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Studies on hypoglycaemic and analgesic activities of *Chlorophytum borivilianum* Sant & Ferz.

S. K. Panda[1]*, S. C. Si[1], S. P. Bhatnagar[2]

1. Institute of Pharmacy & Technology, Salipur, Cuttack, Orissa.
2. Dept. of Pharmaceutical Sciences, Birla Institute of Technology, Mesra, Ranchi - 835 215.

Abstract

Objective: To study the anti-hyperglycaemic and analgesic activity of the root extracts of *Chlorophytum borivilianum* Sant. & Ferz. on rats and mice respectively. Materials and methods: Anti-hyperglycaemic activity of the methanol extract of the roots (100mg & 200mg/kg body weight, p.o.) was evaluated by using both normoglycaemic and alloxan induced hyperglycemic rats of six numbers in a group. The analgesic activity was assessed by using methanol extract of the roots (100mg & 200mg/kg body weight, p.o.) on mice of six numbers in a group by Tail flick and Tail immersion method. Results: The methanol extract was found to produce significant reduction of blood glucose concentration between 2-4 hr. of administration in alloxan induced hyperglycemic rats at tested dose levels. However, in normoglycaemic animals, the extract at 200mg/kg produced significant reduction of blood glucose between 2-4 hr. of administration. In the analgesic study, the root extract showed significant activity. In tail flick method the extract at 100mg/kg showed activity after 45 minutes whereas in tail immersion test, it showed significant activity after 30 minutes interval. Conclusion: *Chlorophytum borivilianum* Sant. & Ferz. has hypoglycaemic activity and analgesic activity. So it can be recommended for further studies.

Key words: *Chlorophytum borivilianum*, hypoglycaemic, analgesic, Glibenclamide, Pentazocin.

I. Introduction

Chlorophytum borivilianum Sant. & Ferz. (Fam-Liliaceae) is a small, pretty, perennial herb. The plant is having bunches of fleshy tuberous roots. Leaves are linear and flowers are white. The plant finds its application in various folklore medicines for a veraity of ailments around the globe. The popular use of the plant, however mainly refers to its aphrodisiac, galactogogue and useful in bleeding piles, increasing sperms and arthritis [1-3].

An extensive search of the literature reveals no reports on the antihyperglycaemic and analgesic activity of the plant. The vast ethonomedical uses of the plant around the globe for the

* Corresponding author
Email : pharmabarpali@yahoo.co.in

treatment of various diseases and ailments triggered this research investigation to study anti-hyperglycaemic and analgesic property of the tuberous roots.

2. Materials and methods

2.1. Plant material

Fresh tuberous roots were collected from the commercial cultivator M/S Spak Agro, Cuttack. The plant material was authenticated by taxonomist with the help of authentic herbarium species in Department of Botany, Revenshaw College, Cuttack. The collected roots were washed under running tap water to clean the adhering dirt materials and then peeled and dried under shade. The dried peeled roots were coarsely powdered by means of a mechanical grinder and passed through sieve no 40 and used for further studies. A specimen of the plant species is deposited in the Department of Pharmacognosy of the Institute for future reference.

2.2. Preparation of extract

The powdered roots were and charged into the Soxhelet's apparatus and successive hot continuous extraction was carried out using methanol as solvent for 24 h. The liquid extract was concentrated under vacuum to yield dry extract (methanol extract 9.78% w/w with respect to dry material) and preserved on a descicator till further experiments. The methanol extract was dissolved in 2% w/v of gum acacia in distilled water and used for the antidiabetic and analgesic screening.

2.3. Animals used

Adult Wistar albino rats (150-200g) of either sex were used for antidiabetic studies. Whereas adult Swiss albino mice (25-30g) of either sex were used for analgesic studies. A written permission from in house ethical committee has been taken to carry out and complete this study. The animals were kept in standard polypropylene cages at room temperature of 30±2°C and 60 - 65% relative humidity.

3. Experimental

3.1. Anti-diabetic evaluation [6-9]

3.1.1. Using hyperglycaemic rats

The acclimatized animals were kept fasting for 24 h. with water *ad libitum* and injected intraperitoneally a dose of 120mg /kg of alloxan monohytdrate in normal saline. After one hour, the animals were provided feed *ad libitum*. The blood glucose level was checked before alloxanisation and 24 h. after alloxanisation by withdrawing blood from the tip of tail of each rat under mild ether anaethesia. The blood glucose level was measured with haemoglucostrips supplied by M/S. Pulsatum Health Care Pvt. Ltd., Bangalore with the help of a pulsatum blood glucose monitor.

Animals were considered diabetic when the blood glucose level was raised beyond 200mg/ dl of blood. This condition was observed at the end of 48 h. after alloxanisation. The animals were segregated into four groups of six rats in each. Group-I served as negative control and received vechile (2ml/kg) through oral route. Group-II received glibenclamide (2.5mg/kg). Group-III and IV received the methanolic extract at doses of 100mg and 200mg/kg in a similar manner. Blood samples were collected from each rat by cutting the tip of the tail under mild ether anesthesia. Blood glucose level was estimated at 0, 1, 2, 4 and 8 h. (Table No.1).

