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EVALUATION OF *PORTULACA OLERACEA* LINN. FOR HYPOGLYCAEMIC EFFECTS

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ABSTRACT: Hypoglycaemic and anti-hyperglycaemic effects of *Portulaca oleracea* Linn. (POL) was evaluated in experimental rat models in vivo. The aqueous extract of POL was subjected to preliminary toxicity screening by an acute toxicity study in *Wister* albino rats. The POL was found to be safe in the doses used and there was no mortality up to a dose of 3000mg/kg orally after 24 hours. Hypoglycaemic effects of POL extract with doses 200mg, 400mg and 800mg were evaluated on Wistar albino rats by using a standard protocol. Anti-hyperglycaemic effect of POL extract with the same doses i.e. 200mg, 400mg and 800 mg were also studied in alloxan induced diabetic albino rats. The aqueous POL extract at doses 800mg showed significant hypoglycaemic effects as compared with a standard drug like glibenclamide. The same doses of POL also showed anti-hyperglycaemic properties in alloxan induced diabetic rats. The present study showed that the aqueous POL extract has hypoglycaemic as well as anti-hyperglycaemic effects.

INTRODUCTION: *Portulaca oleracea* Linn. (Portulaceae) is a succulent annual herb, which may reach up to 40cm in height, found abundantly throughout the world and known for its numerous traditional medicinal uses [1, 2]. It is known as Lonika in Sanksrit and Leipak-kundo in Manipuri language.

Since time immemorial, the indigenous healers of Manipur use the extract in gonorrhoea, gum and teeth complaints, scurvy and liver diseases. It is also used in kidney and bladder disease, cardiovascular complaints, dysentery, sore nipples and mouth ulcers.

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The roasted seeds are used as diuretic, anti-dysentery and applied in burns and scalds [3].

Chemical extractions have shown it to contain many biologically active compounds like α-linolenic acid and β-carotene, coumarins and monoterpene glycoside, N-transferuloyltyramine, dopamine, DOPA, high concentrations of noradrenaline, ferulic acid and adenosine [4]. This traditional medicine has been reported to be having various beneficial pharmacological effects, including antibacterial [5], analgesic, anti-inflammatory [6], skeletal muscle relaxant [7, 8] and wound healing [9], etc. from the studies conducted around the world.

Very few experimental animal studies were reported on its probable therapeutic potential as an anti-diabetic activity during 2009-2010. However, in recent time, its potential anti-hyperlipidaemic and anti-diabetic activity in various animal models have been reported [2].

The present study was undertaken in 2009, to evaluate the hypoglycaemic effects of the aqueous extract of *Portulaca oleracea* Linn. (POL) in normal albino rats and also in alloxan induced diabetic rat animal models.

MATERIALS AND METHODS: Present study was conducted after obtaining approval from the Institutional Ethics Committee (IEC), Regional Institute of Medical Sciences, Imphal, Manipur, India.

Preparation of extract: The aqueous extraction of *Portulaca oleracea* Linn. was done by a modification of the method described by Verma and Agarwal [10]. *Portulaca oleracea* Linn. (Whole plant) were collected during the month of June and July, 2009. Sample was authenticated by Prof. P. Kumar Singh, Department of Life Sciences, Manipur University. A plant sample was deposited at the Department herbarium and allocated MUH No. 003419. Collected samples were cleaned with water and air dried in shade for several days. The shade dried plants were powdered using a mixture grinder. The powdered material (42.6gm) was then Soxhleted with roughly ten times its volume of distilled water. The water was then heated to boil and subjected to extraction for 30 minutes. On evaporation of water from the filtrate, a deep brown residue (14.34gm) was obtained which was stored in porcelain jar at 4^{o}C for future use. The aqueous extract of *Portulaca oleracea* Linn. (POL) obtained by this method was used as the study material in the entire study, for both the hypoglycaemic study model and alloxan induced diabetic rat model.

