

## Research Article



## Evaluation of *In Vivo* and *In Vitro* Anti-diabetic Activity of *Roylea cinerea*

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### ABSTRACT

In the present study, we investigated *In vivo* & *In vitro* anti-diabetic activity of different fractions (petroleum ether, ethyl acetate and methanolic) of aerial part of *Roylea cinerea*. *In vivo* method Glibenclamide, an oral hypoglycemic agent was used as a positive control against alloxan-induced diabetic rats and *In vitro* method all three fractions of *Roylea cinerea* were evaluated for antidiabetic activity by determining their inhibitory effect on alpha amylase and alpha-glucosidase enzyme. The methanolic extract of aerial part of *Roylea cinerea* was found to exhibit potent antidiabetic activity *In vitro* as well as *In vivo*. The methanolic extract showed maximum reduction (41.20% *In vivo* and 80% *In vitro*) in blood glucose levels.

**Keywords:** Blood glucose, GLB, Diabetes, *Roylea cinerea*, Alloxan

### INTRODUCTION

Diabetes mellitus is one of the chronic, worldwide heterogeneous and life-threatening diseases. Its prevalence will be 5.4% by the year 2025, with the global diabetic population reaching to 300 million. Among all the WHO regions<sup>1</sup>, Southeast Asian region is the highest affected with maximum global burden of the disease and, by the year 2025, there will be nearly 80 million diabetics in the region. Given the pandemic spread of type II diabetes, the identification of new therapeutic avenues in the treatment of all pathological aspects of this disorder remains a major challenge for current biomedical research<sup>2</sup>. A number of plants present in nature possess marked anti-diabetic activity. *Roylea cinerea* (family-Lamiaceae) commonly known as Karui, is used locally to cure ailments such as fever, malaria, skin diseases and diabetes<sup>3,4</sup>. But, none of the pharmaceutical companies are using *Roylea cinerea* as a drug component. Therefore, this herb can be a potential new source for the cure for several diseases particularly in case of diabetes. Thus objective of our study is to investigate the aerial part of *Roylea cinerea* for its *in vivo* and *in vitro* antidiabetic activity.

### MATERIALS AND METHODS

#### Collection and Identification of Plant Sample

The fresh aerial parts of *Roylea cinerea* were collected from adjoining area of Village Ringoal Ganv (Dist- Tehri Uttarakhand) in the month of August-November 2013. The plant was authenticated by botanist Dr. C. S. Rana, Department of Botany and the voucher specimen number is GUH 2917, H. N. B. Garhwal (A Central University) Srinagar Garhwal, Uttarakhand India.

#### Preparation of Plant Extract

The plant material was separated into its selected aerial part, air dried, ground to moderately fine powder and

soxhlet extracted with increasing polarity solvent (petroleum ether, chloroform, ethyl acetate, acetone, methanolic, ethanolic and water)<sup>5</sup>. Each extract was evaporated to dryness under reduce pressure using rotary evaporator. The coarse powder of aerial part was subjected to successive hot continuous extraction with various solvent each time before extracting with next solvent the powdered material will be air dried (weight of crude extract 300gm). The various concentrated extracts were stored in air tight container for further studies.

#### Chemicals and Instrument

Glibenclamide 100mg/kg was purchased from local market, Alloxan ALX (0.5% w/v in Tween 80 solution). All other chemicals used for the study were of research grade.

#### Experimental Animals

Mature adult albino rats weighing (180-210g) were purchased from Central animal house facility (CPCSEA Regd. No. 245/CPCSEA, dated 11 March 2015) and acclimatized for seven days to faculty animal house, and maintained at standard conditions of temperature and relative humidity, with a 12-hour light dark cycle. Water and commercial rat feed *ad libitum* were provided. The current study was carried out with prior sanction from Institutional Animal Ethical Committee and proposal no. 583.

#### Method for *In vitro* Anti-diabetic Activity

##### *Inhibition of Alpha-amylase Enzyme*

Starch solution (0.1% w/v) was prepared by stirring 0.1g of potato starch in 100 ml of 16 mM of sodium acetate buffer. The enzyme solution was prepared by mixing 27.5 mg of alpha-amylase in 100 ml of distilled water. The colorimetric reagent was prepared by mixing sodium potassium tartarate solution and 3, 5 Di-nitro salicylic acid solution at 96 mM concentration. Both control and plant



extracts were separately added with starch solution and left to react with alpha- amylase solution under alkaline conditions at 25°C. Their action was measured after 3 minutes. The generation of maltose was quantified by the reduction of 3, 5 Di-nitro salicylic acid to 3-amino-5- nitro salicylic acid. This reaction is detectable at 540 nm<sup>6</sup>.

