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Evaluation of Hypoglycemic Effect of Chloroform Extracts of Stem of Benincasa hispida in Male Wistar Rats

Dr. T. Jayasree*, Dr. N. Chandrsekhar¹, Dr. Rohit Dixit¹

* Professor, Department of Pharmacology, Mamata Medical College, Khammam, Andhra Pradesh, India

¹ Post graduates, Department of Pharmacology, Mamata Medical College, Khammam, Andhra Pradesh, India

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ABSTRACT

The aim of the present research work was to evaluate the hypoglycemic activity of Benincasa hispida stem extract in normal male wistar rats and to compare its activity with control and standard drug glybenclamide. A total of 20 male adult wistar rats weighing around 150–200gms were taken. Rats were divided into four groups of 5 rats each. First group served as control and received 2 ml of normal saline, second group as standard received glybenclamide 0.1mg/kg, third and fourth groups as test received 200mg, 400mg of the extract respectively. Blood glucose levels of all the animals were observed at 0 hrs with the help of glucometer. Then normal saline was given to control group, glybenclamide to group 2 and the test extract to group 3 and 4 in the doses of 200mg, 400mg/kg body weight respectively. The blood glucose levels were taken at 2hrs, 4hrs, 6hrs and 8 hrs intervals. Blood was taken from the distal part of rat tail vein. Statistical analysis shows maximum reduction in blood glucose levels with stem extract of Benincasa hispida at 200mg/kg dose which was at 4hrs interval when compared to control group. The stem extract of Benincasa hispida has significant hypoglycemic activity. It can be tried in cases of newly diagnosed non-insulin dependent diabetic patients along with diet restriction, exercise etc.

Key Words: Benincasa hispida, Hypoglycemic effect, Diabetes Mellitus, Glybenclamide, Sugar, Antidiabetic.

INTRODUCTION

Modern technology enables in producing synthetic drugs even up to hybridization techniques synthesizing monoclonal antibodies. But even today plants are indispensable source of medicinal preparations. It is an era of phytomedicines which link traditional and modern medicines. Since diet forms the mainstay in the management of diabetes mellitus, there is scope for exploiting the anti-diabetic potency of vegetables to the maximum extent. Such plant food adjuncts possessing hypoglycemic activity appear to hold promise as potential anti-diabetic agents. The use of traditional plant for treatment of diabetes mellitus is widely practiced in world. Many plant species are known in folk medicine of different cultures to be used for their hypoglycemic properties and therefore used for treatment of diabetes mellitus¹. The world health organization has recommended that use of herbs should be encouraged² in India; herbal medicines are widely used to treat this condition. Several marketed polyherbal products are available in the market for the treatment of diabetes. The fruit of Benincasa hispida (Thumb) Cogn, commonly called as ash guard, belonging to cucurbitaceous family is employed as a main ingredient in kusmanda lehyam, in Ayurvedic

system of medicine. The leyham is used as rejuvenate agent and also numerous nervous disorders. For centuries it has been used in many empirical applications in India for various ailments such as GIT problems like dyspepsia and burning sensation, heart disease, vermifuge, diabetes and urinary disease^{3,4}. However, some scientific studies carried out reveal its anti-inflammatory⁵, diuretic⁶, hypoglycemic⁷, anti-Alzheimer's⁸, antidiarrheal⁹, antioxidant¹⁰, antiulcer^{10, 11}, anti-obesity¹², antihistaminic⁴ and anticancer¹³ activities. It is also used in disorders related to urinary tract. The major constituents of this fruits are triterpenoids, flavanoids, glycosides, saccharides, carotenes, vitamins, β sitosterin and uronic acid¹⁴⁻¹⁶. Different parts of Benincasa hispida such as fruit, the rind, the pulp, seeds, leaves and roots were shown to have hypoglycemic and anti-hyperglycemic effects. But studies on hypoglycemic effect of stem of the plant were not documented so this study is done to reveal the above effect.

MATERIALS AND METHODS

Collection of the Plant

The stem of Benincasa hispida was obtained from a village named Konijerla near Khammam, Andhra Pradesh. The identification and authentication of the stem of the plant was done at the department of Botany government degree college, Khammam.

Extraction Procedure

The preparation of extract of stem of Benincasa hispida was done at the department of Pharmacology, Mamata Medical College, Khammam. Shade dried stem was cut into small pieces. The pieces were then finely powdered. The powdered stem was extracted with chloroform water by process of simple maceration¹⁷.

