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EVALUATION OF HYPOGLYCEMIC ACTIVITY OF *MOLLUGO PENTAPHYLLA* AND *GLINUS OPPOSITIFOLIUS* (L)

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ABSTRACT

Diabetes mellitus is a metabolic disorder affecting carbohydrate, fat and protein metabolism that affects nearly 10% of the population every year. The treatment of diabetes mellitus has been confined to use of oral hypoglycemic agents and insulin, the former being reported to possess serious side effects. This leads to increasing demand for herbal products with antidiabetic factor with little side effects. This article describes the antihyperglycaemic activity, of the ethanolic extract of the aerial parts of *Mollugo pentaphylla and Glinus oppositifolius*. The extracts produced significant decrease in the blood glucose level when compared with the controls in alloxan induced hyperglycemic, normoglycemic and oral glucose tolerance test in suitable rat models and is comparable with the standard drug glibenclamide.

Keywords: *Mollugo pentaphylla; Glinus oppositifolius*; Hypoglycemic activity; Glibenclamide.

INTRODUCTION

Diabetes mellitus is a metabolic disorder affecting carbohydrate, fat and protein metabolism. A worldwide survey reported that diabetes mellitus is affecting nearly 10% of the population every year[1]. The treatment of diabetes mellitus in clinical practice has been confined to use of oral hypoglycemic agents and insulin, the former being reported to be endowed with characteristic profiles of serious side effects[2]. This leads to increasing demand for herbal products with antidiabetic factor with little side. effects. A large number of plants have been recognized to be effective in the treatment of diabetes mellitus[3]. *Mollugo pentaphylla* Linn. (family- Aizoaceae) is commonly known as carpet weed. It is an erect slender, much branched annual herb, up to 30 cm. high, commonly found in dry as well as moist areas. Leaves are falsely whorled or opposite, linear-lanceolate to obovate. Flowers are white, greenish, orange or pink, in terminal compound cymes. It is also having numerous applications in traditional medicine as stomachic, aperient, antiseptic, emmenagogue and is also used in poultices for sore legs. An infusion of the plant is given to women to promote the menstrual discharge. Leaves are bitter and antiperiodic, they are warmed after smearing with oil and applied to the ear to relieve earache[4]. It has been reported that the plant possesses antimicrobial[5], whooping cough[6], hepatitis[7], anticancer[8], spermicidal[9], antibacterial[10] and antifungal activity[11]. *Glinus oppositifolius* Linn. (family- Molluginaceae)[12] is an annual or perennial sub shrub rarely dioecious, glabrous or rarely hairy, Stems erect or prostrate, Stem simple, alternate, rarely opposite, Flowers bisexual, Petals absent or few to many, white, pink, or purple[13]. Traditionaly *G. oppositifolius* is used in the treatment of skin disease, increase appetite, cures kapha, piles, leucoderma, tonic to intestine, urinary infections, fever, cough, liver problem and also used as antioxidant due to its excellent properties and potent phytoconstituents[14]. Activities like free radical scavenging and antioxidant activities[15], hepatoprotective effect[16], antiprotozoal activity[17], immunomodulating activity of *G. oppositifolius*[18] has been reported. Therefore, this present study was under taken to evaluate the antihyperglycaemic activity, of the ethanolic extract of the aerial parts of both *M. pentaphylla and G. oppositifolius.*

EXPERIMENTAL

Materials and Methods

Plant material

Both the *plant M. pentaphylla and G. oppositifolius.* were collected from the rural belt of Rayagada (Odisha) during the month of January and was authenticated by the Taxonomist of Botanical Survey of India, Howrah. The collected plant materials were washed under running tap to remove adhered dirt, and then shed dried. Then the aerial part was ground in to coarse powder.

Preparation of extract

The powered plant material was defatted with petroleum ether (60-80°c) and then extracted with 80% ethanol using soxhlet apparatus. The solvent was removed under reduced pressure to obtain dry extract, which gave a greenish-black coloured sticky residue. The extracts were stored in desiccators for further use.

Phytochemical Screening

In this research work the ethanolic extract of both *M. pentaphylla and G. oppositifolius* were qualitatively tested for the presence of chemical constituents. It shows the presence of carbohydrates, alkaloids, gums, saponins, flavanoids, tanins and steroids[19].

Animals

Swiss albino mice (20–25 g) of either sex were used for acute toxicity study and adult wistar albino rats (150-200 g) of either sex were used for evaluation of pharmacological studies. The animals were kept in standard polypropylene cages at room temperature of 34 ± 2 ^{o}C and at 60-65 % relative humidity during the experimental work. The experiment has been performed in the CPCSEA approved laboratory of Institute of Pharmacy and Technology, Salipur (Regd. No. 1053/ac/07/CPCSEA) with the permission of Institutional animal ethics committee.

