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BIOCHEMICAL & PHARMACOLOGICAL INVESTIGATION OF BAUHINIA VARIEGATAON FRUCTOSE INDUCED METABOLIC SYNDROME IN RATS

Seema Gupta [1], Gazala Parveen [2], Rajesh Ramesh Patil [3], B. Sanjeeb Kumar Patro [4], Shilpy Shakya [5], Sonam Bhutia [6], Ramenani Hari Babu [7], Pulipati Sowjanya [8]*

1. Professor, R. R. College of Pharmacy, Chikkabanavara, Hesaraghatta main road, Bangalore 560090
2. Professor, College of Pharmacy, Knowledge University Erbil, Kurdistan Region of Iraq-446015
3. HEAD, Pimpri Chichwad University, School Of Pharmacy, PCET's Pimpri Chinchwad University, School of Pharmacy, Gut No. 44, 46, 48, 49 and 50, Sate, Maval (PMRDA) Dist. – Pune-412106, Maharashtra, India.
4. Professor, Department of Pharmacology College of Pharmaceutical Sciences, Brahmapur
5. Assistant Professor, Department of Zoology, Government Post Graduate College, Fatehabad, Agra 283111
6. Assistant Professor, Government Pharmacy College Sajong, Government of Sikkim, Sikkim University, Rumtek Sajong,Sikkim,India-737135
7. Professor, M B School of Pharmaceutical Sciences, Mohan Babu University, Tirupati
8. Professor, Vignan Pharmacy College, Vadlamudi, Guntur (Dt), 522213

Corresponding Author: Dr. Pulipati Sowjanya
Affiliation and Designation: Professor, Vignan Pharmacy College, Vadlamudi, Guntur (Dt), 522213
Email Id: sowjypulipati@gmail.com

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ABSTRACT:
Widespread metabolic syndrome (MBS) is closely linked to poor diet and insufficient exercise, which can lead to more severe disorders like obesity, cardiovascular disease, and type 2 diabetes mellitus. The purpose of this study was to investigate the potential mechanisms underlying the therapeutic effects of Bauhinia variegata extract against a few metabolic and hepatic symptoms seen in MBS rats. Rats were fed a high-fructose diet for 12 weeks in order to induce MBS. For four weeks, BVE (50 mg/kg) was given orally to rats that were MBS-free and normal. The liver index and the hepatic expression of glucose transporter 2 (GLUT2) were measured using liver tissues. Additionally measured were the systolic blood pressure, metabolic parameters, oxidative stress indicators, and the fat/muscle ratio. Our findings shown that giving fructose diet rats BVE dramatically lowered their high systolic blood pressure. Additionally, the alterations in serum insulin, blood glucose, serum lipid profile, and oxidative stress markers were roughly restored to normal levels. Furthermore, BVE therapy reduced blood liver enzyme activity, fat/muscle ratio, and liver index. Here, we demonstrated that, in the experimental model, Bauhinia variegata had a superb effect against metabolic diseases.
KEYWODS: Metabolic syndrome (MS), Bauhinia variegate, Liver, Fructose

1. INTRODUCTION

Gerald Reaven identified metabolic syndrome (MS), also known as insulin resistance syndrome, as the primary global metabolic condition that poses a threat to human health (1, 2). Actually, between 20 and 30 percent of people on the planet may have MS (3). Many animal species, including dogs (4), cattle (5), laboratory animals (6), and horses (7), may also be affected. The primary characteristics of MS include obesity, insulin resistance, hyperglycemia, dyslipidemia, and hypertension (6). Since the pathophysiology of MS was not fully understood, several theories were put forth. The important one is that diet, lifestyle, and genetics all interact to cause multiple sclerosis (MS); yet, insulin resistance continues to be the primary cause of this illness (9, 10). Due to the negative energy balance caused by the high mass of adipose tissue in MS patients, a significant amount of lipids, especially free fatty acids (FFAs), were released into the bloodstream, which inhibited insulin-mediated glucose uptake and caused the muscles to respond negatively to insulin. Thus, hyperglycemia stimulates the pancreatic beta cells to secrete more insulin, leading to hyperinsulinemia (11). Conversely, because insulin primarily functions to suppress lipolysis, insulin resistance and increased blood levels of FFAs hinder glycogenesis and lead to increased levels of hyperglycemia and hypertriacylglycerolemia. Once more, insulin resistance causes increased lipolysis, which produces more FFAs and advances the harmful cycle (10).

