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Research Article

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Antidiabetic activity of *Rephanus Sativus* L, leaves extracts on alloxan induced diabetic rats

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ABSTRACT

The different solvent extracts of Raphanus Sativus L. leaves (Family: Brassicaceae) were tested for antidiabetic activity using alloxan induced diabetic rats and compared with standard. The results expressed that aqueous extracts had shown significant protection and maximum reduction in blood glucose was observed in alloxan induced diabetic rats ($p<0.001$). The results of this comprehensive study reveal that R. sativus L leaves showed statistically significant Antidiabetic activity in comparison to the standard glibenclamide.

Key words: *Rephanus sativus* L., Antidiabetic activity, Alloxan, Glibenclamide.

INTRODUCTION

Traditionally, herbs used for the treatment of disease and disorders. Among other disorders, Diabetic mellitus is a chronic disorder and is a major public heath problem in the developed as well as developing countries caused by partial or complete insulin deficiency, resulting in hyperglycaemia leading to acute and chronic complications[1]. Synthetic drugs are likely to give serious side effects and in addition they are not suitable for intake during conditions like pregnancy[2-4]. *R. sativus* L. (Radish) is used in the treatment of intestinal parasites, though the part of the plant used is not specified. The leaves, seeds and old roots are used in the treatment of asthma and other chest complaints. The juice of the fresh leaves is diuretic and laxative. The seed is carminative, diuretic, expectorant, laxative and stomachic. It is taken internally in the treatment of indigestion, abdominal bloating, wind, acid regurgitation, diarrhoea and bronchitis[5]. The root is antiscorbutic, antispasmodic, astringent, cholagogue, digestive and diuretic. It is crushed and used as a poultice for burns, bruises and smelly feet. Radishes are also an excellent food remedy for stone, gravel and scorbutic conditions. The plant contains raphanin, which is antibacterial and antifungal. Radish preparations are useful in liver and gall bladder troubles. The roots are said to be useful in urinary complaints, piles and in gastrodynia. The present work has been carried out to evaluate the antidiabetic effect of the different extracts of *R. sativus* L. leaves

EXPERIMENTAL SECTION

Chemicals and solvents:
Alloxan monohydrate and Glibenclamide (Sigma-Aldrich Company, St. Louis, Missouri, USA), Ascorbic acid

(Universal laboratories, Mumbai), Hydrogen peroxide (S.S.Pharm Hanamkonda), Glucometer kit (Taidoc Technology Corporation, San-Chung, Taipei country, Taiwan) were procured from local market. All the solvents and other chemicals were procured from E. Merck (Mumbai) and they were of analytical grade quality.

Plant material:
Dried leaves of *R. sativus* L. were purchased from commercial supplier of rural Nalgonda, Andhra Pradesh, India. The plant was authenticated by Prof. Dr.K. Raju, Head of Department of Botany, Kakatiya University, Warangal, India.

Preparation of extracts:
The extracts of leaves of *R. sativus* L. were prepared by maceration with various solvents viz., petroleum ether, chloroform, methanol and water. In the present study, the leaves of *R. sativus* L. were collected, dried and subjected to size reduction to get uniform coarse powder. The shade dried leaf powder was packed into closed vessels and kept in shaker using double maceration techniques. Extraction was successively made using petroleum ether, chloroform and ethanol as the extracting solvents. Finally, dried marc was extracted with chloroform-water for 24 hours shaking to obtain the aqueous extract[6,7]. Each strained and expressed solvent mixed extract was filtered then concentrated by evaporating the solvent on the water-bath and the obtained extracts were weighed. The physical characteristics and percentage yield of various extracts were measured and reported.

Experimental animals:
Albino Wistar rats (weighing 180-230 g) of either sex were fed with a standard diet and water *ad libitum*. The animals were housed in spacious polypropylene cages bedded with rice husk. The animal room was well ventilated and maintained under standard experimental conditions (temperature 27^0C and 12 hours light/dark cycle) throughout the experimental period. Animal experiments were carried out following the guidelines of the animal ethics committee of the institute.

