

Original Research Article

An Assessment of Anti-diabetic Activity of *Benincasa hispida* Extract in Alloxan-induced Rat Model

Abstract

Humans have used herbal medicine to cure medical conditions since the beginning of human civilization. This research aims to investigate the effectiveness of *Benincasa hispida* as an anti-diabetic agent as well as its lipid profile. Utilizing the alloxan-induced diabetic approach, we evaluated the effectiveness of the anti-diabetes medication. In terms of the efficiency of the medication against diabetes, the dosage of 900 mg/kg was the only one that demonstrated statistical significance ($p < 0.05$) when compared to the dosages of 300, 600, and 900 mg/kg. Despite a slight decrease in HDL, LDL, and total cholesterol, none of the groups exhibited statistically significant results. This held true despite the fact that the parameters were declining. When the doses of 900 mg/kg were administered to group 6, statistically significant outcomes ($p < 0.05$) were achieved for triglyceride levels ($97.70 \pm 7.50^*$), SGPT, and SGOT. The results of the kidney function test showed that Group 5 had statistically significant outcomes ($p < 0.05$), with a mean score of $93.24 \pm 9.23^*$. All groups received doses of 600 mg/kg.

Keywords: *Benincasa hispida*, Herbal medicine, Antidiabetic, Creatinine, Diabetes mellitus.

Introduction

Diabetes mellitus (DM) is a metabolic disorder characterized by persistent hyperglycemia resulting from insufficient insulin action, synthesis, or both in target tissues. Persistent microvascular problems (retinopathy, neuropathy, and nephropathy) and macrovascular problems (coronary and peripheral artery diseases and stroke) associated with chronic hyperglycemia distinguish all forms of diabetes mellitus. These issues result in organ damage and fatality, sometimes identified at a late stage or without sufficient medical supervision [1]. The liver, the biggest glandular organ, governs most physiological functions in the human body. The liver is the organ responsible for absorbing an individual's entire blood volume multiple times daily. It is essential for human metabolic processes [2]. Excessive alcohol intake, substance dependence, exposure to some hazardous substances, or infection by viruses or parasites may result in increased levels of reactive oxygen species (ROS), including OH, H₂O₂, and O₂ [3]. This might result in hepatocellular damage. The Centers for Disease Control and Prevention performed a study involving 1,492 doctors providing ambulatory care in non-governmental institutions. The study revealed that hyperlipidemia ranks as the second most prevalent chronic illness among these physicians, with hypertension being the only condition they encounter more frequently [4]. The research indicates that the main cause of hyperlipidemia is the excessive intake of high-fat meals [5]. The liver is essential for the metabolism of frequently prescribed anti-hyperlipidemic medications, including atorvastatin, pravastatin, fluvastatin, simvastatin, lovastatin, and rosuvastatin. As a result, the bioavailability of these pharmaceuticals is very low [6]. Statins may temporarily block the enzyme 3-hydroxy-3-methylglutaryl-coenzyme A reductase (HMG-CoAR). This enzyme lowers cholesterol levels. This enables the reduction of cholesterol production inside the cells. Statins may penetrate hepatocytes and inhibit HMG-CoAR, which accounts for their pharmacological actions [7]. Statin-associated muscle symptoms (SAMS), sometimes referred to as muscular complications, are the predominant adverse effects that restrict the use of statins. Two other potentially adverse outcomes include the development of diabetic mellitus (DM) and problems impacting the central nervous system [8]. These synthetic medications not only exhibit significant side effects but are also costly, possibly imposing financial burdens on patients who need prolonged use during the whole treatment regimen [9]. Consequently, it is essential to formulate highly efficacious antihyperlipidemic

agents with minimum adverse effects. Plants are crucial in the discovery and synthesis of innovative medicines [10]. They function as a valuable and plentiful source of naturally occurring compounds for medicinal purposes. Experts in the field suggest that certain chemical compounds derived from medicinal plants have therapeutic capabilities. Consequently, researchers continuously seek innovative herbal treatments and other plant-derived medications to successfully address diverse conditions [4].

Many countries globally have historically used traditional medicines as cures sourced from plants, dietary supplements, and alternative medical practices. In recent years, the use of traditional medicine has markedly risen, with several individuals nationwide relying on it as a primary mode of healthcare [11]. Medicinal plants include a variety of chemical compounds, allowing them to produce a wide array of pharmacological and therapeutic effects. These compounds include many elements, such as tanning agents, glycosides, alkaloids, saponins, polysaccharides, essential oils, terpenoids, resins, and plant lipids [12–14]. Genetically modified plants enable precise regulation of chemical concentrations, ultimately achieving the intended medicinal effect. Reverse genetics has several potential applications, one of which is the enhancement of secondary metabolite production, including the synthesis of alkaloids [15]. Global improvements in scientific study have resulted in a heightened exploration of the medicinal properties of plant species [16]. Plants are gaining popularity due to their intrinsic safety, powerful pharmacological attributes, and economic advantages over manufactured medications.

