Antiobesity Activity of *Bauhinia purpurea* Extract: Effect on Hormones and Lipid Profile in High Calorie Diet Induced Obese Rats

Tiruttani Kuppireddy Padmaja¹, Parim Brahma Naidu¹, Ghali Eswara Naga Hanuma Kumar¹, Saravanan Ganapathy², Meriga Balaji¹*

¹Department of Biochemistry, Sri Venkateswara University, Tirupati, India
²Department of Biochemistry, Center for Biological Sciences, K.S.R. College of Arts & Sciences, Tiruchengode, India

Email: *balaji.meriga@gmail.com*

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**Abstract**

Obesity is well recognized as a global public health concern and the quest continues to explore natural product-based novel therapeutics. *Bauhinia purpurea* finds its place in traditional medicine as an effective anti-ulcer, antiplatelet, wound healing and hypolipidemic agent. The present study investigated the effects of ethanolic extract of *Bauhinia purpurea* (EEB) on the changes in body weight, lean mass, fat free mass, fat percent, tissue and plasma lipid profiles, plasma glucose, insulin and insulin resistance as well as on the activity of amylase, lipase, leptin and adiponectin levels in high caloric diet (HCD) induced obese rats. After induction of obesity with HCD, rats were administered orally with EEB (100, 200 and 300 mg·kg⁻¹ BW) or orlistat (5 mg·kg⁻¹ BW) once daily for 42 days. HCD substantially increased the body weight, fat free mass, fat percent, tissue and plasma lipid profiles, plasma glucose, insulin and insulin resistance as well as on the activity of amylase and lipase. EEB at a dose of 300 mg·kg⁻¹ BW exerted its therapeutic effects similar to orlistat in decreasing body weight, insulin resistance, levels of glucose, leptin, and the activities of amylase and lipase. EEB at a dose of 300 mg·kg⁻¹ BW exerted its therapeutic effects similar to orlistat in decreasing body weight, insulin resistance, levels of glucose, leptin, and the activities of amylase and lipase. EEB at a dose of 300 mg·kg⁻¹ BW exerted its therapeutic effects similar to orlistat in decreasing body weight, insulin resistance, levels of glucose, leptin, and the activities of amylase and lipase. EEB at a dose of 300 mg·kg⁻¹ BW exerted its therapeutic effects similar to orlistat in decreasing body weight, insulin resistance, levels of glucose, leptin, and the activities of amylase and lipase.

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Keywords
Obesity, Leptin, Fat Free Mass, Dyslipidemia, Insulin Resistance

1. Introduction
Obesity is a common health disorder of lipid and carbohydrate metabolism which results from excessive fat accumulation in adipose tissue, liver, skeletal muscle etc. (WHO 2011) [1]. The prevalence of obesity is escalating across the world cutting across age groups, sex and ethnicity. Obesity is one of the major contributors to morbid conditions like type-2 diabetes, hypertension, cardiovascular disorders, and non-alcoholic fatty liver metabolic syndrome [2] [3]. Though the etiology of obesity is diverse, the root cause is energy disparity: more calories consumed than expended. It is characterized by the unwarranted increase of adipose tissue which is regulated by caloric rich nutritional factors and genetics [4].

Along with lifestyle modifications, the pharmacological approach to weight management includes reduction of nutrient absorption, modulating lipogenesis-lipolysis, regulating adipose signals and appetite centers [5]. Although a few FDA-approved drugs like orlistat and sibutramine are available in the market, they are not preferred for long-time usage owing to considerable adverse effects. Epidemiological studies have recommended the utilization of natural product-based drugs to reduce the risk of obesity and associated complications [6]. Therefore, attempts are being made to develop plant-based safe and effective drugs to treat obesity. Bauhinia purpurea, a member of Leguminosae, is native to India and Southern China. Almost all parts of the plant are used in traditional medicine for the treatment of various ailments like asthma, leprosy, piles, snake bite and liver complaints [7]-[8]. Besides this, its extracts have been found to have antifungal activity [9]. Various well-reported pharmacological activities of B. purpurea include anti-ulcer, antiplatelet, hypotensive and hypolipidemic actions [10]. A good number of phytoconstituents from B. purpurea have been reported in literature, in addition to bauhinastatins [7]. However, there is scanty of literature pertaining to antiobesity effects of B. purpurea. Diet intervention study in rodents is a good model to evaluate the anti obesity efficacy of a drug/formulation. Rats fed on high calorie diet (HCD) develop dyslipidemia and obesity which bear close resemblance to human obesity [11].

