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Effect of an Isolated Compound (BM-1) from *Bacopa Monnieri* (L.) Wettst. Leaves on Serum Lipids in Normal and Diabetic Rats

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Abstract
An active compound (BM-1) was isolated from leaves of *Bacopa monnieri* (L.) Wettst. It was given orally in low (10 mg/kg) and high (50 mg/kg) doses to normal and alloxan-induced diabetic rats for two weeks. Serum lipids like cholesterol, triglycerides, HDL, LDL and VLDL were estimated. Results showed that the active compound (BM-1) produced a significant fall in serum cholesterol, triglycerides, LDL and VLDL in normal rats. In diabetic rat BM-1 also decreased the raised levels of serum cholesterol, triglycerides, LDL and VLDL but increased HDL cholesterol. BM-1, thus, may be used to treat hyperlipidemia in diabetics.

Keywords: *Bacopa monnieri* (L.) Wettst, serum lipids, alloxan, diabetes, isolated compound (BM-1)

Introduction
Bacopa monnieri (L.) Wettst. (family : Scrophulariaceae) is a perennial creeping herb, grows in wet places and under shade. It is distributed throughout India generally reared up to 5000 ft. The plant is known as 'Bramhisak' in Bengali. In Hindi it is called 'Brahmi' and in Sanskrit as 'Bharati'. 'Water hyssop' is the English name of the plant. October and December are the flowering and fruiting times of *Bacopa monnieri* (L.) Wettst respectively.

Bacopa monnieri (L.) Wettst. is a well-known plant in Ayurveda. The plant is bitter and pungent in taste. It has heating, emetive, laxative, aphrodisiac, diuretic and eperient properties used in curing diseases such as ulcers, tumours, ascities, enlargement of spleen, indigestion,

inflammation, fever, diarrhoea etc. Hysteria cases are cured by the use of this plant and are also found to be maturant and expectorant. The plant is also used as nervine tonic useful in insanity, epilepsy and hoarseness. The plant has further been described as cardiac tonic also used for dermatosis, anaemia and diabetes [1,2].

In modern research, *Bacopa monnieri* (L.) Wettst. is said to have capacity of improving intellectual activity [3] and memory capacity [4]. It also enhances immune function [5] and is useful in the treatment of epilepsy and asthama [6]. *Bacopa monnieri's* extract has also been shown to impact the oxidative stress cascade by scavenging reactive oxygen species, inhibiting lipoxygenase activity and reducing divalent metals [7]. Many active compounds like alkaloid, saponins, flavonoids, sterols etc. are the ingredients of *Bacopa monnieri* (L.) Wettst.

With this in view, we undertook studies on isolation of active compound(s) from *Bacopa monnieri* (L.) Wettst. as our laboratory is engaged in the research work of medicinal plants of Darjeeling and Sikkim Himalayas[8 – 18].We had isolated an active compound (BM-1) from *Bacopa monnieri* (L.) Wettst. and was interested to see whether the compound has any beneficial effect on serum lipids in normal and diabetic rats.

Materials and Methods

Collection of *Bacopa monnieri* (L.) Wettst leaves

Bacopa monnieri (L.) Wettst leaves were collected from the garden of medicinal plants of the University of North Bengal during September, 2012 and identified by Prof. A.P. Das of the department of Botany, University of North Bengal. A voucher specimen was kept in the department for future reference.

Bacopa monnieri (L.) Wettst

Isolation of the active compound (BM-1) from leaves of *Bacopa monnieri* (L.) Wettst

Following steps were done :

1. Fresh plant leaves were dried under sun and grounded into fine powder.
2. 60g of this powder was then extracted with 300 ml of water - chloroform mixture (1:1,V/V) for 1 hour using the soxhlet apparatus at a temperature of 37^0C.
3. The extract was concentrated under reduced pressure using a rotary evaporator to a volume of 10 ml **. 2**
4. This was then subjected to column chromatography using silicic acid as adsorbent. Elution was done by 50% ethanol-chloroform mixture.
5. Eluted material of the second fraction was evaporated to dryness and extracted with 10 ml chloroform.
6. The chloroform extract was further subjected to column chromatography using silica gel G mesh (200-400 size) as adsorbent.
7. The third fraction obtained after elution with chloroform was subjected to repeated crystallization when a compound was crystallized.

