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PHYTOCHEMICAL AND ANTIDIABETIC SCREENING FOR METHANOLIC EXTRACT OF CURCUMA PSEUDOMONTANA J. GRAHAM. RHIZOMES AGAINST STREPTOZOTOCIN INDUCED DIABETES RAT MODEL

Balakrishnaiah P*, Srinivasa Rao Y, Gana manjusha K, Jeevitha K

Vignan Institute of Pharmaceutical Technology, Duvvada, Visakhapanam-530049, A.P, India.

*Corresponding author E-mail: **balakrishnaiahp@gmail.com**

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ABSTRACT

Diabetes mellitus is a chronic metabolic disorder associated with relative deficiency in release and act of insulin on the metabolism of carbohydrates,fats and protiens. In present study, methanolic extracts of *Curcuma pseudomontana* was investigated for its antihyperglycemic activity in streptozotocin-induced diabetic mice. The animal model (mice) were divided into different groups as normal control, glibenclamide treated (standard drug) and test groups (plant extract treated 100, 200 and 400 mg/kg body weight). Blood glucose concentration of all the study animals was determined by Glucose auto analyser. Qualitative phytochemical analysis of the plant extract was also performed with standard procedure. It was investigated that treatment of streptozotocin-induced diabetic rats with methanolic extract(400 mg/kg body weight) of plant showed highly significant ($P < .001$) on reduction in blood glucose levels at 8^{th} hour which is compared with standard drug glibenclamide(0.45 mg/kg body weight) at 4^{th} hour in a dose dependent manner. It could be concluded that studied medicinal plant have antihyperglycemic activity.

INTRODUCTION

Diabetes mellitus is a chronic metabolic disorder characterized by derangement in carbohydrate, fat and protein metabolism due to absolute or relative deficiency of insulin secretion and with varying degree of insulin resistance[1]. In diabetes, the deficiency of insulin leads into a complex series of reactions, which are clinically manifested as hyperglycemia and is characterized by a loss of glucose homeostasis[2,3]. *Curcuma pseudomontana* J. Graham., also known as hill turmeric,belongs to zingiberaceae family is endemic to the Western and Eastern Ghats of peninsular India and is grown as a potential ornamental species in Karnataka, Maharashtra, and Andhra Pradesh. Traditionally Savara, Bagata, Valmiki tribes of Munchingiputtu Mandal, Visakhapatnam district, Andhra Pradesh use rhizome extracts to cure jaundice and Bagata tribes use this plant for Diabetes[4]. It is also beneficial against leprosy, dysentery, cardiac diseases. *Curcuma pseudomontana* is used medicinally for the treatment of snakebites, contraceptive purposes, blood purifier, and treatment of swellings [5,6]. Rhizome powder are useful in leucoderma, scabies, small pox and intestinal worms as well juice as strong remedy against rheumatism and in combination of ginger used for smooth delivery in North East India[7]. Many steroids,

tannins, alkaloids, and flavonoid compounds have also been reported from the rhizomes of *Curcuma pseudomontana* [8].It has been reported to have anticancer activity , Anti-tubercular activity and Antibacterial activity [9,10]. Based on the previous literature, there is no scientific evidence available about antihyperglycemic activity of *Curucuma pseudomontana*. The present study was designed with an objective to evaluate antidiabetic acivity of methanolic extract of *Curucuma pseudomontana* rhizomes in an streptozotocin-induced diabetic rat model.

2.MATERIALS AND METHODS

2.1 Preparation of crude extracts

Fresh samples of rhizomes of *Curcuma pseudomontana* were collected from Munchingiputtu Mandal, Visakhapatnam district, Andhra Pradesh (Figure 2.1). The plant was identified and authenticated using herbarium collection at Botany research laboratory,Andhra University and specimen is deposited in Vignan Institute of Pharmaceutical technology, Visakhapatnam. Fresh rhizomes were washed thoroughly under running tap water followed by sterile distilled water and dried under shade. The material was ground into coarse powder using mechanical grinder. The powder was stored in airtight containers at room temperature. Dried powder of rhizome sample was extracted in Soxhlet extractor apparatus at 65^0 C for 6-8 hours, methanol used as solvent and prepared sample was stored in amber colour bottle for phytochemical screening and antihypoglycemic activity.

