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Antihyperglycemic effect of *Portulaca pilosa* Linn. in streptozotocin-induced diabetic rats

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ABSTRACT

The antihyperglycemic activity of ethanolic extract of *Portulaca pilosa* Linn. was evaluated against streptozotocin-induced diabetic rats. The blood glucose levels were calculated at various time intervals of 0, 1, 2 and 3 h after the administration of ethanolic extract of *P.pilosa*. The ethanolic extract reduced the blood glucose levels significantly when it was administered at three doses of 100, 200 and 400 mg/kg. It reduces the blood glucose level from 334.67±5.27 to 134.57±6.49 at 3h after the administration of 200 mg/kg of ethanolic extract. The antihyperglycemic activity was evaluated in comparison of the administration of an oral hypoglycemic agent, Tolbutamide.

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INTRODUCTION

Diabetes mellitus is a collection of metabolic problems characterised by hyperglycemia. These metabolic problems include in the alterations in carbohydrate, protein and fat metabolisms. The basic symptoms of diabetes mellitus are polyphagia, polydipsia, pruritis, polyuria and weight loss (Jaykar & Suresh, 2003). Apart from hyperglycemia, other factors such as hyperlipidemia and dyslipidemia are also involved in the development of both macro and microvascular complications (Arul *et al.,* 2006), those are primary reasons of morbidity and mortality (Randle *et al*., 1963). Even though many antidiabetic agents are available in the market (Holman and Turner 1991; Prout TE 1974. Kameswara Rao 1997; Bei and David 2000), the search for new antidiabetic agents is still continuing and the patients are looking for a natural product to reduce the side/adverse effects due to the continuous intake of existing agents. The literature indicates that around 400 plant species are having the antidiabetic activity (Jaykar & Suresh, 2003) and several pharma industries are involving in the isolation of new antidiabetic agents from herbal plants (Rai, 1995), which are traditionally used to cure but not yet scientifically validated.

Portulaca pilosa Linn. belonging to the family Portulaceae, is native to America. The plant juices are traditionally used as antidiuretics, antipyretics, and analgesics. Its antityrosinase activity (Rocha *et al*., 1994), urinary potassium and sodium excretion (Baurin *et al.*, 2002) renal effects (Lans, 2006) and anticancer (Kothai *et al*., 2017) were already reported. So, in this present work, an attempt was made to study the antihyperglycemic activity of

ethanolic extract of *P.pilosa* against streptozotocin-induced (Arul *et al*., 2005) Wistar rats.

MATERIALS AND METHODS

Plant material

The leaves of the plant *P.pilosa* was collected from the Thrissur, Kerala, in June 2017 and cleaned to remove the debris (Arul *et al.,* 2005). The collected leaves were identified and authenticated by a botanist Dr A. Balasubramanian, ABS Botanical garden, Salem. The voucher specimen (PPS-1) has been kept in our museum for future reference. The leaves were shade dried for 10 days and coarsely powdered to pass through sieve No. 40.

Preparation of the extract

The leaves of the plant *P.pilosa* was extracted by continuous hot extraction process, using soxhlet apparatus with various solvents such as a pet. Ether, chloroform, acetone, alcohol and aqueous by their increasing order of polarity. After the extraction, the extracts were dried under reduced pressure. The marc of extracted crude drug powder was once again subjected to successive extraction with the other polar solvents, and their extractive values were calculated. The dried extracts were subjected to various chemical tests to identify the phytoconstituents (Manivannan *et al.,* 2014).

Animals

Swiss albino mice of either sex weighing about 20-25g and Wistar albino rats of 175-200 gm of approximately the same age was used for the acute toxicity studies (Isah *et al.*, 2007) and antihyperglycemic studies, respectively. The animals were housed in their polypropylene cages and fed pellets and water ad *libitum*. The animals were exposed to an alternative cycle of 12 h darkness and light each. The animals have fasted for at least 12h before each test. The experimental protocols were subjected to the institutional animal ethics committee and were cleared by the same (Arul *et al*., 2005).

Acute toxicity studies

The acute toxicity studies were conducted in Swiss albino mice, as per the OECD guideline (423) and the animals were observed up to 48 h for any mortality and the LD50 was calculated (Arul *et al.*, 2006).

Induction of diabetes

The Wistar rats were kept fast for 24 h before induction of diabetes and the freshly prepared streptozotocin (45 mg/kg, i.p.,) was administered (Siddique *et al*., 1987; Arul *et al.,* 2004). After 7 days, rats those have fasting blood glucose of more than 300 mg/dl were selected for the study (Arul et al., 2006).

Effect of *P. pilosa* on glucose tolerance in rats

The fasted animals were divided into three groups each consisting of six. The first group acted as a control and received only water for injection. II and III groups received an oral dose of the ethanolic extract of *P. pilosa* and tolbutamide at 200 mg/kg and 100 mg/kg, respectively. After 30 min of administration of the extract, all the rats were treated orally with 2 g/kg of glucose solution (Archana *et al.,* 2001). The blood samples were withdrawn periodically at 0, 0.5, 1, 1.5 and 2 hrs after the administration of glucose. The blood samples were analysed (Arul *et al*., 2006) by the O-toluidine method (Fings *et al.*, 1970) for the blood glucose level.