Animals and Hypoglycaemic study: The method of Babu *et al* [11] was followed to investigate the hypoglycaemic effect with some modification. Healthy albino rats of either sex weighing 100-180 gm were obtained from central animal house, RIMS, Imphal and kept in the Departmental polypropylene cages and acclimatized for 10 days. They were fed with standard laboratory diet and water *ad libitum*. 12 hours dark-light cycle was maintained. The animals were fasted for 18 hours prior to the experiment. Care was taken to avoid coprophagy but water was allowed *ad libitum*. Fasting blood glucose was estimated using glucometer kit (Lifescan Inc, Milipitas, USA) [12] for each rat. The blood was collected from the orbital sinus using a capillary tube. Animals with a blood sugar of 80-110mg/dl were included for the study. The animals were divided into five groups of six animals each and were given the treatment as shown in **Table 1.**

TABLE 1: GROUPING OF ANIMALS IN THE HYPOGLYCAEMIC STUDY AND ALLOXAN INDUCED DIABETIC ALBINO RATS STUDY:

Group	Treatment
I (control)	2% gum acacia suspension
II (test drug)	Aqueous extract of POL – 200mg in 2% gum acacia suspension
III (test drug)	Aqueous extract of POL – 400mg in 2% gum acacia suspension
IV (test drug)	Aqueous extract of POL – 800mg in 2% gum acacia suspension
V (standard)	Glibenclamide (Adventis) – 0.5mg/kg

Animals were treated accordingly with the study materials for 7 days. On the 8^{th} day, the blood glucose was estimated using a glucometer at fasting and the results was recorded. Then the above treatments were given to each rat again. Blood glucose was estimated at 30 minutes, 1 hour and at 2 hours after the treatment and the results was recorded.

Study in alloxan induced diabetic albino rats: the anti-hyperglycaemic effect of *Portulaca oleracea* Linn was studied in alloxan induced diabetic albino rats following the method of Antia *et al* [13] with some modifications. Alloxan monohydrate was injected intraperitoneally (i.p) to overnight (18 hours) fasted rats at the dose of 150mg/kg body weight. The blood glucose was estimated after 72 hours and animals with blood glucose of 150-250mg/dl were included in the study.

Induced diabetic rats were then grouped into five groups of six animals each and were given treatment daily for a period of 7days, similar to the hypoglycaemic study as shown in table 1.

After 7 days, the rats were fasted for 18 hours. The blood glucose was estimated at fasting and the results recorded. The animals were given its specific treatment again. Blood glucose was then estimated at 30 minutes, 1 hour and at 2 hours after the treatment and the results was recorded.

Results obtained from the current study were analyzed using "analysis of variance (ANOVA)" and Dunnet`s `t` test. A level of confidence measuring 5% was considered significant.

RESULTS AND OBSERVATION:

Toxicity study: the aqueous extract of POL was found to be safe in the doses used. No mortality was observed between 0 – 24 hours during feeding of an increasing oral dose upto 3000mg/kg

Hypoglycaemic study: The results obtained on blood glucose levels in each group of rats are tabulated in **Table 2.**

TABLE 2: STUDY OF HYPOGLYCAEMIC EFFECTS IN ALBINO RATS: THE BLOOD GLUCOSE LEVELS IN DIFFERENT GROUPS OF EXPERIMENTAL RATS:

Group		Blood Glucose (mg/dL)			
		0 min	30 mins	1 hour	2 hours
I		91.16±3.66	89.67±2.95	90.33±2.86	92.33±3.91
II		90.50±3.46	89.50±3.28	86.50±4.81	80.00±2.16
III		91.00±2.52	89.00±3.07	83.00±2.93α	77.33±1.92α
IV		90.16±2.89	88.50±2.73	78.50±1.77#	75.00±1.71#
V		86.0±2.43	82.66±1.81	67.33±2.05*	61.00±2.63*
One Way ANOVA	Df	4.25	4.25	4.25	4.25
	F	0.58	1.08	14.14	27.53
	p	>0.10	>0.10	<0.01	<0.01

*p <0.001, #p <0.01, α p<0.05 compared to control, n=6 in each group

Results can be summarized as follows:

i. Fasting: the mean fasting blood glucose level of the control group, the test drug group treated with 200mg/kg, 400mg/kg, 800mg/kg and that of the standard group treated with glibenclamide (0.5mg/kg) were (91.16±3.66, 90.50+ 3.46, 91.00±2.52, 90.16±2.89 and 86.00±2.43 respectively).