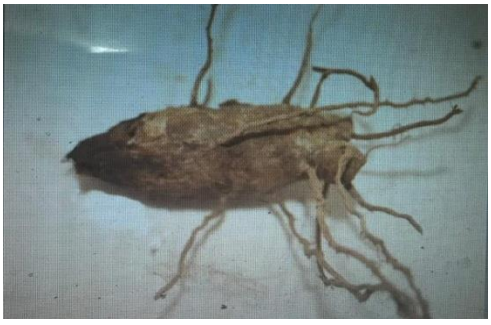


Figure 2.1 Rhizomes of Curcuma pseudomontana

2.2 Phytochemical Study: The different phytochemical tests methanolic extract of the rhizome were carried out for the the detection of secondary metabolites by adopting various procedures[11].

2.3. Dose Selection and Preparation: The methanolic extract rhizomes was prepared in solution form at concentrations of 100, 200 and 400mg/kg body weight of animals in 1 mL normal saline (pH 7.4) for in vivo study on mice model. The prepared plant extract solutions were used for intraperitoneal (IP) injection at a dose of 0.1 mL/kg body weight into the experimental rat.

2.4. Induction of Diabetes: Diabetes was induced by a single intraperitonial dose of 60 mg/kg of b. w of streptozotocin *(*STZ*)* (Sigma-Aldrich labs) dissolved in 0.1M fresh cold citrate buffer (pH 4.5) into 12 hr fasted rats[12,13].

2.5. Animal Grouping and Experimental Design: The experimental albino rats were used for this study weighing of 25 (2.5) g. They were subjected to standard diet and water ad libitum. The rats were acclimatized for the adoptive environment to handlers for 3 days before the start of the experiments. The experimental protocol was approved by the institutional animal ethics committee of Andhra university, Vishakhapatnam, which was registered with Committee for the purpose of control and supervision of experiments on animal (CPCSEA), Govt. of India (registration no.516/01/A/CPCSEA). The rats were divided into three groups: group I (n = 6; untreated rats) were given 0.1 mL/kg body weight normal saline; group II (n = 6; diabetic rats) was given glibenclamide at a dose of 0.45 mg/kg body weight; and group III (n = 18; diabetic rats) was given plant extracts at 3 dose levels (100, 200 and 400 mg/kg body weight).

2.5. Collection of blood samples and serum glucose estimation: The blood samples (0.5ml) were collected for every time intervals of 0, 2, 4, 8,12, 18, and 24th hr in 1ml Eppendorf's tubes.Serum was separated by centrifuging at 3000 rpm for 10 minutes. 30 µl of serum sample and 3 ml of working glucose reagent were taken in to a dry and clean test tube and incubated for 10 minutes at 37 ^{o}C. The pink color developed was measured by using auto analyzer[14].

2.6. Statistical analysis: The obtained data were subjected to statistical analysis for the

determination of significance using analysis of variance. The analyzed data were presented as means (standard deviation [SD]). Statistical significance is expressed by *P* values less than .05[15].

3. RESULTS AND DISCUSSION

3.1.Phytochemical screening of rhizome extract of *Curcuma pseudomontana* J. Grahm., showed the presence of glycoside, flavonoids, tannin, carbohydrates and proteins in methanolic portion which was shown in Table 3.1.1 . Rhizome extract showed the absence of steroids alkaloid and terpenoids respectively.

