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Hypoglycemic Activity of the Fruits of *Benincasa hispida* Found in State of Tripura, India

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ABSTRACT

The ethyl acetate fraction from the methanolic crude extract of the fruits of *Benincasa hispida* growing in state Tripura, India, showed no toxic effect on haematological parameters, though able to show significant hypoglycemic activity in mice against alloxan induced diabetes.

Keywords: Fruits, *Benincasa hispida*, Alloxan, Hypoglycaemic.

INTRODUCTION

Diabetes mellitus is a metabolic disorder characterised by hyperglycemia, glycosuria, hyperlipidemia, negative nitrogen balance and sometimes ketonemia [1]. Oral hypoglycemic drugs are commonly used to treat or control diabetes, but they fail to alter the complication related to hyperglycemia [2]. In India, indigenous remedies have been used in the treatment of diabetes, since the time of Charaka and Sushruta (6th Century B.C.) [3]. Many plants and their product (active natural principle and crude extract) that have been mentioned/used in Indian traditional system of medicines and shown experimental or clinical antidiabetic properties [4]. *Benincasa hispida* (Thumb.) is a large climbing or trailing herb with stout, angular, hispid stems, cultivated as a vegetable throughout India upto a altitude of 1200 m. Various parts of the plants are used in different disease indigenously. The taxonomic position is as- Kingdom: Plantae, Phylum: Spermatophyta, Division: Angiospermae, Class: Dicotyledon, Order: Cucurbitales, Family: Cucurbitaceae. *B. hispida* is widely used vegetable in India and other topical countries. A decoction of the fruits of *B. hispida* is styptic, laxative, diuretic, aphrodisiac, antiperiodic, specific for hemoptysis, used in cure of internal haemorrhage and disease of respiratory tract [5]. In this compilation, the hypoglycemic activity of the fruits of *B. hispida* growing in state Tripura, India, is reported.

MATERIALS AND METHODS

Chemicals, solvents etc. were used of Analytical Grade. Mature fruits of *B. hispida* were purchased from local market in Agartala, Tripura, india, during the month of October and November, 2017, and duly identified as well as authenticated by the subject experts of Women's College. The cuticle of the fruit was removed and then from the pulp of the fruits, the seeds were removed. The juice from the pulp was extracted with the help of an electric juicer. For preparation of the methanolic extract, one litre of fresh juice/slurry was mixed with 2.5 l of methanol and was kept in conical flask for 48 hours at room temperature. The slurry was stirred intermittently after 6 h and left overnight.

The mixture was first filtered with muslin cloth to remove course materials and then further filtered through Whatmann filter paper. The filtrate was dried in vacuum oven at a temperature of 40 degree Celsius to obtain the final form of extract, which was stored in desiccator. The yield obtain was 3.52% w/w. A part of the crude methanolic extract was used for pharmacological evaluation purpose. The other part was successively extracted with ethyl acetate and methanol to yield ethyl acetate soluble fraction and methanolic soluble fraction. These fractions were evaporated to dryness below 40 degree Celsius in vacuum oven. The crude methanolic extract (CMET), ethyl acetate fraction (EAF) and methanolic fraction (MF) of *B. hispida* were used for pharmacological experiments.

The study was conducted on albino Swiss mice weighing 20-35 g. Animals were acclimatized for one week before experimental procedure. Animals were housed in plastic cage and were provided with food and water *ad libitum*. The food was comprised of corn flour, dry fish and wheat flour, which were mixed in equal ratio to form dough, then made into pellets by induction of diabetes with the procedures follows- After overnight fasting, animals were intraperitoneally treated with single dose of alloxan monohydrate in 150 mg/Kg body weight. A resting period of

seven days was given to stabilize blood sugar. Alloxan monohydrate was freshly prepared by dissolving it in distilled water. The fasting glucose level (FGL) was determined and animals with moderate diabetes were selected for experimental study [6].

Animals were divided into different groups as per protocol; each group comprising of 8 animals. The route of administration of drugs and extract was intraperitoneal (i.p.). Group 1-Animals were administered distilled water (0.5 ml/kg): Normal Control Group (NC). Group 2-Diabetic animals received distilled water once daily for seven consecutive days: Diabetic control group (DC). Group 3-Diabetic mice treated with standard drug Metformin (150 mg/kg) once daily for seven consecutive days (DSD). Group 4-Animals were administered Crude methanolic extracts (250 mg/kg) by dissolving it in a measured amount of distilled water once daily for seven consecutive days (CMET). Group 5-Diabetic ethyl acetate fraction treated group (EAF) received ethyl acetate fraction (250 mg/kg) once daily for seven consecutive days. Group 6-Diabetic methanol fraction treated group (MF) received methanol fraction (250 mg/kg) once daily for seven consecutive days. After 2 h of the last injection on 7th day at fasting condition, blood was collected from retro orbital plexus and plasma glucose level was determined by GOD-POD kit (Qualigens). Body weight of each animal was also determined [7].