According to Kennedy et al. (2010), a crucial genetic model for multiple sclerosis (MS) caused by spontaneous mutation is the leptin-deficient mouse (Lepob/ob). These days, mice can be used to intentionally induce it. In contrast, Bezerra and Oliveira (2013) have shown

that mature adipocytes secrete higher levels of proinflammatory cytokines, such as interleukin-6 (IL-6), tumour necrosis factor alpha (TNF-alpha), leptin, and adiponectin when obesity is present. Adipokines are cytokines that are delivered from adipose tissues and have been linked to insulin resistance. Hepatic fatty acid production and lipolysis are facilitated by TNF-alpha and IL-6. (12) By adversely interacting directly with insulin receptors, they are also increasing insulin resistance. Recent proteomic analysis of specific liver proteins, such as fatty acid synthase, 1-pyruvate dehydrogenase, fructose 1, 6-bisphosphatase, and Acyl-CoA synthetase 1, has revealed varied variations in the metabolism of lipids and carbohydrates, as well as associated pathways (13). In actuality, MS is not fully known and requires much more research (10), i.e., the evaluation of the biochemical alterations associated with experimentally produced MS in rats as a model for this illness has received relatively little attention. Actually, the purpose of this effort is to demonstrate the associated metabolic profiles and induce MS in rats in a straightforward manner.

In Ayurvedic literature, thousands of herbal plants and their medicinal properties have been documented. BV is a member of the Leguminosae family, which is widely distributed throughout India and to high altitudes in the Himalayas. It is grown in tropical and warm climates worldwide (14). Lupeol, caempferol-3-glucoside, beta-sitosterol, and 5.7 dihydroxy and 5.7 dimethoxy flavanone-4-O-a-L rhamnopyrosyl-β-D-glycopyranosides are all produced by BV stem bark (15). There was a lot of interest in the possible health benefits of BV and its active phytochemicals due to their biological activities, which included hepatoprotective (16), anti-inflammatory (17), antioxidant (18, 19), nephroprotective (20), and cardioprotective (21), as well as their ability to enhance insulin release, hypoglycemic and antidiabetic activity (22, 23). Due to the significant side effects of traditional drugs used in various complications, the focus has now shifted to an herbal and medicinal plant-based therapeutic approach, with this objective being investigated in the present study wasBauhinia variegata on fructose induced metabolic syndrome in rats.

MATERIALS AND METHODS:

Preparation of plant extract: B. variegata L. stem bark were freshlycollected from …….. then dried in an incubator at 50°Cfor 72 hours. The dried stem bark was consequently crushed intopowdered materials using an electric blender then percolated withdifferent solvents (water,ethanol, methanol, and ethyl acetate)according to the protocol at room temperature for 3 days. The residues were obtainedthrough evaporating the different extracts in oven at 37-40°C. (24)

Drugs and Chemicals: Fructose was purchased from Sigma-Aldrich Chemical Co. All other chemicals and solvents were ofthe highest grade commercially available. Bauhinia variegata was freshly preparedin a 0.5% aqueous solution of carboxymethyl cellulose.

Animals and experimental design: The Basic & Clinical Pharmacology & Toxicology (BCPT) policy for experimental and clinical investigations was followed in the conduct of the study [19]. Eight weeks old, adult male Wistar rats weighing between 180 and 200 grammes were taken from the animal house…….. Rats were housed in stainlesssteel cages with free access to water and standard laboratory foodand left to acclimatizefor a week before the study under controlled laboratoryconditions of normal light/dark cycle and temperature. The studyprotocol was approved by the Pharmacology and Toxicology Department,……..

Rats were randomly assigned in four groups of 6 animalseach. The first group was fed standard chow diet and served asa control group. Animals in the second group were fed a normaldiet throughout the study and received BVE (50 mg/kg/day, orally) in the last four weeks, and served as a control BVEgroup.Animals in the third and fourth groups were fed high fructosediet, then started administration of BVE in for four weeks asfirst and second groups. For the fructose diet, we used an adapteddiet standardized by Botezelli et al. (25) composed of 60% fructose(W/W) in diet for 12 weeks. The control group received thevehicle (0.5% aqueous solution of carboxymethyl cellulose). Thelast group was given BVE (50 mg/kg/day, orally) (26).