3.2. Antihyperglycemic activity of *Curcuma pseudomontana* J. Grahm., rhizomes

The mean blood glucose levels of control and drug treated animals after oral administration of different doses (100, 200 and 400 mg/kg b.w) of methanolic extract of *Curcuma pseudomontana* J. Grahm., rhizomes at various time intervals (0, 2, 4, 8, 12, 18 and 24 hrs) were shown in **Table 3..2.1** and **Figure 3.2.1**. The statistical significance of decrease in blood glucose levels was calculated with respect to initial blood glucose levels. Oral administration of 1% Sodium CMC suspension did not change the blood glucose levels of rats. The blood glucose levels of diabetic rats treated with Glibenclamide (0.45 mg/kg b.w) showed significant ($P<0.05$) decrease in blood glucose levels at 8 &12^{th} hrs, more significant ($P<0.01$) decrease in blood glucose levels at 2^{nd} hr and highly significant ($P<0.001$) decrease in blood glucose levels at 4^{th} hr. Nevertheless, the reduction in mean blood glucose levels was no significant at 18 & 24 hrs. After the oral administration of standard drug the mean blood glucose levels were 349.96 ±11.09, 259.20± 7.32, 197.93± 4.20, 271.65± 14.62 and 299.27 ±3.74 mg/dl at 0, 2, 4, 8 and 12^{th} hr respectively. Administration of 100 mg/kg b. w of plant crude drug produced no significant ($P>0.05$) decrease in blood glucose levels at all the time intervals.However administration of 200 mg/kg b.w of methanolic extract of *Curcuma pseudomontana J. Grahm* rhizomes produced no significant ($P>0.05$) decrease in blood glucose levels up to 4^{th} hr after oral administration. However, it showed significant ($P<0.05$) decrease in blood glucose levels at 8^{th} and 18^{th} hr respectively after the oral administration of the extract. It also showed more significant ($P<0.01$) decrease in blood glucose levels at 12^{th} hr after the administration of the extract. The mean blood glucose levels at 0,8,12 and 18 hrs after the oral administration of 200 mg/kg b. w of methanolic extract of *Curcuma pseudomontana J. Grahm* rhizomes were 354.34± 14.15, 291.12± 7.54, 278.86 ±9.51 and 291.31± 5.49 mg/dl respectively. The oral administration of 400 mg/kg b. w of methanolic extract of *Curcuma pseudomontana J. Grahm* rhizomes showed significant ($P<0.05$) decrease in blood glucose levels up to 18^{th} hr, more significant ($P<0.01$) decrease in blood glucose levels at 4^{th} and 12^{th} hr and highly significant ($P<0.001$) decrease in blood glucose levels at 8^{th} hr respectively. The mean blood glucose levels were 342.12 ±6.07, 291.51± 9.75, 215.59± 9.41, 279.38± 9.09 and 296.25± 11.46 mg/dl at 0, 4, 8, 12 and 18^{th} hr respectively after the administration of 400 mg/kg b. w of methanolic extract of *Curcuma pseudomontana J. Grahm* rhizomes. The lowest blood glucose levels were observed at 12^{th} and 8^{th} hr after oral administration of 200 and 400 mg/kg b. w of methanolic extract of *Curcuma pseudomontana J. Grahm* respectively. It also showed antihyperglycemic activity in dose dependent manner in STZ induced diabetic rats. The blood glucose levels at 18^{th} hr after the oral administration of 200 and 400 mg/kg b. w of methanolic extract of *Curcuma pseudomontana J. Grahm* rhizomes were significantly lower compared to initial level. Whereas the standard drug Glibenclamide did not lower the blood glucose levels significantly at 18^{th} hr compared to initial glucose level which shows that there might be impact of metabolism of the drug in the body. The percent decrease in blood glucose levels after the oral administration of different doses (100, 200 and 400 mg/kg b.w) of methanolic extract of *Curcuma pseudomontana J. Grahm* rhizomes was shown in **Table 3.2.2** and **Figure. 3.2.2**. The administration of standard drug Glibenclamide showed 25.47±4.23 %, 42.41±2.94 %, 21.65±5.62 % and 13.81±3.41 % reduction in blood glucose levels at 2, 4, 8 and 12 hrs respectively.