3.1.2. Using normoglycaemic rats

The animals were fasted for 18 h, but were allowed free access to water before and throughout the duration of experiment. At the end of the fasting period, taken as zero time (0 h), blood was withdrawn from the tip of the tail of each rat under mild ether anesthesia and the blood glucose was estimated.

The normal rats were then divided into four groups of six animals in each. Group-I served as negative control to which the vehicle (2% w/v gum acacia) was administered through oral route. Group-II received glibenclamide (2.5mg/kg) and served as reference control. Group-III and IV vehicle received the methanolic extract at doses of 100mg and 200mg/kg in a similar manner. Blood glucose levels were monitored after 1, 2, 4 and 8 h. of administration of single dose of test samples (Table No.2).

3.1.3. Statistical analysis [10]

Results were expressed as mean ± SD from six observations. One way ANOVA ($F_{3,20}$) is followed by Dunnet's test. The minimum level of significance was fixed at $P<0.01$.

3.2. Analgesic activity evaluation [5-6]

3.2.1. Tail flick method [11-12]

Before the study, swiss albino mice were screened for sensitivity test by placing the tip of the tail on the radiant heat source. Any animal that held to withdraw its tail in 5 second was rejected from the study. The selected animals were then divided into four group of six mice each. Group-II received pentazocin (30mg/kg) and Group-I received 2% w/v of gum acacia (2ml/kg) in normal saline intraperitoneally. Group-III and Group-IV received methanolic extract in 2% w/v gum acacia in normal saline intraperitoneally at a dose of 100 and 200 mg/kg respectively. Analgesia was assessed with a tail flick apparatus (Analgesiometer).

The basal reaction time was measured initially and another set of four measures were taken at 15, 30, 45 and 60 minutes interval and the reaction of the animal considered as the post-drug reaction time. A cut-off period of 10 sec. was observed to prevent tissue damage of the tail of the animal (Table No.3).

Table No.1 Effect of methanolic extract of the roots of *C. borivillianum* Sant. & Ferz. on the blood glucose concentration in alloxan induced hyperglycaemic rat.

Group	Treatment	Dose	Blood glucose conc. (mg/dl)					$F_{(3,20)}$
			0h	1h	2h	4h	8h	value
I	2% w/v Gum acacia (vehicle)	2ml/kg	270.17 ±5.53	272.67 ±5.92	268.50 ±6.41	275.83 ±7.03	273.50 ±9.89	0.97
II	Glibenclamide	2.5mg/kg	274.67 ±8.04	200.83* ±6.77	139.83* ±5.85	91.33* ±4.18	82.67* ±3.08	1132.23*
III	Methanolic extract	100mg/kg	277.50 ±7.34	232.83* ±6.11	197.17* ±5.56	179.83* ±6.82	162.33* ±7.74	276.31*
IV	Methanolic extract	200mg/kg	276.17 ±5.53	246.33 ±6.09	177.17* ±4.83	136.67* ±4.27	108.83* ±4.67	1155.66*

Results are expressed as mean ± SD. One way ANOVA followed by Dunnet's t-test is performed.
The blood glucose conc. at 0 hr. is compared with that at 1 h, 2 h, 4 h & 8 h.

* signified $P<0.01$.

3.2.2. Tail immersion Test

Prior to the analgesic experiment, the animals were screened for the sensitivity test by immersing the tail of the mice gently in hot water maintained at 55°C-55.5°C. The animal which lifted the tail from hot water within 5 seconds was selected for the study. The selected mice were then divided into four groups of six mice each. Group-III and IV received methanolic extract in 2% w/v gum acacia in normal saline intraperitoneally at a dose of 100 and 200mg/kg respectively. Group-II received pentazocin (30mg/kg) and group-I received 2% w/v of gum acacia (2ml/kg) in normal saline in similar manner. After administration of the test drugs, the reaction time was measured at 0, 15, 30, 45 and 60 minutes (Table No. 4).

3.3. Statistical analysis [10]

Results were expressed as mean ± SD from six observations. One way ANOVA ($F_{3,20}$) is followed by Dunnet's test. The minimum level of significance was fixed at P<0.01.

Table No.2 Effect of methanolic extract of the roots of *C. borivillianum* Sant. & Ferz. on the blood glucose concentration in alloxan in normoglycaemic rats.

