



Anti-diabetic and wound healing potential of *Benincasa hispida* in streptozotocin-induced diabetic rats

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ABSTRACT

Benincasa hispida (*B.hispida*) is a potential bioactive herb with a wide range of pharmacological actions. Diabetes wound healing effect of effect of *B.hispida* is not clear. Hence, the study was planned to investigate the antidiabetic and wound healing activities of methanolic and petroleum ether of *B.hispida* on streptozotocin-induced diabetes mellitus in Wistar rats. Diabetes was induced by a single intra peritoneal injection of Streptozocin (60mg/kg). The antidiabetic activity was studied at 50, 100 and 200 mg/kg body weight (BW) of plant extracts. In this study, STZ intoxicated rats displayed increased blood glucose level, lipid peroxidation and decreased level of antioxidants. Further, lipid profiles such as total cholesterol, triglycerides, LDL and VLDL were significantly increased and HDL was significantly decreased in STZ diabetic rats. Treatment with *B.hispida* methanolic and petroleum extracts at the dose of 100 and 200mg/kg showed significant antidiabetic activity. Further, in wounded rats, *B.hispida* significantly increased the wound contraction rate and shorten the period of epithelization. In conclusion, *B.hispida* showed a significant antidiabetic and wound activities mediated through its antioxidant effect.



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INTRODUCTION

Diabetes is becoming something of a pandemic, and despite the recent surge in new drugs to treat and

prevent the condition, its prevalence continues to soar. Perhaps the most worrying aspect of all is that the rise is even reflected in children. Besides hyperglycemia, several other factors, including hyperlipidemia or hyperlipidemia, are involved in the development of micro and macrovascular complications of diabetes which are the major cause of morbidity and death (Thiruvengkatasubramaniam and Jayakar, 2010).

Although several drugs targeted for carbohydrate hydrolyzing enzymes (psuedosaccharides), the release of insulin from pancreatic β -cells (sulphonyl ureas), glucose utilization (biguanides), insulin sensitizers, PPAR γ agonists (glitazones) are in clinical practice, the growing diabetes market observes several changes. Some of these drugs are linked to

liver toxicity (troglitazone), including a few deaths from hepatic failure and raising the symptoms and risk factors of heart disease leading to heart failure (rosiglitazone).

Therefore, as the long term of risk and effect on the complications of diabetes-related with these drugs are not at clear. On the other hand, traditional medicinal plants with various active principles and properties have been used since ancient times by physicians and laymen to treat a great variety of human diseases such as diabetes, coronary heart disease and cancer.

The beneficial multiple activities like manipulating carbohydrate mechanism by various mechanism, preventing and restoring integrity and function of β -cells, insulin-releasing activity, improving glucose uptake and utilization and the antioxidant properties present in medicinal plants offer exciting opportunity to develop them into novel therapeutics. The multifactorial pathogenicity of diabetes demands a multimodal therapeutic approach. Thus, future therapeutic strategies require a combination of various types of multiple agents. Thus, plant-based herbal drugs and botanicals with free radical scavenging activity are emerging as the primary components of holistic approaches to diabetes management (Büyükbalci and El, 2008; Sabu and Kuttan, 2002).

Wound healing is the process of repair that follows injury to the skin and other soft tissues. Following injury, an inflammatory response occurs, and the cells below the dermis (the deepest skin layer) begin to increase collagen (connective tissue) production. Later, the epithelial tissue (the outer skin layer) is regenerated (Nayak and Pereira, 2006). There are three stages to the process of wound healing: inflammation, proliferation, and remodeling. The proliferative phase is characterized by angiogenesis, collagen deposition, epithelialisation and wound contraction. Current methods used to treat chronic wounds include debridement, irrigation, antibiotics, tissue grafts and proteolytic enzymes, which possess major drawbacks and unwanted side effects (Nayak et al., 2009).