Benincasa hispida (Thunb) Cogn. (Family: Cucurbitaceae) is often known as white pumpkin, wax gourd, or ash gourd. The fruit *B. hispida* is an essential ingredient in Kusmandalehyam (Ayurvedic medicine), often used in the treatment of neurological disorders. The fruits and seeds of *B. hispida* have many pharmacological properties, functioning as a laxative, tonic, diuretic, aphrodisiac, and antiperiodic, and are used in the management of hemoptysis, internal hemorrhages, insanity, epilepsy, and other neurological disorders [17]. The fruit comprises carotenes, flavonoids, glycosides, saccharides, proteins, vitamins, minerals, volatile oils, β -sitosterol, and uronic acid. The incorporation of terpenes, flavonoid C, glycosides, and sterols makes it a potent antioxidant [18].

The point of this study is to look into whether an ethanolic extract from *Benincasa hispida* can help people with diabetes and what its lipid profile is like.

MATERIALS AND METHODS

Drugs, Chemicals, and Instruments

Ethanol and alloxan were procured from Sigma Aldrich in Germany. Healthcare Pharmaceutical Limited supplied us with a free sample of metformin, a widely used drug for diabetic management. The blood serum analysis kits for many biomarkers were obtained from Plasmatic Laboratory Products Ltd. in the United Kingdom. This research used the Alere Inc. glucometer. We obtained it from Shahbag in Dhaka, Bangladesh. We evaluated the biochemical parameters with the Humalyzer 3000, a semiautomated clinical chemistry analyzer.

Plant Collection and Extract Preparation

Benincasa hispida plants were collected from three diverse places in Bangladesh: North Bengal, a hilly region, and a lowland area. The next phase included authentication and taxonomic classification. The National Herbarium of Bangladesh maintained the plant specimen in accordance with applicable rules. The leaves were desiccated in a shady location for seven to ten days, then finely ground. The powdered leaves were agitated for 96 hours while immersed in a 70% ethanol solution. Subsequent to the soaking operation, the extract was filtered, and the resultant liquid was collected. We condensed it with a rotary evaporator. We collected and refrigerated the dried extract for future use. Ethanol and alloxan were procured from Sigma Aldrich in Germany. Healthcare Pharmaceutical Limited supplied us with a free sample of metformin, a widely used drug for diabetic management. The blood serum analysis kits for many biomarkers were obtained from Plasmatic Laboratory Products Ltd. in the United Kingdom. This research used the Alere Inc. glucometer. We obtained it from Shahbag in Dhaka, Bangladesh. We evaluated the biochemical parameters with the Humalyzer 3000, a semiautomated clinical chemistry analyzer.

Experimental Animal Handling

Fifty male Wistar rats, weighing between 125 and 200 grams, were acquired from the Pharmacy Department of Jahangirnagar University in Dhaka, Bangladesh. The rats were housed in a regulated environment at the Institute of Nutrition and Food Science, University of Dhaka, with a 12-hour light/dark cycle and a stable temperature of 25 °C. We consistently supplied the participants with a standardized pellet diet and fresh water. The rats were placed at the facility to

acclimatize before the trial began. The rat studies complied with the regulations established by the Institutional Animal Ethics Committee (IAEC). The Department of Zoology at Dhaka University granted ethical permission under issue number 147/pharm.science.ewu. The researchers attended to and administered the animals in compliance with the protocols established by the Swiss Academy of Medical Sciences (SAMS) and the Swiss Academy of Sciences (SCNAT).

Experimental Design

We categorized the rats into groups according to their body weight and then assessed them for antihyperglycemic efficacy (Table 1). The rodents were classified into groups based on their body weight, with five rats per group. Table 1 depicts the alloxan control group, including rats that underwent just alloxan treatment. N/A signifies the lack of therapeutic intervention in this cohort.

Table 1: Anti-hyperglycemic Activity Analysis

Group number	Group Status	Treatment specimen	Dose of treatment specimen (mg/kg)	Group Abbreviation
1	Negative Control	Physiological Saline	10 mL/kg	N
2	Alloxan control	Alloxan	150 mg/kg	A
3	Alloxan + Metformin	Alloxan + Metformin	150 mg/kg + 100 mg	A + M100
4	Alloxan + <i>Benincasa hispida</i>	Alloxan + <i>Benincasa hispida</i> extract low dose	150 mg/kg + 300 mg/kg	A + BH ₃₀₀
5	Alloxan + <i>Benincasa hispida</i>	Alloxan + <i>Benincasa hispida</i> extract medium dose	150 mg/kg + 600 mg/kg	A + BH ₆₀₀
6	Alloxan + <i>Gymnemasylvestre</i>	Alloxan + <i>Benincasa hispida</i> extract high dose	150 mg/kg + 900 mg/kg	A+ BH ₉₀₀
7	Metformin	Metformin	100 mg/kg	M

