POTENTIAL HYPOGLYCEMIC EFFECT OF ESSENTIAL OIL OF *CITRUS RETICULATA* IN WISTAR RATS

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ABSTRACT

The essential oil of *Citrus reticulata* is reported to have antidiabetic activity, but there are no reports on their interaction with commonly used hypoglycemic agent like glibenclamide. The present study was planned to elucidate hypoglycemic effect of essential oil of *Citrus reticulata* (ECR) and its interaction with glibenclamide in euglycemic and hyperglycemic rats. Euglycemic and alloxan induced hyperglycemic male Wistar rats were orally administered, the essential oil, glibenclamide (0.9mg/kg), subhypoglycemic dose (SHD) of ECR and SHD of glibenclamide (0.45mg/kg) in single dose separately in different groups blood glucose was estimated using glucometer at 0, 2, 4, 6, 8 and 12hrs. The essential oil of *Citrus reticulata* exhibited significant hypoglycemic activity, while it did not potentiate the hypoglycemic activity of glibenclamide.

Keywords: *Citrus reticulata*; Glibenclamide; Hypoglycemia.

Introduction

The ancient Indian literature has prescribed various herbs for cure of diabetes mellitus, several plants have been investigated and reported to possess hypoglycemic activity *Aegle marmelos [1] ,Allium cepa* [2]*Brassica oleraceae* [3], *Euginea jambolica* [4], *Psidium guajav* [5] *etc.* Similarly *Citrus reticulata* is commonly known as santre. It is one of the essential oil bearing plant belonging to family Rutaceae, it is a perennial bushy tree with globose fruit. This plant finds its place in indigeneous traditional medicinal system. The medicinal properties of this plant being mainly due to the essential oil produced by the secondary metabolism. In traditional medicine the essential oil from citrus fruit rind was advised for cuteneus complaints, hemiplagia,snake bite, fever loss of taste, chronic rheumatism, stomach ache, menorrhagia, spleenomegaly, edema and cardiac diseases [6].

Therefore it is interesting to investigate the fruit rind oil for their hypoglycemic activity. Present study was therefore planned to confirm the hypoglycemic activity of essential oil of *Citrus reticulata* and to explore effect of fruit rind oil on glucose in euglycemic and alloxan induced hyperglycemic Wistar rats. It was also planned to elicit their interaction with commonly used hypoglycemic agent like glibenclamide.

Materials and methods

Preparation of *Citrus reticulata* essential oil:

Fresh mature fruits were obtained from the local market were identified and confirmed by the botanist Mrs R. Rashinwadkar and the samples were preserved at M. M. College Botany dept with voucher no. MMC47. The fruit rind was removed and pale yellow colored essential oil was obtained by hydro distillation using Clevenger type apparatus.

Animals:

The complete course of the experiment was carried out using healthy male rats of Wistar strain, reared and maintained at the animal house of the institution and were fed on commercial laboratory animal feed (Amrut brand, Sangli) and water ad libitum. The rats weighing between 150-250 g were housed for about a week for acclimatization under 12:12 light – dark cycle. The animals were starved overnight with water ad libitum prior to the day of experimentation. Ethical clearance was obtained from Institutional Animal Ethics Committee constituted as per CPCSEA guidelines.

Dose determination:

In various groups (n=6, in each) of euglycemic animals ECR in the dose of 500, 1000, 1500 and 2000 mg/kg body weight was administered orally. Accordingly from the preliminary results as 500 mg/kg of the extract failed to show significant hypoglycemia, while 1500 and 2000mg/kg showed significant hypoglycemia. In the present study 1500mg/kg of the extracts was selected as hypoglycemic dose while 500mg/kg as subhypoglycemic dose (SHD). Glibenclamide used as standard was suspended with 2% gum acacia and administered orally. Rat equivalent dose of glibenclamide was calculated using conversion table devised by Paget and Barnes [7] and was 0.9mg/kg body weight, while subhypoglycemic dose through different experiments was determined to be 0.45mg/kg.