Benincasa hispida (Thunb) Cogn. (Family: Cucurbitaceae) is commonly known as white pumpkin or wax gourd or ash gourd. The fruit *B.hispida* is an important ingredient of Kusmanda lehyam (Ayurvedic medicine), which is widely used in nervous disorders. The fruits and seeds of *B.hispida* possess a number of pharmacological properties and uses laxative, tonic, diuretic, aphrodisiac, antiperiodic, haemoptysis, other internal hemorrhages in insanity, epilepsy and other nervous

disorders (Chopra, 1956). In this backdrop, the present study was conducted to evaluate the antidiabetic and wound healing activity of methanol and petroleum ether extracts of *B. hispida* leaves on streptozocin induced diabetes and excision wound models.

MATERIALS AND METHODS

Plant extraction

Fresh leaves of *B. hispida* were collected locally and dried under shade. They were identified and authenticated by Dr. Madhavachetty, Head of Department, Botany, Sri Venkateshwara University, Tirupathi, Andhra Pradesh. The dried leaves were pulverized and then the coarse powder was subjected to methanol and ethylacetate extraction using cold maceration. Then the resultant product is filtered, dried in a desiccator and stored until further use.

Animals

Healthy adult male Wistar rats weighing 250–300 g were used in this study. They were maintained on a standard laboratory pellet chow diet, provided water ad libitum and were kept under standard conditions at 23–25° C, 35 to 60% humidity, 12hr light /dark cycle. The mice were acclimatized to the laboratory conditions a week prior to the experiment. The experimental protocol was duly approved by an institutional animal ethics committee (IAEC). The animal experimentation was carried out under CPC-SEA registration.

Acute toxicity studies

The acute toxicity studies for methanolic (MEBH) and petroleum ether (PEMD) of *B. hispida* were done according to the OECD guidelines No. 423. The extract did not produce any signs of toxicity when given in doses up to 2000 mg/kg by an oral route. Hence, for further studies, 100 & 200 mg/kg dose of the extract were selected.

Streptozocin induced diabetes

A freshly prepared solution of STZ (60 mg/kg in 0.1 M citrate buffer, pH 4.5) was injected intraperitoneally to overnight-fasted rats exhibited hyperglycemia within 48 h of STZ administration (Vogel, 1051). The rats having fasting blood glucose (FBG) values of 250 mg/dl or above were considered for the study.

Study design

The experiment was carried out in groups of six rats each:

Group I- Normal control rats received saline.

Group II- Diabetic control rats received streptozocin (60mg/kg)

Group III- Diabetic rats treated with standard drug, Metformin (50 mg/kg).

Group IV- Diabetic rats treated with MEBH (50 mg/kg in 0.5% CMC).

Group V- Diabetic rats treated with MEBH (100 mg/kg in 0.5% CMC).

Group VI- Diabetic rats treated with MEBH (200 mg/kg 0.5% CMC).

Group VII-Diabetic rats treated with PEBH (50 mg/kg 0.5% CMC).

Group VIII-Diabetic rats treated with PEBH (100 mg/kg 0.5% CMC).

Group IX- Diabetic rats treated with PEBH (200 mg/kg in 0.5% CMC).

Serum glucose was estimated on day 1, 7, 14 and 21 by the Glucose oxidase method and the absorbance was measured at 505 nm by UV-Spectrophotometer (ELICO-SI-159). On the 22nd day, the animals were sacrificed, and the liver was removed and homogenized for the estimation of antioxidants parameters.

Preparation of liver homogenate

The liver was quickly removed and perfused immediately with ice-cold saline (0.9% NaCl). A portion of the liver was homogenized in chilled Tris-HCl buffer (0.025 M, pH 7.4) using a homogenizer. The homogenate obtained was centrifuged at 5000 rpm for 10 min, the supernatant was collected and used for the estimation of antioxidants Estimation of antioxidants.

The hepatic levels of superoxide dismutase (SOD), catalase (CAT) and malondialdehyde (MDA), an index of lipid peroxidation was estimated by standard kits.

Estimation of lipid profiles

The serum levels of total cholesterol, LDL, HDL, VLDL and triglycerides were estimated by standard biochemical kits.

