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HYPOGLYCEMIC ACTIVITY OF *PUTRANJIVA ROXBURGHII* WALL. IN ALLOXAN INDUCED DIABETIC RATS

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ABSTRACT

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Diabetes mellitus is the most common endocrine disorder that impairs glucose homeostasis resulting in severe diabetic complications including retinopathy, angiopathy, nephropathy, neuropathy and causing neurological disorders due to perturbation in utilization of glucose. In the present study diabetes was induced in albino rat models with alloxan monohydrate. *Putranjiva roxburghii* Wall. has been claimed to possess antidiabetic properties by many investigators. The present study was undertaken to screen the hypoglycemic activity of ethanol extracts of leaves of *Putranjiva roxburghii*. The results showed that it has significant antihyperglycemic effect in experimental model of diabetes mellitus.

INTRODUCTION: Diabetes is a major degenerative disease in the world today ¹, affecting at least 15 million people and having complications which include hypertension, atherosclerosis and microcirculatory disorders ². Diabetes mellitus is also associated with long-term complications, including retinopathy, nephropathy, neuropathy and angiopathy and several others ³. India has today become the diabetic capital of the world with over 20 million diabetics and this number is set to increase to 57 million by 2025 ⁴. Diabetes mellitus (DM) is a multifactorial disease which is characterized by hyperglycemia ⁵, lipoprotein abnormalities ⁶, raised basal metabolic rate ⁷, defect in reactive oxygen species scavenging enzymes ⁸ and high oxidative stress induced damage to pancreatic beta cells ⁹. Diabetes mellitus is ranked seventh among the leading causes of death and is considered third when its fatal complications are taken into account ¹⁰.

Plants are well known in traditional herbal medicine for their hypoglycaemic activities, and available literature indicate that there are more than 800 plant species showing hypoglycaemic activity ¹¹. There has been increasing demand for the use of plant products with antidiabetic activity due to low cost, easy availability and lesser side effects. Therefore, plant materials are continuously scrutinized and explored for their effect as hypoglycemic agents. One such plant is *Putranjiva roxburghii* which has been used in traditional system of medicine for treating azoospermia, diuretic, catrrah, ophthalmopathy and constipation ¹². In addition, this plant is considered to anti-inflammatory, analgesic and antipyretic ¹³. The present research programme was aimed to investigate antidiabetic activities of leaf extract of *Putranjiva roxburghii* in alloxan induced diabetic rats.

MATERIALS AND METHODS:

Plant material: The leaves of *Putranjiva roxburghii* were collected in the month of August from Kanpur (U.P.) and authenticated by Dr. D. V. Amla, National Botanical Research Institute (NBRI), Lucknow (U.P.), with ref. no., NBRI/CIF/112/2009. The leaves were dried in shade at room temperature. The dried leaves were powdered by using grinder to coarse powder, packed into Soxhlet column and the extracted 70% ethanol for 48 hrs. The excess of solvent was removed using rotatory flash evaporator. The obtained crude extract was stored in airtight container in refrigerator below 100C for further studies.

Experimental Animals: Male albino rats of (180-210 g) were used throughout the experiments. The animals were procured from Institute of Pharmacy, Bundelkhand University Jhansi (U.P.). Before initiation of experiment, the rats were acclimatized for a period of 7 days. Standard environmental conditions such as temperature (26+20C), relative humidity (45-55%) and 12hrs dark/light cycle were maintained in the quarantine. All the animals were fed with rodent pellet diet (Gold mohur, Lipton India Ltd.) and water was allowed *ad-libitum* under strict hygienic conditions. Ethical clearance for performing the experiments on animals was obtained from Institutional Animal Ethics Committee (IAEC).

Induction of diabetes: Alloxan (2, 4, 5, 6-tetraoxypyrimidine; 2, 4, 5, 6-pyrimidinetetrone) is an oxygenated pyrimidine derivative ¹⁴ and was originally isolated in 1818 by Brugnatelli and got its name in 1838 by Friedrich Wöhler and Justus von Liebig ¹⁵. Alloxan is a toxic glucose analogue, which selectively destroys insulin-producing cells in the pancreas when administered to rodents and many other animal species. This causes an insulin-dependent diabetes mellitus (called "Alloxan Diabetes") in these animals, with characteristics similar to type 1 diabetes in humans (Lenzen, 2008).

Anti-diabetic activity: Fasting blood glucose was determined after depriving food for 16 hrs with free access of drinking water. Hyperglycemia was induced by a single i.p. injection of 120 mg/kg of alloxan monohydrate in sterile saline. After 5 days of alloxan injection, the hyperglycemic rats (glucose level >250mg/dl) were separated and divided into different groups comprising of 6 rats each for the anti-diabetic study. The treatment (p.o.) was started from the same day except normal control and diabetic control groups for a period of 10 days. During this period, animals in all groups had free access to standard diet and water. Body weight and blood glucose levels were estimated on 4th, 7th and 10th day of the treatment. On the 10th day, blood samples were collected from overnight fasted rats by cardiac puncture under mild ether anesthesia for biochemical estimation.

