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# Evaluation of weight reduction and anti-cholesterol activity of Punarnava root extract against high fat diets induced obesity in experimental rodent

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## ABSTRACT

**Objective:** To evaluate the weight reduction and hypercholesteremic potential of punarnava root extract induced with high fat diets in experimental rodents by administrating oral doses (100, 200 and 400 mg/kg). **Methods:** Thirty six female Sprague–Dawley rats were divided in six groups and each group had six animals. Group–I administered laboratory diet, control obese group–II induced with high fat diet (HFD), group–III standard drug plus HFD rats (5 mg/kg) and *Boerhaavia diffusa* root extract (BDRE) were administered (100, 200 and 400 mg/kg) plus HFD rats (group–IV, V and VI). After the end of experimental period (60 days) the body weight, organ fat pad weight, lipid profile, liver and kidney marker enzyme were estimated along with histopathological examination in experimental groups of animals. **Results:** The obtained results showed that the significant reduction on administration of BDRE (200 and 400 mg/kg) in body weight, visceral fat pad ( $P<0.01$ ), lipids level ( $P<0.01$ ), AST and ALT ( $P<0.01$ ), urea ( $P<0.01$ ) and creatinine ( $P<0.01$ ) when compared with HFD groups II. The biochemical estimation were supplemented by histopathological examinations. **Conclusions:** The results of this study scientifically supported its traditional uses of *Boerhaavia diffusa* as antiobesity activity by normalizes the elevated body weight and organ fat pad weight as well as antilipidemic property by lowering the altered levels of lipid profile in female Sprague–Dawley

## 1. Introduction

Obesity is one of the most widespread metabolic disorders in contemporary society. It is associated with the development of type two diabetes mellitus, coronary heart disease, cancer, respiratory complications and osteoarthritis[1]. Recently, natural and alternative anti-obesity agents, in the form of beverages or teas, have been used for the treatment of obesity. These could attenuate the clinical adverse effects of chemical anti-obesity agents[2]. Leptin is a hormone secreted by adiposities that provides a negative feedback signal to the brain to decrease energy intake. Deficiency in leptin as well as genetic defects in the leptin receptor are known to cause obesity in mice and have been proposed to play a role in obesity in humans[3]. Obese persons are at increased risk for developing serious medical

conditions such as diabetes mellitus, hypertension, and cardiovascular disease, and obese persons are at increased risk of death from cardiovascular disease, diabetes, kidney disease, and cancer[4]. *Boerhaavia diffusa* Linn. (Family: Nyctaginaceae) is an herbaceous plant, cultivated in fields[5], spreading vine widely distributed in the tropical and subtropical regions in the world[6] and contain a number of constituents mainly as alkaloids, others are flavonoids, saponins and steroids[7]. The superabundance of reports has been published. In Punjab region, the drug is useful for the eye disease and in Bombay used for dropsical swellings. The leaves juice is used in jaundice and the root is generally used in infusion in internal inflammation, laxative, and also in urinary disease[8]. The depletion of the germinal epithelial lining of the seminiferous tubules with enhance number of germinal cell, decreased sperm counts with increase percentage of tail and head abnormalities and increases in both pre and post-implantation tissue[9] also showed the methanolic extracts of the plant were effective in reducing metastases formation in some melanoma

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cells<sup>[10]</sup>. Most investigations on the plant have centred on the root, whereas significant differences in the chemical composition of the root and the leaves have been reported<sup>[11]</sup>. The plant is also reported to have adaptogenic and antistress activity and roots have anti-inflammatory, fibrinolytic and anticonvulsant activities<sup>[12]</sup>. Ethanol extract showed potent inhibitory effect on gram positive bacteria except *M. luteus* and gram-negative bacterial<sup>[13]</sup>. Among currently available drugs, synthetic drugs do have potential adverse reactions and which can be minimized to greater extent through natural compounds<sup>[14]</sup>.

