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Evaluation of Anti-Diabetic Potential of Ethanolic Extract of *Benincasa hispida* in Alloxan Induced Diabetic Rats

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Abstract: Diabetes mellitus is becoming increasingly common in both developed and developing countries around the world. Existing treatments, particularly synthetic pharmaceuticals, frequently fail to restore normal glycemic control without side effects. A variety of herbal medications are being studied as an alternative therapy for diabetes. The goal of this study was to test one such herbal extract of *Benincasa hispida* seed for anti-diabetic efficacy in diabetic rats that had been artificially generated. The extract at a dose 500mg/kg/day was administered to Alloxan induced diabetic animals for 15 consecutive days. Blood glucose levels and body weight were measured for the evaluation of its anti-diabetic effect. Our study indicates that ethanolic extract of *Benincasa hispida seed* has a promising effect in controlling blood glucose levels and also aid in counteracting the derangement of lipid profile, a major concern in diabetes mellitus.

Keywords: *Benincasa hispida,* Ethanolic extract, Wistar rats.

I. INTRODUCTION

Diabetes mellitus is a chronic, progressive metabolic illness that affects every organ system and is linked to consequences such cardiovascular disease, retinopathy, neuropathy, and nephropathy. It is the largest cause of mortality and disability in the world. Its global frequency was around 8% in 2011 and is expected to increase to 10% by 2030. Diabetes affects over 80% of adults in low- and middle-income nations. In 2014, the global prevalence of diabetes among adults aged 18 and up was predicted to be 9%. Diabetes was directly responsible for 1.5 million fatalities in 2012.[1] If current trends continue, 592 million people, or one out of every ten adults, will have diabetes by 2035. The global diabetes epidemic has had a significant impact on India, the world's second most populated country. According to the International Diabetes Federation (2013), only three nations account for roughly half of all diabetics: China (98.4 million), India (65.1 million), and the United States (24.4 million). In most high-income nations, it is the fourth or fifth greatest cause of death, and there is evidence that it is becoming an epidemic in many low- and middle-income countries. Diabetes will undoubtedly be one of the most difficult health issues of the twenty-first century. The conventional major abnormalities responsible for the development and progression of diabetes mellitus are impaired insulin secretion (beta-cell), increased hepatic glucose synthesis (liver), and decreased peripheral (muscle) glucose consumption.[2, 3]

Various synthetic chemical agents are currently available for diabetes control and therapy, but comprehensive recovery and avoiding consequences from a disturbed lipid profile remain a serious issue. In addition to insulin, the treatment of diabetes primarily entails a prolonged reduction in hyperglycemia by the use of biguanides, thiazolidinediones, sulfonylureas, D-phenylalanine, and -glucosidase inhibitors. However, the efficacies of these compounds are questionable due to undesired side effects, and there is a demand for novel compounds for the treatment of diabetes. As a result, better and safer alternative compounds for the treatment of diabetes mellitus are constantly being evaluated. One such method is to seek out and carefully research traditional South Asian plants and fruits that have been used for over 1000 years. Traditional herbal remedies should be researched further, according to a WHO expert committee on diabetes. As a result, plants have been recommended as a rich source of potentially helpful anti-diabetic medications that has yet to be studied. Many traditional herbal diabetic remedies are utilised all over the world. Plant-based medications and herbal formulations are often thought to be less harmful and free of adverse effects than manufactured drugs. These plants' anti-hyperglycemic properties stem from their capacity to restore pancreatic tissue function by raising insulin secretion, inhibiting glucose absorption in the intestine, and facilitating metabolite transport in insulin-dependent activities. As a result, herbal medicine treatment protects -cells while also smoothing out fluctuations in glucose levels.[5, 4]

Benincasa hispida is a member of the Cucurbitaceae family, which is used in Ayurvedic medicine to treat dyspepsia, heart illness, vermifuge, and urinary disease. Anti-inflammatory, diuretic, hypoglycemic, anti- alzheimer's, anti-

