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Exploring the Therapeutic Efficacy of Ethanolic Extract of *Benincasa hispida* in Alloxan Induced Diabetic Rats

Ikram Wahid Toha [a*], Shafia Islam Promi [b], Sampa Barai [c], Abdullah Al Mamun [d], Shabira Sagir [e], Sadia Tasnim [f], Ambia khatun Sony [g] and Israt Jahan Rasna [h]

[a] Department of Pharmacy, University of Chittagong, Bangladesh.
[b] Department of Pharmacy, University of Science and Technology Chittagong, Bangladesh.
[c] Department of Clinical Pharmacy and Pharmacology, University of Asia Pacific, 74/A Green Road, Dhaka 1205, Bangladesh.
[d] Department of Pharmacy, Islamic University, Kushtia -7003, Bangladesh
[e] Department of Pharmaceutical Technology, University of Asia Pacific, 74/A Green Road, Dhaka 1205, Bangladesh.
[f] Department of Chemistry and Chemical Engineering, University of New Haven, United States.
[g] Department of Pharmacy, University of Asia Pacific, 74/A Green Road, Dhaka 1205, Bangladesh
[h] Department of Botany, University of Chittagong, Bangladesh.

Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

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**Corresponding author: E-mail: ikramtohabd@gmail.com;*

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ABSTRACT

Since the dawn of civilization, humans have used herbal medicine to treat medical ailments. This study seeks to evaluate the efficacy of *Benincasa hispida* as an anti-diabetic agent and its impact on lipid profiles. We assessed the efficacy of the anti-diabetes medication using the alloxan-induced diabetic model. Only the 900 mg/kg dosage exhibited statistically significant antidiabetic effects ($p < 0.05$) when compared to the 300 and 600 mg/kg dosages. Notwithstanding a slight reduction in these parameters, no groups demonstrated statistically significant results regarding HDL, LDL, and total cholesterol. The triglyceride value of 97.70 ± 7.50* yielded statistically significant results ($p < 0.05$). Conversely, SGPT and SGOT exhibited no statistically significant effects at dosages of 300, 600, or 900 mg/kg. Group 5 exhibited statistically significant results ($p < 0.05$) in the kidney function test in case of urea, with a value of 93.24 ± 9.23* after the administration of an extract at a dose of 600 mg/kg. No statistically significant results were seen in any groups concerning creatinine levels.

Keywords: Benincasa hispida; herbal medicine; antidiabetic; creatinine; Diabetes mellitus.

1. INTRODUCTION

Diabetes mellitus (DM) is a metabolic condition characterized by persistently elevated blood sugar levels due to improper insulin production or response by cells. Long-lasting microvascular problems (like retinopathy, neuropathy, and nephropathy) and macrovascular problems (like heart and peripheral artery diseases and stroke) linked to high blood sugar define all types of diabetes mellitus. These problems, sometimes detected too late or without adequate medical care, have the potential to cause organ damage and death (Yikna et al. 2021). The liver, which is the body's largest digestive organ, controls most of its processes. The liver is responsible for absorbing a person's whole blood several times a day. It is necessary for the body's cellular processes (Himi et al. 2024). Reactive oxygen species (ROS), such as OH, H_2O_2, and O_2, can accumulate when an individual consumes excessive amounts of alcohol, develops a drug addiction, encounters hazardous substances, or contracts viruses or bugs (FM et al. 2023). This could lead to hepatocellular damage. A study by the Centers for Disease Control and Prevention looked at 1,492 doctors who provided outpatient care in non-government organizations. The research showed that hyperlipidemia is the second most common long-term illness among these doctors. The only disease these doctors see more frequently is hypertension (Baroi et al. 2023). According to the study, eating too many high-fat foods is the main reason for hyperlipidemia (Zhang et al. 2021). The liver must break down medications such as atorvastatin, pravastatin, fluvastatin, simvastatin, lovastatin, and rosuvastatin to treat high cholesterol. As a result, these drugs do not function effectively in the body (Srinivasa et al. 2011). The enzyme 3-hydroxy-3-methylglutaryl-coenzyme Statins may temporarily block HMG-CoAR. This enzyme makes cholesterol levels go down. This slows down the production of cholesterol inside the cells. Statins have the ability to penetrate hepatocytes and inhibit the function of HMG-CoAR, which is one of their main drug effects (Schachter 2005). The main side effects that complicate the use of statins are statin-associated muscle symptoms (SAMS), also known as muscular problems. Other potential side effects include the development of diabetes mellitus (DM) and issues affecting the central nervous system (Thompson et al. 2016). Not only do these man-made drugs have serious side effects, but they are also very expensive, which could put a financial strain on patients who have to use them for a long time during their treatment (Rupak et al. 2022). Because of this, it is important to make antihyperlipidemic drugs that work very well and have few side effects. Plants are crucial for finding and making new medicines (Islam et al. 2022). They provide a convenient and effective method for obtaining naturally occurring chemicals for medicinal purposes. According to experts in the field, some chemical compounds that come from medical plants may be able to help people. As a result, experts are always looking for new herbal treatments and other medicines made from plants that can effectively treat a wide range of diseases (Baroi et al. 2023).