## 2. Materials and Methods

### 2.1. Chemicals

Diethyl ether, trichloroacetic acid, Disodium ethylenediamine tetra acetate, Total cholesterol (TC), triglyceride (TG), low and very low density lipoprotein (LDL and VLDL), Aspartate aminotransferase (AST), alanine aminotransferase test kits were purchased from clinical chemistry division.

### 2.2. Collection and preparation of plant extract

*Boerhaavia diffusa* roots was collected in the month of September 2010 from area Aminabad market Lucknow Uttar Pradesh India. Root was identified by their vernacular names and latter validated at the department of Botany NBRI Lucknow, India used and the voucher specimen (031006). Roots were air dried at room temperature for 3 weeks to get consistent weight. The dried plants were later ground to crude powder. Two hundred grams of crude powder plant material were shaken with hydro alcoholic for 24 hrs on an orbital shaker at room temperature. Extracts were filtered using a Buckner funnel and Whatman No 1 filter paper. Each filtrate was concentrated to dryness under reduced pressure at 40°C through evaporator. The extract was resuspended in the respective solvent<sup>[15]</sup>.

### 2.3. Animals

The female Sprague–Dawley rats body weight (130–140 ± 5) g were purchase from the Central Drug Research Institute Lucknow (India). They were housed for 1 week under a 12/12 h light/dark cycle in a temperature (25±2) °C and humidity (60±5)% controlled room and freely fed standard laboratory chow with water ad libitum. The standard laboratory chow contained. All studied were performed as per CPCSEA guideline, India (Reg. No.1213/ac/08/CPCSEA/IU).

### 2.4. Experimental schedule

*Boerhaavia diffusa* root extract (BDRE) at a dose of 100,

200, 400 mg/kg and 5 mg/kg standard (Sibutramine) drugs daily administered orally using animal feeding needles at the same time of day for a total 60 days. There were six groups and each groups have six animals. Briefly animals were divided into normal control (Group–I) administered laboratory diet, control obese group induced with high fat diet [HFD (National Institute of Nutrition, Hyderabad, India)] (Group–II), standard drug plus HFD (Group–III) and BDRE (100, 200 and 400 mg/kg, respectively) plus HFD rats (Group–IV, V and VI).

### 2.5. Effects of BDRE on lipid profile

Rats were anaesthetics with diethyl ether the blood sample was collected from tail vein at the end of the experiment. The serum level of TC, LDL, VLDL and TG were estimating by methods<sup>[16]</sup>.

### 2.6. Effects of BDRE on AST, ALT levels in rats

At the end of experiments rats were anesthetized with diethyl ether and blood was collected by tail vein and estimation of AST and ALT were perform according to Maheshwari *et al*<sup>[17]</sup>.

### 2.7. Effects of BDRE on blood urea nitrogen (BUN) and creatinine

At the end of experiments rats were anesthetized with diethyl ether and blood was collected by tail vein into append drop tubes and estimation of blood urea nitrogen and serum creatine were commercially available span diagnostic kits<sup>[18]</sup>.

### 2.8. Histological studied

The liver and kidney was collected and fixed in 10% formalin, dehydrated in graduated ethanol (50–100%), cleared in xylene, and embedded in paraffin. Sections 4–5 μm thick were prepared and then stained with hematoxylin and eosin (H–E) dye and examined for histopathological changes under the microscope (Leica DMIL, Leica Microsystems AG, Wetzlar, Germany). The images were taken using a Leica DFC 280 CCD camera (Leica Microsystems Digital Imaging, Cambridge, UK) at original magnification of 200×. The sections were examined microscopically for histopathology changes<sup>[19]</sup>.

### 2.9. Statistical analysis

The data were expressed as mean±SEM (Standard Error Means) and the results were analyzed by One–way ANOVA followed by Dennett's test (graph pad version–3.0). The value of  $P < 0.05$  was considered significant.