Therefore, in the present study, we carried out LC-MS analysis to find out the possible bioactive compounds present in the ethanolic extract of Bauhinia purpurea (EEB) and investigated the protective effects of EEB on body weight, fat free mass, fat-%, glucose, insulin resistance, tissue and plasma lipid profiles, leptin, adiponectin and the activities of amylase and lipase in HCD-fed obese rats and sought possible mechanisms of action.

2. Materials and Methods
2.1. Chemicals
Fasting blood glucose and insulin were measured by using kits procured from Stanbio Laboratory USA, Bio-Merieux, RCS, Lyon, France respectively. Orlistat (Cat No. 04139) was obtained from Sigma-Aldrich. All other reagents used in the experiments were of analytical grade and of the high purity.

2.2. Collection and Extraction of Plant Material
The bark of Bauhinia purpurea was collected from Seshachalam forests of Rayalaseema region, A.P., India. Its identity was authenticated by a taxonomist, department of Botany, S.V. University, Tirupati, voucher number 136, and a specimen has been preserved at the departmental herbarium. The bark of B. purpurea was shade dried, pulverized to a coarse powder and sequentially extracted with hexane, ethylacetate, ethanol and water. The respective filtrates obtained were evaporated to dryness in a rotary vacuum evaporator to obtain respective dry extracts. Based on preliminary studies and phytochemical analysis, ethanolic extract was selected for further studies.

2.3. LC-ESI-MS/MS Analysis B. purpurea Ethanolic Extract
LC-ESI-MS/MS analysis of ethanolic extract of B. purpurea was carried out on 6520 Accurate Q-TOF (Agilent...
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Santa Clara, CA) mass spectrometer coupled to HPLC equipped with a UV-Vis detector. The column used was Zobax SB C18 rapid resolution, 4.6 mm × 150 mm, 3.5 μm particle size (Column-SL, Model G1316B). The conditions were; mobile phase consisted of binary mixture of solvent (A) formic acid (0.1%) and 10 mM ammonium phosphate, (B) acetonitrile + 0.1% formic acid; gradient (in solvent B) with linear gradient programme as follows: Starting from 95% solvent A and 5% solvent B increasing to 35% solvent B over 12 min, then increased to 90% over 35 min, back to 5% solvent B over 1 min and finally isocratic for 10 min. The sample wavelength was set at 240 - 360 nm. A sample injection volume of 10 μl in methanol and a constant flow rate of 0.3 ml/min were used for the analysis of sample. The mass spectra were acquired with ESI source (Agilent Santa Clara, CA). Nitrogen was used as the sheath and auxiliary gas and helium was used as the collision gas. The ESI MS spectra were acquired in positive ion mode and a spray voltage of 4 kV was employed. The temperature of the heated transfer capillary was 325˚C. The mass spectrometer was scanned from m/z 100 to 1200 in full scan mode, gas flow 10 L/min; Nebulizer 40 psi [2].

2.4. Animals and Diets

Male Sprague-Dawley rats, normal diet and high caloric diet were obtained from National Centre for Laboratory Animal Sciences (NCLAS), National Institute of Nutrition (NIN), Hyderabad, India. Normal diet contained pellet chow of standard composition containing all the recommended macro and micronutrients (56% carbohydrate, 18.5% protein, 8% fat, 12% fiber and adequate levels of minerals and vitamins). HCD contained 29.5% beef tallow, 22.0% casein, 23.0% starch, 17.9% cellulose, 4.0% L-cystine, 0.3% choline chloride, 1.8% vitamin mixture (AIN-93 VIX) and 1.5% salt mixture. During the course of the experimental period (20 weeks), rats were fed with either normal diet or freshly prepared HCD (15 g/rat/day) as mentioned below and water ad libitum. Experimental animals were maintained under standard laboratory conditions (temperature: 22˚C ± 2˚C; humidity: 40% - 60%). Rats initially weighing 180 - 200 g were randomly divided in to six groups of six each (n = 6). After induction of obesity, to test the activity of EEB, rats were treated with different doses of EEB (100, 200, or 300 mg∙kg⁻¹ BW), suspended in 0.5% carboxy methyl cellulose (CMC), for 42 days (15th to 20th week), using an intragastric tube. All experimental protocols were followed as per institutional animal ethical committee guidelines (Resolution No: 55/2012/(i)/a/CPCSEA/IAEC/SVU/MBJ, Dt: 08.07.2012).