Table 3.1.1 Identification tests for active metabolites

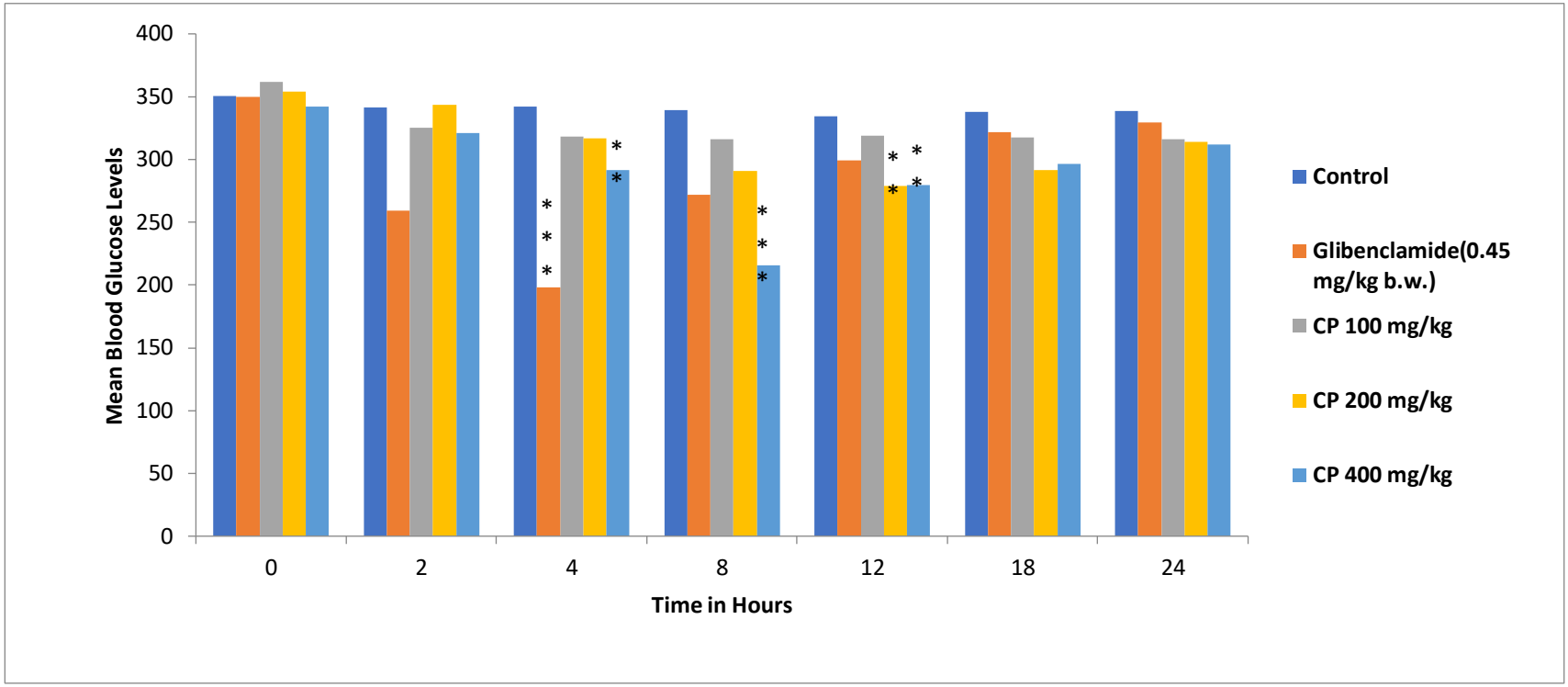
Sl No	Active metabolite	Chemical tests	Response
1	Glycoside	Fehlings test for the presence of glycon upon hydrolysis	Positive
2	Flavonoid	Shinoda Test	Positive
		Alkaline Reagent Test	Positive
3	Tannin	Ferric chloride test	Positive
		Goldbeater's skin test	Positive
4	Steroids	Liebermann Buchard Test	Negative
5	Alkaloids	Dragendorff's reagent test	negative
		Wagner's reagent test	negative
		Hager's reagent test	negative
6	Terpenoids	Salkowski test	negative
7	Carbohydrates	Molisch's test	Positive
8	Protiens	Biuret test	Positive

Table 3..2.1 Effect of Methanolic extract of *Curcuma pseudomontana* J. Grahm rhizomes on blood glucose levels (mg/dl) in STZ induced diabetic rats

Group (n=6)	Treatment mg/kg b.w.	Time in hours						
		0	2	4	8	12	18	24
1	Control	350.21± 13.12	341.28± 11.31	342.02± 13.53	339.10± 11.14	334.17± 11.27	338.12± 10.72	338.45± 9.67
2	Glibenclamide (0.45 mg/kg b.w.)	349.96 ±11.09	259.20± 7.32**	197.93± 4.20***	271.65± 14.62*	299.27 ±3.74*	321.95± 5.09	329.58± 6.97
3	CP 100	361.56± 6.39	325.35± 5.13	318.48 ± 5.82	315.87± 7.74	318.69± 6.74	317.65± 6.30	316.42± 7.81
4	CP 200	354.34± 14.15	343.23± 12.98	316.85± 7.47	291.12± 7.54*	278.86 ±9.51**	291.31± 5.49*	313.68± 3.73
5	CP 400	342.12 ±6.07	321.16± 10.47	291.51± 9.75**	215.59± 9.41***	279.38± 9.09**	296.25± 11.46*	311.78± 14.65

N.S: No significant difference as compared to zero hr (P>0.05); *: significant decrease as compared to zero hr (P< 0.05); **: More significant decrease as compared to zero hr (P<0.01); ***: Highly significant decrease as compared to zero hr (P< 0.001).