Animals

Wistar albino rats of male sex of age 10 to 12 weeks, weighing between 150 – 200 gm were obtained from central animal house, Mamata Medical College, Khammam. Protocol was approved by IAEC, Mamata Medical College, Khammam. These animals were used for the study of hypoglycemic activity of the Benincasa hispida stem extract. The animals were stabilized for 1 week; they were maintained in standard condition at room temp; $60 \pm 5\%$ relative humidity and 12 hr light dark cycle. They were given standard pellet diet and water ad-libitum.

Selection of Doses

Acute toxicity studies were done with different doses of stem extract of Benincasa hispida which showed no toxic effects like morbidity and mortality. For the assessment of hypoglycemic activity, two dose levels were chosen in such a way that, one dose was approximately one tenth of the maximum dose during acute toxicity studies and a high dose, which was twice that of one tenth dose (200mg/kg and 400mg/kg)

Experimental Design

Four groups of fasted rats, five rats in each, were used. Group one (1) was treated with 2 ml normal saline which served as control. Group two (2) with Glybenclamide 0.1mg/kg body weight which served as standard. Group three (3) and group four (4) were given per oral Benincasa hispida stem extract 200mg/kg and 400mg/kg of body weight respectively. Blood samples were collected at 0 hr (prior to the administration of the extract) 2hrs, 4hrs, 6 hrs and 8hrs after administration of extract for blood glucose level estimation with the help of Glucometer (Accucheck, Go) and readings were tabulated. No food was given throughout the experiment.

Statistical analysis was done with Student 't' test.

RESULTS

Table-1

The blood glucose level at 0 hr with normal saline was 84 ± 2.47 , with Glybenclamide it was 119.2 ± 2.15 , with 200mg Benincasa hispida stem extract it was 85 ± 2.25 , with 400mg dose it was 91.2 ± 5.09 .

After administration of the drugs, the blood glucose levels at 2 hrs with normal saline was 78 ± 3.03 , P-value being 0.164. With Glybenclamide it was 88.4 ± 1.77 , $P=0.000$, with 200mg of stem extract of Benincasa hispida it was 66.2 ± 4.52 , $P=0.006$, with 400mg it was 72.2 ± 2.57 , $P=0.010$. The reduction in blood glucose

levels with both 200mg and 400mg of stem extract of *Benincasa hispida* are significant when compared with Glybenclamide and highly significant with normal saline.

At 4th hr the blood glucose level with normal saline 76.4 ± 2.33 , $P=0.056$. With Glybenclamide it was 87.2 ± 1.77 , $P=0.00$. With 200mg of stem extract of *Benincasa hispida* it was 61.8 ± 5.12 , $P=0.003$, with 400mg it was 68.4 ± 2.52 , $P=0.04$. It showed again significant reduction in blood glucose level with both the doses when compared with Glybenclamide and highly significant with normal saline.

At 6th hr the blood glucose level with normal saline was 78.2 ± 2.76 , $P=0.156$. With Glybenclamide 92.8 ± 1.59 , $P=0.000$. With 200mg of stem extract of *Benincasa hispida* it was 67.6 ± 3.72 , $P=0.004$, with 400mgs the blood glucose levels were 69.8 ± 2.67 , $P=0.015$. There was significant reduction in blood glucose level with 200mg dose when compared with Glybenclamide and not significant with 400mg dose.

The 8th hr blood glucose level with normal saline was 80.2 ± 2.24 , $P=0.288$. With Glybenclamide it was 96 ± 1.73 , $P=0.00$. With 200mgs of stem extract of *Benincasa hispida* it was 74 ± 1.14 , $P=0.02$ which is significant when compared with Glybenclamide and normal saline. With 400mgs dose the blood glucose level was 81.2 ± 2.47 , $P=0.11$ which is not significant.

Table-2 and Bar Diagram-1

The percentage of reduction in blood glucose level with normal saline, Glybenclamide and two doses of *Benincasa hispida* was compared at 2nd, 4th, 6th and 8th hour and shown in table-2 and bar diagram. The percentage of reduction in blood glucose level with 200mgs of *Benincasa hispida* at 2nd hr, 4th hr and 6th hr was comparable with that of Glybenclamide since there were no statistically significant differences between the groups. But maximum reduction was seen at 4th hr.