Evaluation of antidiabetic activity of more active fraction was also carried out in presence of insulin. Group 7-Diabetic Mice received subcutaneously fast acting insulin (0.1 IU/kg) once daily for seven consecutive days: Diabetic insulin treated group (DIT). Group 8-Mice received ethyl acetate fraction (250 mg/kg) simultaneously with insulin: Diabetic insulin treated and ethyl acetate fraction (more active fraction) treated group (DIEAF). Fasting plasma glucose level was determined initially and finally i.e. before the treatment and after the treatment. Body weight of each mouse was also determined.

Further the R.B.C. and W.B.C. count with the Haemoglobin content were also carried out [7]. All the data reported here are expressed as mean \pm S.E.M. Statistical analysis was performed using student t-test and analysis of variance (ANOVA) followed by Tukey multiple comparisons. The value was considered to be significantly difference when the p value was less than 0.05 compared to the respective control. All statistical tests were performed with Jandel sigma plot. Statistical software version 2.0 (1992-1995). The results are presented in Table 1.

Table 1: Plasma Glucose level (PGL) in mg/dl, body weight (BW) in gm and haematological data of the treated mice of different groups

S. No.	Group of Mice	PGL in 0 day	PGL in 7 day	% Reduction in PGL	BW in 0 day	BW in 7 day	R.B.C. in 0 day (million / Cmm)	R.B.C. in 7 day (million / Cmm)	W.B.C. in 0 day (x 1000 / Cmm)	W.B.C. in 7 day (x 1000 / Cmm)	Hb (mg/100 ml) in 0 day	Hb (mg/100 ml) in 7 day
1	NC	91.45 \pm 1.52	92.16 \pm 1.51	-0.78	25.57 \pm 1.90	25.48 \pm 1.00	3.31 \pm 0.21	3.34 \pm 0.18	5.73 \pm 0.30	5.95 \pm 0.31	13.25 \pm 0.5	13.5 \pm 0.43
2	DC	193.4 \pm 2.86	195.0 \pm 2.13	-0.82	27.5 \pm 0.95	30.0 \pm 1.64	3.00 \pm 0.23	3.02 \pm 0.17	6.1 \pm 0.34	6.21 \pm 0.9	12.83 \pm 0.60	12.75 \pm 0.58
3	DSD	198.4 \pm 2.47	135.1 \pm 6.9	31.8	26.88 \pm 0.92	31.25 \pm 0.82	-	-	-	-	-	-
4	CMET	194.3 \pm 2.08	186.9 \pm 2.00	7.92	25.36 \pm 1.18	27.55 \pm 1.59	-	-	-	-	-	-
5	EAF	195.5 \pm 3.08	150.7 \pm 4.41	22.89	24.38 \pm 1.75	25.25 \pm 1.98	-	-	-	-	-	-
6	MF	198.7 \pm 3.06	174.4 \pm 5.07	12.2	25.63 \pm 1.80	27.5 \pm 0.95	-	-	-	-	-	-
7	DIT	201.1 \pm 2.22	117.4 \pm 2.63	41.63	30.0 \pm 1.34	29.38 \pm 1.52	3.04 \pm 0.30	3.05 \pm 0.18	5.74 \pm 0.40	5.40 \pm 0.48	13.63 \pm 0.90	12.99 \pm 0.34
8	DIEAF	195.7 \pm 2.58	110.1 \pm 3.5	43.76	26.88 \pm 1.32	29.41 \pm 1.49	3.40 \pm 0.13	3.26 \pm 0.12	5.29 \pm 0.18	5.62 \pm 0.26	14.13 \pm 0.37	14.12 \pm 0.54

Each value is represented as Mean \pm SEM, where n=number of animal per group i.e.8; Hb: haemoglobin; NC: Normal control; DC: Diabetic control; DSD: Standard drug; CMET: Crude extract; EAF: Ethyl acetate fraction; MF: Methanolic extract; DIT: Diabetic insulin treated; DIEAF: Diabetic insulin treated and ethyl acetate fraction treated.

RESULTS AND DISCUSSION

The mice of normal control group did not show any significant fluctuation in Plasma glucose level, body weight and in haematological parameters. The mice of diabetic group showed the significant induction of diabetes and weight increase, but the significant fluctuation of plasma glucose level was not recorded. The haematological changes were not also found in case of DC group. Standard drug was able to reduce the plasma glucose level significantly, but the weight gained remained as such. Among the CMET, EAF and MF, it was observed that EAF i.e. Ethyl acetate fraction was able to reduce the plasma glucose level significantly, but not at par to the standard drug. But, it is observed that EAF was almost able control the increase of body weight.

After one week treatment, PGL was determined and DC group was compared with DIT and DIEAF treated group. There was significant reduction in PGL in DIT group and DIEAF treated animal when compare with DC group. The percentage reduction in PGL of DIT and DIEAF are 41.63% and 43.76% respectively which are statistically significant. But DIEAF was not able to control the increase of body weight, whereas DIT showed its control in body weight. Haematological parameters were not observed to be changed significantly. It was indicated that the ethyl acetate fraction of the methanolic crude extract of the fruits of *B. hispida* growing in state Tripura, India, had no toxic effect on haematological parameters even after seven days treatment, though showed significant hypoglycemic activity.

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