Figure 3.2.1. Bar diagram showing effect Methanolic extract of *Curcuma pseudomontana* J. Grahm rhizomes on blood glucose levels (mg/dl) in STZ induced diabetic rats



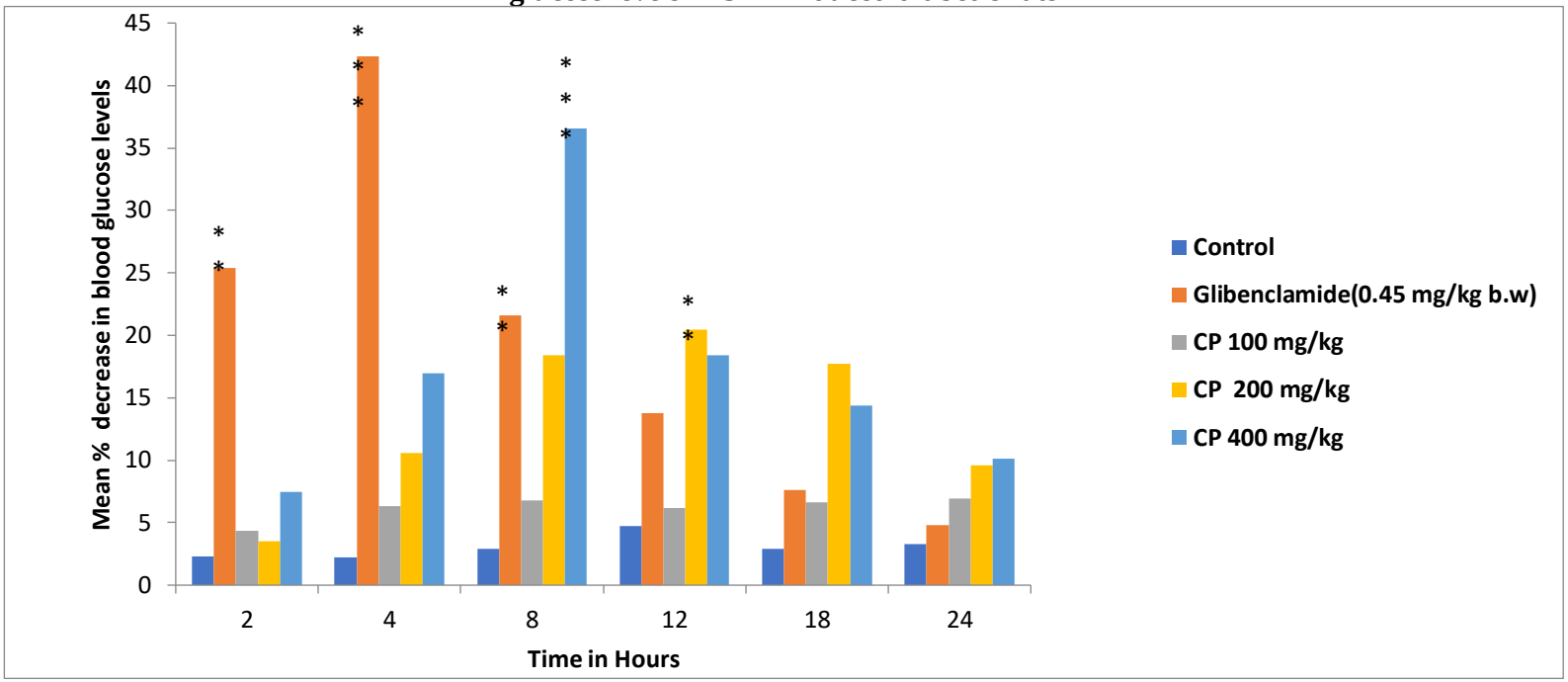
N.S: No significant difference as compared to control (P>0.05); *: significant decrease as compared to control (P< 0.05); **: More significant decrease as compared to control (P<0.01); ***: Highly significant decrease as compared to control (P< 0.001).