Acute toxicity study

The acute toxicity of ethanolic extract of *M. pentaphylla and G. oppositifolius* were determined as per the CPCSEA guideline no. 420 (fixed dose method). It was observed that the test extracts shows no mortality even at 2000 mg/kg dose hence, 1/10th (200 mg/kg) and 1/5th (400 mg/kg) of this dose were selected for further study.

Screening for hypoglycaemic activity (Single dose study)

Hypoglycemic activity of ethanolic extract (200 and 400 mg/kg, p.o.) of aerial part of both *M. pentaphylla and G. oppositifolius* were performed on Wistar Albino rats. Glibenclamide (2.5 mg/ kg, p.o.) was used as reference standard.

Using normoglycemic rats

In this test the animals were allowed for free access to water before and throughout the duration of experiment was allowed the acclimatized animals were fasted for 18 h[20]. The end of the fasting period was taken as zero time (0 h), and the collection of blood was done by tail vein method of each rat under mild anesthesia[21]. The blood glucose level was measured with Senso card blood glucose meter supplied by M/s Avecon Health Care Pvt. Ltd., Himachal Pradesh. The normal rats were then divided into six groups of six animals in each. Negative control was designated as group I and received vehicle (2 ml/kg) through oral route. Group-II received glibenclamide (2.5 mg/kg p.o.). Group-III received ethanolic extract of *M. pentaphylla* (200 mg/kg, p.o.), Group-IV received ethanolic extract of *M. pentaphylla* (400 mg/kg, p.o.), Group-V received ethanolic extract of *G. oppositifolius* (200 mg/kg, p.o.) and Group-VI received ethanolic extract of *G. oppositifolius* (400 mg/kg, p.o.). After 1, 2, 4 and 8 h of administration of single dose of test samples blood glucose levels were measured (Table-1).

Oral glucose tolerance test (OGTT) in rats

In this test fasted rats were divided into six groups of six rats each group. Group I served as a control and received only vehicle (2 ml/kg) through oral route. Group-II received glibenclamide (2.5 mg/kg, p.o). Group-III received ethanolic extract of *M. pentaphylla* (200 mg/kg, p.o.), Group-IV received ethanolic extract of *M. pentaphylla* (400 mg/kg, p.o.), Group-V received ethanolic extract of *G. oppositifolius* (200 mg/kg, p.o.) and Group-VI received ethanolic extract of *G. oppositifolius* (400 mg/kg, p.o.). After 30 min of treatment, rats of all groups were loaded orally with glucose (2 g/kg, p.o). Blood

samples were collected before and at 30, 60, 150 and 180 min after glucose administration as per the method described earlier[22] (Table-2).

Using hyperglycemic rats

In this methode acclimatized animals after fasting for 24 hours with water *ad libitum* and then intraperitoneal injection of a dose of 150 mg/kg of alloxan monohydrate in normal saline was given. The animals were provided standard laboratory diet *ad libitum* after one hour. Under mild anesthesia the blood was withdrawn from the tip of the tail of each rat and the blood glucose level was checked before alloxanisation and 24 h after alloxanisation. The blood glucose level was measured as stated above. Rats having the blood glucose level above 225 mg/dl were selected and grouped into six groups consisting of six animals each[23]. This condition was observed at the end of 48 h after alloxanisation. Orally 1% Tween 80 solution (2 ml/kg p.o) was received by the Group-I which served as diabetic control, glibenclamide (2.5 mg/kg) was received by Group-II, Group-III received ethanolic extract of *M. pentaphylla* (200 mg/kg, p.o.), Group-IV received ethanolic extract of *M. pentaphylla* (400 mg/kg, p.o.), Group-V received ethanolic extract of *G. oppositifolius* (200 mg/kg, p.o.) and Group-VI received ethanolic extract of *G. oppositifolius* (400 mg/kg, p.o.). After 1, 2, 4 and 8 h of administration of single dose of test samples, blood glucose levels were measured (Table-3).

Screening for hypoglycaemic activity (Multi dose study)