Wound healing activity

Animals were under light ether anaesthesia through the surgical procedure. An impression of 2.5 cm diameter (500 sq mm) as described by Morton and Malone was made after leaving at least 5 mm complete space from the ears. The skin of the impressed area was excised carefully to the complete thickness, and a wound of 500 sq mm was formed. Homeostasis was achieved by the application of a normal saline solution. The rats were kept individually in separate cages. The physical attributes of wound

healing viz wound closure (contraction) and epithelization were recorded. The wound contraction was studied by tracing the raw wound area on transparent paper on 0,3rd, 6th, 9th, 12th, 15th and 18th day. (Morton and Malone, 1972) The criterion for complete epithelization was fixed as the formation of a scar with an absence of raw wound area. The animals were grouped as follows,

Group I: Control, applied topically (0.5 g), simple ointment.

Group II: Standard, applied topically (0.5g), 5% w/w Povidone ointment.

Group III: Excision Wound.

Group IV: Treated with MEBH 2% w/w ointment (0.5g), topically.

Group V: Treated with MEBH 5% w/w ointment (0.5g), topically.

Group VI: Treated with MEBH 10% w/w ointment (0.5g), topically.

Group VII: Treated with PEBH 2% w/w ointment (0.5g), topically.

Group VIII: Treated with PEBH 5% w/w ointment (0.5g), topically.

Group IX: Treated with PEBH 10% w/w ointment (0.5g), topically

Statistical analysis

The data were expressed as mean \pm SEM. The results were analyzed by SPSS version 19 using a one-way analysis of variance. The differences between mean values were considered significant at $P < 0.05$.

RESULTS AND DISCUSSION

Acute toxicity studies

Acute oral toxicity studies revealed the non-toxic nature of the MEBH and PEBH. There was no lethality observed and any profound toxic reactions found at a dose of 2000mg/kg b.wt. p.o. which indirectly pronounces the safety profile of the plant extract.

Antidiabetic activity of *B.hispida* on STZ induced diabetes

In this study, a single intraperitoneal injection of streptozocin (60mg/kg; b.wt) had displayed noxious biochemical changes. Whilst treatment with MEBH and PEBH significantly attenuated the toxic manifestation and thus inhibited the state of diabetes. In the present study, STZ intoxicated rats displayed significant ($p < 0.001$) elevation of blood glucose level on the 1st, 7th, 14th and 21st day as that of the control rats. Meanwhile, MEBH and PEBH at the

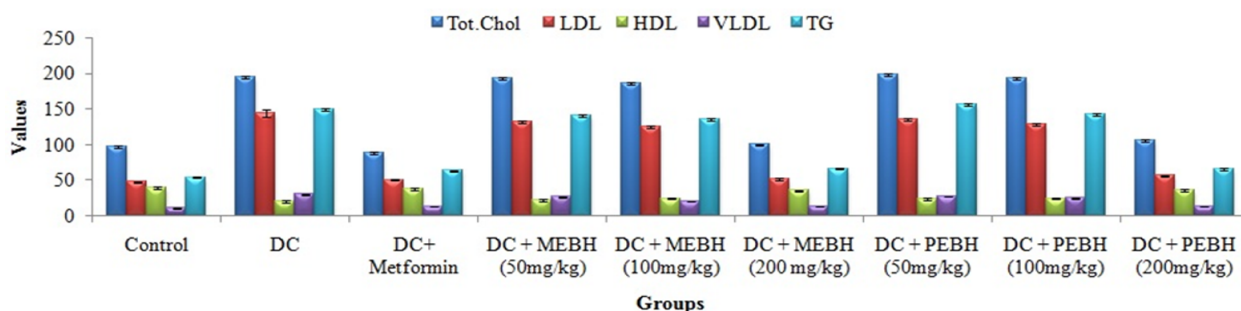


Figure 1: Effect of methanolic and petroleum ether extracts of *B.hispida* leaves on lipid profiles in STZ induced diabetic rats