Table 3.2.2.Effect of Methanolic extract of *Curcuma pseudomontana* J. Grahm rhizomes on percent decrease blood glucose levels in STZ induced diabetic rats

Group (n=6)	Treatment mg/kg b.w.	Time in hours					
		2	4	8	12	18	24
1	Control	2.29±2.01	2.21±2.65	2.89±2.59	4.75±2.89	2.87±2.53	3.27±2.31
2	Glibenclamide (0.45 mg/kg b.w)	25.39±4.19**	42.32±2.91***	21.61±5.60*	13.78±3.39**	7.59±4.54	4.82±4.15
3	CP 100	4.32±1.58*	6.33±1.18**	6.79±2.76	6.18±3.20	6.65±3.38	6.90±3.11
4	CP 200	3.54±1.17*	10.59±2.13**	18.37±3.32**	20.48±4.21**	17.74±4.62*	9.63±5.01
5	CP 400	7.47±3.28	16.93±3.26**	36.57±3.32***	18.39±2.89**	14.41±3.14*	10.15±3.62*

N.S: No significant difference as compared to control (P>0.05); *: significant decrease as compared to control (P< 0.05); **: More significant decrease as compared to control (P<0.01); ***: Highly significant decrease as compared to control (P< 0.001).

Figure 3.2.2 Bar diagram showing effect Methanolic extract of *Curcuma pseudomontana* J. Grahm rhizomes on percent decrease blood glucose levels in STZ induced diabetic rats



N.S: No significant difference as compared to control (P>0.05); *: significant decrease as compared to control (P< 0.05); **: More significant decrease as compared to control (P<0.01); ***: Highly significant decrease as compared to control (P< 0.001).

The percent decrease in blood glucose level at 24th hr after the administration of Glibenclamide was not significant (P>0.05). Administration of 100 mg/kg b. w of methanolic extract of *Curcuma pseudomontana* rhizomes produced significant (P<0.05) decrease in blood glucose levels at 2nd hr and more significant (P<0.01) decrease in blood glucose levels at 4th hr. Administration of the 100 mg/kg b.w of methanolic extract of *Curcuma pseudomontana J. Grahm* rhizomes showed 4.37±1.60 % and 6.34±1.21 % reduction in blood glucose levels at 2nd and 4th hr. Administration of 200 mg/kg b. w of methanolic extract of *Curcuma pseudomontana J. Grahm* rhizomes produced significant (P<0.05) decrease in blood glucose levels at 2nd, 18th hr and more significant (P< 0.01) decrease in blood glucose levels at 4, 8 and 12th hr. Administration of 200 mg/kg b. w of methanolic extract of *Curcuma pseudomontana J. Grahm* rhizomes showed 3.52±1.19 %, 10.62±2.16%, 18.45±3.73%, 20.50±4.18% and 17.28±4.75% reduction in blood glucose levels at 2, 4, 8, 12 and 18 hr respectively. Administration of 400 mg/kg b. w of methanolic extract of *Curcuma pseudomontana J. Grahm* rhizomes produced significant (P<0.05) decrease in blood glucose level at 18 and 24th hr, more significant (P<0.01) decrease in blood glucose levels at 4 and 12th hr and highly significant (P<0.001) decrease in blood glucose levels at 8th hr. Administration of 400 mg/kg b. w of methanolic extract of *Curcuma pseudomontana J. Grahm* rhizomes showed 16.39±3.28%, 36.46±3.35%, 18.46±2.95%, 14.37±3.82% and 10.13±3.68% reduction in blood glucose levels at 4, 8, 12, 18 and 24 hrs respectively. The maximum percent reduction in blood glucose levels was observed at 12th and 8th hr after oral administration of the 200 and 400 mg/kg b. w of methanolic extract of *Curcuma pseudomontana J. Grahm* rhizomes. In addition, the reduction in blood glucose levels was found to be dose dependent in STZ induced diabetic rats. The reduction in blood glucose levels at 18th and 24th hrs after the oral administration of 200 and 400 mg/kg b. w of methanolic extract of *Curcuma pseudomontana J. Grahm* rhizomes were significant when compared with the control group at identical times. Whereas the reduction in blood glucose caused by the Glibenclamide was not significant at 18 and 24th hr. It could be assumed that there are specific active metabolites that have mimic effect in the form of pure substance in the drug that are responsible for decreasing the levels of glucose in the blood. These chemical constituents might gradually start the synthesis of insulin and its release from the pancreatic β cells to target cells in streptozotocin-induced diabetic rats and act like insulin.

4. CONCLUSION

It is concluded that antidiabetic potential of methanolic extract of Curcuma *pseudomontana* against streptozotocin-induced diabetes is scientifically proven based on the usage of plant in the treatment of diabetes by tribal people. Presence of glycosides, flavanoids and tannins could be responsible the observed effects acted in synergism. Further studies to formulate and standardise the extract in a combination form available in ayurvedic preparations. However, more research is required to identify and isolate the phytochemical constituents responsible for antihyperglycemic effects of studied medicinal plants.

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