8	<i>Benincasa hispida</i>	Alloxan + <i>Benincasa hispida</i> extract low dose	300 mg/kg	BH ₃₀₀
9	<i>Benincasa hispida</i>	Alloxan + <i>Benincasa hispida</i> extract medium dose	600 mg/kg	BH ₆₀₀
10	<i>Benincasa hispida</i>	Alloxan + <i>Benincasa hispida</i> extract high dose	900 mg/kg	BH ₉₀₀

Biological Sample Collection

Blood glucose levels were measured by obtaining blood samples via puncturing the tip of the rat's tail. Blood was swiftly extracted from the deceased animal after a cardiac puncture and transferred to a microcentrifuge tube. The collected samples were centrifuged for 5 minutes at 5,000 rpm to extract the supernatant fluid. The fluid was then transferred to a different microcentrifuge tube to enable biochemical analysis. The kidneys and liver were promptly excised from the animal's body post-sacrifice and meticulously cleaned with an ice-cold saline solution for further functional examination. We classified the rats into several groups based on their body weight and then performed procedures to assess their antihyperglycemic efficacy (Table 1). We categorized the rodents according to their body weight, with 5 rats each group. The alloxan control group in Table 1 exclusively administered alloxan to the rats. This group does not get therapeutic intervention when N/A is specified.

Estimation of Biochemical Parameters

By using a glucometer, the blood glucose level was ascertained. The Humaluzer 3000 was one of many tests administered, along with those for the lipid profile (HDL, LDL, Cholesterol, triglyceride), kidneys (Urea, Creatinine), and liver (SGPT and SGOT). We also tested liver and kidney samples for gluconeogenic and glycolytic enzyme activity

Statistical Analysis

All of our findings (raw data) in terms of numerical parameters were recorded and analyzed on a broadsheet using the MS Excel application. The gathered data were subjected to descriptive statistics, with the findings reported as mean SD. To evaluate statistical significance, we used the

SPSS 16 software's "One-way Anova test" to interpret inter-group heterogeneity in terms of several biological factors. The occurrences are considered statistically significant since the 'p' value was less than 0.05($p < 0.05$).

Results and discussion

Herbal medicine is the use of medicinal plants to prevent and cure sickness, which may range from traditional and popular therapies found in all cultures to the use of standardized and triturated herbal extracts. In this study, we looked at the anti-diabetic properties and lipid profile of the herb *Benincasa hispidain* mice. Diabetes is one of the most serious health challenges of the 21st century. It is one of the leading causes of death, and diabetic macro- and microvascular complications result in increased disability and significant healthcare expenses. Only the 900 mg/kg dosage showed statistically significant ($p < 0.05$) antidiabetic effectiveness compared to the 300, 600, and 900 mg/kg doses. Several investigations found comparable results [19-21]. In terms of HDL, LDL, and total cholesterol, no groups demonstrated statistically significant outcomes, despite a minor drop in these parameters. Statistically significant results ($p < 0.05$) were obtained for triglyceride ($97.70 \pm 7.50^*$), SGPT, and SGOT in group 6, with dosages of 900 mg/kg. However, SGPT and SGOT did not exhibit any statistically significant consequence in any of the doses of 300, 600, and 900 mg/kg. Other studies on comparable studies reached the same findings [22]. Group 5 exhibited statistically significant outcomes ($p < 0.05$) with $93.24 \pm 9.23^*$ in the kidney function test, with dosages of 600 mg/kg across all groups. Additional investigation into such an experiment revealed the same results [23].