The compound was given a trivial name BM-1. The compound was preserved for acute toxicity study as well as for its effect on serum lipid in normal and diabetic rats, if any.

Test drug

Isolated compound (BM-1) was used as the test drug.

Chemicals

Alloxan monohydrate was procured from Sigma Ltd. USA. All other chemicals used were of analytical grade.

Experimental animals

Wister strain rats of either sex, body weight between 150g and 180g, were used for this study. Animals were housed individually in polypropylene cages, maintained under standard conditions e.g. 12h light and 12h dark cycle, 20-30 degree centigrade, 35-60 % humidity. Rats were fed with standard rat pellet diet (Hindustan Lever Ltd., Mumbai, India) and provided water *ad libitum*. Rats were divided into two groups of 30 each. First group was normal while the second group was diabetic. The animals were made diabetic by a single intraperitoneal injection of alloxan monohydrate 5% w/v in normal saline in a dose of 150mg/kg body weight after overnight fasting by the method of Dheer and Bhatnagar [19]. Rats with blood glucose level higher than three times the normal after three days were included in this study. Both the two groups were further subdivided into three groups of 10 animals each.

In normal as well as in diabetic rats the first sub group was treated as control while the second and third sub groups were treated as test. Animals of these two test groups received BM-1 once daily for two weeks in the dose of 10mg/kg and 50 mg/kg respectively through oral route by using a feeding tube in addition to normal diet and water.

Estimation of serum lipids

After overnight fast, samples of blood from rats were withdrawn through cardiac puncture under pentobarbitone sodium (40mg/kg, i.m.) anaesthesia. Blood samples, taken before and after 2

weeks of BM-1 treatment , were estimated for total serum cholesterol , serum triglycerides , high density lipoprotein as well as for low density and very low density lipoproteins by the methods of Jasmin & Daisy [20] and Banz *et al*., [21].

Acute oral toxicity study

This was done by the method of Ghosh M N [22].

Acute toxicity studies were carried out on Swiss albino mice. Isolated compound (BM-1) from *Bacopa monnieri* (L.) Wettst. leaves was given orally at doses of 10, 50, 100 and 200 mg/kg to five groups of mice, each group containing six animals. After administration of the compound, the animals were observed for the first three hours for any toxic symptoms followed by observation at regular intervals for 24 hours up to two weeks. At the end of the study, the animals were also observed for general organ toxicity, morphological behavior and mortality.

Statistical analysis

The values were expressed as mean ± SEM and were analyzed using one-way analysis of variance (ANOVA) using Statistical Package for Social Sciences (SPSS). Differences between means were tested employing Duncan's multiple comparison test and significance was set at $p < 0.05$.

Clearance from ethics committee

This work has clearance of ethics committee of the North Bengal Medical College.

Results and Conclusion

Acute toxicity studies

Acute toxicity studies revealed that BM-1 did not produce any toxic symptoms when administered orally to mice in doses of 10, 50, 100 and 200 mg/kg. Animals were healthy, cheerful and behaved normal throughout the experimental period. No death of animal was recorded during two weeks of experiment.

Effect of BM-1 on serum lipid levels in normal rats

Results are given in Table-1. No significant change in serum lipid profile was observed in control group of rats after 2 weeks. There was significant fall ($p < 0.05$) in serum total cholesterol, triglycerides, LDL and VLDL cholesterol after two weeks of BM-1 (10mg/kg) treatment. The decrease was more significant ($p<0.001$) in higher dose of BM-1 (50mg/kg). Concentration of HDL cholesterol, however, did not show any change in control as well as in BM-1 treated groups.