Herbal medicine shows great potential in managing diabetes, particularly in regulating blood sugar levels and to boost overall health. The drastic changes in modern lifestyle, which include irregular sleeping patterns, bad diet regimes, smoking, alcoholism etc., may cause an imbalance between insulin production and blood

glucose leading to development of diabetes mellitus. Despite extensive research into diabetes and its complications, there is no single theory that can be used to treat diabetes and its complications completely. 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 (Chowdhury et al. 2024). 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 (Lima et al. 2023, Chowdhury et al. 2024, Saxena et al. 2023). 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 (Sakib et al. 2024). Global improvements in scientific study have resulted in a heightened exploration of the medicinal properties of plant species (Pracheta et al. 2011). 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. hipsida is an essential ingredient in Kusmanda lehyam (Ayurvedic medicine), often used in the treatment of neurological disorders. The fruits and seeds of B. hipsida 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 (Al-Snafi 2013). The fruit comprises carotenes, flavonoids, glycosides, saccharides, proteins, vitamins, minerals, volatile oils, ß-sitosterol, and uronic acid. The incorporation of terpenes, flavonoid C, glycosides, and sterols makes it a potent antioxidant (Doharey et al. 2021).

This manuscript highlights the potential of *Benincasa hispida* as a natural alternative for diabetes management, addressing the limitations of synthetic drugs. Its findings provide valuable insights into plant-based therapeutics, encouraging further exploration of traditional medicine for safer and cost-effective treatments.

2. MATERIALS AND METHODS

2.1 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.

2.2 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.

2.3 Experimental Animal Handling

100 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).

2.4 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.

2.5 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.

2.6 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.

2.7 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 heterogenicity in terms of several biological factors. The occurrences are considered statistically significant since the 'p' value was less than 0.05($p<0.5$).

3. RESULTS AND DISCUSSION

Traditional and popular therapies found in all cultures, as well as the use of standardized and tritated botanical extracts, are all examples of herbal medicine, which is the use of medicinal plants to prevent and cure illness. This study examined the anti-diabetic properties and lipid profile of the herb *Benincasa hispida* in mice. Diabetes is one of the most severe health challenges of the 21st century. It is a primary cause of mortality, and diabetic macro- and microvascular complications lead to significant healthcare expenses and increased disability. In comparison to the 300, 600, and 900 mg/kg doses, only the 900 mg/kg dosage demonstrated statistically significant antidiabetic efficacy ($p < 0.05$). Numerous investigations yielded identical outcomes (Ahmed et al. 2010, Kumar et al. 2017, Kang et al. 2012, Jauharah et al. 2024). Despite a minor decrease in these parameters, no groups exhibited statistically significant outcomes in terms of HDL, LDL, and total cholesterol. The triglyceride value of 97.70 ± 7.50* yielded statistically significant results ($p < 0.05$). On the other hand, SGPT and SGOT did not show any statistically significant effects at 300, 600, or 900 mg/kg. Other comparable investigations arrived at the same conclusions (Mishra & Pancholi 2013). Group 5 demonstrated statistically significant outcomes ($p < 0.05$) in the kidney function test with a value of 93.24 ± 9.23* for urea, administered extract at a dosage of 600 mg/kg. However, no statistically significant outcomes were observed in any of the categories