#### **Inhibition of Alpha-glucosidase Enzyme**

The inhibitory activity of alpha-glucosidase enzyme was determined by incubating 1 ml solution of starch substrate (2 % w/v maltose or sucrose) with 0.2 M Tris buffer pH 8.0 and plant extracts separately for 5 minutes at 37°C. Their action was initiated by adding 1 ml of alpha-glucosidase enzyme (1U/ml) to it followed by incubation for 40 minutes at 35°C. Then the reaction was terminated by the addition of 2 ml of 6N HCl. Then the intensity of the colour was measured at 540nm<sup>7</sup>.

#### **Method for *In vivo* Anti-diabetic Activity**

##### **Acute Toxicity Study**

To determine the minimum lethal dose, acute oral toxicity studies were performed as per OECD guidelines<sup>8</sup>. Adult albino rats of either sex weighing 180-210gm were used. The animals were divided into six groups, the group I was given 2 ml of 1% Tween and group II received 2 ml of 1% vanillin both acted as control. The other four groups were administered 50, 100 and 150 mg/kg bw of the methanolic extract with 2 ml of 1% vanillin orally using intra Gastric Catheter respectively. All the experimental rats were fasted overnight. They were observed continuously for any gross behavioral changes and toxic manifestations like hyperactivity, grooming, convulsions, sedation, hypothermia and mortality during the first three hours. Thereafter the animals were continuously monitored at regular intervals for 7 days. No adverse effect or mortality was detected in this study up to 500 mg/kg bw dose. Hence sub-lethal doses of 50, 100 and 150 mg/kg bw doses of the extract were selected for the following experiments.

##### **Induction of Diabetes to Experimental Animals**

Alloxan was commonly utilized as an animal model of diabetes. The animals were fasted for 12h prior to the induction of diabetes with slight modification. Alloxan (ALX), freshly prepared in 0.5% Tween 80 was administered intra-peritoneal at single dose of 140mg/kg body weight<sup>9</sup>. Development of diabetes was confirmed by measuring blood glucose concentration 5 days after the administration of ALX. Rats with blood glucose level of above 200 mg/dl were considered to be diabetic and used for the studies.

##### **Experimental Design**

In the present experiment, a total of 30 rats (24 diabetic surviving rats; 6 normal rats) were used. The extracts were diluted to prepare the specific doses. The rats were randomized into five groups comprising of six animals in each group as given below.

##### **Group I**

Normal control (NC) rats received distilled water 10ml/kg body weight, p.o.

##### **Group II**

Diabetic rats received standard drug GLB (10 mg/kg p.o.), 5 days after ALX treatment.

##### **Group III**

Diabetic rats given pet ether extract of *Roylea cinerea* prepared in distilled water (100 mg/kg p.o.), 5 days after ALX treatment.

##### **Group IV**

Diabetic rats given ethyl acetate extract of *Roylea cinerea* prepared in distilled water (100 mg/kg p.o.), 5 days after ALX treatment.

##### **Group V**

Diabetic rats given methanolic extract of *Roylea cinerea* prepared in distilled water (100 mg/kg p.o.), 5 days after ALX treatment.

##### **Biochemical Assay (Glucose levels)**

Blood samples were collected from retro-orbital plexus of each rat under mild anesthesia at 0, 1, 2 and 4h (Acute study) as well as on 0<sup>th</sup>, 7<sup>th</sup>, 14<sup>th</sup> and 21<sup>st</sup> days after administration (Chronic study) of extracts.

Blood glucose level was estimated by enzymatic glucose oxidase method.

The reduction in blood glucose level was calculated with reference to the initial level. The body weight of all animals was quantified on the 0, 7<sup>th</sup>, 14<sup>th</sup> and 21<sup>st</sup> days after 1h of treatment with the plant extracts and GLB<sup>9</sup>.

##### **Glucose Tolerance Test**

Five days before the termination of the experiment, the oral glucose tolerance test (OGTT) was performed to evaluate the ability to respond appropriately to a glucose challenge.

For this purpose, overnight fasted rats (control and treated rats) were feed glucose (2g/kg body weight) orally and blood was collected at 0, 30, 60 and 120 minutes interval from orbital sinus for glucose estimation using a glucometer.

## **RESULTS AND DISCUSSION**

Plants are important source of medicinal uses and potentially bioactive constituents for the development of new chemotherapeutic agents. The first step towards this goal is the *in vitro* and *in vivo* antidiabetic activity assay.