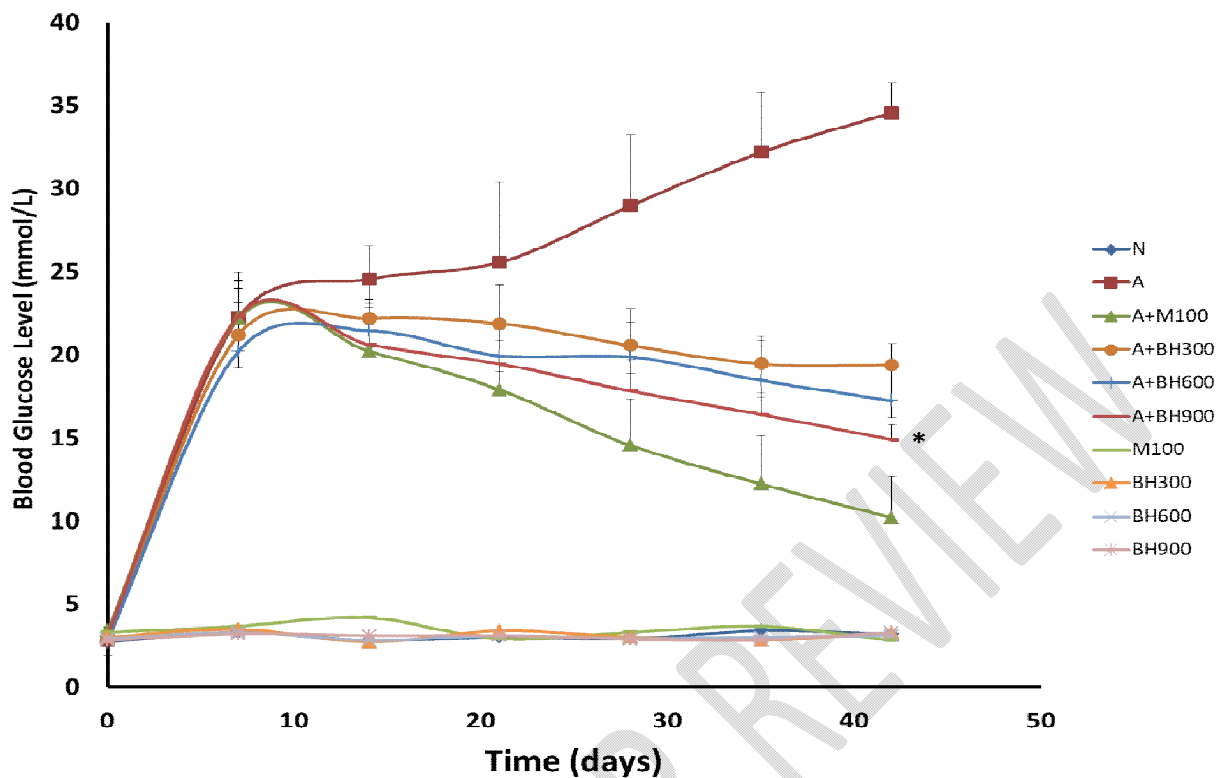


Figure 1: Antidiabetic activity of different dose of *Benincasa hispida*

Table 2: Lipid profile after administration of different dose of *Benincasa hispida*

	Total Cholesterol	HDL	LDL	Triglyceride	SGPT	SGOT	Urea	Creatinine
N	114.24±6.24	83.75±6.29	28.70±3.29	54.21±5.21	40.24±4.19	43.29±3.52	35.29±3.82	0.7±0.024
A	202.37±6.91	50.79±4.29	126.29±11.29	113.21±7.29	113.25±11.70	120.53±10.47	109.73±9.22	2.4±0.397
A+M ₁₀₀	140.72±7.92	77.21±4.20	50.91±6.29	62.75±6.73	65.29±8.12	61.44±8.23	59.15±6.24	1.1±0.082
A+BH ₃₀₀	190.24±8.27	54.29±5.21	121.77±12.29	110.25±8.32	108.23±10.32	110.21±9.04	104.25±8.91	2±0.075
A+ BH ₆₀₀	181.47±7.59	60.22±6.70	114.77±8.28	106.29±7.63	101.30±10.74	99.23±9.74	93.24±9.23*	1.8±0.069

A+ BH ₉₀₀	172.90±8.21	67.22±5.08	100.29±9.70	97.70±7.50*	92.10±9. 63	90.24±8. 73	84.70±5.91	1.4±0.059
M ₁₀₀	110±3.99	82.08±6.02	30.21±2.82	56.29±4.20	41.24±3. 25	44.27±4. 50	34.25±4.91	0.8±0.026
Bh ₃₀₀	114.24±4.79	79.23±5.83	24.97±4.21	53.20±5.33	37.30±3. 52	47.31±4. 37	38.22±5.74	0.75±0.05 6
BH ₆₀₀	118±4.28	70.20±6.16	28.28±3.21	58.17±4.86	38.47±4. 50	43.27±3. 99	34.33±3.75	0.83±0.07 4
BH ₉₀₀	112.77±4.89	77.28±5.71	27.29±3.91	58.53±5.17	37.14±3. 91	45.28±4. 40	35.70±3.82	0.75±0.06 1

Note: The results were expressed in Mean±SEM (standard mean error) *p< 0.05, **p< 0.01, and ***p< 0.001 were considered as statistically significant. The statistical analysis followed by one-way analysis of variance (Dunnett's test) compared to the control.

Conclusion

The findings of this research indicate that an ethanol extract from the plant *Benincasa hispida* may help protect against diabetes, high cholesterol, liver damage, and impaired kidney function. Despite having anti-diabetic and anti-hyperlipidemic characteristics, the plant extract had no meaningful effect on the desired result. More research is needed to determine the anti-diabetic and anti-hyperlipidemic active components in the entire extract. Upon identification of the active chemicals, a comprehensive investigation can commence.

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