ii. After 30 minutes of treatment: The mean blood glucose levels observed in animals of different groups as above respectively were 89.67±2.95, 89.50±3.28, 89.00 ±3.07, 88.50±2.73 and 82.66±1.81. Thus, there was no significant reduction in the blood sugar level after 30 minutes of treatment for the entire group including that of the glibenclamide treated group

iii. After 1 hour of treatment: The mean blood glucose level recorded in these groups was 90.33±2.86, 86.50±4.81, 83.00±2.93, 78.50±1.77 and 67.33±2.05 respectively. There was a significant reduction for the group treated with the aqueous extract of POL at the doses of 400mg/kg ($p<0.05$) and 800mg/kg ($p<0.01$) and the group treated with glibenclamide is found to be highly significant ($p<0.001$) when compared to the group treated with the vehicle. However, there was no significant reduction in the blood glucose level for the test group treated with the aqueous extract of POL at the dose of 200mg/kg.

i. ii. After 2 hours of treatment: The mean blood glucose level recorded in these groups were 92.33±3.91, 80.00±2.16, 77.33±1.92, 75.00±1.71 and 61.00±2.63 respectively in these groups 2 hours after the treatment. Reduction in blood glucose was highly significant for the test group treated with 400mg/kg ($p<0.05$) and 800mg/kg ($p<0.01$) of the aqueous extract of POL and that of the group treated with glibenclamide when compared with the control group ($p<0.001$). However, there was no significant reduction for the group treated with 200mg/kg body weight.

Study of alloxan induced diabetic albino rats: The results obtained on blood glucose levels in each group of rats are tabulated in **Table 3.**

TABLE 3: STUDY IN ALLOXAN INDUCED DIABETIC ALBINO RATS: THE BLOOD GLUCOSE LEVELS IN DIFFERENT GROUPS OF EXPERIMENTAL RATS:

Group		Blood Glucose (mg/dL)			
		0 min	30 mins	1 hour	2 hours
I		229.00±5.72	227.16±6.62	227.50±7.55	230.50±13.2
II		213.00±3.93	211.33±3.09	206.50±6.68α	204.66±7.42#
III		211.00±6.44	209.83±7.43	202.83±4.17#	196.16±4.92*
IV		205.00±7.16	202.33±7.43#	195.83±7.39*	188.33±6.32*
V		186.16±5.68	182.83±5.92*	163.50±5.09*	140.66±5.53*
One Way ANOVA	Df	4,25	4,25	4,25	4,25
	F	6.45	6.24	15.04	32.97
	p	<0.01	<0.01	<0.01	<0.01

*p <0.001, #p <0.01, α p<0.05 compared to control, n=6 in each group

Results can be summarized as follows:

i. **Fasting:** The mean fasting blood glucose level of alloxan induced diabetic rats of the control group, the test drug group treated with 200 mg/kg, 400 mg/kg, 800 mg/kg and that of the standard group treated with glibenclamide (0.5 mg/kg) were 229.00±5.72, 213.00±3.93, 211.00±6.44, 205.00±7.16 and 186.16±5.68 respectively.

ii. **After 30 minutes of treatment:** The mean blood glucose level recorded among these groups was 227.16±6.62, 211.33±3.09, 209.83±7.43, 202.33±7.43 and 182.83±5.92 respectively. Reduction in blood glucose was significant for the group treated with 800 mg/kg of aqueous extract of POL (p<0.01) and the group treated with glibenclamide (p<0.001) while the group treated with the aqueous extract of POL at the dose of 200 mg/kg and 400 mg/kg were not significant when compared with the control group treated with the vehicle.

iii. **After 1 Hour of treatment:** The mean blood glucose level recorded among these groups was 227.50±7.55, 206.50±6.68, 202.83±4.17, 195.83±7.39 and 163.50±5.09 respectively. There was significant reduction in the blood glucose level for all the groups treated with the aqueous extract of POL 200 mg/kg (p<0.05), 400 mg/kg (p<0.01) and 800 mg/kg (p<0.001) and that of the group treated with glibenclamide (p<0.001) when compared with the control group.

iv. **After 2 Hours of treatment:** The mean blood glucose level recorded among these groups was 230.50±13.2, 204.66±7.42, 196.16±4.92, 188.33±6.32 and 140.66±5.53 respectively. After 2 hours of treatment, the groups treated with the aqueous extract of POL at the doses of 400 mg/kg, 800 mg/kg and glibenclamide (0.5 mg/kg) were all significant at the level of p<0.001, while the group treated with 200 mg/kg of the aqueous extract of POL was significant at the level of p<0.01 when compared with the control group treated with vehicle.

DISCUSSION: In our present study, we tried to evaluate hypoglycaemic effects of the aqueous extract of *Portulaca oleracea* Linn. in normal albino rats as well as the anti-hyperglycaemic effects in alloxan induced diabetic rat animal models.

