day) distilled water and served as diabetic control for 1-14 days.

Gr 3: STZ-induced diabetic rats received standard drug glibenclamide (5 mg/kg) for 1-14 days.

Gr 4: STZ-induced diabetic rats received the plant extract (200 mg/kg BW) for 1-14 days.

Gr 5: STZ-induced diabetic rats received the plant extract (400 mg/kg BW) for 1-14 days.

BW was assessed in all rats prior to the onset of diabetes and on the 4th, 7th, and 14th days of treatment. Blood glucose levels were determined on the 1st, 7th, and 14th days through tail tip cutting. Upon completion of the experiment on the 14th day, an adequate amount of blood was obtained via retro-orbital bleeding from all animals while under anaesthesia for the evaluation of biochemical parameters (blood glucose and BW). [26]

Statistical analysis

ANOVA was used to analyze all the parameters, [27] followed by Dunnett's test. The mean ± SD was used to express the results. The statistical analysis was conducted using Graph and Prism, version 6.0 software.

RESULTS

Phytochemical screening

The phytochemical analysis of the extracts of (CR) showed the presence of Alkaloids, Glycosides, Saponins, Flavonoids, Steroids/Triterpenoids, Tannins/Polyphenolics, Carbohydrate/Reducing sugars and Amino acids/Proteins shown in Table 1. [28]

Acute toxicity study

Acute toxicity study revealed non-toxic nature of the compound at 2000 mg/kg. No lethality or toxic reactions were observed such as tremor and motor activity and after 14 days, no discernible toxic signs were seen. Hence 200 mg/kg (1/10th of the-dose) was selected as the therapeutic- dose for further Pharmacological studies. [29,30]

***In vivo* anti-diabetic effect**

Results relating to the effects of CR leaves extracts (200 mg/kg and 400 mg/kg) and glibenclamide (5 mg/kg) to the diabetic rats are shown in Table 2. The mean BW showed a

decrease in Gr 2, Gr 3, Gr 4, and Gr 5 on 14th day on comparing with 1st day. Vehicle control animals were found to be almost stable in their BW, but diabetic-induced rats showed a significant reduction in BW ranging from 172.54±5.15 g (1st day) to 152.76±6.45 g (14th). Gr 2 (diabetic rats) showed a significant reduction in BW $p<0.05$ when compared to the groups. The administration of (CR) leaves extract and glibenclamide (5 mg/kg) to the diabetic rats restored the changes in the BW from 173.91±5.74 g (1st day) to 161.36±5.13g (14th day) for 200 µg kg of dose and 172.50±5.63 g (1st day) to 169.76±4.21 g (14th day) for 400 µg kg of dose of (CR) leaves extract and 172.70±4.63 g (1st day) to 169.76±4.21 g (14th day) for glibenclamide (5 mg/kg)-treated animals. Results relating to the effects of (CR) leaves extracts (200 mg/kg and 400 mg/kg) and glibenclamide (5 mg/kg) to the diabetic rats are shown in Table 3**.** Effect of methanolic extract of (CR) on whole blood glucose in STZ-induced diabetic rats Diabetic rats (Gr 2) showed a significant increase in blood glucose. This fall in fasting blood glucose level progressively increases until the end of 3rd week. When the reduction glucose level with 200 mg/400 mg was compared, there was a statistically significant reduction in blood glucose levels ($p<0.05$) with 400 mg of the extract (Fig 3).