Effect of BM-1 on serum lipid levels in diabetic rats

Results are given in Table – 2. Significant increase ($p<0.05$) in serum total cholesterol, triglycerides, LDL and VLDL cholesterol and significant decrease ($p<0.05$) in HDL cholesterol were observed after two weeks in diabetic rats.

BM-1 (10mg/kg) treatment caused significant fall ($p<0.05$) in serum total cholesterol, triglycerides, LDL and VLDL cholesterol and significant rise ($p<0.05$) in HDL cholesterol after two weeks. The corresponding decrease and increase were more significant ($p< 0.001$) in higher

dose of BM-1 (50mg/kg).

Our results showed that the compound BM-1 collected from *Bacopa monnieri* (L.) Wettst. leaves had hypolipidemic effects. It lowered total serum cholesterol, triglycerides, LDL and VLDL cholesterols in normal rats. The effect was dose dependant. BM-1 in the dose of 10mg/kg could lower total serum cholesterol, triglycerides, LDL and VLDL cholesterols by 23.1%, 20.2%, 32.9% and 40.4% respectively while in the dose of 50mg/kg the figures were 40%, 35.8%, 44.9% and 50.0% respectively after two weeks of treatment. Thus BM-1 isolated from *Bacopa monnieri* (L.) Wettst. may have possible use in treating hyperlipidemia in normal individuals.

HDL cholesterol in normal rats, however, did not change significantly after two weeks of treatment by BM-1 though an increasing trend was noticed.

It is known that there is often hyperlipidemia in diabetes [23]. Total serum cholesterol, triglycerides, LDL and VLDL cholesterols are increased in diabetes. In the present study we also observed hyperlipidemia in diabetic rats. Total serum cholesterol, triglycerides, LDL and VLDL cholesterols were increased significantly during diabetes. Serum HDL cholesterol, however, was found significantly decreased in diabetic rats. This is in confirmation of the earlier findings of Gupta *et al*.[24].

Treatment with BM-1 collected from *Bacopa monnieri* (L.) Wettst. leaves had hypolipidemic effects in diabetic rats. It lowered total serum cholesterol, triglycerides, LDL and VLDL cholesterols significantly ($p<0.05$) when compared to the control values in diabetic rats after two weeks of diabetes. BM-1 in the dose of 50 mg/dl could lower total serum cholesterol, triglycerides, LDL and VLDL cholesterols almost to the normal values. Serum HDL cholesterol was found significantly increased in diabetic rats after two weeks of treatment with BM-1.The effect was found dose dependant. BM-1 in the dose of 10mg/kg could increase HDL cholesterol by 46.8% while in the dose of 50mg/kg the increase was 56.4%.

From the present result it may be concluded that total serum cholesterol, triglycerides, LDL and VLDL cholesterols which are increased in diabetes may be lowered significantly and HDL cholesterol which was decreased in diabetes may be increased significantly by the treatment of BM-1 collected from *Bacopa monnieri* (L.) Wettst. leaves.

Diabetes mellitus is the most important non-infective epidemic to hit the globe in the present millennium. By the year 2025, India shall have the maximum number of diabetics in the world making it the "Diabetic capital of the world" [25]. Despite the great strides, made in understanding and management of diabetes, the disease and disease-related complications are increasing unabated due to multiple defects in its pathophysiology [26].

Diabetes is often associated with hyperlipidemia. Simpson *et al*.,[27] have shown increase in serum triglycerides and LDL cholesterol during diabetes. Hyperlipidemia has association with atherosclerosis [28]. Atherosclerosis, in turn, could take life of a diabetic. As the present study showed hypolipidemic activity of BM-1 collected from *Bacopa monnieri* (L.) Wettst. leaves, BM-1 may be used to treat hyperlipidemia in diabetics. Further, BM-1 increased serum HDL cholesterol markedly which was significantly decreased during diabetes. It is to be remembered that HDL cholesterol has protective effect for atherosclerosis [24, 29].