Acute toxicity test:
The different extracts of *R sativus* L. were screened for acute toxicity, following the standard method (OECD/OCDE No: 425)[8]. Animals were maintained on normal diet and water prior to and during the course of experiment. The dose of all extract was prepared with 5% acacia and was administered orally. The acute toxicity was tested at the doses of 300 and 2000mg/kg.

Evaluation of antidiabetic activity in alloxan induced diabetic wistar rats fed with the various extracts of *R. sativus* L.leaves.
The rats of either sex were randomly divided in to 7 groups (6 rats /group) and were fasted overnight (18hrs). Animals in group I were treated with acacia (5%) as control, remaining groups animals were treated with freshly prepared aqueous solution of alloxan monohydrate in a dose of 150mg/kg body weight through intraperitonial route[9,10]. Then 5% dextrose was administrated to combat the immediate hypoglycemia. Group II was kept as a diabetic control. After 18 hrs, Group III animals were treated with standard glibenclamide (600 µg/kg body weight) through oral route. Groups IV, V, VI and VII animals were treated through oral route with 400mg/kg of the petroleum ether, chloroform, methanol and aqueous *R. sativus* L. leaf extracts respectively. Blood samples were taken from the tail vein at 0, 1, 2, 3, 4, 5, 6 and 7 hs. The blood glucose concentration was measured using glucometer and recorded[11].

RESULTS AND DISCUSSION

Table. 1. Percentage yield and physical appearance of different extracts

No.	Extract (Solvent used)	Nature of Extract	Color	Yield (%w/w)
1.	Petroleum ether	Semi Solid/Resinous	Dark green	6.4
2.	Chloroform	Hard Semi Solid	Blackish green	4.2
3.	Ethylacetate	Semi Solid/Resinous	Green	3.2
4.	Methanol	Semi Solid/Resinous	Green	6.9
5.	Water	Solid	Brown	20.4

The percentage yields and nature of extracts were given in Table 1. The acute toxicity studies reported that the doses of 300 and 2000mg/kg were not produced any toxic effect to animals and animals used for toxicity studies were

surveyed. Based on the acute toxicity studies, the dose of plant extract was selected for further studies.

Fig. 1 and Table 2 represent the evaluation of antidiabetic activity of the tested extracts of *R. sativus* L. (petroleum ether, chloroform, methanol and water) on alloxan induced diabetic rats fed (400mg/kg) with these extracts, where, the changes in the levels of blood glucose in group II, III, IV, V, VI and VII are represented. *R. sativus* L. aqueous extract showed the maximum reduction in blood glucose level calculated by comparing the blood glucose level at 7th hr with the 0 hr of its respective groups. Group II, III, IV, V and VII showed significant ($p< 0.001$) reduction in blood glucose level at 7 hrs compared to zero hour to its respective group.

Table. 2 Blood glucose levels in rats before and after treatment with different leaf extracts of *R. sativus* L

Group	Blood glucose level (mg/dl) (Mean ± SD)							
	0 hr	1 hr	2 hr	3 hr	4 hr	5 hr	6 hr	7 hr
I. Control	86.16 ± 5.1	85.33± 7.2	96.6 ± 7.5	98.6 ± 7.4	98.3 ± 9.7	96.6 ± 8.3	97.0 ± 6.0	97.1 ± 6.9
II. Diabetic control	455.8 ± 42.2	450.6 ± 43.5	453.6 ± 46.5	450.5 ± 46.5	450.1 ± 48.4	451.0 ± 46.8	451.3 ± 48	450.6 ± 49.5
III.Standard (glibenclamide)	342.3 ± 20.7	130.8 ± 15.4[a]	94.6 ± 7.5[b]	96.6 ± 7.4[b]	96.3 ± 9.7[b]	94.6 ± 8.3	95.7 ± 6.03	96.9 ± 6.9 (71.7)[c]
IV. Pet.ether extract (400mg/kg)	315.6 ± 45.3	239.6± 26.2	217.8 ± 34.5	213.8 ± 47.0[a]	181.1 ± 41.6[b]	213.6 ± 42.7	215.5 ± 49.1	220.5 ± 50.0 (30.2)[c]
V. Chloroform extract (400mg/kg)	342.3 ± 20.7	144.6 ± 17.9	132.5 ± 20.1	128.8 ± 21.4[a]	109.3 ± 17.1[b]	123.8 ± 30.5	127.3 ± 18.9	132.0 ± 16.7 (61.5)[c]
VI. Methonolic extract (400mg/kg)	254.5 ± 31.9	129.1 ± 19.9	128.5 ± 21.4	129.5 ± 12.5	133.3 ± 20.8[b]	143.0 ± 20.4	147.5 ± 21.9	149.5 ± 18.4 (41.3)[c]
VII. Aqueous extract (400mg/kg)	303.8 ± 48.8[b]	112.6 ± 42.8[a]	96.6 ± 42.6[b]	98.5 ± 42.7[b]	98.3 ± 33.0[b]	99 ± 40.6[b]	96.3 ± 39.1[b]	99.8 ± 39.3[b] (67.0)[c]