The results of *in vitro* and *in vivo* antidiabetic activity were tabulated in table 1, 2, 3 & 4 and fig. 1, 2, 3 & 4 which are evaluated against two enzymes (alpha-amylase and alpha-glucosidase) and standard drug GLB (Glibenclamide).



## Antidiabetic Activity

*Roylea cinerea* aerials extract showed significant activity (80.0%, 76.56% & 75.43%) against alpha-amylase and alpha-glucosidase enzymes in different extracts, the order of the extract based on total antidiabetic activity is as follows: methanolic > ethyl acetate > pet. ether.

**Table 1:** *In vitro* anti-diabetic effect of different extract of *Roylea cinerea* in  $\alpha$  amylase enzyme activity.

Extract Name	Solubility	Percent reduction in $\alpha$ amylase enzyme activity
Pet ether	Petroleum Ether	57.12
Ethyl acetate	Ethyl acetate	75.43
Methanol	Methanol	80.0

**Table 2:** *In vitro* anti-diabetic effect of different extract of *Roylea cinerea* in  $\alpha$  glucosidase enzyme activity.

Extract Name	Solubility	Percent reduction in $\alpha$ glucosidase enzyme activity
Pet ether	Petroleum Ether	55.23
Ethyl acetate	Ethyl acetate	74.43
Methanol	Methanol	76.56

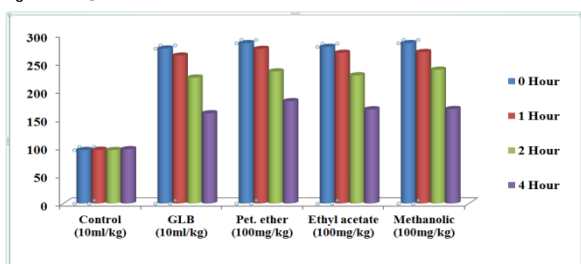
**Table 3:** Acute study: *In vivo* anti-diabetic effect of different extract of *Roylea cinerea* in alloxan Induced albino rats.

Treatment/Group	Dose mg/kg	Blood glucose level (mg/dl)				Total Reduction % (0-4 Hour) %
		0 Hour	+1 Hour	+2Hour	+04Hour	
Normal Control/ Group I	10ml/kg	95.12 $\pm$ 0.45	95.56 $\pm$ 0.56	95.08 $\pm$ 0.62	96.53 $\pm$ 0.73	-
GLB Treated/Group II	10ml/kg	275.67 $\pm$ 1.12*	263.16 $\pm$ 2.43**	224.06 $\pm$ 1.20**	160.53 $\pm$ .43**	41.76 $\pm$ 0.05
Pet. ether extract/Group III	100mg/kg	285.34 $\pm$ 1.12*	275.11 $\pm$ 2.12**	235.01 $\pm$ 1.23**	182.05 $\pm$ .86**	36.20 $\pm$ 0.05
Ethyl acetate extract/Group IV	100mg/kg	278.67 $\pm$ 1.25*	268.12 $\pm$ 1.45**	228.03 $\pm$ 0.88**	167.52 $\pm$ .83**	39.90 $\pm$ 0.05
Methanolic extract/Group V	100mg/kg	285.37 $\pm$ 1.25*	269.52 $\pm$ 1.45**	238.02 $\pm$ 0.88**	168.02 $\pm$ .83**	41.20 $\pm$ 0.05

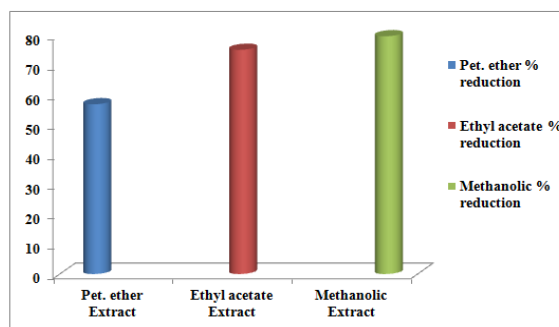
**Table 4:** Chronic study: The effect of different extracts of *Roylea cinerea* on alloxan induced albino rats