### 3. Results

#### 3.1. Effects of BDRE on body weight and visceral fat pad

High fat diet fed rats showed significant increase in 87% body weight between 1st initial week and 8th final week compare to normal control group of animals. Administration at a dose of 5 mg/kg standard, BDRE 200 and 400 mg/kg daily showed significantly reduced in body weight ( $P<0.01$ ,  $P<0.01$  and  $P<0.05$ ) when compare with HFD group. But administrations of BDRE 100 mg/kg daily indicate not significant when compare with HFD group (Table 1). There was significant increased in fat pad of internal organs like heart, liver, spleen, kidneys perirenal and uterine fat pads in HFD rats when compare to normal control group. After

the administration of standard (5 mg/kg) and BDRE (200, 400 mg/kg) daily showed significant ( $P<0.01$ ,  $P<0.01$  and  $P<0.05$ ) reduced in weight of heart, spleen, kidneys perirenal and uterine fat pads and 100 mg/kg daily administered it showed non significant compare with their obese control group (Table 2).

#### 3.2. Effects of BDRE on serum TC levels

Figure 1 showed that the change in total cholesterol level concentration significantly increase induced by HFD fed in rats compared to the normal control group. Administration of 5 mg/kg/day sibutramin showed significant decrease ( $P<0.01$ ) in TC ( $126.41\pm3.35$ ), LDL ( $54.96\pm1.92$ ) and VLDL ( $12.48 \pm 0.25$ ) levels in group III. On treatment of BDRE 200 mg/kg

**Table 1**  
BDRE effects on Body Weight in SD rats.

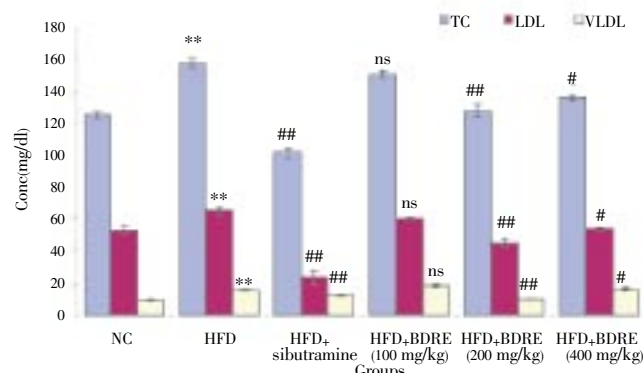
Parameters	Normal Control (NC)	High Fat Diet (HFD)	Sibutramin 5 mg/kg	BDRE 100 mg/kg	BDRE 200 mg/kg	BDRE 400 mg/kg
Initial Body Weight (g)	134.17±2.21	135.00±2.58	136.67±2.74	138.34±3.33	134.17±3.01	136.67±3.80
Final Body Weight (g)	207.92±5.42	326.25±8.96**	231.67±6.49##	284.26±5.94 <sup>ns</sup>	242.34±6.73##	255.00±7.36 <sup>#</sup>
Body weight gain (g)	73.75±2.43	192.58±7.09	110.0±3.92	146.92±6.27	125.66±4.26	136.41±5.37
Food intake Ratio (g/d)	55.29±3.97	129.28±7.89**	80.61±5.86##	106.00±7.06 <sup>ns</sup>	94.56±6.63##	83.75±4.37 <sup>#</sup>

All values are expressed as Mean±S.E.M. for six animals. (n=6)  
<sup>ns</sup>: non significant, when compared with obese control (group II)  
<sup>\*\*</sup> $P<0.01$ = highly significant when compared with normal control (group I)  
<sup>#</sup> $P<0.05$ , <sup>##</sup> $P<0.01$ = significant when compared with obese control (group II)

