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Hypoglycemic and Anti-hyperglycemic Effect of Alcoholic Extract of *Benincasa hispida* in Normal and in Alloxan Induced Diabetic Rats

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ABSTRACT - The hypoglycemic and anti-hyperglycemic effect of alcoholic extract of *Benincasa hispida* was investigated in normal and alloxan induced diabetic rats. A single oral administration of alcoholic extract of *Benincasa hispida* at doses 50,100,200mg/kg produced a significant blood glucose reduction in a dose dependent manner. A bioguided extraction and fractionation of alcoholic extract of the stem of *Benincasa hispida* afforded three compounds, ß-sitosterol, α-amyrin, quercetin. The alcoholic extract at 200mg/kg significantly reduced the blood glucose levels and equipotent with that of the standard drug Tolbutamide.

KEY WORDS: Diabetes mellitus, *Benincasa hispida*, Alloxan.

INTRODUCTION

Diabetes mellitus is a group of syndromes characterized by hypoglycaemia, altered metabolism of lipids, carbohydrates and proteins, it is an increased risk of complications from vascular diseases (1). Chronic hyper glycemia during diabetes causes glycation of body proteins that in turn leads to secondary complications effecting eyes, kidneys, nerves and arteries (2). These may be delayed, decreased or prevented by maintaining blood glucose values close to normal. The increasing number of aging population, consumption of calories rich diet, obesity and sedentary life style have lead to tremendous increase in the number of diabetics worldwide.

According to W.H.O projections, the prevalence of diabetes is likely to increase by 35%. Currently there are over 150 million diabetics worldwide and this is likely to increase to 300 million or more by the year 2025. Statistical projections about India suggest that the number of diabetics will rise from 15 million in 1995 to 57 million in the year 2025 making it the country with the highest number of diabetics in the world (3,4). It is apparent that due to the side effects of the currently used drugs, there is a need for safe agents with minimal adverse effects, which can be taken for long duration. Recently, the search for appropriate hypoglycemic agents has been focused on plants used in traditional medicine partly because of leads provided by traditional medicine to natural products that may be better treatments than currently used drugs (5). India is a country with a vast reserve of natural resourses and rich history of traditional medicine (6). In the indigenous system of medicine (Ayurveda), a mention was made on many number of plants for the cure of diabetes or 'madhumeha'and Some of them have been experimentally evaluated and the active principles were isolated (7, 8, 9, 10, 11). How ever, search for novel anti diabetic drugs continues.

The family contains 119 genera and contains around 825 species, Plants belonging to the *Benincasa* species have been the subjects of many investigations for their biologically active components Some species of *Benincasa* have been used as medicinal plants for the treatment of diabetes, urinary infection, summer fever, (12) epilepsy, insanity and other nervous diseases, gonorrhoea, demulcent facilitates pus drainage.(13,14).The rind of the fruit is used as diuretic(15).

MATERIALS AND METHODS

Plant

The stem of the plant *Benincasa hispida* was collected from a place called Gujarathi peta, Srikakulam dist, and A.P, India in August-2001. It was confirmed by Associate professor, Dr.M.Venkaiah (Department of Botany, A.U, Vishakapatnam). A voucher specimen (BGR-2006) is deposited in herbarium of Andhra University, Visakhapatnam.

Animals

Laboratory breed Sprague Dawley rats of either sex weighing 200-225 g were employed for the study. All animals were procured from National Institute of Nutrition, Hyderabad. The rats were maintained under standard laboratory conditions at 25±2°C, relative humidity 50±15% and normal photo period (12hr dark /12hr light). Commercial pellet diet (Rattan Brothers, India) and water were provided adlibitum. The experimental protocol has been approved by the Institutional Animal Ethics Committee and by the regulatory body of the government (Reg No-516/01/A/CPCSEA).

Drugs

Alloxan mono hydrate was purchased from Sigma Chemicals (St Louis,U.S.A). All other chemicals used for this study were of analytical grade.

Extraction and Isolation Procedure

Freshly collected plant material stem was cut into small pieces and shade dried. The dried stems were powdered in Willey mill .The powdered stem (700g) was extracted with methanol (4L) by process of continuous extraction (soxhlation). The crude extract was evaporated to dryness in a rotary film evaporator.1 g of alcoholic extract equivalent to 30.33 g of crude drug was obtained. β-sitosterol was crystallized from hexane as colourless fine needles with m.p136 to 138^{oC},α-amyrin was obtained from the fractions of hexane:ethylacetate(90:10) of colum chromatography from methanol as colourless needles with m.p 186 to 187^{oC}, β-sitosterol-3-o-β-D-glucoside was obtained from fractions of 51-60 of colum chromatography of ethylacetate extract and crystallized from acetone as white shining crystalline needles with m.p 289-292^{oC} ,Quercetin was soluble in ether acetone ethylacetae and methanol.The compound was crystallized twice from methanol as yellow needles with m.p 310^{oC}.All the above compounds were confirmed by melting point and Co-TLC studies with the authentic sample.