Determination of total polyphenols and antioxidant activity: The biological efficiency was evaluated throughquantifying the total polyphenolic compounds, total antioxidant capacity, andtotal reducing power in addition to percentage ofthe antioxidant activity that was assayed using 2,2-diphenyl-1-picryl-hydrazyl-hydrate (DPPH) as free radicals initiator in the different plant extracts to select themost suitable and effective one. (27)

Measurement of blood pressure by non-invasivemethod: Systolic blood pressure (SBP) was determined at the start ofthe study and every 2 weeks during the induction of MBS andthen, weekly during BVE treatment by a tail-cuff method withan automatic sphygmomanometer.

Blood and Tissue Samples: Rats were exposed to light anesthesia by diethyl ether andblood samples were collected from retro-orbital sinus veins viaglass capillaries before (0 time) and at 30, 60, 90, and 120 minafter oral glucose loading (2 g/kg). Glucose concentration wasdetermined with an automatic blood glucose meterfor the determination of oral glucose tolerancetest (OGTT).On the second day, rats were weighed, and then overnightfasted rats were sacrificed, then blood samples were collectedfrom neck vessels by decapitation. Clear sera were separated andkept at –80°C until an assessment of various parameters. Theliver was rapidly dissected out, blotted dry, weighed, and then theliver index was calculated according to the formula: (weight/bodyweight) × 100. Liver tissues were divided into two parts. The firstpart was put in 10% formalin for histopathology examination,while the second part was kept at –80°C for biochemical analysis.

Effect of BVE on metabolic syndrome complications in rats: The visceral fat pads and the gastrocnemius muscles were excised,blotted dry and weighed and the ratio of visceral fat to thegastrocnemius muscle (g/g) was considered as an index of body/muscle ratio (28).

Biochemical measurements: Serum alanine aminotransferase (ALT) and aspartate aminotransferase(AST) activities were determined by using availablecommercial kits (Bio-diagnostic, Cairo, Egypt). Fasting bloodglucose level was measured using an automatic blood glucose meter(Super Glucocard) and fasting serum insulin was determinedusing ultrasensitive rat insulin ELISA kit (Bio-Source, EuropeS.A., Nivelles, Belgium). Insulin resistance was determined usingthe homeostasis model assessment index for insulin resistance(HOMA-IR) utilizing the following formula: HOMA-IR index =[fasting glucose (mg/dl) × fasting insulin (□U/ml)/405] accordingto Matthews et al. (29)

Serum total cholesterol (TC), high-densitylipoprotein cholesterol (HDL-C) and triglycerides (TG) weredetermined using commercially available colorimetric kits (Biodiagnostic,Cairo, Egypt). Serum level of low density lipoproteincholesterol (LDL-C) was calculated using the formula that wasreported by Friedewald et al. (30)

Histopathology examination: Liver specimens were fixed in 10% formalin and processed forparaffin sections of 4 µm thickness. Sections were stained withhematoxylin and eosin for routine histopathological examination.All histological examinations were performed by an experiencedpathologist who was blinded to the experimental groups.

Statistical analysis: Results were expressed as means ± standard error of the mean(SEM) and were analyzed for statistically significant differencesusing one-way analysis of variance (ANOVA) followed by theTukey-Kramer post-analysis test to compare all groups. P-values less than 0.05 were considered significant. (31)

RESULTS AND DISCUSSION

Selection of the most effective B. variegata extract: As revealed in Table 1, it was found that the ethanolic B. variegata extract was the most effective extract noticed with the highest concentration of total polyphenols (745.76 ± 3.78 mg gallic acid/100 g), total antioxidant capacity (6.12 ± 0.34 mg gallic acid/g), iron reducing power (13.67 ± 0.38 µg/mL), and free radical scavenging activity (78%).

Table 1. Polyphenol concentration, total antioxidant capacity and free radical scavenging activity in different B. variegata extracts.

Solvent	Polyphenol(mg gallic acid/100gm)	Totalantioxidant capacity(mg gallic/gm)	Reducing power (µg/mL)	Antioxidant activity(%)
Methanol	725.89 ± 4.87	4.03 ± 0.068	10.67 ± 1.29	73.1 %
Ethanol*	745.76 ± 3.78	6.12 ± 0.34	13.67 ± 0.38	79%
Ethyl acetate	719.61 ± 4.18	1.71 ± 0.03	8.99 ± 0.54	65%
Water	623.61 ± 8.62	0.76 ± 0.01	9.56 ± 1.23	75%

Values expressed as mean ± SE of four replicates, *: The most effective extract with respect to the others.