**Table 2**  
BDRE effect on visceral fat pad in SD rats.

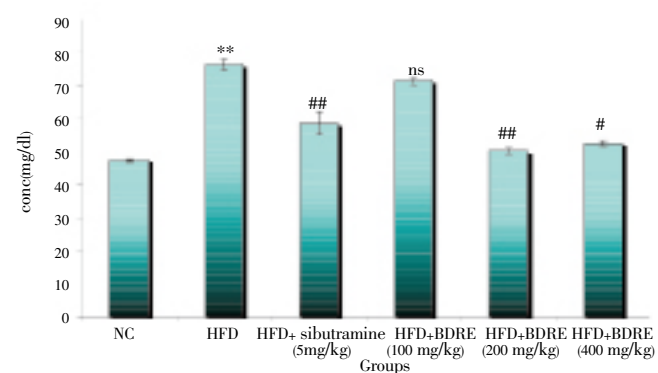
Treatment	Heart (g)	Kidney (g)	Liver (g)	Uterus Fat (g)
Normal	1.77±0.06	1.16±0.04	1.56±0.11	1.45±0.07
HFD	2.31±0.09**	1.57±1.23**	2.36±0.13**	2.70±0.10**
HFD + Sibutramin (5 mg/kg)	1.87±0.09##	1.18±0.39##	1.59±0.12##	1.45±0.04##
HFD + BDRE (100 mg/kg)	2.04±0.11 <sup>ns</sup>	1.34±1.15 <sup>ns</sup>	1.96±0.34 <sup>ns</sup>	2.53±0.11 <sup>ns</sup>
HFD + BDRE (200 mg/kg)	1.89±0.08##	1.28±0.74##	1.61±0.14##	1.44±0.04##
HFD + BDRE (400 mg/kg)	1.98±0.10 <sup>#</sup>	1.34±0.71 <sup>#</sup>	1.89±0.16 <sup>#</sup>	2.35±0.13 <sup>#</sup>

All values are expressed as Mean±S.E.M. (n=6)  
<sup>ns</sup>: non significant, when compared with obese control (group II)  
<sup>\*\*</sup> $P<0.01$ = highly significant when compared with normal control (group I)  
<sup>#</sup> $P<0.05$ , <sup>##</sup> $P<0.01$ = significant when compared with obese control (group II)



**Figure 1.** Effects of BDRE on serum levels of total cholesterol (TC), low density lipoprotein (LDL) and very low density lipoprotein (VLDL) in experimental groups of animals.

All values are expressed as Mean±S.E.M. for six animals.  
<sup>ns</sup>: non significant, when compared with obese control (group II).  
<sup>\*\*</sup> $P<0.01$ = Significant when compared with normal control (group I).  
<sup>#</sup> $P<0.05$ , <sup>##</sup> $P<0.01$ = Significant when compared with obese control (group II).



**Figure 2.** Effect of BDRE on triglyceride level in experimental groups of animals.

All values are expressed as Mean± S.E.M. for six animals.  
<sup>ns</sup>: non significant, when compared with obese control (group II).  
<sup>\*\*</sup> $P<0.01$ = Significant when compared with normal control (group I).  
<sup>#</sup> $P<0.05$ , <sup>##</sup> $P<0.01$ = Significant when compared with obese control (group II).

and 400 mg/kg/day showed significantly decrease ( $P<0.01$  and  $P<0.05$ ) in serum TC ( $131.52\pm 3.70$  and  $135.90\pm 1.61$ ), LDL ( $55.18\pm 3.07$  and  $56.02\pm 1.04$ ), VLDL ( $9.52\pm 0.09$  and  $10.07\pm 0.16$ ) in groups V and VI. When administration of 100 mg/kg daily administered, observed non significant of TC ( $145.51\pm 2.18$ ), LDL ( $79.78\pm 3.48$ ) VLDL ( $15.73 \pm 0.36$ ) group IV and compare with obese control group-II.