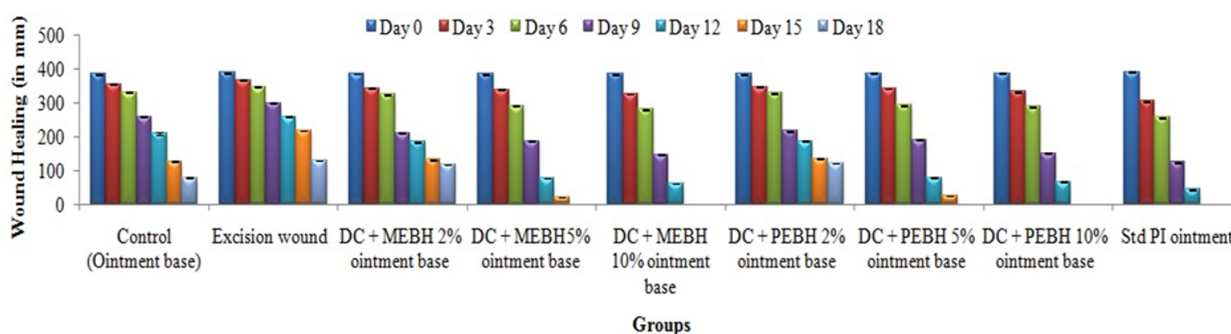


Figure 2: Effect of methanolic and petroleum ether extracts of *B.hispida* leaves on Wound healing in STZ induced diabetic rats

Table 1: Effect of methanolic and petroleum ether extracts of *B.hispida* leaves on blood glucose levels in STZ induced diabetic rats

Treatment	Blood Glucose level (mg/dl)			
	1 day	7 th day	14 th day	21 st day
Group I Control 0.5% CMC (1ml/kg; p.o)	85.12 ± 1.87	86.12 ± 2.12	86.54 ± 1.92	87.12 ± 1.24
Group II Diabetic Control	271.76 ± 4.90 ***	280.45 ± 3.87***	282.78 ± 4.32 ***	286.56 ± 4.65***
Group III DC+ Metformin (250 mg/kg, b.wt; p.o)	272.24 ± 4.6 ^{NS}	104.25 ± 2.34***	98.98 ± 1.65***	89.21 ± 0.87***
Group IV DC + MEBH (50mg/kg, b.wt; p.o)	268.28 ± 4.52 ^{NS}	256.24 ± 4.67	254.65 ± 3.87 ^{NS}	250.34 ± 3.12 ^{NS}
Group V DC + MEBH (100 mg/kg, b.wt; p.o)	253.20 ± 4.61 ^{NS}	248.80 ± 5.64	245.20 ± 5.70 ^{NS}	242.00 ± 2.96
Group VI DC + MEBH (200 mg/kg, b.wt; p.o)	249.00 ± 5.02 ^{NS}	150.65 ± 3.72*	135.82 ± 2.12**	115.32 ± 1.76***
Group VII DC + PEBH (50mg/kg, b.wt; p.o)	267.87 ± 5.67 ^{NS}	256.65 ± 4.28 ^{NS}	254.26 ± 5.12 ^{NS}	252.45 ± 3.35 ^{NS}
Group VIII DC + PEBH (100 mg/kg, b.wt; p.o)	262.00 ± 5.04 ^{NS}	248.70 ± 4.32 ^{NS}	246.00 ± 3.86	245.70 ± 2.28
Group IX DC + PEBH (200 mg/kg, b.wt; p.o)	265.50 ± 9.93 ^{NS}	181.00 ± 3.72**	152.80 ± 1.96**	127.20 ± 2.48***

Table 2: Effect of methanolic and petroleum ether extracts of *B.hispida* leaves on lipid peroxidation and antioxidant levels in STZ induced diabetic rats