Treatment/Group	Dose mg/kg	Blood glucose level (mg/dl)			
		4th day	7th day	14 <sup>th</sup> day	21th day
Normal Control/ Group I	10ml/kg	182.12 $\pm$ 0.45	185.01 $\pm$ 0.56	189.23 $\pm$ 0.2	197.56 $\pm$ 0.73
GLB Treated/Group II	10ml/kg	195.45 $\pm$ 1.12*	192.34 $\pm$ 2.43**	195.67 $\pm$ 1.20**	185.34 $\pm$ 1.43**
Pet. ether extract/Group III	100mg/kg	198.16 $\pm$ 1.12*	197.56 $\pm$ 2.12**	193.34 $\pm$ 1.23**	155.23 $\pm$ 0.86**
Ethyl acetate extract/Group IV	100mg/kg	198.82 $\pm$ 1.25*	197.56 $\pm$ 1.45**	186.53 $\pm$ 0.88**	150.22 $\pm$ 0.83**
Methanolic extract/Group V	100mg/kg	195.23 $\pm$ 1.25*	183.45 $\pm$ 1.45**	175.46 $\pm$ 0.88**	155.43 $\pm$ 0.83**

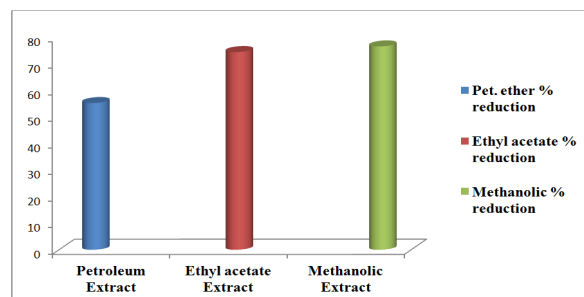
The data are expressed in mean  $\pm$  S.E.M. n = 6 in each group. P values were analyzed using One way ANOVA [\*p<0.05- less significant; \*\*p<0.05- most significant]



**Figure 3:** Acute Study: The effect of different extracts of *Roylea cinerea* on alloxan induced albino rats.



**Figure 1:** Percent reduction in  $\alpha$  amylase enzyme activity by different extracts.

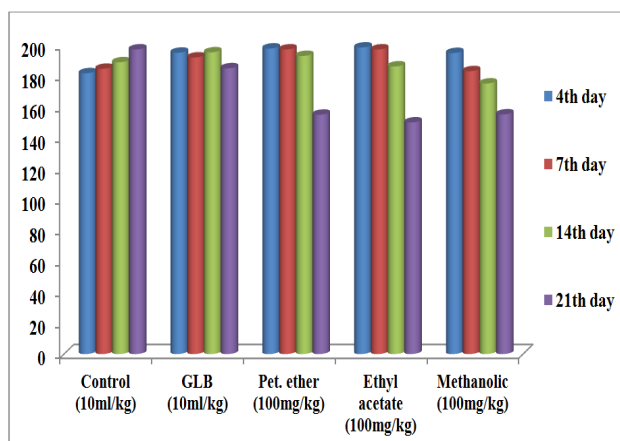


**Figure 2:** Percent reduction in alpha-glucosidase enzyme activity by different extracts

## Anti-diabetic activity in Alloxan-induced diabetic rats (Chronic study)

As per chronic study, it was observed that repeated dose administration of diabetic rats with GLB (10 mg/kg) and extracts (100 mg/kg) in separate specific groups with an interval of 7 days had progressively reduced the blood glucose level in a dose dependent manner over a period of 3 weeks. The results were found to be most significant of extracts at 4<sup>th</sup>, 7<sup>th</sup>, 14<sup>th</sup> and 21<sup>st</sup> days of treatment. It was observed that with the passage of time of dosing and

performing repeated dosing of extracts, the blood glucose level gets decreases in comparison to the positive control/GLB. The results are shown in Table 4 and Figure 4.



**Figure 4:** Chronic Study: The effect of different extracts of *Roylea cinerea* on alloxan induced albino rats

### CONCLUSION

The present finding reveals that *Roylea cinerea* efficiently inhibits both  $\alpha$ -amylase and  $\alpha$ -glucosidase enzyme *in vitro* in a dose dependent manner. The methanolic and ethyl acetate extract of *Roylea cinerea* shows 80.01%, 76.56% and 75.43% reduction in  $\alpha$ -amylase and  $\alpha$ -glucosidase enzyme activity.

The *in vivo* experiments of the methanolic extract of *Roylea cinerea* shows maximum reduction value of blood glucose level of diabetic rats as compare to Glibenclamide a standard drug (Chronic study). The active ingredient in the extract that reduces the blood sugar is not known at present.

There is ongoing research to isolate and characterize the bioactive compound (s) responsible for the anti-diabetic activity of *Roylea cinerea*.

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