2.5. Experimental Design

Group 1: Normal Diet Control (Normal Diet).
Group 2: High Caloric Diet Control (HCD).
Group 3: HCD + Orlistat (5 mg∙kg⁻¹ BW).
Group 4: HCD + EEB (100 mg∙kg⁻¹ BW).
Group 5: HCD + EEB (200 mg∙kg⁻¹ BW).
Group 6: HCD + EEB (300 mg∙kg⁻¹ BW).

2.6. Measurement of Body Weight

Once in a week, the body weight of experimental animals was measured. The total amount of food consumed was recorded daily (data are not shown). At the end of the experiment, blood was collected from overnight fasted animals under inhalation of anaesthesia by retro-orbital puncture method. Plasma was separated by centrifugation at 2500 rpm for 15 min and was used for further experiments.

2.7. Body Composition

Body composition of experimental animals was assessed at the end of experimental period by Total Body Electrical Conductivity (TOBEC) using small animal body composition analysis system (EM-SCAN, Model SA-3000 Multi detector, Springfield, USA). Lean body mass, fat free mass and total body fat and fat percentage were calculated as described previously [11] [43].

2.8. Blood Glucose, Insulin and Insulin Resistance

At the end of the experiment, blood was collected from overnight fasted rats under inhalation of anaesthesia by
retro-orbital puncture method. Plasma was separated by centrifugation at 2500 rpm for 15 min. Plasma glucose was estimated using kits (Cat No. 1060-500, Stanbio laboratory, USA). Plasma insulin was determined using kits obtained from Bio-Merieux, RCS, Lyon, France. Insulin resistance was calculated using the homeostasis model assessment [12].

2.9. Oral Glucose Tolerance Test (OGTT)
To test glucose tolerance, OGTT was performed. At the end of the experiment, after overnight fasting, glucose was administered orogastrically at a dose of 2.0 g·kg⁻¹ BW to rats and blood samples were collected from supra orbital sinus at 0, 30, 60 and 120 minutes and glucose level was estimated.

2.10. Estimation of Leptin and Adiponectin
Plasma leptin and adiponectin levels were measured by using enzyme linked immunosorbent assay kits (Crystal Chem, Downer’s Grove, IL, USA), performed in duplicate, as per the manufacturer’s guidelines and were expressed in ng·mL⁻¹.

2.11. Estimation of Plasma Lipid Profiles
For estimation of lipid profiles, blood samples were centrifuged at 4000 rpm/min for 10 min to separate plasma which was then stored at −80°C for further biochemical analysis. Total cholesterol, HDL and triglyceride levels were estimated by CHOD-PAP method and GPO-PAP method [11]. LDL levels were calculated by the method of Johnson et al. (1997) [13].

2.12. Estimation of Tissue Lipid Profiles
Tissue lipids were extracted from experimental animals by the method of Floch et al. (1957) [14]. The liver tissues were rinsed with ice cold physiological saline, dried, homogenized in cold chloroform-methanol (2:1, v/v) and contents were extracted after 24 h. The extraction was repeated four times. The combined filtrate was washed with 0.7% KCl and the aqueous layer was discarded. The organic layer was made up to a known volume with chloroform and used for tissue lipid analysis.

2.13. Estimation of Pancreatic Lipase and Amylase Activity
Pancreatic lipase and amylase activities were determined by kinetic method using commercial kits (Bioclin®, Minas Gerais, Brazil) following the guidelines of the manufacturer.

2.14. Statistical Analysis
All the results were expressed as the mean ± S.D. for six animals in each group. All the grouped data were statistically evaluated with SPSS10.0 software. Hypothesis testing methods included one way analysis of variance (ANOVA) followed by Least Significant Difference (LSD) test. Significance level at $p < 0.05$, $0.01$, $0.001$ were considered to indicate statistical significance.