The hypoglycaemic effect was studied by the method described by Babu et al. [11]In our study, the basal blood glucose level of the control was found to be 91.16±3.66 which was consistent with the finding of Farswan et al [14] (90±0.8). At 30 minutes reading, the aqueous extract of *Portulaca oleracea* Linn. at doses of 200 mg/kg, 400 mg/kg and 800 mg/kg showed no significant reduction in blood glucose level of normal fasted rats. The standard drug glibenclamide also failed to produce a significant reduction in blood glucose at 30 minutes. The extract at the dose of 200 mg/kg failed to reduce the blood glucose after 1 hour while that of 400 mg/kg and 800 mg/kg showed a significant reduction (p <0.05 and p<0.01 respectively). But at the end of 2 hours, the test drug at the doses of 400 mg/kg and 800 mg/kg showed significant reduction in the blood glucose level (p<0.05 and p<0.01 respectively).

Maximum reduction in the blood glucose level was seen at the dose of 800mg/kg. However, the reduction in the blood glucose produced by the test drug at 1 hour and 2 hours were less than that of the standard drug. The reduction in blood sugar of the standard drug was 29.06% after 2 hours which was consistent with the finding of Das et al [15] (28.24%).

Antihyperglycaemic effect of *Portulaca oleracea* Linn. was evaluated following the methods of Antia *et al* [13] as already mentioned. Alloxan monohydrate at the dose of 150 mg/kg was administered intraperitoneally to produce diabetes in the present study. In our study the basal blood glucose level of the control group of alloxan induced diabetic rats was 229.00±5.72 which was in agreement with the finding of Antia *et al* [13] (222.1±1.3). At 30 minutes, only the aqueous extract of *Portulaca oleracea* Linn. at the dose of 800 mg/kg produced significant($p<0.01$) reduction in the blood glucose level of the diabetic rats.

After 1 hour and 2 hours of treatment, the aqueous extract of *Portulaca oleracea* Linn. at different doses (200, 400 and 800 mg/kg)produced significant reduction in the blood glucose level of alloxan induced diabetic rats. Maximum reduction in the blood glucose level was seen at the dose of 800 mg/kg. However, the reduction in the blood glucose level of even the most effective dose of the extract of Portulaca oleracea Linn. (800 mg/kg) was much less than that of the standard drug, glibenclamide. In the present study, the reduction in blood glucose level of diabetic rats by the standard drug at the end of 2 hours was 24.44% which was in consistent with the finding of Ghosh et al [16](25.4%).

Present study shows that the aqueous extract of *Portulaca oleracea* Linn. has hypoglycaemic as well as anti-hyperglycaemic properties. The effect was dose dependent and maximum reduction in the blood glucose level was found at the dose of 800 mg/kg. The hypoglycaemic effect was not seen after 30 minutes of treatment. However, the aqueous extract of *Portulaca oleracea* Linn. at the dose of 800 mg/kg produced a significant ($p<0.01$) reduction of blood glucose level in diabetic rats even at 30 minutes. The reduction in the blood glucose level was more after 2 hours than at 1 hour.

Portulaca oleracea Linn. contains a variety of biologically active compounds –alpha-tocopherol, ascorbic acid, beta-carotene and glutathione and flavonoids [17, 18]. The hypoglycaemic effects observed in our study may be due to the presence of one or more of these compounds in the aqueous extract. A plausible mechanism of action is that the aqueous extract of *Portulaca oleracea* Linn. may suppress the hyperglycaemia, probably by stimulating the secretion of insulin in normal rats and may also stimulate the residual pancreatic β-cell function in case of the alloxan induced diabetic rats. There is also the possibility that they may produce hypoglycaemia through an extra-pancreatic mechanism, probably increasing the peripheral utilization of glucose.

CONCLUSION: In the present study, we found that the aqueous extract of *Portulaca oleracea* Linn. has significant hypoglycaemic and anti-hyperglycaemic effects. The present study on an experimental model of rats re-establishes the already reported beneficial pharmacological effects of the age old traditional medicine as an agent capable of lowering blood sugar level. This warrants a further study to explore the active composition and its mechanism of action for potential drug development in future. Its effect on lipid metabolism and hepatic enzymes are also required to be studied. More research is required on this traditional plant with much medicinal value.

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CONFLICT OF INTEREST: There are no conflicts of interest to declare.

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