Using hyperglycaemic rats

In this method the acclimatized animals after fasting for 24 hours with water *ad libitum* received intraperitoneal injection of a dose of 150 mg/kg p.o of alloxan monohydrate in normal saline. The animals were provided standard laboratory diet *ad libitum* after one hour. Under mild anesthesia the blood was withdrawn from the tip of the tail of each rat and the blood glucose level was checked before alloxanisation and 24 h after alloxanisation [24]. The blood glucose level was measured as stated above. Rats having the blood glucose level above 225 mg/dl were selected The blood glucose level was measured with Senso card blood glucose meter supplied by M/s Avecon Health Care Pvt. Ltd., Himachal Pradesh. Wistar albino rats of weighing 125-150g were graded to six animals per group. Group I served as control, which received only vehicle (2 ml/kg, p.o.), Group II received glibenclamide (2.5 mg/kg, p.o.), Group-III received ethanolic extract of *M. pentaphylla* (200 mg/kg, p.o.), Group-IV received ethanolic extract of *M. pentaphylla* (400 mg/kg, p.o.), Group-V received ethanolic extract of *G. oppositifolius* (200 mg/kg, p.o.) and Group-VI received ethanolic extract of *G. oppositifolius* (400 mg/kg, p.o.). The samples under test were administered to the selected animals once daily for 21 days and blood glucose was measured on 1^{st}, 7^{th}, 14^{th} and 21^{th} days respectively (Table-4).

Statistical analysis

All the results were statistically analyzed using one way ANOVA followed by Dunnet's t-test. Values are expressed as mean±S.E. (n=6). *P<0.05 and **P<0.01 compared with control was considered as significant.

RESULTS AND DISCUSSION

Reports of the normoglycaemic study (Table-1) reveals that both the plant extracts exhibited significant reduction in blood glucose concentration in a dose dependant manner as compare to control. It was observed that ethanol extracts at the doses 400 mg/kg significant reduction in blood glucose concentration from 2 h respectively where as glibenclamide showed reduction in blood glucose concentration in rats after 1 h treatment. The effect of ethanolic extracts on oral glucose tolerance test in normal rats is shown in Table-2. At 30 min after glucose administration the peak of blood glucose level increased rapidly from the fasting value and then subsequently decreased. The test extract exhibited significant hypoglycaemic effect in a dose dependant manner but glibenclamide (2.5 mg/kg, p.o.) and ethanolic extract (400 mg/kg, p.o.) significantly reduced the peak of blood glucose level from 60 min after glucose loading.

In antihyperglycaemic study, the rise in the blood glucose level was observed after 24 h of alloxanization to the animals. Single dose administration of ethanolic extract of aerial part of *M. pentaphylla* and *G. oppositifolius* at the tested dose level (200 and 400 mg/kg, p.o.) in diabetic rats showed significant reduction in blood glucose level (55.66% and 56.15% respectively) at 400 mg/kg after 8 h. Glibenclamide (2.5 mg/kg, p.o.) showed maximum reduction (58.67% decrease blood glucose levels) after 8 h. shown in

Table-3. Where as in multi dose study of ethanolic extracts of both the plants also shows the most improving effect in a dose dependent manner as shown in Table-4.

CONCLUSION

The results of the present study justify the use of the aerial part of both the plant *M. pentaphylla* and *G. oppositifolius* for treating diabetes as suggested in the folklore remedies. The comparable effect of the extracts with glibenclamide may suggest similar mode of action, since alloxan permanently destroys the pancreatic ß-cells and the extract lowered blood sugar level in alloxanised rats, indicating that the extract possesses extra pancreatic effects. The exact biological active constitutent(s) responsible for the said effect and the exact mode of action of the hypoglycaemic activity were not reported earlier.

Table-1: Blood Glucose Concentration (mg / dl) of single dose treated normoglycemic rats.

Group	Treatment	Dose (mg/kg)	Fasting	1 hour	2 hour	4 hour	8 hour
I	Control	2ml/kg	96.66±2.71	97.83±2.93	98.66±2.27	97.33±2.31	98.33±1.45
II	Glibenclamide	2.5	96.83±3.45	75.50±4.63**	55.50±1.68**	49.83±1.51**	44.33±2.09**
	% Reduction			22%	42.68%	48.53%	54.21%
III	Ethanolic extract of M.p	200	97.83±2.21	94.50±3.30	85.50±2.40**	76.83±2.21**	67.50±2.49**
	% Reduction		--	3.40%	12.60%	21.46%	31.00%
IV	Ethanolic extract of M.p	400	98.33±1.49	84.66±3.10*	72.83±3.30**	65.50±2.68**	49.66±2.99**
	% Reduction			13.90%	25.93%	33.38%	49.49%
V	Ethanolic extract of G.p	200	97.16±1.22	94.00±3.05	84.83±2.12**	75.00±2.74**	65.83±2.78**
	% Reduction			3.25%	12.69%	22.80%	32.24%
VI	Ethanolic extract of G.p	400	96.83±1.37	82.00±2.54**	71.50±3.04**	64.16±2.27**	51.66±3.24**
	% Reduction		--	15.31%	26.15%	33.73%	46.64%

Values are expressed as mean±S.E. (n=6). *P<0.05 and **P<0.01 compared with vehicle control (ANOVA followed by Dunnet's t-test) *M.p - M. pentaphylla, G.p – G. Oppositifolius*

Table-2: Blood Glucose Concentration (mg / dl) of single treated oral glucose tolerance test.