3. Results
3.1. Phytochemical Analysis
Phytochemical analysis of ethanolic extract of B. purpurea was carried out by LC-Q-TOF MS/MS to identify the compounds present in EEB. MS/MS spectra of the mass to charge ratio of ESI scanned spectra results are shown in Figure 1. The major compounds identified in EEB were 2,4'-Dihydroxy-4-methoxydihydrochalcone, Isoliquiritigenin 2’-methyl ether, 4'-Hydroxy-7,3'-dimethoxyflavan, 2,3-Dihydroxy propyl olate, Bauhiniastatin-1, 5,7,3',4',5'-Pentamethoxyflavone, Preracemosol B and 5,7,3',4'-Tetramethoxyflavone. Among these, Bauhiniiastatin-1 and 5,7,3',4'-Tetramethoxyflavone (Kaempferol) were found to be most abundant (12.36% and 15.48% respectively). Remaining compounds were present in concentrations of less than 2%.
3.2. Body Weight and Body Composition

Figure 2 depicts the changes in body weight and body composition of experimental rats. Consumption of HCD for 20 weeks produced a substantial increase in body weight (468 ± 8.3 g), total fat (75.9 ± 9.4 g), fat % (16.1 ± 3.8), and fat free mass (173.1 ± 2.8 g) in HCD control group when compared to normal control group of rats whose body weight, total fat, fat % and fat free mass were 367 ± 11.6 g, 26.9 ± 4.8 g, 7.3% ± 1.2% and 153.4 ± 6.6 g respectively. Oral administration of EEB (100, 200, 300 mg∙kg−1 BW) for 42 days (from 15th to 20th week) considerably reduced body weight and body composition in a dose dependant manner. Among the three doses administered, EEB at a dose of 300 mg∙kg−1 BW, showed significant (p < 0.05) therapeutic effect. At 300 mg∙kg−1 BW of EEB, the body weight, total fat, fat % and fat free mass were 387.1 ± 6.7 g, 40.2 ± 3.3 g, 10.97% ± 2.6% and 155 ± 4.9 g respectively.

3.3. Estimation of Plasma Glucose, Insulin, Insulin Resistance

The levels of plasma glucose, insulin and insulin resistance in control and experimental obese rats are shown in...
Figure 2. Effect of Bauhinia purpurea on body weight (Figure 1(a)); Lean mass (Figure 1(b)); Total fat (Figure 1(c)); Fat-% (Figure 1(d)) and Fat free mass (Figure 1(e)) in normal and experimental obese rats. Values are mean ± S.D., n = 6. Values are statistically significant at *p < 0.05. a* Significantly different from normal control; b* Significantly different from HCD control.

Table 1. There was a significant (p < 0.05) elevation in plasma glucose, insulin and insulin resistance in HCD control obese rats when compared to normal control rats. Oral administration of different doses of EEB could bring these changes to near normalcy in a dose dependent manner, the most significant effect being observed at 300 mg·kg\(^{-1}\) BW.

### 3.4. Oral Glucose Tolerance Test

Figure 3 depicts the results of oral glucose tolerance test performed on control and experimental obese rats. In the normal control group of rats, blood glucose level reached its maximum value at 60 min after glucose load and declined to near basal level at 120 min, whereas, in HCD-induced obese rats, the peak increase in blood glucose level was noticed even after 60 min and remained high over the next 60 min. Administration of EEB (300 mg·kg\(^{-1}\) BW) or orlistat to obese rats elicited a significant decrease in blood glucose level at 60 min and beyond when compared with HCD control rats.

### 3.5. Leptin and Adiponectin Levels

Leptin and adiponectin are two major adipocytokines of adipose tissue. Figure 4 explains the levels of leptin and adiponectin in control and experimental obese rats. There was a marked elevation in leptin and decrease in
Table 1. Effect of EEB on plasma glucose, insulin and insulin resistance in normal and experimental obese rats.