DISCUSSION:

The quality of the plant can be assessed by the phytochemical screening. Colored reactions of the bioactive compounds show the presence or absence of the compounds. Curative activity against several human problems is produced by the phytoconstituents in medicinal plants. During analysis it was observed that a wide range of active phytoconstituents except carbohydrates was found to be present in methanol-extract, hence this present study of methanolic extract of the plant (200 mg/kg BW) & extract (400 mg/kg BW) were consider for biological evaluation. Methanol is one of the good solvents in plant extractions, which include low toxicity, easy evaporation at low heat, preservative action, and inability to cause the extract to complex or dissociate. Hence, the presence of phytochemicals in the plant extracts might serve in the prevention of diabetes mellitus along with protection from free radicals produced in the body systems due to various metabolic activities. Natural products have been used as promising sources of novel agents for the treatment of various disorders due to their less toxic effect. Moreover, the lethal dosage not only designates the toxic level of a particular extract but also helps in determining the effective dosage that can be used for the experiment. There was no lethality or any toxic reactions in the present study, found in the animals at any of the doses selected until the end of the investigation period. The results of the acute toxicological studies revealed that the administration of methanolic extract of CR by oral route up to 2000 mg/kg BW did not produce any mortality and it was tolerated. On comparing the doses of 200 mg/kg with 400 mg/kg, there was a significant difference in BW ($p<0.05$) for a dose of 200 mg/kg. The dose-dependent antidiabetic property of the methanolic extract of CR exhibited improvement in BW. The study indicated that the methanolic extracts of treated groups produced a fall in the blood glucose level when compared to diabetic rats. The extracts might be enhancing glucose utilization and glucose metabolism. In the present study, the STZ-induced diabetic rats (Gr 2) showed a significant rise in blood glucose to a level when compared to normal control rats (Gr 1). On the contrary, diabetic rats treated with methanolic extracts and standard drug glibenclamide for 14 days, exhibited a decrease in blood glucose level. It was observed that methaolic extracts reversed these effects in diabetic animals. These results imply that the methanolic extract of CR can reduce the complications

of BW and associated cardiovascular risk factors during diabetes.

Fig.1 *Cleome Rutidosperma* Whole Plant

Fig.2 Leaf of *Cleome Rutidosperma* Plant

Table-1. Results of phytochemical screening of extracts of Cleome rutidosperma:				
Test for chemical groups	Pet. Ether -extract	Chloroform - extract	Methanol - extract	Aqueous - extract
Alkaloids.	--	+	+	+
Glycosides.	--	--	+	+
Saponins	--	--	+	+
Flavonoids.	--	--	+	+
Steroids / Triterpenoids	+	--	+	--
Tannins/Polyphenolics	--	--	+	+
Carbohydrate/Reducing sugars	--	--	--	+
Amino acids/Proteins	--	--	+	+
*(+) denotes present; (--) denotes absent				

Table-2. Effect of Methanol-extract of the leaves of *Cleome rutidosperma* on body weight in streptozotocin-induced diabetic rats.					
Groups & Treatment		Body weight in (g)			
		Day 0	**Day 1**	**Day 7**	**Day 14**
Gr-1	Control group (DW)	171.14±7.00	171.20±7.00	173.54±7.00	179.45±4.13
Gr-2	STZ-induced Diabetic rats	173.04±6.35	172.54±5.15	160.04±5.24	152.76±6.45
Gr-3	Standard group (GL- 05 mg/kg)	172.70±4.63	172.70±4.23	171.70±3.72	170.57±5.87
Gr-4	(CR-ME) extract (200 mg/kg)	173.91±5.74	171.91±5.24	168.91±4.64	161.36±5.13
Gr-5	(CR-ME) extract (400 mg/kg)	172.50±5.63	171.30±5.42	170.40±6.73	169.76±4.21
Values are expressed as mean±SD (n=6), Values are statistically significant at p<0.05 using one-way ANOVA followed by Dunnett's test. CR ME :- Cleome rutidosperma Methanol-extract, DW:- Distilled water, STZ- Streptozotocin,					