Group	Treatment	Dose (mg/kg)	Fasting	30 min.	60 min.	150 min.	180 min.
I	Control	2ml/kg	91.66±0.88	126.16±2.34	147.16±2.13	159.66±7.33	153.50±3.04
II	Glibenclamide	2.5	93.50±0.99	127.50±3.41	105.83±1.44**	93.66±4.09**	81.16±4.96**
	% Reduction				17.00%	26.54%	36.34%
III	Ethanolic extract of M.p	200	92.66±1.02	129.66±3.01	116.33±3.53**	107.16±6.70**	103.16±3.66**
	% Reduction				10.28%	17.35%	20.43%
IV	Ethanolic extract of M.p	400	93.50±0.76	128.16±2.86	106.16±2.74	101.66±5.49**	85.66±3.95**
	% Reduction				17.16%	20.43%	33.16%
V	Ethanolic	200	93.33±0.84	129.16±3.29	118.66±2.47**	109.50±6.13**	102.66±4.28**

	extract of G.p						
	% Reduction				8.12%	15.22%	20.51%
VI	Ethanolic extract of G.p	400	93.16±0.90	127.83±3.26	108.66±2.64**	99.66±4.65**	84.16±3.10**
	% Reduction		--	--	15.00%	22.03%	34.16%

Values are expressed as mean±S.E. (n=6). *P<0.05 and **P<0.01 compared with vehicle control (ANOVA followed by Dunnet's t-test). *M.p - M. pentaphylla, G.p – G. oppositifolius*

Table-3: Blood glucose concentration (mg / dl) of hypoglycemic test.

Group	Treatment	Dose	Fasting	1 hour	2 hour	4 hour	8 hour
I	Control	2ml/kg	238.83±2.05	250.83±3.59	252.16±3.62	254.16±3.36	257.33±2.87
II	Glibenclamide	2.5 mg/kg	241.16±2.80	202.50±3.06**	152.66±2.10**	113.83±1.74**	99.66±2.12**
	% Reduction			16%	36.69%	52.79%	58.67%
III	Ethanolic extract of M.p	200 mg/kg	239.16±2.30	211.83±2.35**	196.33±2.36**	185.33±1.83**	165.66±2.60**
	% Reduction			11.42%	17.90%	22.50%	30.73%
IV	Ethanolic extract of M.p	400 mg/kg	238.33±1.81	199.50±2.57**	189.16±1.92**	163.83±2.91**	105.66±3.33**
	% Reduction			16.29%	20.63%	31.25%	55.66%
V	Ethanolic extract of G.p	200 mg/kg	239.16±2.45	210.16±2.40**	195.66±2.49**	186.16±2.13**	163.83±3.02**
	% Reduction			12.12%	18.18%	22.16%	31.49%
VI	Ethanolic extract of G.p	400 mg/kg	238.33±1.02	198.33±1.49**	188.83±2.89**	165.33±2.84**	104.50±2.78**
	% Reduction			16.78%	20.76%	30.62%	56.15%

Values are expressed as mean±S.E. (n=6). *P<0.05 and **P<0.01 compared with vehicle control (ANOVA followed by Dunnet's t-test). *M.p - M. pentaphylla, G.p – G. oppositifolius*

Table-4: Blood glucose concentration (mg / dl) of multi dose treated hyperglycemic rats.

Group	Treatment	Dose (mg/kg)	1st day	7th day	14th day	21st day
I	Control	2ml/kg	254.66±4.20	271.83±7.22	288.16±3.09	294.16±4.67
II	Glibenclamide	2.5	235.50±2.81**	181.16±9.05**	150.33±2.69**	104.33±3.67**
	% Reduction		--	23.07%	36.16%	55.69%
III	Ethanolic extract of M.p	200	234.83±4.68**	221.20±2.57**	179.66±3.51**	122.50±4.00**
	% Reduction		--	5.80%	23.49%	49.76%
IV	Ethanolic extract of M.p	400	236.66±4.77**	208.66±3.96**	162.16±4.26**	114.66±3.80**
	% Reduction		--	11.83%	31.47%	51.55%
V	Ethanolic extract of G.p	200	234.66±3.69**	218.16±2.85**	175.50±4.41**	123.16±4.08**
	% Reduction		--	7.03%	25.21%	47.51%
VI	Ethanolic	400	235.83±4.72**	207.5±4.26**	160.83±5.12**	113.50±4.99**

	extract of G.p					
	% Reduction		--	12.01%	31.80%	51.87%

Values are expressed as mean±S.E. (n=6). *P<0.05 and **P<0.01 compared with vehicle control (ANOVA followed by Dunnet's t-test). *M.p - M. pentaphylla, G.p – G. Oppositifolius*

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