DISCUSSION

Diabetes is possibly the world's fastest growing metabolic disease¹⁸. Diabetes mellitus is not a single disease entity but rather a group of metabolic disorders characterized by defective regulation of carbohydrate, fat and protein or in other words it is a metabolic disorder characterized by hyperglycemia, glycosuria, hyperlipidemia, negative nitrogen balance and sometime ketonemia¹⁹.

The use of traditional plant for treatment of diabetes mellitus is widely practiced in world. Many plant species are known in folk medicine of different cultures to be used for their hypoglycemic properties and therefore used for treatment¹⁹. Mankind has a long history in the use of herbal medicines. Rig-Veda and Ayurveda (4500-1600 BC) reveal that ancient Indians had a rich knowledge of the use of medicinal plants. India unquestionably occupies the top most position in the use of herbal drugs since ancient times utilizing nearly 600 plant species in different formulations. Great majorities of people in India have been depending on crude drugs for the treatment of various diseases as evidenced from well-documented indigenous system of medicines, Ayurveda and Unani. The Materia Medica of these systems contains a rich heritage of indigenous herbal drugs¹⁴.

Since diet forms the mainstay in the management of diabetes mellitus, there is scope for exploiting the anti diabetic potency of vegetables to the maximum extent. Such plant food adjuncts possessing hypoglycemic activity appear to hold promise as potential anti diabetic agents.

The active principles of plants are often definite substances but in other cases they are complicated mixtures. The first classes of these substances with medicinal properties are vegetable bases which include amines and alkaloids. A considerable number of medicinal drugs owe their curative properties to these bitter alkaloids. Another class of these active principles included glycosides, essential or volatile oils, resins and antibiotic each are having their own functional significance.

This study also revealed that the maximum hypoglycemic effect was produced only at four hrs after administration of extract to the fasted animals. This indicates that it takes about 2 hrs for the active ingredient(s) or its (their) metabolites in the extract to enter into the circulation and reach target tissues to bring about hypoglycemic effect at 2nd hr and maximum at fourth hour which is maintained for at least 2 to 4hrs. The reduction in blood glucose level with two doses of *Benincasa hispida* is comparable to Glybenclamide. Significant reduction in blood glucose were seen with 200mg/kg dose when compared with control group at 2nd, 4th hr, 6th hr and 8th hr, where as with 400mg/kg dose of stem extract of *Benincasa hispida* there was significant reduction in blood glucose level at 2nd and 4th hour only but not significant at 6th and 8th hour. It is to be assumed that the 200mg/kg body weight of stem extract of *Benincasa hispida* is the effective dose. With 400mg/kg dose the decreased effect on glucose level at 6th hr may be due to saturable kinetics.

From the photochemical analysis it was found that the major chemical constituents of extract were carbohydrate, alkaloid and saponins and *Benincasa hispida* may be active due to the presence of these compounds²⁰.

CONCLUSION

Different parts of *Benincasa hispida* plant has been studied extensively for their hypoglycemic and anti-hyperglycemic effects but not that of stem. In this study there was hypoglycemic effect with stem extract of *Benincasa hispida* plant in dose of 200mg/kg body weight in wistar rats. The major chemical constituents are carbohydrate, alkaloid and saponins which have hypoglycemic effect. The stem extract assumed having the same hypoglycemic effect. As all parts of *Benincasa* fruit, leaves and other edible parts are not toxic; the stem extract is also assumed to be non toxic and can be used in adjuvant therapy of diabetes.

It can be concluded that the stem extract of *Benincasa hispida* can be tried in cases of newly diagnosed non insulin dependent diabetic patients along with diet restriction, exercise etc, then it can also be suggested as an adjuvant therapy along with other hypoglycemic drugs in both insulin dependent and non insulin dependent diabetic patients. So, further evaluation has to be done with stem extract of the plant *Benincasa hispida* for its hypoglycemic and for anti-hyperglycemic effect.