Treatment	SOD	CAT	MDA
Group I Control 0.5% CMC (1ml/kg; p.o)	24.77 ±0.79	27.04 ±2.79	3.33 ±0.41
Group II Diabetic Control (DC)	12.95 ±0.56***	15.87 ±1.36***	12.58 ±0.56***
Group III DC+ Metformin (250 mg/kg, b.wt; p.o)	23.32 ±0.82***	26.15 ±2.45***	4.54 ±0.45***
Group IV DC + MEBH (50mg/kg, b.wt; p.o)	13.45 ±0.42 ^{NS}	17.43 ±1.65	11.98 ±0.48
Group V DC + MEBH (100 mg/kg, b.wt; p.o)	14.02 ±0.73	18.51 ±2.70 ^{NS}	10.35 ±0.52
Group VI DC + MEBH (200 mg/kg, b.wt; p.o)	22.69 ±0.47**	23.12 ±2.87**	5.12 ±0.42**
Group VII DC + PEBH (50mg/kg, b.wt; p.o)	14.68 ±0.48	16.58 ±1.92 ^{NS}	10.24 ±0.42
Group VIII DC + PEBH (100 mg/kg, b.wt; p.o)	15.35 ±0.65 ^{NS}	18.54 ±3.12 ^{NS}	11.12 ±0.47
Group IX DC + PEBH (200 mg/kg, b.wt; p.o)	24.12 ±0.75***	25.34 ±2.76***	5.75 ±0.38***

Table 3: Effect of methanolic and petroleum ether extracts of *B.hispida* leaves on Period of epithelization in STZ induced diabetic rats

Groups	Period of epithelialization in days
Control (Ointment base) Excision wound	21.56±0.57 23.76±0.65
DC + MEBH 2% ointment base	21.35±0.52 ^{NS}
DC + MEBH 5% ointment base	15.87±0.98**
DC + MEBH 10% ointment base	14.65±0.43***
DC + PEBH 2% ointment base	22.96±0.56
DC + PEBH 5% ointment base	18.12±0.65**
DC + PEBH 10% ointment base	14.86±0.68***
Standard Povidone-iodine ointment	14.12±0.65***

doses of 5 100mg/kg had not display any significant alteration in the blood glucose level as that of the diabetic control. However, treatment with MEBH and PEBH at the dose of 200 mg/kg showed a significant ($p<0.001$) reduction in the blood glucose level on the 7th, 14th and 21st day as compared to the diabetic control (Table 1).

The values are expressed as Mean \pm SEM, $n=6$. * $p<0.05$, ** $p<0.01$, *** $p<0.001$, extract-treated groups were compared with diabetic control group. ^{NS} non-significant One-way ANOVA followed by Dunnett's t-test. *** $p<0.001$, the diabetic control group was compared with the normal group.

Effect of *B.hispida* on antioxidant enzymes and lipid peroxidation in STZ induced diabetic rats

In the present study, STZ intoxicated rats displayed a significant ($p<0.001$) reduction in the level of antioxidant enzymes, superoxide dismutase (SOD) and catalase (CAT) and an increase in the MDA in the liver tissue of diabetic rats. However, treatment with MEBH and PEBH at the dose of 100mg/kg had not displayed effects, but at the dose of 200mg/kg, there was a significant increase in the level of antioxidants and a decrease in lipid peroxidation (Table 2).

The values are expressed as Mean \pm SEM, $n=6$. * $p<0.05$, ** $p<0.01$, *** $p<0.001$, extract-treated groups were compared with diabetic control group. ^{NS} non-significant One-way ANOVA followed by Dunnett's t-test. *** $p<0.001$, the diabetic control group was compared with the normal group.

Effect of *B.hispida* on lipid profiles in STZ induced diabetic rats

In this study, STZ intoxicated rats displayed significant ($p < 0.001$) elevation in the level of total cholesterol, LDL, VLDL triglycerides and a decrease in HDL in serum. Meanwhile, treatment with MEBH and PEBH at the dose, 100 mg/kg, displayed a minimal restoration of lipid profiles, but it was statistically non-significant. However, MEBH and PEBH at the dose of 200mg/kg showed a significant ($p < 0.001$) decrease in the level of total cholesterol, LDL, VLDL and triglycerides with an increase in HDL level and thus restored the lipid profiles to normalcy (Figure 1)

Wound healing activity of *B.hispida* on excision wound models

On day 0 till day 3 there were no significant wound contractions observed in both control and wound-induced and extracts treated experimental groups. On the 6th day, a significant wound contraction process started in experimental animals treated with 5 and 10% ointment of MEBH and PEBH. On day 12, the wound contraction rate was rapid in 10% ointment of MEBH and PEBH as that of the 2% and 5% ointment of MEBH and PEBH. The complete healing was observed in 10% ointment of MEBH and PEBH on 15th day, whilst in 5% ointment of MEBH and PEBH, the complete healing was observed only on the 18th day (Figure 2).