Effect of BE on fat/muscle ratio, liver index and liver enzyme activities: The results in Table 2 revealed a significant ($p < 0.05$) elevationin the fat/muscle ratio, liver index and liver enzyme activities(ALT and AST) in the MBS group compared to the controlgroup. On the other hand, the above-mentioned parameters weresignificantly ($p < 0.05$) reduced after the treatment with BVEwhen compared to non-treated MBS group. These results indicatethat BVE improves hepatic steatosis through reversing of liverenzyme levels. Typically the range for normal AST is reportedbetween 10 to 40 U/L and ALT between 7 to 56 U/L.

Table 2. Effect of BVE on body/muscle ratio, liver index, serum ALT and serum AST in fructose-induced metabolic syndrome in rats

Groups	Body/muscle ratio (g/g)	Liver index (%)	AST (IU/L)	ALT (IU/L)
Control	2.56 ± 0.37	2.54 ± 0.14	63.98 ± 2.45	33.2 ± 3.11
BVE	2.68 ± 0.48	2.42 ± 0.09	61.7 ± 4.67	33.5 ± 1.33
MBS	4.98 ± 0.42a	4.34 ± 0.21a	125.4 ± 2.12a	76.2 ± 3.95a
MBS + BVE	4.11 ± 0.27a,b	3.32 ± 0.54b	84.6 ± 5.43a,b	56.4 ± 2.11a,b

Data are represented as mean ± SEM (n = 6 rats). ALT, alanine aminotransferase; AST, aspartate aminotransferase; MBS, metabolic syndrome; BVE, Bauhinia variegata extract. a,b Significantly different from normal control and MBS groups, respectively at $p < 0.05$.

Effect of BVE on blood glucose level, serum insulin, insulin resistance, OGTT and lipid profile: Significantly ($p < 0.05$), the treatment of BVE brought insulin resistance measures down to almost control levels (Table 3). When it came to OGTT, the MBS group's area under the curve (AUC) value was considerably higher than that of the control group after receiving the fructose diet for 12 weeks (Fig. 1).However, when compared to the control group, the AUC value in the BVE-treated group was considerably lower. Table 3 illustrates how the fructose diet caused a severe disruption in the lipid profile in the MBS group, as evidenced by the considerable ($p<0.05$) rise in serum levels of TC, TG, and LDL-C, and the significant ($p<0.05$) decrease in serum HDL-C.These data suggested that oral administration of BVE might attenuatethe fructose diet-induced dyslipidemia in the MBS group(Table 3).

Table 3. Effect of BVE on metabolic parameters in fructose-induced metabolic syndrome in rats

Groups	Blood glucose (mg/dl)	Serum insulin (µIU/ml)	HOMA-IR index	TC (mg/dl)	TG (mg/dl)	HDL-C (mg/dl)	LDL-C (mg/dl)
Control	93 ± 6.4	9.5 ± 0.26	2.4 ± 0.06	86 ± 3.9	101 ± 2.6	41 ± 1.9	22 ± 2.5
BVE	90 ± 3.5	10.9 ± 0.76	2.3 ± 0.97	80 ± 2.8	99 ± 3.7	46 ± 1.4	20 ± 0.8
MBS	154 ± 5.4a	16.8 ± 0.78a	6.3 ± 0.53a	149 ± 5.2a	211 ± 9.7a	23 ± 1.6a	82 ± 2.4a
MBS+BVE	143 ± 2.1a,b	11.6 ± 0.41a,b	4.5 ± 0.54a,b	104 ± 4.8a,b	168 ± 3.4a,b	46 ± 2.7b	36 ± 1.6b

Data are represented as mean ± SEM (n = 6 rats). MBS, metabolic syndrome; BVE, Bauhinia variegata extract; TC, total cholesterol; TG, triglycerides; HDL-C, high density lipoprotein cholesterol; LDL-C, low density lipoprotein cholesterol. a,b Significantly different from normal control and MBS groups, respectively at $p < 0.05$. Homeostasis Model Assessment-Insulin Resistance (HOMA-IR) = [fasting glucose (mg/dl) x fasting insulin (µIU/ml)]/405.

