SEMICARPUS ANACARDIUM FRUIT AND SEED EXTRACTS POTENTIATE HYPOGLYCEMIC ACTIVITY OF GLIBENCLAMIDE IN WISTAR RATS.

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Summary

The ethanolic extract of the *Semecarpus anacardium* seed is reported to have antidiabetic activity, but there are no reports on their interaction with commonly used hypoglycaemic like glibenclamide. The present study was planned to elucidate hypoglycemic effect of *Semecarpus anacardium* aqueous as well as alcoholic fruit and seed extract, and their interaction with glibenclamide in euglycemic and hyperglycemic rats. Euglycemic and alloxan induced hyperglycemic male Wistar rats were orally administered, the aqueous/alcoholic seed/ fruit extracts alone seperately, glibenclamide (0.9mg/kg), subhypoglycemic dose (SHD) of extracts and SHD of glibenclamide (0.45mg/kg) in single dose. Blood glucose was estimated using glucometer at 0, 2, 4, 6, 8 and 12hrs. The seed/ fruit aqueous and alcoholic extracts exhibited significant hypoglycemic activity, while it did not potentiate the hypoglycaemic ativity of glibenclamide.

Keywords: *Semecarpus anacardium;* Glibenclamide; Hypoglycemia.

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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 guajava*[5] *etc.* Similarly ethanolic extract of the *Semecarpus anacardium* seed is reported to have antidiabetic activity[6,7,8]. The seeds are used in sweet dishes to increase the delicacy. Such sweets and seeds as such are consumed by normal individuals while the diabetic patients usually deprived of these seeds as the sweets are not freely permitted to them. However in traditional practice such patients are advised to consume seeds and fruits, but as fruits being available seasonally, seeds are routinely used. It is not known whether fruits possess antidiabetic activity, though traditional practitioners and patients claim its efficacy in diabetes. Therefore it is interesting to investigate the fruits for their hypoglycemic activity (if any). Present study was therefore planned to confirm the hypoglycemic activity of seed extracts and to explore effect of fruit extracts on glucose in euglycemic and alloxan induced hyperglycemic Wistar rats. It was also planned to elicit their interaction with commonly used hypoglycaemic agent like glibenclamide.

Materials and methods

Preparation of *Semecarpus anacardium* Seed and fruit Extract:

Semecarpus anacardium seeds and fruits obtained from the local market were identified and confirmed by the taxonomist Dr. Harsha Hegde and the samples were preserved at ICMR's RMRC with voucher no. RMRC470. Shade dried seeds and fruits were powdered to moderately coarse grade. Water and ethanol extracts of fruit and seeds were obtained using soxhlet, the extraction was continued for 12 cycles or until the solvent in the thimble was clear. After evaporating the solvent, the extracts were kept in an air tight container at 4^0c for future use. Extracts were reconstituted in water to obtain desired concentration for administration in rats.

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 lib* 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 aqueous as well as ethanolic extracts of seed and fruit in the dose of 500, 1000, 1500 & 2000 mg/kg body weight were administered orally. Accordingly from the preliminary results as 500 mg/kg of the extracts 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[9] and was 0.9mg/kg body weight, while sub hypoglycemic 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 & 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 aqueous/ ethanolic extract of seed, aqueous /ethanolic extract of fruit. Vehicle treated groups acted as control while glibenclamide (effective dose) treated group served as standard. Similarly for interaction studies different groups were treated with SHD of individual extracts *ie* aqueous/ ethanolic extracts of fruit/ seed with SHD of glibenclamide. Glucose was estimated at 0, 2, 4, 6, 8, 10 & 12hrs. Standard kits (Beacon diagnostic) were used to estimate Fasting blood glucose.

Statistical analysis

The results were analysed 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 fruit aqueous extract significantly ($p<0.01$) decreased the blood glucose at 2, 4, 6 and 8 hrs in euglycemic rats, while alcoholic extract of the same failed to do so. Whereas both aqueous as well as alcoholic extracts of seed significantly ($p<0.05$) decreased blood glucose levels at 4, 6, 8 and at 8 hr respectively.(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 aqueous extracts of both fruit as well as seed showed significant ($p<0.01$) decrease in blood glucose level at 4, 6 and 8 hrs. while alcoholic extract of fruit and seed showed significant ($p<0.05$) decrease in blood glucose at 6hrs.(Table 2)

In interaction studies the subhypoglycemic dose of aqueous or alcoholic extracts of either fruit or seed coadministered with that of glibenclamide did not significantly lower the blood glucose level.(Table 1&2)