Effect of *B.hispida* on a period of epithelization on wound-induced rats

In this study, the epithelialization period was significantly ($p < 0.01$; $P < 0.001$) lower in 10% and 5 % ointment of MEBH and PEBH as that wound-induced group (Table 3).

The values are expressed as Mean \pm SEM, $n = 6$. ** $p < 0.01$, *** $p < 0.001$, extracts treated groups were compared with a wound-induced group. ^{NS} non-significant One-way ANOVA followed by Dunnett's t-test.

DISCUSSION

The present investigation discusses the antidiabetic and wound healing potential of the leaves of the latex of *A. megalacantha* in STZ-induced diabetic mice. The use of STZ to induce DM in rodent models is widely accepted, and STZ-induced diabetes is reported to resemble human DM (Adisa et al., 2011), which is characterized by glycosuria, hyperglycemia, polyphagia, polydipsia, and body weight loss when compared with normal rodents (Kumar et al., 2010). Metformin is often used as a standard antidiabetic drug in STZ-induced moderate diabetes

to compare the antidiabetic effects of a variety of bioactive compounds (Ramkumar et al., 2011).

The *B.hispida* and metformin-treated groups showed a reduction of the glucose levels compared to diabetic control. The decrease of the glucose levels may be due to the plasma insulin levels elevation or the enhancement of the blood glucose transportation in the peripheral tissue (Petchi et al., 2014). Glibenclamide could enhance the insulin secretion from the pancreatic beta cells by the closure of K_{ATP} channels. As a result, the membrane will be depolarized and cause the activation of the voltage-dependent Ca^{2+} channels. The influx of Ca^{2+} to the cells will initiate the secretion of insulin (Fridlyand et al., 2013). The production of insulin could lower down the glucose level and reverse back the glycemic control. There was a significant elevation of the glucose levels in the diabetic control and this may due to the damage of the beta cells of the pancreas.

Diabetic rats also showed increased levels of serum cholesterol, TG, LDL, VLDL, and decreased HDL levels. Increased in levels of TC, LDL and VLDL indicate the development of hyperlipidemia in rats, and this may be due to an increase in the activity of hormone-sensitive lipase, which catalyzes the mobilization of fatty acids from triacylglycerols stored in adipocytes (Ahmadian et al., 2007).

Wound healing is a process by which damaged tissue is restored as closely as possible to its normal stage and wound contraction is a process of shrinkage of an area of the wound. It depends upon the reparative abilities of the tissue, type and extent of the damage and general state of health of the tissue. The granulation tissue of the wound is generally composed of fibroblasts, collagen, edema and small new blood vessels (Singh et al., 2005). Since *B.hispida* enhanced wound contraction, it would have either enhanced the contractile property of myofibroblasts or increased the number of myofibroblasts recruited in the wound area. In the excision wound model, *B.hispida longifolia* fasten the period of epithelization. It appears that *B.hispida* was able to promote epithelization either by a proliferation of epithelial cells or by increasing the viability of epithelial cells.

CONCLUSION

The present study indicates the significant antidiabetic effect of the various extracts of leaves extracts of *Benincasa hispida* and supports its traditional usage in the control of diabetes. And also concluded that the leaves extracts of *B.hispida* has a strong effect on wound healing in albino rats. Further studies are required for the detailed studies

in isolation of the compounds and pharmacological investigations to explore the exact mechanism of action.

Conflict of Interest

The authors declare that they have no conflict of interest for this study.

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