Cardioprotective Efficacy of *Bacopa monniera* in Experimental Diabetes Mellitus: Biochemical and Histopathological Assessment

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**ABSTRACT**

**INTRODUCTION**: Herbs have been used as medicines since ancient times and it has been observed that human body is well suited to herbal remedies. In the present study, the myocardial salvaging effects of *Bacopa monniera* (L) Pennell (Scrophulariaceae) (BM), a medicinal herb was evaluated in diabetes mellitus.

**Materials and methods**: Type II diabetes mellitus was induced chemically in rats using streptozotocin (STZ) (65 mg/kg). Wistar rats were randomly allocated to sham, STZ control and BM treated groups. Lyophilized hydro-alcoholic extract of BM (75 mg/kg) was administered once a day orally to the rats for 21 days. On the 22nd day, biochemical parameters (fasting blood sugar and creatinine phosphokinase [(CPK), CPK-mB] and histopathological assessment of myocardium was undertaken to evaluate the cardioprotective efficacy of BM.

**Results**: Pretreatment of BM to experimental rats restored the raised fasting blood sugar levels, CPK, CPK-mB activity and preserved the histopathological architecture of pancreas, heart, liver and kidney as compared to the STZ control group. BM demonstrated significant cardioprotective effects in the experimental model of diabetic mellitus.

**Conclusion**: BM demonstrated significant myocardial salvaging effects in the presence of diabetes mellitus. Histopathological assessment of myocardium shows the cardioprotective effects of BM in the STZ model of diabetic mellitus.

**Keywords**: Cardioprotection, Medicinal plant, Streptozotocin, Antidiabetic, Antioxidants.


**Source of support**: Nil

**Conflict of interest**: None

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**INTRODUCTION**

Diabetes mellitus is a chronic metabolic disorder characterized by hyperglycemia, caused by insulin deficiency, often combined with insulin resistance. Oxidative stress due to increased production of free radicals and compromised antioxidant defense mechanisms is known to play a significant role in the pathogenesis of diabetes mellitus. Diabetes mellitus is an independent risk factor for cardiovascular disease and is associated with increased susceptibility to cardiovascular complications. The majority of diabetes-related deaths arise from cardiovascular complications, such as myocardial infarction, stroke and peripheral vascular disease.

Herbs have been used as medicines since ancient times and it has been observed that human body is well suited to herbal remedies. Therefore, the search for an ideal antidiabetic drug has been extended to medicinal herbs with antidiabetic as well other beneficial effects that may be useful to address the various macrovascular and microvascular complications of diabetes mellitus. These herbs if found effective, may be used as adjuncts to modern conventional medicines. *Bacopa monniera* (Bm), is commonly known as brahmi in Ayurveda, the Indian system of medicine. It has gained world-wide recognition as a memory booster and is used for the treatment of epilepsy and bronchial asthma. The whole plant is therapeutically used and the active ingredients bacosides are mainly responsible for its antioxidant, immunomodulatory and adaptogenic properties. The present study was undertaken to evaluate the cardioprotective activity of BM in the streptozotocin (STZ) model of diabetes mellitus. Further, following STZ induced injury, the safety profile of the medicinal herb was evaluated by studying the histopathological architecture of the liver, kidney, heart and pancreas.

**MATERIALS AND METHODS**

**Experimental Animals**

Adult male Wistar rats, 10 to 12 weeks old, weighing 150 to 200 gm were obtained from the Central Animal Facility of MGM Medical College, Navi Mumbai, India, and were...
maintained under standard laboratory conditions in the
department animal house. The study protocol was reviewed
and approved by the Institutional Animal Ethics Committee
and conforms to the Indian National Science Academy
Guidelines for the Use and Care of Experimental Animals
in Research. Rats were kept in polyacrylic cages (38 × 23 ×
15 cm) with not more than four animals per cage and housed
in an air-conditioned room and were kept under natural light
and dark cycles (approximately 14 hours light/10 hours dark)
with 60 ± 5% humidity and an ambient temperature of 25 ±
2°C. The animals were allowed free access to standard diet
(Anmut Laboratory Animal Feed, Maharashtra) and tap water
ad libitum and allowed to acclimatize for 1 week before the
experiments. Commercial pellet diet contained 24% protein,
5% fat, 4% fiber, 55% carbohydrates, 0.6% calcium, 0.3%
phosphorous, 10% moisture and 9% ash w/w. All chemicals
were of analytical grade. Streptozotocin was purchased from
HIMEDIA, Mumbai chemicals. Double distilled water was
used in all biochemical assays. Hydro-alcoholic lyophilized
extracts of leaves of Bacopa monniera was procured from
Dabur Research Foundation, New Delhi, India.