3.3. Effects of BDRE on serum TG levels in obese rats

HFD animals showed significant increase ( $P<0.01$ ) the serum triglyceride when compare with normal control group. Administration of 5 mg/kg/day sibutramin showed significantly decrease ( $P<0.01$ ) in TG ( $58.77\pm 3.51$ ) levels in group III. Figure 2 explained the treatment of BDRE 200 mg/kg and 400 mg/kg/day showed significantly decrease ( $P<0.01$  and  $P<0.05$ ) in serum TG ( $47.56\pm 0.84$  and  $50.35\pm 0.81$ ) in groups V and VI, when administration of 100 mg/kg daily administered, observed non significant of TG ( $71.47\pm 1.07$ ) in group IV and compare with obese control group-II.

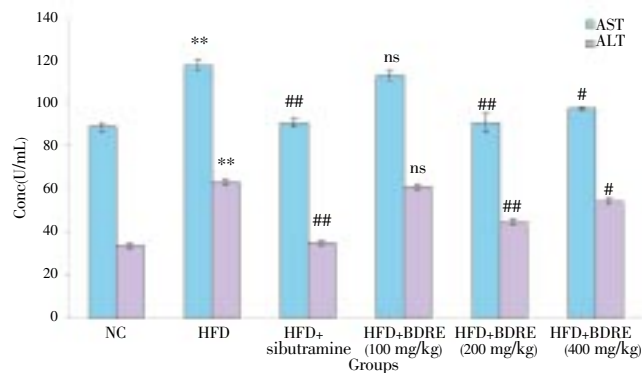


Figure 3. Effects of BDRE root extract on serum alanine and aspartate in experimental groups of animals. All values are expressed as Mean  $\pm$  S.E.M. for six animals. ns: non significant, when compared with obese control (group II). \*\* $P<0.01$ = Significant when compared with normal control (group I). # $P<0.05$ , ## $P<0.01$ = Significant when compared with obese control (group II).

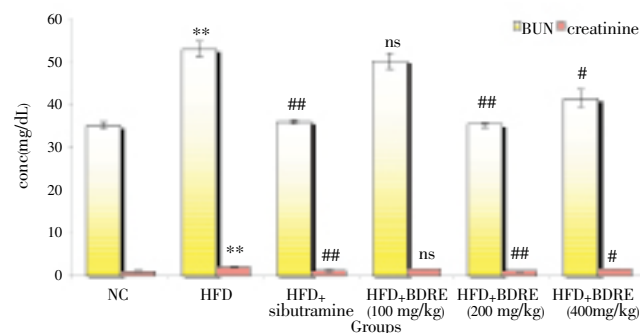


Figure 4. Effects of BDRE on blood urea nitrogen and creatinine levels in experimental groups of animals. All values are expressed as Mean  $\pm$  S.E.M. for six animals. ns: non significant, when compared with obese control (group II). \*\* $P<0.01$ = Significant when compared with normal control (group I). # $P<0.05$ , ## $P<0.01$ = Significant when compared with obese control (group II).

3.4. Effects of BDRE on serum AST and ALT levels

The results was showed the serum AST and ALT levels significantly increased ( $P<0.01$ ) in HFD induced obese animals when compare to normal control group. Treatment of 5mg/kg/day sibutramin showed significantly decrease ( $P<0.01$ ) in AST and ALT ( $81.91\pm 2.85$  and  $45.47\pm 1.23$  U/ mL) levels in group III. Figure 3 showed that the orally administrating of BDRE (200 and 400 mg/kg/day) showed significantly decrease ( $P<0.01$  and  $P<0.05$ ) serum AST and ALT levels ( $83.90\pm 2.37$ ,  $87.52\pm 2.81$  and  $48.39\pm 1.47$ ,  $51.68\pm 1.38$ ) in groups V and VI, whereas administration of 100 mg/kg/d showed non significant of AST ( $112.68\pm 3.33$ ) and ALT ( $54.68\pm 1.04$ ) levels in groups IV when compared with obese control group-II.