Toxicity Evaluation in Mice

The alcoholic extract was tested for its acute and short-term toxicity (if any) in mice. To determine acute toxicity, a single oral administration of the alcoholic extract at doses of 0.25,0.5,0.75,1.0 g/kg were administered to different groups of mice (2 mice were used for each group, control mice received 1%sodiumCMC) mortality and behavior of the animals were observed periodically for 48hr. The animals were observed continuously from the initial at the 4th, 6 th, 24 th, 48 th hours following drug administration. The parameters observed were grooming hyperactivity, sedation, loss righting, respiratory rate and convulsion. To study short-term toxicity, 3groups of mice each containing 6male mice (20-25g body weight) were used.

Group1 was kept as control and Group2,Group3 received 200and 400mg/kg alcoholic extract respectively in 1%sodiumCMC. The drug was administered daily for 14 days (*p.o*) control group received 1%SodiumCMC in an identical manner. The behaviors of the animals were observed daily for 1hr in the forenoon (10to 11a.m) for 14days. Initial and final body weights, water and food intake, state of tool and body temperature was observed.

Induction of Diabetes

Animals were allowed to fast 18hr and were injected with alloxan monohydrate dissolved in sterile normal saline at a dose of 140mg/kg body weight intraperitoneally. After 2 weeks, diabetic rats (250-350mg/dl) were used for the experiment.

Experimental Design

In the experiment a total number of 60 (30 normal and 30diabetic) surviving rats were used. The rats were divided into 10 groups, each group consisting of 6 animals.Group1 Normal rats treated with vehicle (1% sodium CMC) and served as normal control,Group2 Normal rats treated with alcoholic extract of *B.hispida* at dose of 50 mg/kg , Group3 Normal rats treated with alcoholic extract of *B.hispida* at dose of 100 mg/kg, Group 4 Normal rats treated with alcoholic extract of *B.hispida* at dose of 200 mg/kg, Group 5treated with Tolbutamide 40mg/kg. Group6 Diabetic rats treated with vehicle (1% sodium CMC) served as diabetic control. Group7 Diabetic rats were treated with alcoholic extract of *B.hispida* at doses of 50mg/kg , Group8 Diabetic rats were treated with alcoholic extract of *B.hispida* at doses of 100mg/kg, Group9Diabetic rats were treated with alcoholic extract of *B.hispida* at doses of 200mg/kg and Group 10 treated with Tolbutamide 40mg/kg. All the doses were administered orally.

Estimation of Blood Glucose

The rats were fasted for 18hr and blood samples were collected by puncture of retro-orbital plexus immediately with capillary tube under ether anesthesia into glass vials containing a small quantity of a mixture of potassium oxalate and sodium fluoride as an anticoagulant at 0hr (before treatment) and 2, 4, 6, 8, 12, 24hr (after treatment). The plasma blood glucose levels were determined by using GOD-POD method (16).

Statistical Analysis

All values were expressed as Mean±S.E.M. The differences were compared using one way analysis of variance (ANOVA) followed by Dunnett's t test. p values <0.05 were considered as significant.

RESULTS

The effect of different doses of alcoholic extract of *Benincasa hispida* on fasting blood sugar levels in normal rats were assessed at different time intervals. The maximum percentage blood glucose reduction with 50, 100 and 200mg /kg doses of *B.hispida* at 6hr were 21.32%, 45.87% and 66.06% respectively. Tolbutamide 40mg/kg dose produced 64.89%blood glucose reduction in normal rats and results were shown in table1.The *anti hyperglycemic* effect of different doses of alcoholic extract of *B.hispida* on fasting blood glucose levels in diabetic rats were assessed at different time intervals. The percentage blood glucose reduction with 50, 100 and 200mg/kg dose of *B. hispida* at 6hr were 52.3%, 54.25% and 61.9% respectively. Tolbutamide (40mg/kg) produced 64.89% blood glucose reductions in alloxan induced diabetic rats were shown in table2.

DISCUSSION

The present study was conducted to evaluate the hypoglycemic and anti hyperglycemic activity of *Benincasa hispida* which is a very new herbal drug that was firstly identified by us to get a berth in the group of antidiabetic herbal drugs. In this study, the alcoholic extract of *Benincasa hispida* produced a dose dependent percentage blood glucose reduction in normal and diabetic group. In normal treated groups a significant percentage blood glucose reduction was observed up to 24hr and maximum percentage blood glucose reduction was observed at 6hr, where as in diabetic groups also significant reduction in blood glucose was maintained up to 24hr and maximum at 6hr. The percentage blood glucose reduction produced by the extract at 200mg/kg in diabetic groups is highly significant($p<0.001$), greater than the percentage reduction observed in Tolbutamide(standard) treated groups.