The animals were assigned to the following experimental
groups. There were ten animals in each group.

**Group 1: Sham Group-Sham Control**
Experimental rats were administered 0.9% normal saline
orally once a day for 21 days.

**Group 2: Streptozotocin Group-STZ Control**
The rats of this group were administered 0.9% normal saline
orally once a day for 21 days. Diabetes was induced to all
the rats of this group by a single dose of STZ (65 mg/kg)
intraperitoneally on day 0.

**Group 3: Treated Group-Bm-75**
* Bacopa monniera* 75 mg/kg body weight was orally fed
once in a day to healthy experimental animals for 21 days.
On day 0, diabetes induced to experimental rats by a single
dose of STZ (65 mg/kg, IP).

**Pilot Study**

**Dose Selection Study for Bacopa monniera**
Previous studies from our laboratory shows that a dose of
75 mg/kg of Bm treatment significantly prevented leakage
of myocardial enzyme CPK and preserved the myofiber
architecture as compared to the ISP induced myocardial
necrosis group. Hence, Bm (75 mg/kg) dose was selected
for further evaluation of its cardioprotective effects in the
presence of diabetes mellitus.9

**Streptozotocin Induced Diabetes Mellitus**
STZ is a chemical agent that is specifically cytotoxic to beta
cells of the pancreas. Diabetes was induced by a single STZ
injection (65 mg/kg body wt, i.p. dissolved in 0.01 M citrate
buffer, pH 4.5). Serum glucose was estimated (fasting blood
sugar > 200 mg/dl) periodically (day 0, 3, 7, 14, 21) from the
tail vein to confirm the status of diabetes mellitus.

**PARAMETERS EVALUATED IN THE
PRESENT STUDY**

**Estimation of Biochemical Parameters**
Blood glucose estimation was done on zero, 3rd, 7th,
14th and 21st day after administrating STZ (65 mg/kg,
intraperitoneally) to experimental rats using One Touch Basic
Blood Glucose Monitor (Omnitest). The rat blood samples
were collected from retro-orbital vein on the 22nd day. Serum
was separated by centrifugation at the speed of 300 rpm for
15 minutes for the estimation of creatine phosphokinase
(CPK) and CPK-MB. CPK-MB activity was estimated by
the method.10 Average body weight (gm/day) was recorded
every week and the change in body weight was calculated
after 21 days feeding. At the same time, mortality if any
during the 21 days of oral administration of the respective
drugs was also monitored.

**Histopathological Studies**
Myocardial, hepatic, renal and pancreatic tissue: at the
end of the experiment on the 22nd day the animals were
sacrificed. The myocardium, pancreas, liver and kidney were
immediately fixed in 10% buffered neutral formalin solution.
The tissues was carefully embedded in molten paraffin with
the help of metallic blocks, covered with flexible plastic
molds and kept under freezing plates to allow the paraffin
to solidify. Cross sections (5 µm thick) of the fixed tissues
were cut. These sections were stained with hematoxylin and
eosin and visualized under light microscope to study the light
microscopic architecture of the myocardium, pancreas, liver
and kidney. The investigators performing the histological
evaluation were blind to biochemical and hemodynamic
results and to treatment allocation.

**RESULTS**

**Fasting Blood Glucose (FBS) Levels**
The FBS levels in rats of different experimental groups are
depicted in Graph 1. Blood glucose levels in sham group rats
were found to 80 ± 6.9, 78.1 ± 6.7, 79.4 ± 6.98, 77.8 ± 5.8
and 78.6 ± 6.28 mg/dl at 0, 3, 7, 14, 21 days respectively.
Following STZ injection, in the control group rats, blood
glucose levels, were elevated from 80.25 ± 7.72 (0 day)
to 278.75 ± 33.38, 262 ± 26.57, 233.5 ± 9.48 and 226.5 ± 32.02 mg/dl at 0, 3, 7, 14, 21 days respectively. Oral feeding of Bm (75 mg/kg) for 21 days restored the elevated blood glucose levels as compared to the STZ control group. The values were found to be 84 ± 2.82, 151.25 ± 24.748, 149.25 ± 17.367, 130.13 ± 5.514 and 125.1 ± 4.65 mg/dl at 0, 3, 7, 14, 21 days respectively.