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 +100mg	A + M100
4	Alloxan + *Benincasa hispida*	Alloxan + *Benincasa hispida* extract low dose	150 mg/kg + 300 mg/kg	A + BH_{300}
5	Alloxan + *Benincasa hispida*	Alloxan + *Benincasa hispida* extract medium dose	150 mg/kg + 600 mg/kg	A + BH_{600}
6	Alloxan + *Benincasa hispida*	Alloxan + *Benincasa hispida* extract high dose	150 mg/kg +900 mg/kg	A+ BH_{900}
7	Metformin	Metformin	100 mg/kg	M
8	*Benincasa hispida*	Alloxan +*Benincasa hispida* extract low dose	300 mg/kg	BH_{300}
9	*Benincasa hispida*	Alloxan +*Benincasa hispida* extract medium dose	600 mg/kg	BH_{600}
10	*Benincasa hispida*	Alloxan + *Benincasa hispida* extract high dose	900 mg/kg	BH_{900}

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±0.056
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±0.074
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±0.061

*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.*

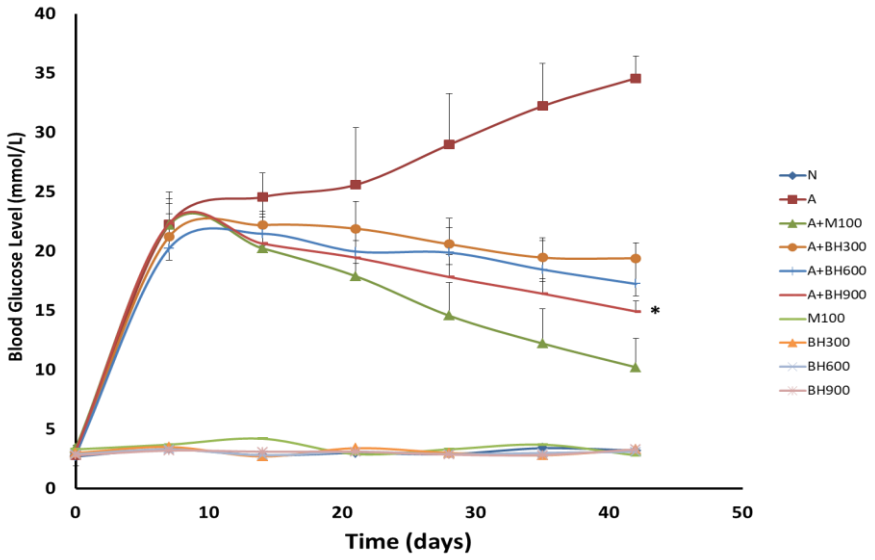

Fig. 1. Antidiabetic activity of different dose of *Benincasa hispida*

with respect to creatinine levels. Further investigation into such an experiment revealed the same results (Kishore & Singh 2017).

4. 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.

DISCLAIMER (ARTIFICIAL INTELLIGENCE)

Author(s) hereby declare that NO generative AI technologies such as Large Language Models (ChatGPT, COPILOT, etc) and text-to-image generators have been used during writing or editing of this manuscript.

CONSENT

It is not applicable.

ETHICAL APPROVAL

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.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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