Discussion

Findings of the present study clearly indicate that acute (single dose) treatment with aqueous or alcoholic extract of *Semecarpus anacardium* seed showed significant hypoglycemic activity, which is in agreement with the earlier studies.[7,8] The aqueous as well as alcoholic extract of its fruit extract also showed significant hypoglycemic activity and reports on such activity could not be traced in the available literature. Whereas the single dose administration of these extracts in sub hypoglycemic doses 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 aqueous and alcoholic extracts of *Semicarpus anacardium* fruit and also to confirm that of seeds. Though the present study was not aimed to elicit the mechanism of hypoglycemic activity of the extracts, the phytochemical constituents of *Semicarpus anacardium* biflavanoids, jeediflavone, semecarpetin, semecarpuflavanone, galluflavanone [10] 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.[11]

If the findings of the present study extrapolated to clinical situation, the fruit or the seeds are good for consumption by the diabetics and by patients on oral hypoglycemics as it is not expected to develop sever hypoglycaemia. However the impact of such consumption on chronic basis by the patients on oral hypoglycemics needs to be explored clinically and experimentally.

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

Groups	*0 hr*	*2 hr*	*4 hr*	*6 hr*	*8 hr*	*10 hr*	*12 hr*
Glibenclamide	89.42±1.42	82.76±1.54*	80.21±1.13*	79.92±2.35**	78.63±3.71*	76.31±2.41**	72.11±2.13**
WFE	83.34±2.82	70.83±2.99*	69.16±3.72*	61.66±4.71**	65.67±2.09**	72.67±1.86	79.5±2.94
AFE	89.83±4.91	83.16±5.58	78.16±3.75	75.00±3.36	78.5±4.50	83.83±4.35	88.15±5.93
WSE	77.5±2.69	68.66±3.28	57.66±3.23**	52.00±2.54**	60.00±1.48**	68.33±1.76	74.34±2.78
ASE	77.34±3.57	70.00±3.59	66.67±3.41	65.33±2.85	67.00±3.29*	72.34±3.13	78.00±3.29
WSE$^{\infty}$+GLB$^{\infty}$	90.17±4.756	87.5±3.35	89.66±4.97	89.00±4.58	86.00±4.16	88.67±2.47	90.5±4.12
ASE$^{\infty}$ +GLB$^{\infty}$	91±1.63	93±3.17	88.17±1.74	88.16±1.60	91.83±2.06	91.00±1.34	92.5±2.75
WFE $^{\infty}$+GLB$^{\infty}$	83.16±3.98	83.35±3.90	82.33±2.36	78.16±4.62	82.16±2.68	84.66±3.92	87.83±3.48
AFE $^{\infty}$+GLB$^{\infty}$	93.34±5.806	91.05±5.778	89.67±4.863	89.5±5.915	90.84±6.872	93.5±5.787	96.0±6.072

(WFE: Fruit Aqueous Extract , AFE: Fruit Alcoholic Extract, WSE: Seed Aqueous Extract, ASE: Seed Alcoholic Extract)
ANOVA followed by Dunnet's test, *p<0.05, **p<0.01. $^{\infty}$ Sub anti-convulsant dose

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

Groups	*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**
WFE	388.3±5.39	374.7±4.76	360.2±5.94*	324.8±6.93**	345.5±9.08**	366.3±9.63	388.8±5.78
AFE	358.83±5.82	349.5±4.84	354.67±6.12	330.16±6.58*	337.5±6.46	353.36±4.79	364.0±5.01
WSE	403.2±4.47	395.5±2.47	387.9±3.19**	371.0±2.11**	385.5±2.69**	394.7±2.73	401.4±5.74
ASE	402.7±12.69	390.2±14.94	371.2±14.46	340.5±14.42*	356.2±14.84	383.3±13.72	404.0±14.81
WSE$^{\infty}$+GLB$^{\infty}$	323.2±21.70	322.5±20.47	338.5±22.82	357.5±22.56	322.2±21.04	327.5±22.06	307.6±20.17
ASE$^{\infty}$ +GLB$^{\infty}$	327.3±27.09	328.3±26.74	328.7±28.66	325.5±28.61	325.5±28.78	330.5±27.12	339.6±31.95
WFE $^{\infty}$+GLB$^{\infty}$	302.5±17.19	288.5±13.85	288.5±18.01	286.2±15.78	289.7±15.67	289.8±15.94	285.2±13.14
AFE $^{\infty}$+GLB$^{\infty}$	305.3±25.33	293.7±23.47	291.3±24.45	284.7±23.53	290.5±24.74	288.5±23.80	294.0±27.38

(WFE: Fruit Aqueous Extract , AFE: Fruit Alcoholic Extract, WSE: Seed Aqueous Extract, ASE: Seed Alcoholic Extract)
ANOVA followed by Dunnet's test, *p<0.05, **p<0.01. $^{\infty}$ Sub anti-convulsant dose

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