<table>
<thead>
<tr>
<th>Groups</th>
<th>Glucose (mg·dl$^{-1}$)</th>
<th>Insulin (µU·ml$^{-1}$)</th>
<th>Insulin Resistance</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>81.9 ± 4.3</td>
<td>5.5 ± 0.7</td>
<td>3.3 ± 0.07</td>
</tr>
<tr>
<td>HCD Control</td>
<td>160.9 ± 12.2***</td>
<td>14.8 ± 1.5*</td>
<td>5.3 ± 1.6*</td>
</tr>
<tr>
<td>HCD + Orlistat</td>
<td>128.5 ± 13.1**</td>
<td>18.0 ± 1.2**</td>
<td>3.1 ± 0.8**</td>
</tr>
<tr>
<td>HCD + EEB (100 mg·kg$^{-1}$ BW)</td>
<td>137.4 ± 10.6***</td>
<td>15.4 ± 1.9**</td>
<td>4.4 ± 1.2**</td>
</tr>
<tr>
<td>HCD + EEB (200 mg·kg$^{-1}$ BW)</td>
<td>132.1 ± 2.6**</td>
<td>15.8 ± 0.8**</td>
<td>3.8 ± 1.2**</td>
</tr>
<tr>
<td>HCD + EEB (300 mg·kg$^{-1}$ BW)</td>
<td>129.7 ± 15.3**</td>
<td>16.6 ± 0.7**</td>
<td>3.2 ± 0.6**</td>
</tr>
</tbody>
</table>

Values are mean ± S.D., n = 6; Values are statistically significant at *$p < 0.05$, **0.01***0.001; a*Significantly different from control; b*Significantly different from HCD control.

Figure 3. Effect of Bauhinia purpurea on glucose tolerance in control and experimental obese rats. Values are mean ± S.D., n = 6. Values are statistically significant at *$p < 0.05$. a*Significantly different from normal control; b*Significantly different from HCD control.

Figure 4. Effect of Bauhinia purpurea on leptin and adiponectin in control and experimental obese rats. Values are mean ± S.D., n = 6. Values are statistically significant at *$p < 0.05$. a*Significantly different from normal control; b*Significantly different from HCD control.
adiponectin levels in HCD-fed obese rats over their normal control rats. Interestingly, treatment with EEB (300 mg·kg⁻¹ BW) or orlistat has significantly (p < 0.05) restored their levels to normalcy.

3.6. Assay of Amylase and Lipase

The activities of amylase and pancreatic lipase of normal and experimental obese rats are exemplified in Figure 5. There was a two fold increase in the activities of lipase and amylase in HCD control rats when compared to normal control group of rats. Administration of EEB has brought down their activities in a dose dependant manner. A significant (p < 0.05) reduction in their activities was noted at 300 mg·kg⁻¹ BW of EEB.

3.7. Estimation of Lipid Profiles

The levels of total cholesterol, FFAs, TGs, Phospholipids (PLs), HDL, LDL and VLDL were measured in plasma and are depicted in Figure 6(a). The concentrations of total cholesterol, FFAs, TGs, PLs, LDL and VLDL were markedly elevated while HDL level decreased in experimental obese rats as compared to the normal rats. Oral administration of EEB has reversed these alterations in a dose dependant manner, the most profound effect being noted at a dose of 300 mg·kg⁻¹ BW.

Liver tissue lipid profiles (total cholesterol, FFAs, TGs and PLs) of control and HCD-induced obese rats are shown in Figure 6(b). Supplementation of HCD, led to profound increase in tissue levels of total cholesterol, FFAs, TGs and PLs. Treatment with EEB (300 mg·kg⁻¹ BW) or orlistat significantly (p < 0.05) reduced liver tissue lipids in obese rats.

4. Discussion

Although there is a growing interest for herbal remedies across the globe, lack of adequate scientific evidences on plants and herbs is limiting their usage. Hence, more detailed herbal studies are needed on suitable animal models. The development of an ultimate animal model for obesity studies that would closely imitate the accepted metabolic features of human obesity is very exigent. Among the animal models of obesity, rats that are fed a high-calorie diet are proved to be suitable for testing antiobesity actions. In HCD-fed rats, we observed an increase in body weight and fat per cent, which is a hallmark of obesity. Long-term HCD feeding resulted in obesity which was linked with increased body weight, lean mass, total fat, fat percentage and fat free mass [15]. This might be due to consumption of a diet rich in calories in the form of sugars and fats and its accumulation in various parts of the body, leading to excessive growth of adipose tissue [16].