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diarrheal, antioxidant, antiulcer, anti-obesity, antihistaminic, and anti-cancer properties have been discovered through scientific studies. Long-term insulin deficiency or dysfunction results in metabolic abnormalities marked by aberrant glucose, triglyceride, and cholesterol levels in the blood, as well as glycogen content in the liver and muscle. Drugs that can entirely or partially restore the broken mechanism should be able to show a change in the blood levels of these metabolites, which can be utilised as a metric in its assessment. Although several investigations have revealed *Benincasa hispida*'s hypoglycemic action, only a few studies have confirmed its relationship with correcting lipid metabolic dysregulation using histological evaluation of the pancreas.[6]

Figure 1: *Benincasa hispida*

Figure 2: *Benincasa hispida*'s seed

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II. MATERIALS AND METHODS

A. Collection of Plant Material

The fresh fruits of "*Benincasa hispida*" were collected in the month of October 2020 from rural area of Garibpur, North 24 Parganas, West Bengal, India. The fruits were identified by Head of the Depertment, Department of Pharmacognosy, Bharat technology, Uluberia, Howrah.

B. Preparation of Ethanolic Extract [7, 8]

Fresh fruit were cut into small piece and collected the seed. Seeds were washed by distill water and shade dried. After drying seeds were produce in coarse powder by a dry mixture grinder. The coarse powder is collected and weighted, 50gm of coarse powder is added to 100ml petroleum ether and left for 36 hrs with occational shaking. The mixture is filter and the filter cake is collected, dried under the fan. The coarse the powder was extracted by Soxhlet apparatus using 100ml of ethanol. Removed solvent from extract by simple distillation. After removal of solvent, it was subsequently partitioned with Chloroform. Then the drug is collected in Eppendorf tube and storage in the refrigerator (5-8 ^{0}C) until the use.

C. Phytochemical Screening [9]

The ethanolic extract of seeds of *Benincasa hispida* were subjected to preliminary phytochemical screening for the detection of major active compound. The result of different chemical tests on the ethanolic seed extract of *Benincasa hispida* showed the presence of alkaloids, amino acids, carbohydrate, flavonoids, glycosides, protein, tannins, and phenolic compounds.

D. Selection and Maintenance of Animals

Healthy adult albino rats (Wistar rats) of either sex, weighing between 140 gm and 190 gm were obtained from M/S Mondal Enterprises, Kolkata, West Bengal, India. The animals were acclimatized under laboratory condition in a polypropylene cage for 2 weeks before the starting of experiments. They were provided with standard diet and water and maintained under standard conditions of temperature (24 ± 1 ◦ C) and humidity (49%) with an alternating 12 h light/dark cycles. All the studies were conducted in conformity with the proper guidance for care and standard experimental animals study ethical protocols.

E. Induction of Diabetes Mellitus

Before receiving Alloxan monohydrate, all rats were fasted overnight. Diabetes was produced by administering 120

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mg/kg of Alloxan monohydrate into the tail vein intravenously. The rats were housed for 5 days with free access to food and water after being injected with alloxan. The rats were starved for 12 hours on the sixth day and their blood glucose levels were measured.

F. Acute Toxicity Test [10]

Benincasa hispida shown toxic effect of ethanolic extract of seeds on rats in high dose. Albino mice selected by random sampling technique were employed in this study. The animals were fasted for 3 hr. with free access to water only. The extract administered orally at a dose of 4 mg/kg initially and observed mortality, all mice were live normally then 50 mg/kg and 400 mg/kg also resulted that all mice were normally alive, after final toxicity pass 2000 mg /kg.. The final acute toxicity test is passed out the ethanol fraction (Alkaloid) of *Benincasa hispida* was 2000mg/kg orally.

G. Experimental Design

After 14 days accilmatization, male wistar rats will be randomly divided into three groups (n=6). The wistar will be pre-treated daily with extract seeds of *Benincasa hispida* (500mg/kg), then wistar rats will be treated with Glimepiride 5 mg/kg,p.o. Each group will receive the following treatment.