Methadology

Animals were rendered hyperglycemic by injecting freshly prepared alloxan 60mg/kg i.v. in the tail vein and 100mg of glucose given i.v. after 6hrs and blood glucose was estimated after 24hrs using glucometer, only the animals showing blood glucose 200mg/dl or more were included and divided in different groups (n=6) to receive fruit rind oil. Vehicle treated group acted as control while glibenclamide (effective dose) treated group served as standard. Similarly for interaction studies different groups were treated with SHD of extract of fruit rind oil with SHD of glibenclamide. Glucose was estimated at 0, 2, 4, 6, 8, 10 and 12hrs. Standard kits (Beacon diagnostic) were used to estimate blood glucose.

Statistical analysis

The results were analyzed by ANOVA followed by post hoc Dunnet's posthoc test and $p \leq 0.05$ was considered as significant.

Results

Euglycemic rats: As expected saline treatment did not alter the glucose level significantly, while glibenclamide in therapeutic equivalent dose significantly ($p<0.05$, $p<0.01$)

decreased the glucose level. The ECR significantly ($p<0.01$) decreased the blood glucose at 2, 4, 6 and 8 hrs in euglycemic rats. (Table 1)

Hyperglycemic rats: As expected saline treatment did not alter the glucose level significantly, while glibenclamide in therapeutic equivalent dose significantly ($p<0.05$, $p<0.01$) decreased the glucose level. The ECR showed significant ($p<0.01$) decrease in blood glucose level at 4, 6 and 8 hrs. (Table 2) In interaction studies the subhypoglycemic dose of essential oil of fruit co administered with that of glibenclamide did not significantly lower the blood glucose level. (Table 1 and 2)

Table 1. Effect of various treatments on blood glucose levels in euglycemic rats.

Treatment	0 hr	2 hr	4 hr	6 hr	8 hr	10 hr	12 hr
Glibenclamide	87.12±1.12	81.76±1.44*	78.21±1.23*	76.88±2.15**	77.63±3.43*	75.31±2.35**	72.11±2.13**
ECR	78.4±2.59	69.56±3.38	58.46±3.23**	52.00±2.44**	59.00±1.46**	67.43±1.76	75.34±2.86
ECR+GLB	89.17±4.76	86.5±3.35	88.67±4.87	89.00±4.56	87.00±4.16	86.00±4.16	90.5±4.12

ESR- Essential oil of *Citrus reticulata* ESR+GLB- Subhypoglycemic dose of ESR+ Subhypoglycemic dose of GLB

ANOVA followed by dunnets test *p<0.05, **p<0.01

Table 2. Effect of various treatments on blood glucose levels in hyperglycemic rats.

Treatment	0 hr	2 hr	4 hr	6 hr	8 hr	10 hr	12 hr
Glibenclamide	345.42±3.12	313.43±2.21*	271.14±3.51*	232.92±2.51**	200.63±3.56**	189.51±2.65**	178.51±2.72**
ECR	401.2±3.24	394.2±2.57	382.9±3.19**	370.0±2.14*	384.5±2.59**	392.7±2.77	402.4±5.64
ECR+GLB	348.2±22.70	342.5±20.27	348.5±27.82	357.5±22.56	342.2±21.04	337.5±22.06	327.6±20.17

ESR- Essential oil of *Citrus reticulata* ESR+GLB- Subhypoglycemic dose of ESR+ Subhypoglycemic dose of GLB

ANOVA followed by dunnets test *p<0.05, **p<0.01

Discussion

Findings of the present study clearly indicate that acute (single dose) treatment with essential oil of *Citrus reticulata* showed significant hypoglycemic activity and reports on such activity could not be traced in the available literature. Whereas the single dose administration of the oil in sub hypoglycemic dose with that of Glibenclamide did not show any significant hypoglycemic activity, such interaction reports are not found in the literature. As eluded earlier the objective of the study was to investigate hypoglycemic activity of essential oil of *Citrus reticulata*. Though the present study was not aimed to elicit the mechanism of hypoglycemic activity of the extract, the phytochemical constituents of *Citrus reticulata* bioflavonoid, sterols, D-limonene, linalool could be responsible for its hypoglycemic activity by virtue of their antioxidant property. Antioxidants have been reported to exert beneficial effects on pancreatic β-cell function by preventing or delaying β-cell dysfunction due to glucose toxicity [8]. If the findings of the present study extrapolated to clinical situation, the fruits are good for consumption by the diabetics and by patients on oral hypoglycemic as it is not expected to develop sever hypoglycemia. However the impact of such consumption on chronic basis by the patients on oral hypoglycemic needs to be explored clinically and experimentally.

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