TABLES AND FIGURES

Table -1: Comparison of fasting blood Glucose levels with *Benincasa hispida* stem extract with normal saline and Glybenclamide

Time/Dose	0 hrs	2hrs	4hrs	6hrs	8hrs
NS	84±2.47	783.03	76.4±2.33	78.2±2.76	80.2±2.24
G-0.1mg/kg	119.2±2.15	88.4±±1.77**	87.2±1.77**	92.8±1.59**	96±1.73**
B-200mg/kg	85±2.25	66.2±4.45*	61.8±5.15**	67.6±3.72**	74±1.14*
B-400mg/kg	91.2±5.09	72.2±2.57*	68.4±5.12*	69.8±3.72*	81.2±1.14*

Values represent Mean±SEM, *<0.05, **<0.005

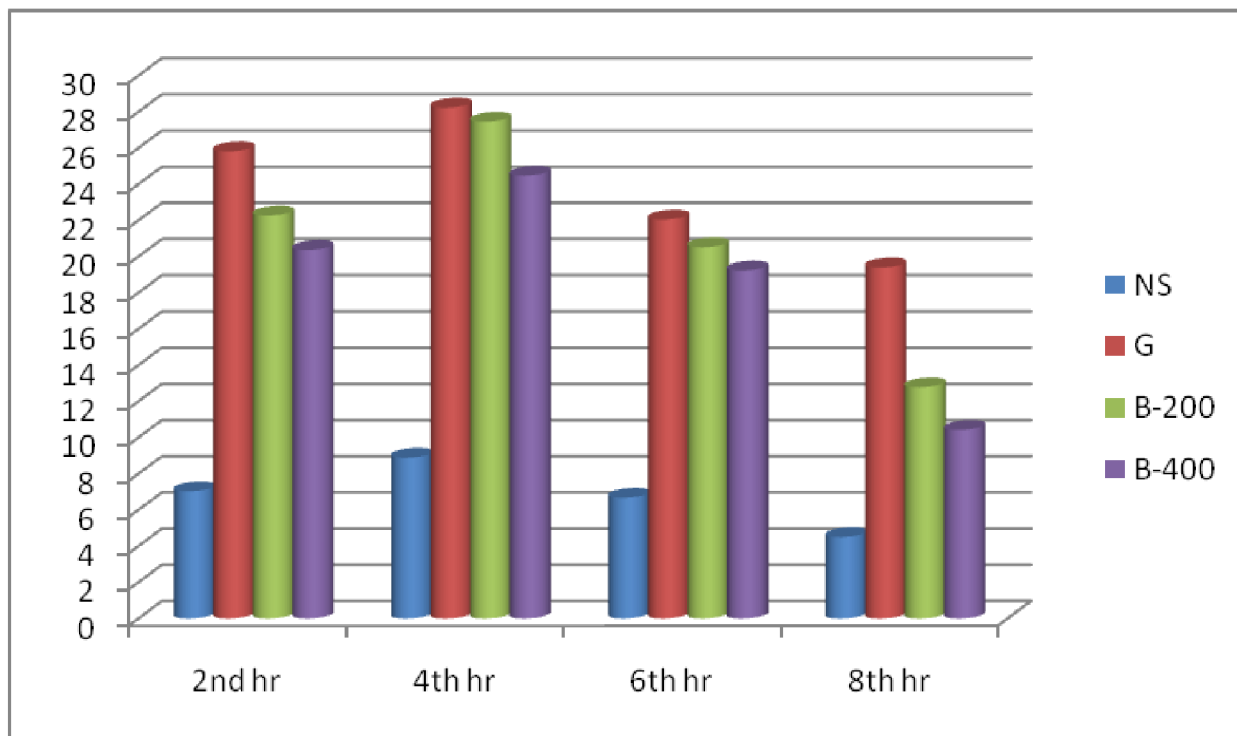
NS=Normal Saline, G=Glybenclamide, B-200, B-400= *Benincasa* 200mg/kg & 400mg/kg

Table-2: Comparison of percentage of reduction in blood glucose levels with stem extract of *Benincasa hispida* with Normal saline and Glybenclamide

Time/Dose	2nd hr	4 th hr	6 th hr	8 th hr
NS	7.02	8.88	6.67	6.673
G-0.1mg/kg	25.81	28.22	22.03	22.03
B-200	22.26	27.44	20.51	20.51
B-400	20.36	24.47	19.22	19.22

NS=Normal Saline, G=Glybenclamide, B-200, B-400= *Benincasa* 200mg/kg & 400mg/kg

Bar Diagram 1: Comparison of percentage of reduction in blood glucose levels with stem Extract of Benincasa hispida with Normal saline and Glybenclamide at various time intervals



NS=Normal Saline, G=Glybenclamide, B-200, B-400= Benincasa 200mg/kg & 400mg/kg

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***Corresponding Author:** Dr. T. Jayasree
Department of Pharmacology,
Mamata Medical College, Khammam (507002),
Andhra Pradesh, India
Contact No.: +91 9848127116
Email id: drtjayasreemd@gmail.com