Table-3. Effect of different extracts of the leaves of Cleome rutidosperma on blood glucose levels in streptozotocin-induced diabetic rats.							
Groups & Treatment	**BGL (mg/dl)**						
	Day 0	**Day 1**	**Day 2**	**Day 4**	**Day 7**	**Day 14**	**F value**
I	236.25 ±13.55	241.83 ±12.18	244.33 ±6.40	246.00 ±10.00	249.87 ±11.17	256.85 ±7.55	0.54
II	248.03 ±9.17	226.35 ±10.25**	192.66 ±7.21**	162 10.76**	132.5 ±7.12**	87.66 ±9.30**	0.42
III	240 ±7.34	243.53 ±8.64	246.5 ±5.30	248.15 ±5.57	249.85 ±6.40	254.73 ±8.15	0.90
IV	238 ±8.14	242.5 ±6.72	240.19 ±7.83	245 ±10.32	248.5 ±9.77	251.47 ±7.36	0.71
V	242.15 ±9.10	234.88 ±9.04	221.36 ±9.26**	204.5 ±7.81**	165.16 ±8.28**	146.55 ±10.09**	0.85
*Values expressed as mean ± SD (n=6). The data were statistically analysed by one-way NOVA, followed by Dunnet's t-test. p values less than 0.05 were considered significant. * p <0.05; ** p <0.01. Figure in parenthesis indicates % fall in BGL as compared to 0 day.*							

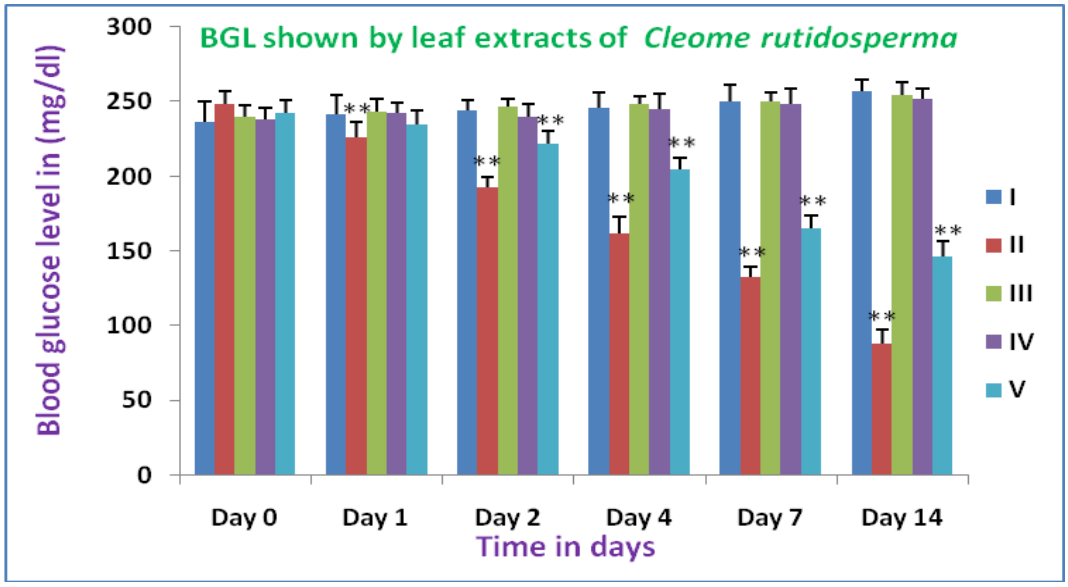

Fig. 3. Effect of different extracts of the leaves of Cleome rutidosperma on blood glucose levels in streptozotocin-induced diabetic rats.

CONCLUSION:

Estimation of glucose was performed for the diagnosis and fall up of diabetes mellitus. In a normal healthy individual, the fasting blood glucose level is between 70 and 100 mg/dL. This level may rise to 500 mg/dL or more in diabetic person which is referred to as hyperglycemia and it mainly occurs due to deficiency of insulin. The continuous treatment for 14 days with the methanolic extract showed a significant reduction in the blood glucose levels. This plant was found to decrease the level of glucose significantly ($p<0.05$) in STZ-induced diabetic rats. The lower dose of *C.R* itself exhibits its activity and the effect was observed to be dose-dependent. The data from the above preliminary phytochemical studies suggest that CR leaves has beneficial effects in diabetes mellitus holding the hope of a new generation for anti-hyperglycemic drugs. This activity is useful in further experimental analysis in the future.

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