Phytochemical analysis of *Benincasa hispida* alcoholic extract shows the presence of alkaloids, flavonoids, saponins and steroids. Different mechanisms of action to reduce blood glucose levels with the help of plant extracts already exist. Some plants exhibit properties similar to the well-known sulfonylurea drugs like Tolbutamide; they reduce blood glucose in normoglycemic animals (17, 18). Flavonoids, sterols/triterpenoids, alkaloids and phenolics are known to be bioactive antidiabetic principles (9,10,19,20). Flavonoids are known to regenerate the damaged beta cells in the alloxan diabetic rats (21). Alloxan induces diabetes by destroying β-cells (22). In the normoglycemicdiabetic rats, the alcoholic extract was effective,which suggests the presence of orally active insulin like compound as reported for *Optunia* species (23). Increased peripheral utilization and inhibition of the proximal tubular reabsorption mechanism for glucose in the kidney, if any,can also contribute to a glucose lowering effect (24).

Table 1. Percentage blood glucose reduction in normal fasting rats after treatment with Benincasa hispida

Hours	Control	Standard (Tolbutamide)	*B.hispida (50mg/kg)*	*B.hispida (100mg/kg)*	*B.hispida (200mg/kg)*
0	0.000±0.000	0.000±0.000	0.000±0.000	0.000±0.000	0.000±0.000
1	1.115±0.644	6.488±3.835 N.S	19.830±10.333 N.S	5.113±0.973[b]	17.285±7.995[a]
2	2.815±0.731	16.910±4.838[a]	15.010±7.536 N.S	12.1±4.742[a]	43.743±2.417[c]
3	3.945±0.303	28.250±7.498[b]	17.610±10.027 N.S	25.337±4.677[c]	30.672±2.081[c]
4	5.932±0.854	44.213±5.971[c]	17.325±8.345 N.S	21.892±4.502[b]	45.278±3.589[c]
6	6.483±0.682	64.893±3.494[c]	21.323±8.244[a]	45.397±4.048[b]	66.075±2.962[c]
8	7.047±0.679	54.228±2.007[c]	13.843±9.231 [b]	20.465±4.413[a]	22.175±4.588[c]
10	8.177±0.491	38.153±1.082[c]	10.474±7.736 N.S	16.878±1.571[c]	8.180±4.361 N.S
12	8.440±0.652	30.323±2.598[c]	10.820±7.521 N.S	9.220±1.799 N.S	6.733±3.803 N.S
24	1.890±0.96	8.541±3.68	6.516± 4.56 N.S	2.122±1.46 N.S	1.046±0.89 N.S

Values of Plasma glucose are expressed mean±S.E.M, N=6.
[a]p<0.05, [b]p<0.01, [c]p<0.001.when compared to control group.
N.S.not significant when compared to control group.

Table 2. Percentage blood glucose reduction in diabetic rats after treatment with Benincasa hispida

Hours	Control	*Standard*	*B.hispida (50mg/kg)*	*B.hispida (100mg/kg)*	*B.hispida (200mg/kg)*
0	0.000±0.000	0.000±0.000	0.000±0.000	0.000±0.000	0.000±0.000
1	1.115±0.644	6.488±3.833 N.S	5.640±3.588 N.S	3.767±1.489 N.S	18.450±9.812 N.S
2	2.815±0.731	16.910±4.838[a]	17.192±6.491[a]	48.675±1.785[c]	39.6±14.246[b]
3	3.945±0.303	28.250±7.498[b]	41.175±5.915[c]	51.825±3.261[c]	49.625±7.370[c]
4	5.932±0.854	44.213±5.971[c]	48.450±4.197[c]	53.775±2.664[c]	56.050±5.313[c]
6	6.483±0.683	64.893±3.494[c]	52.3±3.141[c]	54.525±2.8[c]	61.9±1.807[c]
8	7.047±0.679	54.228±2.007[c]	49.650±2.641[c]	39.2±1.668[c]	30.125±1.059[c]
10	8.177±0.491	28.153±1.082[c]	26.425±2.102[c]	21.425±2.616[c]	27.35±2.908[c]
12	8.440±0.652	20.323±2.598[c]	18.801±1.815[c]	14.45±4.464 N.S	8.25±2.689 N.S
24	2.142± 0.591	2.244±3.653 [N.S]	8.470±1.890[c]	4.26±2.581 N.S	3.68±1.24 N.S

Values of Plasma glucose are expressed mean±S.E.M, N=6.
[a]p<0.05, [b]p<0.01, [c]p<0.001.when compared to control group.
N.S.not significant when compared to control group

Further work is in progress to identify the possible mechanisms of action and to identify the lead molecules responsible for hypoglycemic and anti hyperglycemic activities.

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