[a] P<0.01 [b] P<0.001, compared to 0 hr of their respective group. (n=6)
[c] The number between brackets expresses the percentage reduction of blood glucose.

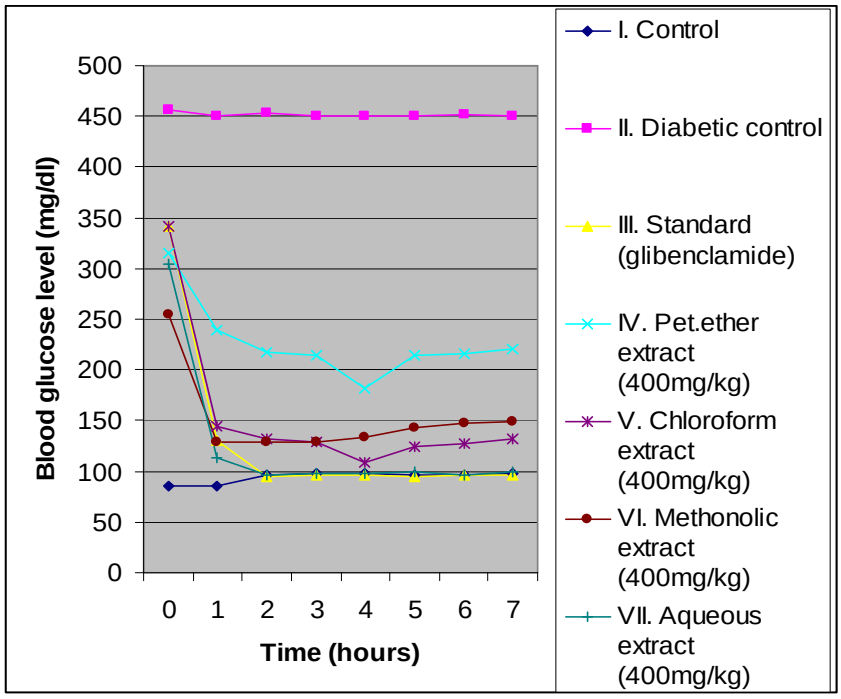

Figure 1 Blood glucose levels in rats before and after treatment with different leaf extracts of *R sativus* L.

Alloxan (beta cytotoxin) induced diabetes in a wide variety of animals by damaging the insulin secreting beta cell resulting in a decrease in endogenous insulin release, which decreased the utilization of glucose by the tissues. The significant antidiabetic activity of leaves of *R. sativus* L. may be due to inhibition of subsequent tissue damage induced by alloxan or potentiation of plasma insulin effect.

Statistical analysis

All data were subjected to analysis of variance (ANOVA). The data (mean±SD) shown are mean value and the significance differences was compared by using Dennett's Multiple comparison test at the $p<0.05$ probability level. ANOVA was carried out by using GRACH-PAD-PRISM version 4.2 software.

CONCLUSION

In conclusion, our findings show that aqueous *R sativus* L. extract more reduction on blood glucose than other extracts and it may be due to the active constituents present within the extract. Petroleum ether *R sativus* L. extract have a least reduction on blood glucose. More studies are required to ascertain the compounds and its mechanism of action, thereby providing a natural hyperglycemic control treatment, and thus decrease risk for diabetes, cardiovascular diseases. However, further studies are needed before *R sativus* L. can be used safely as food additives and supplements.

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