Group	Treatment	Dose	Blood glucose conc. (mg/dl)					$F_{(3,20)}$ value
			0h	1h	2h	4h	8h	
I	2% w/v Gum	2ml/kg	94.67	94.17	95.66	94.17	96.33	0.27
	acacia (vehicle)		±2.66	±4.22	±3.27	±5.38	±6.09	
II	Glibenclamide	2.5mg/kg	96.83	83.67*	74.83	67.16*	65.67*	81.79*
			±2.14	±3.37	±3.66	±4.02	±3.98	
III	Methanolic extract	100mg/kg	92.83	92.17	91.67	91.16	90.17	0.38
			±3.87	±5.42	±2.73	±3.43	±4.17	
IV	Methanolic extract	200mg/kg	92.83	90.16	88.83	82.50*	78.83*	13.97*
			±4.17	±4.35	±4.36	±3.02	±2.71	

Results are expressed as mean ± SD. One way ANOVA followed by Dunnet's t-test is performed. The blood glucose conc. at 0hr. is compared with that at 1 h, 2 h, 4 h & 8 h.

* signified P<0.01.

Table No. 3 Analgesic activity of methanolic extract of the roots of *C.borivilianum* Sant. & Ferz. by Tail flick method in mice.

Group	Treatment	Dose	Basal reaction time (Sec.)	Reaction time (Sec.)				$F_{(3,20)}$ value
				15 min.	30 min.	45 min.	60 min.	
I	2% w/v	2ml/kg	1.67	1.83	1.33	1.50	1.67	0.85
	Gum acacia		±0.52	±0.41	±0.52	±0.55	±0.52	
II	Pentazocin	30mg/kg	1.67	4.33*	5.83*	6.83*	8.50*	102.67*
			±0.52	±0.52	±0.75	±0.75	±0.55	
III	Methanolic extract	100mg/kg	1.67	3.50*	4.67*	5.83*	7.17*	85.79*
			±0.52	±0.55	±0.52	±0.41	±0.75	
IV	Methanolic extract	200mg/kg	1.67	3.67*	4.83*	6.67*	7.83*	
			±0.52	±0.52	±0.41	±0.52	±0.41	156.84*

Results are expressed as mean ± SD. One way ANOVA followed by Dunnet's t-test is performed. The BRT is compared with reaction time after 15 min, 30 min, 45 min & 60 minutes.

* signified P<0.01.

4. Results and discussion

The studies on the methanolic extract of the plant *Chlorophytum borivilianum* revealed that the extract caused significant reduction in the blood glucose levels in the rats. The extract was found to produce marked reduction in blood glucose concentration between 2-4h of administration in alloxan induced hyperglycaemic rats at tested dose levels as depicted in table No. 1.

However, in normoglycaemic animals, the extract at 200mg/kg dose level produced marked reduction of blood glucose between 2-4h of administration which reflected from table No.2 the extract caused noticeable reduction on the blood glucose level in both classes of animals except that the onset of action of glibeclamide was noticed from the first one our while that of the extract was from the 2nd hour at a dose level of 200mg/kg.

The comparable effect of the extract with glibenclamide may suggest similar mode of action, since alloxan destroys the pancreatic β-cells and the extract lowered blood sugar level in alloxanised rats, indicating, that the extract possesses extrapancreatic effects. The exact biological active constituent(s) responsible for the said effect are neither reported nor the exact mode of action of the hypoglycemic activity was reported earlier, with the lone observation that it is used in folklore diabetic treatments.

In analgesic studies, the extract showed significant analgesic activity at all tested dose levels. In tail flick method, the extract at 100mg/kg showed significant activity ($p<0.01$) after 45 minutes But in tail imersion method, the extract showed significant activity at all tested dose levels after 30 minutes interval. The results showed significant analgesic activity against noxious stimuli. From the above studies, it is quite apparent that the methanolic extract of *Chlorophytum borivilianum* possesses significant anti-hyperglycaemic and analgesic activities.

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Table No. 4 Analgesic activity of methanolic extract of the roots of *C.borivilianum* Sant. & Ferz. by Tail imersion method in mice.

Group	Treatment	Dose	Basal reaction time (Sec.)	Reaction time (Sec.) 15 min.	30 min.	45 min.	60 min.	$F_{(3,20)}$ value
I	2% w/v Gum acacia	2ml/kg	1.67 ±0.52	1.67 ±0.52	1.67 ±0.52	1.67 ±0.52	1.5 ±0.55	0.12
II	Pentazocin	30mg/kg	1.67 ±0.52	3.83* ±0.41	5.50* ±0.55	6.50* ±0.55	8.33* ±0.52	149.68*
III	Methanolic extract	100mg/kg	1.67 ±0.52	3.50* ±0.53	4.67* ±0.32	5.83* ±0.41	7.33* ±0.52	41.38*
IV	Methanolic extract	200mg/kg	1.67 ±0.52	3.67* ±0.52	4.83* ±0.41	6.67* ±0.52	7.67* ±0.52	138.38*

Results are expressed as mean ± SD. One way ANOVA followed by Dunnet's t-test is performed.
The BRT is compared with reaction time after 15, 30, 45 & 60 minutes.

* signified $P<0.01$.

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