The various groups used in experiment;

- Group A - Served as normal control and did not receive any treatment.
- Group B - Served as diabetic control and received alloxan monohydrate and vehicle (0.2 ml of 2% aqueous gum acacia)
- Group C - Alloxan monohydrate + Glibenclamide (10 mg/kg, p.o.) and served as Standard.
- Group D - Alloxan monohydrate + Ethanolic extract (100 mg/kg, p.o.)

- Group E - Alloxan monohydrate + Ethanolic extract (250 mg/kg, p.o.)
- Group F - Alloxan monohydrate + Ethanolic extract (500 mg/kg, p.o.)

RESULTS:

Anti-diabetic study: Effect of *Putranjiva roxburghii* Wall. leaf extract on fasting blood glucose level in diabetic rats. Ethanolic extract of *Putranjiva roxburghii* leaves was subjected to anti-diabetic activity in rats where alloxan monohydrate (120 mg/kg b.w., i.p.) used as the diabetogenic agent. A marked rise in fasting blood glucose level observed in diabetic control compare to normal control rats.

Ethanolic extract of *Putranjiva roxburghii* (at 250 and 500 mg/kg) exhibited a dose dependent significant anti-hyperglycemic activity on 4th, 7th and 10th day post treatment. The extract dose of 100 mg/kg also caused reduction in blood glucose level but the results were found statistically insignificant. The antihyperglycemic effect of ethanol extract at was found less effective than the reference standard, Glibenclamide. Glibenclamide produced a significant reduction in blood glucose compare to diabetic control. The results are shown in the **Table 1.**

TABLE 1: EFFECT OF PUTRANJIVA ROXBURGHII LEAF EXTRACT ON FASTING BLOOD GLUCOSE LEVEL IN ALLOXAN INDUCED DIABETIC RATS

Group	Treatment	Fasting blood glucose level (mg/dl)			
		Basal value	4 th day	7 th day	10 th day
A	Normal control	90.46 ± 3.80	92.82±2.92	92.32±1.73	88.29±3.44
B	Diabetic control (Vehicle)	293.8±5.27	286.91±5.05	291.8±5.41	289.41±9.75
C	Alloxan + glibenclamide (10 mg/kg)	285.86±6.92	205.25±7.06***	183.18±6.35***	178.13±6.20***
D	Alloxan + Ethanolic extract (100 mg/kg)	291.76±4.79	277.76±5.65	266.23±8.19	255.42±7.71
E	Alloxan + Ethanolic extract (250 mg/kg)	284.48±5.32	258.23±6.66*	255.85±9.97**	252.06±9.19**
F	Alloxan + Ethanolic extract (500mg/kg)	287.48±5.32	212.61±5.07***	198.36±3.52***	189.83±3.31***

Values are mean ± S.E.M.; n = 6, *P < 0.05, **P < 0.05, ***P < 0.01 and ****P < 0.01 vs Diabetic control; **Animal: Albino Rats, Alloxan: 120 mg/kg, i.p.**

DISCUSSION: Pancreas is the primary organ involved in sensing the organism's dietary and energetic states via glucose concentration in the blood and in response to elevated blood glucose, insulin is secreted². Alloxan is one of the usual substances used for the induction of diabetes mellitus apart from streptozotocin. Alloxan has a destructive effect on the beta cells of the pancreas^{16, 17}. Alloxan causes a massive reduction in insulin release by the destruction of b-cells of the Islets of Langerhans, thereby inducing hyperglycemia¹⁸. Insulin deficiency leads to various metabolic alterations in the animals viz. increased blood glucose, increased cholesterol, increased levels of alkaline phosphate and transaminases^{19, 20}.

The results of the present study indicate that *Putranjiva roxburghii* leaf extract was found to reduce the glucose level in animals made diabetic with alloxan. Alloxan has been shown to induce free radical production and cause tissue injury. The pancreas is especially susceptible to the action of alloxan induced free radical damage. In the present investigation ethanolic extract of *Putranjiva roxburghii* leaf demonstrated the significant anti-diabetic activity. The literature reports reveal that flavonoids and tannins present in the plant extract known to possess antidiabetic activity. In the present investigation also the observed antidiabetic potential of test extract may be due to presence of similar phytoconstitutes which was evident by preliminary phytochemical screening.

CONCLUSION: From this study, we can state that the Ethanolic extract of *Putranjiva roxburghii* has beneficial effects on blood glucose levels. Further pharmacological and biochemical investigations will clearly elucidate the mechanism of action and will be helpful in projecting this plant as a therapeutic target in diabetes research.

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