Oral administration of EEB significantly reduced body weight, lean mass, total fat, fat percentage and fat free mass in experimental rats, compared with that of HCD control rats. This suggests that EEB may inhibit lipid absorption, transportation and accumulation in adipose tissue by inhibiting fatty acid synthase, acetyl CoA carboxylase and other key enzymes involved in lipogenesis. These enzymes are regulated by SREBP-1c at the

![Figure 5. Effect of Bauhinia purpurea on lipase and amylase activities in control and experimental obese rats. Values are mean ± S.D., n = 6. Values are statistically significant at *p < 0.05. a* Significantly different from normal control; b*Significantly different from HCD control.](image-url)
transcriptional level [17]. The therapeutic effect of EEB could be due to the presence of bioactive compounds like bauhiniastatins, kaempferol (5,7,3,4-Tetramethoxyflavone) and related compounds in it. This is in agreement with Tomoko Akase et al. (2011) [18] who suggested that kaempferol, an active polymethoxyflavone found in *Kaempferia parviflora* reduced the accumulation of visceral fat in diet induced obese rats by escalating lipid metabolism through down regulation of SREBPs [19]. The antioxidant and anti hyperlipidemic activity of bauhiniastatins falls in line with the work of Pettit et al. (2005) [20] who reported the antioxidant, anti inflammatory and antineoplastic activity of bauhiniastatins 1-4 in P388 lymphocytic leukemia cell line.

It is well known that insulin can indirectly inspire leptin secretion through its collective effects on the metabolism of nutrients, especially on glucose utilization in adipocytes. Thus, high calorie diet-induced obese rats tend to be an exceptional animal model to study molecular mechanisms for the development of obesity and insulin resistance. Cai-Jie et al. in their studies showed that feeding of HCD for a period of 12 weeks presented hyperglycemia, increased levels of plasma glucose, insulin and insulin resistance [21]. It is evident that longstanding exposure of animals to fed state leads to insulin resistance syndrome as was characterized by the amplified body weight, mild hyperglycemia and/or glucose intolerance, hypertriglyceridaemia. Insulin resistance and hyperglycemia are hallmarks of metabolic syndrome [22]. Insulin inhibits lipolysis which leads to impaired glucose-stimulated insulin secretion that is coupled with an inundate supply of lipids due to improved fat mobi-
Dyslipidemic changes like elevated TGs, VLDL, total cholesterol, PLs, FFAs and LDL, and a declined plasma level of HDL have been observed in the present study upon HCD consumption. Our findings were supported by the work of Park et al. [36]. Thus, alteration of lipid profiles can be used as an index of obesity. There is an augmented discharge of FFA from adipose tissue to other tissues; mainly to the liver is possible during obesity and it may be due to the development of leptin resistance [37]. This leads to the development of fatty liver, decreases FFA oxidation which in turn increases the levels of hepatic and plasma triglycerides [38]. Excessive accumulation of TGs in the form of lipid stores is associated with a number of symptoms of metabolic syndrome [39]. The elevated level of phospholipids in the present study might be due to elevated levels of FFA and total cholesterol that could endorse the production of phospholipids. Prominent TC and elevated LDL levels intensify the risk of coronary heart disease [40]. On the other hand, high HDL is helpful in transporting surplus cholesterol to liver for excretion through bile [41]. Oral supplementation of EEB significantly lowered TC, TGs, FFAs and PLs in both plasma and liver and LDL levels in plasma of HCD-fed obese rats. Our finding are in line with Yongqi et al. (2009) who reported that supplementation of daidzein derivative from soy germ koji to fed animals led to hypolipidemic state by decreasing cholesterol absorption and secretion from the intestine [42] [43].

5. Conclusion

High caloric diet substantially increased the body weight, total fat, fat-%, fat free mass and circulatory lipids in rats. Treatment with EEB has significantly alleviated HCD-induced obesity, hyperlipidemia and associated
changes. The presence of phytoconstituents such as bauhiniasatins, kaempferol and related compounds, as revealed by LC-MS analysis, may play a prominent role in therapeutic activity of EEB. This study offers scientific validation to the traditional use of *Bauhinia purpurea* against several morbidities including hyperlipidemia. The precise mechanisms behind these beneficial effects of EEB need to be further investigated.

**Conflict of Interest Statement**
The authors declare that there are no conflicts of interest.

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