Group I-(Normal control):

Normal healthy rats without treatment. Will receive clean water and normal food. No drug given.

Group III- (Diabetic control):

Diabetic rats treated with normal saline.

Group II-(Standard):

Diabetic rats will receive Glimepiride (5 mg/ kg) for one time.

Group III-(Test):

Diabetic rats will receive extracted drug from seeds of *Benincasa hispida* (500mg/kg; p.o.)

After given the all dose measure the hang over time of all groups animals and take the reading after induced (0day, 3 days, 5 days, and 10 days).

H. Statistical Evaluation

Data were subjected to a Prism. Results were presented as Mean Standard Error of the Mean (SEM). One-way analysis of variance (ANOVA) was used for comparison of the means.

III.RESULTS AND DISCUSSION

A. Effect of administration of Alloxan (120 mg/kg) induced diabetic rats.

The glucose level of Alloxan treated Rat in "0" days (before induced), "3" days (after induced) "7"days and "10" days were found 87.16 ±2.68, 217.5±6.50, 274.5 ± 3.42, 280 ± 1.82, respectively. Thus, alloxan significantly increase the level of glucose against vehicle treated (Group I).

B. Effect of administration of Gilmipride (5 mg/kg) in Alloxan induced diabetic rats.

The glucose level of Gilmipride treated Rat in "0" days (before induced), "3" days (after induced) "7days and 10 days were found to 90±1.82, 192.5 ±13.58, 158.16 ±2.45, 94.66±2.78 respectively. Thus, Gilmipride significantly decrease the level of glucose against Alloxan induced (Group II).

C. Effect of administration of ethanolic extract of seed of *Benincasa hispida* (500mg/kg) in Alloxan induced diabetic Rat.

The glucose level of *Benincasa hispida* treated Rat in "0" days (before induced), "3" days (after induced) "7 days and 10 days were found 88.16±2.34, 185.83±3.89, 117.33±3.14, 82.5±2.36 respectively. Thus, *Benincasa hispida* significantly decrease the level of glucose against Alloxan induced (group II).

Alloxan monohydrate molecular weight 160 and empirical formula of H2N204.H20.It is widely used to insulin depented diabetis mellitus in experimental animalas because of toxic effects on islet beta cells .The diabetogenic action of alloxan monohydrate is the direct result of irreversible damage to the pancreatic beta cells resulting in degranulation and loss of capacity to secreate insulin. The effects of alloxan monohydrate on different organs have been extensively studied. Alloxan monohydrate has various biological actions including the production of acute and chronic cellular injury.[11]

the pancreas when administered ti rodents and many other animal species .This cause an insulin dependent diabetis mellitus in this animal with characteristic similar ti type 1 diabetes in humans Alloxan selectively toxic to insulin producing pancreatic beta cells because it preferentially accumulatesin beta cells through uptake via GLUT2 glucose transpoters Alloxan, in the presence of intracellular thiols, generates reactive reraction with its reduction product dialuric acid. The beta cell toxic action of alloxan is binisiated by free radicals formed in this redox reaction studieds suggests that alloxan does not cause diabetis in human.[12, 13]

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The ethanolic extract of seed of *Benincasa hispida* decrease glucose level in alloxan induced diabetic rat Hence *Benincasa hispida* extraction may effective some pathway which aggrevate the diabetes mellitus Such this experiment indicates *Benincasa hispida* extraction may stimulates the secration of insulin or the activity of insulin increase.

Table 1. Effect of ethanolic extract of seeds of *Benincasa hispida* on Diabetic induced Wistar rats.