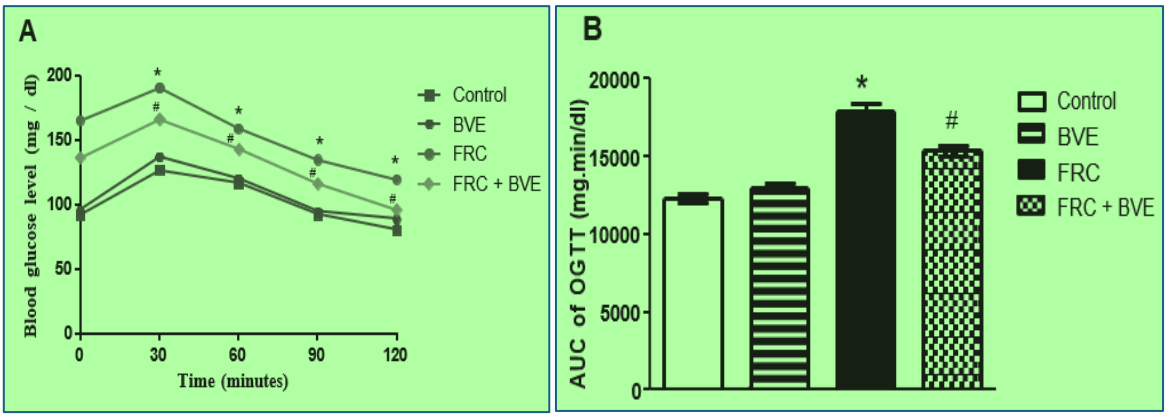


Figure 1. Effect of BVE on OGTT (A) and AUC of OGTT (B) in fructose fed rats. Values are presented as mean ± SEM (n = 6 rats). *,#Significantly different from normal control and MBS groups, respectively at $p < 0.05$.

Effect of BVE on hepatic oxidative stress markers: In the MBS rats, hepatic levels of MDA and NO were significantly($p<0.05$) increased. In the same line, the decrease in GSHlevel and catalase activity were significantly ($p<0.05$) observed ascompared to the

control group. The treatment with BVE couldreduce oxidative stress marker values significantly as comparedto the control group ($p < 0.05$).The results elucidated that the antioxidant capacity of BVEmay be contributed to the protective effects against metabolicdysfunction (Table 4).

Effect of BVE on systolic blood pressure (SBP) changes: At the beginning of our experiment, the SBP value was similarin all groups (119–124 mmHg). Oral ingestion of fructose diet for12 weeks elevated the SBP value significantly ($p < 0.05$) in comparisonto the control group. On the other hand, BVE intakesignificantly ($p < 0.05$) reduced the SBP value as compared to theMBS group (Fig. 2). Such a result assessed the antihypertensiveeffect of BVE.

Table 4. Effect of BVE on hepatic MDA, NO and reduced GSH levels, as well as CAT activity in fructose-induced metabolic syndrome in rats

Group	MDA (nmol/g tissue)	NO (nmol/g tissue)	GSH (mmol/g tissue)	Catalase (U/g tissue)
Control	175 ± 9.9	219 ± 12.8	34 ± 2.9	458 ± 23.7
BVE	177 ± 8.3	211 ± 14.9	35 ± 2.2	421 ± 29.6
MBS	354 ± 15.9a	367 ± 15.6a	17 ± 1.8a	239 ± 14.8a
MBS + BVE	280 ± 12.7a,b	270 ± 14.8a,b	35 ± 1.1b	436 ± 48.5b

Data are represented as mean ± SEM (n = 6 rats). MDA, malondialdehyde; NO, nitric oxide; GSH, glutathione; CAT, catalase; MBS, metabolic syndrome; BVE, Bauhinia variegate Extract. a,b Significantly different from normal control and MBS groups, respectively at $p < 0.05$.