Conclusion

An active compound (BM-1) was isolated from leaves of *Bacopa monnieri* (L.) Wettst. Compound (BM-1) produced a significant fall in serum cholesterol, triglycerides, LDL and VLDL in normal rats. In diabetic rats compound (BM-1) also decreased the raised levels of serum cholesterol, triglycerides, LDL and VLDL but increased HDL cholesterol. Comppound (BM-1), thus, may be used to treat hyperlipidemia in diabetics.

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Table 1- Effect of BM-1 on serum lipid levels (mg%) in normal rats

Group	**Total cholesterol**	**Triglycerides**	**LDL cholesterol**	**HDL cholesterol**	**VLDL cholesterol**
Control					
Before	100.3 ± 6.65	77.2 ± 3.76	48.0 ± 4.67	43.7 ± 4.87	16.2 ± 0.65
After2 weeks	101.0 ± 5.89	76.1 ± 3.65	47.8 ± 4.78	43.6± 3.45	16.5 ± 0.66
BM–1 (10mg/kg)					
Before	102.6± 5.45	76.2 ± 3.45	45.5 ± 3.23	42.6 ± 3.34	17.8 ± 0.66
After2 weeks	78.8± 3.23*	60.8 ± 2.87*	30.5 ± 3.12*	44.2 ± 3.23	10.6 ± 0.34*
BM–1 (50mg/kg)					
Before	101.5± 5.25	78.1 ± 4.51	46.5 ± 3.13	42.3 ± 4.12	17.2 ± 0.51
After2 weeks	60.8± 3.73**	50.1 ± 2.66**	25.8± 3.04**	44.1 ± 5.33	8.6 ± 0.33**

Values are mean SEM, *P<0.05, **P<0.001, Number of rats used in each group : 10

Table 2 - Effect of BM-1 on serum lipid levels (mg%) in diabetic rats

Group	Total cholesterol	Triglycerides	LDL cholesterol	HDL cholesterol	VLDL cholesterol
Control Before	98.3 ± 3.13	72.8 ± 4.11	44.0 ± 3.24	46.1 ± 4.23	18.2 ± 1.66
After2 weeks	131.3± 5.23**	109.9± 5.23**	67.8± 4.18**	30.1± 3.22*	26.1± 2.69*
BM–1 (10mg/kg) Before	102.5± 4.22	76.2 ± 3.45	48.0 ± 3.12	46.1 ± 3.12	18.8 ± 0.62
After2 weeks	111.1± 3.27*	89.1 ± 2.87*	50.1 ± 3.42*	44.2 ± 2.23*	20.8 ± 0.24*
BM–1 (50mg/kg) Before	102.4± 5.32	78.7 ± 4.11	45.5 ± 3.23	47.3 ± 4.62	18.7 ± 0.55
After2 weeks	102.2± 4.78**	78.1 ± 3.32**	45.8± 3.14**	47.1± 2.43**	18.0± 0.43**

alues are mean SEM, *P<0.05, **P<0.001, Number of rats used in each group : 10. Results were compared with the control values after two weeks of diabetes.

Authors Column

Prof. (Dr.) Prasanta Kumar Mitra is a very senior medical teacher and researcher. He has completed thirty six years in medical teaching and about forty years in research. His research area is 'Medicinal plants of India'. He has four Ph.D.s to his credit and published one hundred two research papers in national and international journals. Fifteen students did their Ph.D. work under his guidance. He was co-supervisor of the research projects of five MD students.
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Prof. Mitra is a well known writer, science popularizer. He wrote more than fifteen hundred popular science articles in different newspapers / magazines. He is the recipient of Rajiv Gandhi Excellence award for his academic excellence and outstanding contribution in the field of popularization of science in society.