Sl No.	Group	0 Day	3 Day's	7 Day's	10 Day's
I	Normal Control	85.83±1.94	86.00±1.96	87.5±1,.49	87.0±1.47
II	Diabetic control	87.16 ±2.68	217.5±6.50	274.5 ± 3.42	280 ± 1.82
III	Standard	90±1.82	192.5 ±13.58	158.16 ±2.45	94.66±2.78
IV	Test	88.16±2.34	185.83±3.89	117.33±3.14	82.5±2.36

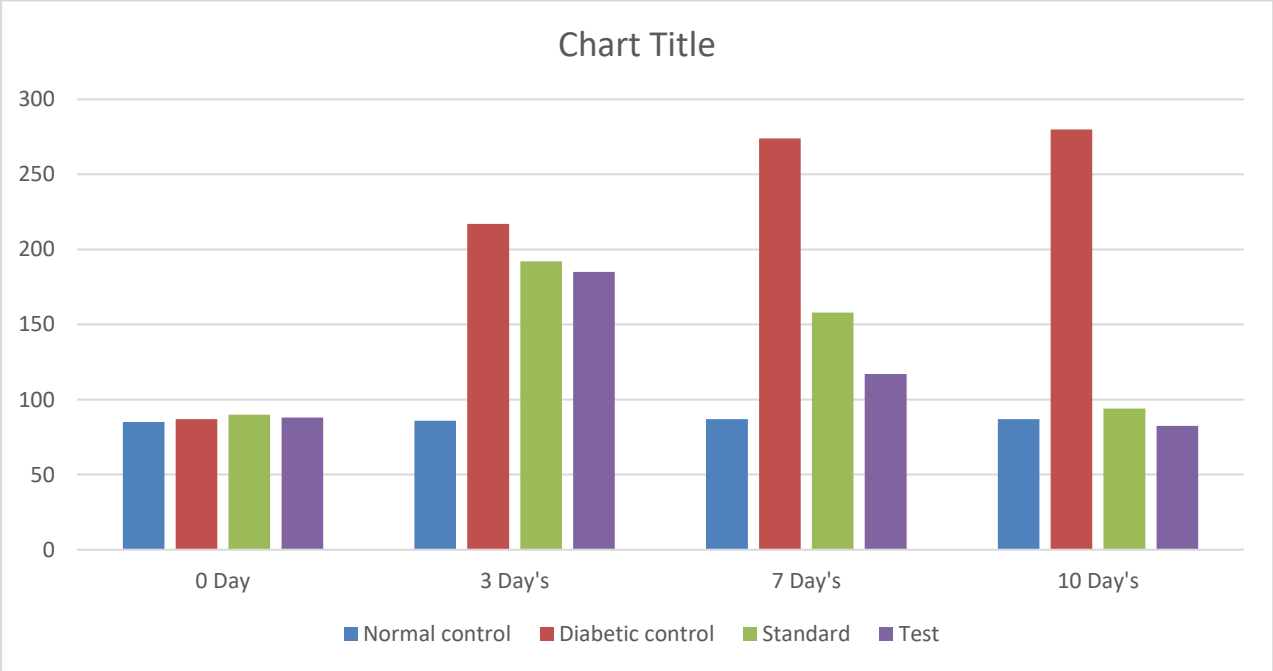

Figure 3: Graphical representation ethanolic extract of seeds of *Benincasa hispida* on Diabetic induced Wistar rats.

Alloxan is an oxygenated pyrimidine and toxic anaiog of glucose .Alloxan selectively destroy insulin producing cells in the pancreas when administered ti rodents and many other animal species .This cause an insulin dependent diabetis mellitus in this animal with characteristic similar ti type 1 diabetes in humans Alloxan selectively toxic to insulin producing pancreatic beta cells because it preferentially accumulatesin beta cells through uptake via GLUT2 glucose transpoters Alloxan, in the presence of intracellular thiols, generates reactive reraction with its reduction product dialuric acid. The beta cell toxic action of alloxan is binisiated by free radicals formed in this redox reaction studieds suggests that alloxan does not cause diabetis in human.[12, 13]

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IV. CONCLUSION

The present study within revels that the ethanolic extract of seeds of *Benincasa hispida* significantly improve the glucose level in alloxan monohydrate induced diabetic rat. Thus.it can be concluded that the ethanolic extract of seeds of *Benincasa hispida* is potential against diabetic mellitus.

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