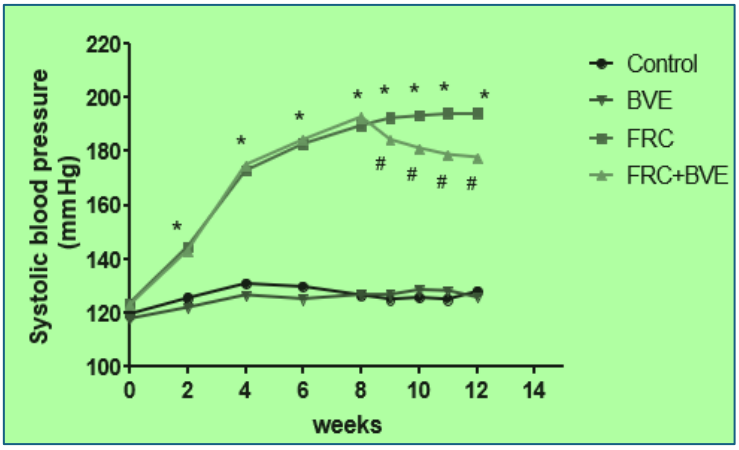
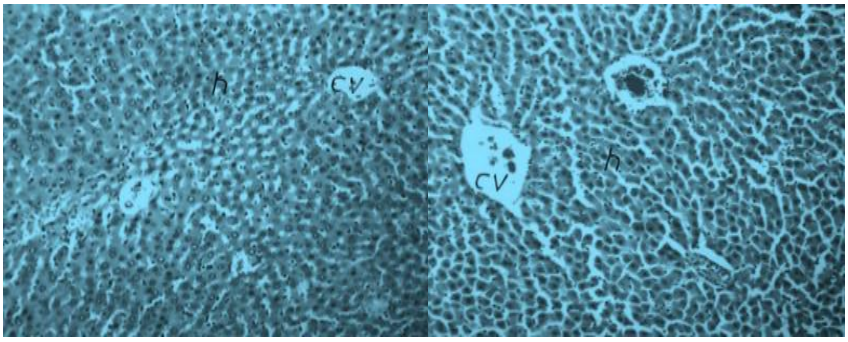
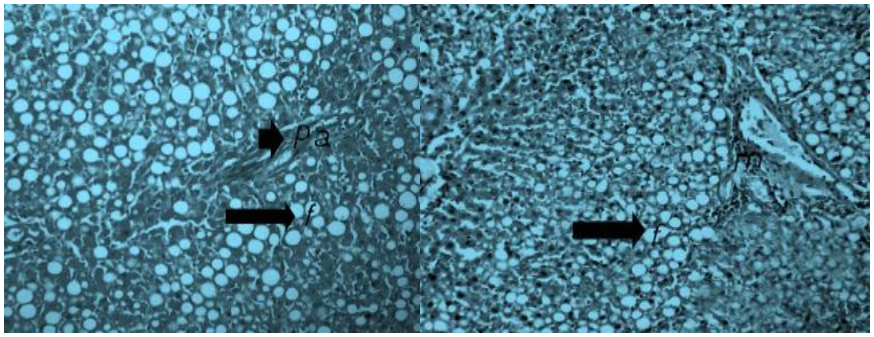


Fig. 2. Effect of BVE on systolic blood pressure in MBS rats. Values are presented as mean ± SEM (n = 6 rats). *,#Statistically significant from normal control and FRC groups, respectively at $p < 0.05$ at each time point (week) by using two-way analysis of variance (ANOVA) followed by Bonferroni post analysis test.

Effect of BVE on Hepatic Histopathology: Figure 3 illustrates the typical histological structure of the central vein (cv) and hepatocytes (h) observed under a light microscope in liver sections of the normal control and control BVE groups. The fructose diet group's hepatic sections displayed notable fatty alterations (f) diffusely throughout the hepatocytes in conjunction with inflammatory cell infiltration and a little amount of fibrosis in the portal area (pa). Controversial: following BVE treatment, the liver tissue of rats given fructose showed a minor infiltration of inflammatory cells in the portal region along with mild fatty alterations in certain hepatocytes.



Control BVE



FRC **FRC +BVE**

Figure3: Light microscopic examination of liver sections

CONCLUSION:

On the basis of the present inquiry, overconsumption of high fructose for prolonged duration may imbalance the alterations in many organ of body metabolism, by intensifying obesity and generating functioning. As a result, the current study offers proof of the possible defence function of BVE against long-term conditions such hypertension, diabetes, and hepatic illnesses brought on by MBS. Morin's capacity to enhance GLUT2 expression and reduce oxidative stress, inflammatory, and fibrotic indicators may be the cause of these effects.

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