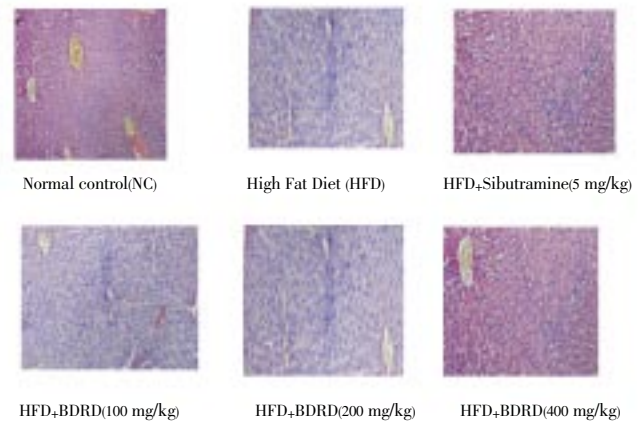


Figure 5. Effect of BDRE stained with hematoxylin and eosin sections of rat liver (10 $\times$ ) in experimental groups of animals.

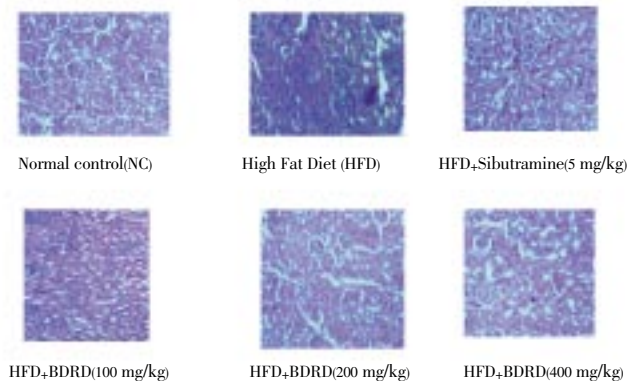


Figure 6. Effect of BDRE stained with hematoxylin and eosin sections of rat kidney (10 $\times$ ) in experimental groups of animals.

3.5. Effects of BDRE on blood urea nitrogen and creatinine

Figure 4 showed the blood urea nitrogen (BUN) and serum creatinine was found to be significantly increased ( $53.05\pm 0.74$  and  $2.06\pm 0.16$ ) when compare with normal control group. Treatment of 5mg/kg/day sibutramin showed significantly decrease ( $P<0.01$ ) the BUN and creatinin ( $36.87\pm 0.49$  and  $1.01\pm 0.11$  mg/dL) levels in group III. On treatment of BDRE



200 mg/kg and 400 mg/kg/d showed significantly decrease ( $P<0.01$  and  $P<0.05$ ) the BUN and creatinin ( $37.28\pm0.47$ ,  $40.57\pm0.68$  and  $1.06\pm0.13$ ,  $1.41\pm0.10$ ) in groups V and VI, while 100 mg/kg/d showed non significant ( $P>0.05$ ) on the level of BUN and creatinine ( $50.36\pm1.79$ ,  $1.67\pm0.02$ ) in groups IV and compare with obese control group-II.

### 3.6. Histopathology

The histopathological observations also support the results obtained from serum biochemical assays. The liver lobules of the normal control animals (Group-I) showed a classical structure with hepatocyte plates directed from the portal triads toward the central vein. Whereas in the liver section fat cells are deposited and cells are degenerated and kidney glomeruli cells was ruptured in HFD treated animals (Group-II) showed changes through out the lobules, cellular vacuolization and dilation of nucleous with focal disruption of the sinusoidal endothelium whereas in kidney glomerulus cells was also changed. The animals (Group-IV and V) treated with BDRE at a dose of 200 and 400 mg/kg and standard at 5mg/kg (Groups III) no cellular vacuolation and better preservation of liver architecture compare with normal control (Group-I) (Figures 5 and 6) while the animals treated with 100 mg/kg observed non significant *i.e.* the liver and kidney cell was ruptured (Group-IV).