Effect of *P. pilosa* on streptozotocin-induced hyperglycemia in rats

Different groups of diabetic-induced rats were used to study the effect of ethanolic extract of *P. pilosa*. The animals were divided into six groups each consisting of six rats (Arul *et al*., 2006). Groups I and II acted like normal and diabetic control and administered water for inj. alone. Third, fourth and fifth groups were diabetic rats treated with ethanolic extract (100, 200 400 mg/kg) of *P. pilosa*. The sixth group of diabetic rats was treated with tolbutamide 100 mg/kg (Jaykar & Suresh, 2003), an oral hypoglycemic agent. The animals were kept fast for overnight and the extracts suspended in 1 % CMC was administered orally. The Blood samples were withdrawn periodically from the tail vein (Jaykar & Suresh, 2003) at 0, 1, 2 and 3 h after administration of the plant extracts and standard drug. The blood samples were analyzed by the O-toluidine method (Fings et al., 1970) for the blood glucose level.

Statistical analysis

All values were expressed as mean ±SEM. The data were statistically analyzed using one way ANOVA followed by Newman Keul's multiple ranges and the difference below $P< 0.05$ are considered as significant (Manivannan *et al*., 2014).

RESULTS AND DISCUSSION

The plant *P. pilosa* was collected from Thrissur, Kerala, air-dried and extracts were prepared by continuous hot extraction method using soxhlet apparatus (Manivannan *et al*., 2014). The percentage yield of ethanolic extract of *P. pilosa* was found to be 3.45 % w/w. The LD50 of ethanolic extract of

Table 1: Effect of ethanolic extract of *P. pilosa* on oral glucose tolerance in rats

Groups	Dose (mg/kg)	Fasting blood glucose (mg/dl) after the treatment				
		0 min	30 min	60 min	90 min	120 min
Control	-	82.15±1.20	158.83±2.64	135.50±2.26	117.17±2.81	105.33±2.55
Ethanolic extract of *P. pilosa*	200	83.58±1.83	124.17±2.19*	112.33±1.32*	99.67±2.83*	84.17±1.32*
Tolbutam-ide	100	84.33±1.26	110.33±2.36*	92.81±2.08*	80.87±1.32*	77.58±2.14*

Values are expressed as mean ±SEM, n=6, * P<0.001 when compared with control.

Table 2: Effect of ethanolic extract of *P. pilosa* on fasting blood glucose levels (mg/dl) in diabetic rats

Groups	Dose (mg/kg)	Fasting blood glucose at different hours after the treatment			
		0 H	1 H	2 H	3 H
Normal	-	82.36±1.31	84.83±1.53	85.23±1.35	85.47±1.28
Diabetic control	-	330.53±7.53	332.83±6.11	328.50±5.20	325.67±3.79
Ethanolic extract of *P. pilosa*	100	332.17±6.51	274.83±7.04	227.67±8.22*	165.33±5.67*
Ethanolic extract of *P. pilosa*	200	334.67±5.27	238.83±7.32	194.38±7.49*	134.57±6.49*
Ethanolic extract of *P. pilosa*	400	330.67±6.33	214.33±6.23	175.33±7.23*	120.21±3.43*
Tolbutamide	100	328.37±6.22	184.33±5.33	128.33±4.71*	110.17±2.41*

Values are expressed as mean ± SEM, n=6, * P<0.001 when compared with control.

P. pilosa was performed as per OECD guideline 423, and it was found to be 2000 mg/kg. The ethanolic extract did not show any toxic effect up to 1000 mg/kg when single i.p dose was administered to mice.

The effect of ethanolic extract *P. pilosa* on glucose tolerance (Kifayatullah & Sengupta, 2016) is shown in Table 1. The ethanolic extract of *P. pilosa* did not allow the blood glucose levels to increase significantly (P<0.001) after the administration of glucose and the maximum tolerance was found in 120 min.

The effect of ethanolic extract of *P. pilosa* on fasting blood glucose level was evaluated in diabetic-induced rats at different time intervals (Arul *et al*., 2006) are shown in Table 2. A significant decrease in blood glucose level was observed, 3 h after administration, in the all the diabetic group of animals treated with *P. pilosa* from 332.17±6.51 to 165.33±5.67, 334.67±5.27 to 134.57±6.49 and 330.67±6.33 to 120.21±3.43 mg/dl, for 100, 200 and 400 mg/kg, respectively, which is comparable to the antihyperglycemic effect (328.37±6.22 to 110.17±2.41) of tolbutamide at the dose of 100 mg/kg.

In this present study, the ethanolic extract of *P.pilosa* was administered in 3 different doses of 100, 200 and 400 mg/kg to determine the dose-dependent activity. Even the starting dose of 100 mg/kg also produced a significant activity after the administration of the crude extracts. But the standard drug tolbutamide caused more significant (Arul *et al*., 2006) antihyperglycemic activity when compared with the extract of all doses. The mechanism of this antihyperglycemic effect of the extracts is not enlightened in this study. Some medicinal plants with antihyperglycemic activities are known to increase circulating insulin level in glycemic rats (Lamela *et al*., 1985). A possible mechanism of action may be the extract stimulated the residual pancreatic mechanism (Jaykar & Suresh, 2003), which is increasing peripheral utilization of glucose (Erah *et al*., 1996). Further investigation is expected to isolate the active principle which is responsible for the activity and elucidate the mechanism of action.

CONCLUSION

From the above study, it may be concluded that the ethanolic extract of *P. pilosa* produced the dose-dependent glucose lowering activity in streptozotocin-induced diabetic rats.

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