**CPK Activity**

In the sham group, the basal levels of CPK were found to be 1779.85 ± 57.57 IU. Following STZ challenge, in the control group rats, CPK was found to be 3483.61 ± 185.4 IU. Treatment with Bm (75 mg/kg), prevented this significant rise in CPK values. Values were reduced to 2800.95 ± 164.7 IU.

**CPK-MB Activity**

In the sham group, a basal level of CPK-MB was found to be 158.4 ± 6.9 IU which increased to 245.7 ± 14.1 IU in the control group, following STZ challenge to rats. Treatment with Bm prevented this significant rise in CPK-MB values. Values reduced to 200.9 ± 18.8 IU.

**Body Weight Gain**

Weight of sham group rats was found to be 187.5 ± 32.73, 198.37 ± 33.16, 215.75 ± 41.73 and 230.625 ± 33.95 gm at 0, 3, 7, 14, 21 days respectively. In the STZ control group rats, weights recorded were significantly less (200 ± 35.25, 176.25 ± 35.53, 156.25 ± 35.12 and 135.62 ± 33.32 gm) respectively at 0, 3, 7, 14, 21 days as compared to sham group. Following oral administration of Bm (75 mg/kg), significant gain in body weight was seen as compared to the STZ control group. The values were found to be 195 ± 24.92, 178.625 ± 30.88, 183.75 ± 34.61 and 200 ± 34.121 gm at 0, 3, 7, 14, 21 days respectively.

**HISTOPATHOLOGICAL ASSESSMENT**

**Heart**

Histopathological assessment of the sham group rat hearts revealed the noninfarcted architecture of the myocardium (Fig. 1A). In contrast, rats subjected to STZ injury, demonstrated marked edema, confluent areas of myonecrosis, separation of myofibrils, congested blood vessels and mild inflammation as compared to the sham group (Fig. 1B). In the Bm treatment group rats, occasional focal myofiber loss, necrosis, edema and inflammation was observed. However, the degree of edema, inflammation and necrosis was less as compared to the STZ control group (Fig. 1C).

**Pancreas**

The pancreas of the sham group were characterized by an organized pattern and showed normal architecture of the islets of Langerhans and the beta cells (Fig. 2A). In contrast, in the STZ control group damaged islets of Langerhans and the beta cells with loss of few nucleus and cytoplasm was observed (Fig. 2B). In the Bm treatment group, few nuclear changes were observed though less compared to STZ-control group (Fig. 2C).
Liver

Histopathological assessment of liver in sham group showed the normal architecture of central veins, periportal veins and hepatocytes (Fig. 3A). In contrast to the sham group, the STZ control group showed degeneration and scattered necrotic cells, multifocal mild degree portal lymphocytic infiltration, swollen cytoplasmic hydropic and microvesicular vacuoles (Fig. 3B). However, treatment with Bm decreased the periportal inflammation and hepatocytes degeneration in diabetic liver as compared to STZ control group. Also no significant congestion in central veins was observed (Fig. 3C).

Kidney

Histopathology of kidney in sham group showed the normal structure of the kidneys. There was absence of congestion of glomerular blood vessels, tubular necrosis, inflammation, cloudy degeneration (Fig. 4A). In contrast, on histological evaluation, rat’s kidney of the STZ control group showed congestion of glomerular blood vessels, tubular necrosis, inflammation and cloudy degeneration as compared to the sham group (Fig. 4B). The treatment with Bm showed the congestion of glomerular blood vessels, tubular necrosis, inflammation, cloudy degeneration but it was less as compared to the STZ control group (Fig. 4C).

DISCUSSION

Diabetes mellitus, a metabolic disorder, is characterized by hyperglycemia, altered metabolism of lipids, carbohydrates and proteins with an increased risk of complication of vascular diseases. Type 2 diabetes mellitus is an independent risk factor associated with increased susceptibility to cardiovascular complications, such as ischemic heart disease, myocardial infarction, stroke and peripheral vascular disease.

A multifactorial approach for the management of diabetes mellitus addressing its microvascular and macrovascular complications is the need of the hour. There is a large subset of diabetic patients with underlying cardiovascular morbidity. Therefore an anti-diabetic drug with additional therapeutic benefits against cardiovascular complications may be beneficial in this group of diabetic patients.