## 4. Discussion

Obesity may induce systemic oxidative stress in accumulated fat is one of the underlying causes of deregulation of adipocytokine and development of metabolic syndrome[20]. *B. diffusa* extract that reduced the food intake in SD rats induced obesity by inhibiting carbohydrate and fatty acids that would have become fat in the liver into the hepatic glycogen[21]. The final body weight gain in 60 days old rats in the HFD group were 87.66% greater than the normal control groups. The food intake of rats in the HFD+BR group was significantly lower than the values for the HFD group ( $P<0.01$ ). Therefore BDRE have the ability to reduced the body weight gain could be due to its combined effects on the metabolic and serotonin pathways[22]. The relative weight of the total visceral fat pad depots were significantly higher in HFD group compare with the ND animals ( $P<0.01$ ) were significantly lower by supplementing HFD with BDRE ( $P<0.01$ ). The perirenal, uterine, spleen, kidney and heart fat-pad weight were significantly reduced by supplemented with BDRE ( $P<0.01$ ) in the HFD group. The BDRE (200 and 400 mg/kg/day) treated hypercholesterolemic rats along with HFD rats for 60 days significantly decrease serum cholesterol, triglycerides and LDL levels ( $P<0.01$  and  $P<0.05$ ), while 100 mg/kg/day of BDRE treated group-IV did not showed any significant reduction in the serum cholesterol, triglycerides and LDL

levels. The phytoconstituents compounds  $\beta$ -sitosterol found in this plant which is structurally similar to cholesterol has been suggested to reduce cholesterol by lowering the level of LDL-cholesterol and cholesterol level decrease significantly in plasma without any side effects[23]. Hypercholesterolemia is one of the major factors associated with atherosclerosis disease in which oxidative modification of human low density lipoprotein[24] play an important roll was reduced LDL level ( $P<0.01$  and  $P<0.05$ ) after administration of BDRE (200 and 400 mg/kg). The hypoglycaemia is characterized by a reduction in insulin-mediated glucose disposal in type 2 diabetes patients[25].

The enzymes AST and ALT are present with higher concentrations in the liver under normal conditions whereas during hepatic necrosis or membrane damage, these enzymes will be released into the systemic circulation, as indicated by elevated serum enzyme levels. The activities of AST and ALT are sensitive indicators of acute hepatic necrosis[26]. ALT is a hepatospecific enzyme that is principally found in the cytoplasm[27]. These results indicated that BDRE have liver protective effect as they significantly reduced ( $P<0.01$  and  $P<0.05$ ) the elevated liver markers (Group-II) levels of both the enzymes on administration of BDRE (200 and 400 mg/kg/d) with HFD groups IV and V. Blood urea nitrogen is derived in the liver protein/amino acid from diet or tissue sources and is normally excreted in the urine. In renal disease, the serum urea accumulates because the rate of serum urea production exceeds the rate of clearance. The plasma creatinine concentrations in normal individuals are usually affected by a number of factors such as the muscle mass, high protein diet, and catabolic state[28]. The histopathological examination of group II animals showed changes in architecture with fatty generation in liver and disarrangement of glomeruli with inflammation were seen in kidney. On administration of BDRE (200 and 400 mg/kg) have liver and kidney protective activity which is comparable with normal control group, whereas 100 mg/kg administered does not shows protective activity on liver and kidney in group-IV. The enzymatic estimation of liver and kidney markers enzymes were supported the histopathological finding.

The present study demonstrated that significant reduction in body weight, visceral fat pad weight as well as lipid profiles, liver and kidney marker enzyme levels exhibited as anti-obese activity and hypolipidemic activity of BDRE in experimental animals supplemented with high fat diets. Further, there is need to identify the phytoconstituents responsible for the activity and to formulate a polyherbal antiobesity preparation containing *Boerhaavia diffusa* root extract as a main ingredient.

### Conflict of interest statement

We declare that we have no conflict of interest.

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