Medicinal plants have been observed to possess numerous activities such as hypolipidemic, antiplatelet, antithrombotic, cardioprotective, anticoagulant and hypoglycemic
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Figs 4A to C: (A) Sham group showed that normal structure of the kidneys. There was no congestion of glomerular blood vessels, tubular necrosis, inflammation, cloudy degeneration, (B) contrastively, on histological evaluation, rats kidney of the STZ control group showed congestion of glomerular blood vessels, tubular necrosis, inflammation and cloudy degeneration as compared to the sham group and (C) the treatment with Bm showed the congestion of glomerular blood vessels, tubular necrosis, inflammation, cloudy degeneration but it was less as compared to the STZ control group

activities with regard to addressing the macrovascular and microvascular complications of diabetes mellitus.9 Thus, the use of plant derived agents represents an important and novel therapeutic target and strategy for the treatment of diabetes with coexisting complications. With this point of view, the study was designed.

STZ is a chemical agent that is specifically cytotoxic to beta-cells of the pancreas. STZ induces partial destruction of beta cells within 24 hours, thereby triggering an inflammatory process leading to macrophage and subsequent lymphocyte infiltration, which is followed by the onset of insulin deficiency results in hyperglycemia rather than insulin resistance. Several studies have demonstrated that STZ (65 mg/kg) induces diabetes mellitus in experimental rats.13 The present study results concur with the previous studies by various authors.14 Diabetes was induced by a single intraperitoneal STZ injection (65 mg/kg body wt). Following STZ injection, a raised fasting blood glucose levels were observed 72 hours after the injection and maintained throughout the experimental duration; confirming the presence of diabetes in the STZ control group. In the Bm (75 mg/kg) treated group, a significant hypoglycemic activity as indicated by a decline in the fasting blood glucose levels was seen as compared to the STZ control group. A negative correlation between rise in fasting blood sugar and gain in body weight was observed in the experimental groups.

Diabetes also induced adverse cardiovascular changes in the rats as indicated by an elevated serum CPK activity of STZ control rats. The leakage of CPK from the myocardium tissue with concomitant rise in the plasma is considered as a marker of myocardial injury.15,16 Leakage of this enzyme is a strong evidence for loss of sarcolemmal integrity due to STZ induced injury to the myocardial cells. In the STZ control group, a significant elevation in the serum CPK, CPK-MB and histopathological assessment of the myocardium confirmed the myocardial injury.

The antihyperglycemic activity of Bm was observed in the STZ model of diabetes mellitus. In addition to its beneficial effect on serum blood glucose, the cardioprotective effect of Bm was also evaluated in the present study. Several authors have previously documented the cardioprotective effects of Bm.9,17,18 Nandave et al 200717 demonstrated that the lyophilized hydro-alcoholic extract obtained from Bm, at doses of 100, 150 and 200 mg/kg, provides significant cardioprotection against ISP-induced myocardial necrosis in rats. Bm (150 mg/kg) produced maximum cardioprotection as evidenced by significant restoration of endogenous antioxidants, CK-MB isoenzyme activities and decrease in malonaldehyde levels. The cardioprotective effects of Bm at the doses 25, 75 and 150 mg/kg in the ISP model of myocardial necrosis was studied previously in our laboratory.9,19

However, this is the first study further exploring the cardioprotective effects of Bm in the presence of diabetes mellitus. In the present study, Bm (75 mg/kg) treatment significantly preserved the myocardial enzyme CPK and myocardial architecture as compared to the STZ control group. Biochemical and histopathological evaluation confirmed the myocardial salvaging effects of Bm (75 mg/kg) in the presence of diabetes mellitus. In addition to the favorable effect of Bm on the myocardial-architecture, Bm treatment also preserved the histopathological structure of the hepatic, renal and pancreatic tissue. Therefore, Bm (75 mg/kg) is a safe efficacious cardioprotective medicinal herb.

Nature has been a source of medicines for thousands of years and plant-derived products continue to play an essential role in the primary healthcare of the world’s population.20 Bm, a medicinal herb traditionally used have been evaluated scientifically in the present study with an aim to define the role of these agents in limiting the deleterious effects of STZ induced diabetes mellitus. The study provides preliminary scientific data to further evaluate Bm as a
promising antidiabetic herb with potential cardiovascular benefits.

CONCLUSION

Bm demonstrated significant myocardial salvaging effects in the presence of diabetes mellitus. Histopathological assessment of the myocardium and CPK levels confirmed the cardioprotective effects of Bm (75 mg/kg) in the STZ model of diabetes mellitus. In addition, Bm (75 mg/kg) pretreatment was safe to the vital organs as it preserved the histopathological structure of the hepatic, renal and pancreatic tissues.

REFERENCES