International Journal of Pharmaceutical and Bio-Medical Science

ISSN(print): 2767-827X, ISSN(online): 2767-830X

Volume 04 Issue 06 June 2024

Page No: 585 -594

DOI: https://doi.org/10.47191/ijpbms/v4-i6-17, Impact Factor: 7.792

Evaluation of Anti-Obesity Effect of Bromhexine using HFD Fed Rats

Navdha Vyas[1], Sneha Bhimani[2], Dr. Tejal Gandhi[3]

[1]Research Scholar, Gujarat Technological University, Anand Pharmacy College, Opp. Town Hall, Rahtlav, Mathiya Chora, Anand-388001, Gujarat, India.

[2]Alumni, Anand Pharmacy College, Opp. Town Hall, Rahtlav, Mathiya Chora, Anand-388001, Gujarat, India.

[3]Principal and Professor, Anand Pharmacy College, Opp. Town Hall, Rahtlav, Mathiya Chora, Anand-388001, Gujarat, India.

ABSTRACT

Obesity is a long-term metabolic disease characterised by an excess of energy stored in adipose tissue, leading to oxidative stress, inflammation, and malfunction of adipocytes. The rising number of non-communicable diseases demands consideration to behavioural risk factors such as elevated body mass index. The aim of this study is to evaluate anti-obesity effect of bromhexine using HFD fed rats. Obesity was induced in male wistar rats by high fat diet. The animals were divided in 5 groups: (I) Control group received rat chow diet (II) Model control received HFD (III) Standard group was administered orlistat (10 mg/kg, p.o.,) along with HFD (IV) Treatment group was administered treatment drug bromhexine (5 mg/kg, p.o.,) + HFD (V) Treatment group was administered treatment drug bromhexine (10 mg/kg, p.o.,) + HFD. Estimation of anthropometric, haemodynamic, left ventricular function, biochemical, pancreatic lipase activity, antioxidant and histopathology were performed. HFD control animals developed the signs of obesity including elevated abdominal fat deposition, hypertension, altered lipid profile (TG, TCHL, HDL, and VLDL), and increased oxidative stress markers. The analysis of left ventricular function (LVEDP, dp/dtmax., dp/dtmin) presented promising results. Bromhexine treatment (5 mg & 10 mg) prevented elevation in blood pressure, oxidative stress and ameliorated lipid profile, liver profile and glucose tolerance indicating the protective effect of bromhexine in improving of lipid, liver enzyme and glucose levels. Pancreatic lipase activity was performed bromhexine (10mg/kg) showed significant inhibition. Furthermore, histopathological studies also indicates that bromhexine ameliorates obesity. Bromhexine dose of 10mg/kg has shown promising results in ameliorating obesity.

KEYWORDS: Obesity, high fat diet, bromhexine

ARTICLE DETAILS

Published On:
28 June 2024

Available on:
https://ijpbms.com/

1.0. INTRODUCTION

Obesity is a severe health problem in the twenty-first century, caused by a complex mix of hereditary and environmental variables such as culture, socioeconomic status, and lifestyle.[1] Being obese raises the risk of a number of illnesses and ailments that are associated with a higher death rate. These comprise non-alcoholic fatty liver disease (NAFLD), depression, obstructive sleep apnea, osteoarthritis, cardiovascular diseases (CVD), metabolic syndrome MetS), chronic kidney disease (CKD), hyperlipidaemia and hypertension.[2] According to estimates from the World Obesity Federation, 800 million people worldwide suffer from obesity today, of which 39 million are children under the age of five (as of 2020) and 340 million are children and adolescents between the ages of five and nineteen. Furthermore, an additional 1 billion people are at danger of being overweight or obese.[3] In obese individual, inflammatory cytokines, including IL-6, TNFα, C-reactive protein (CRP), IL-18, resistin, and visfatin, are circulated at different amounts.[4] Most important culprit for obesity is an excess amount of fats. The fat mainly cholesterol and triglycerides come mostly from the food we eat. The metabolism of triglycerides occurs by three enzymes namely lingual lipase, gastric lipase and pancreatic lipase. The 50-70% (maximum) metabolism occurs by pancreatic lipase.[5] The next step is absorption of triglycerides in the form of

monoglycerides and fatty acids. For transportation of these to various organs, different lipoproteins are synthesized by the liver. Most of the drugs target excess fats after absorption. But researchers are now more interested in curbing the extra fat at the first step itself before absorption. The lipase inhibitors change the conformation as well as catalytic activity of enzymes, rendering less metabolism and absorption of triglycerides from food.[6] Currently, two main medicines are used for obesity namely orlistat which is lipase inhibitor and sibutramine which is appetite suppressant. To contribute more in this field we aimed to target lipase inhibition by drug repurposing. Bromhexine currently acting as mucolytic, has been proven as lipase inhibitor by Gholami et al. The in-silico results proved stable interactions of Bromhexine with residues in the catalytic site of the lipase. The in-vitro study was performed on pseudomonas aeruginosa. The bacteria need lipase enzyme to invade host tissues.[7] Bromhexine showed competitive inhibition of lipase enzyme by making structural changes in it which was studied by fluorescence spectroscopy. With this background, Bromhexine was investigated as an anti-obesity drug in HFD induced obesity in rats.

2.0. MATERIAL AND METHODS

2.1. Drug and Preparation of Solution

The mucolytic drug Bromhexine and Orlistat was obtained as a gift sample from S.G. Bio pharma Pvt. Ltd, Mumbai. Bromhexine and Orlistat solutions were prepared freshly every day by making suspension of the drug in 0.5 % Carboxy Methyl Cellulose (CMC).

2.2. Dose

The dose for the study was converted from a human dose. Two doses are selected 5mg/kg and 10mg/kg for bromhexine.[8] The dose of standard drug orlistat is 10mg/kg.[9]

2.3. Chemicals and Kits:

All the chemicals used in this project were of the analytical grade and have been procured from Merck Chemical, Ahmedabad. All biochemical tests were performed using standard kits purchased from i-chem, Jeev Diagnostic Pvt. Ltd.

2.4. Selection and source of animals

Healthy male wistar rats weighing 150-200g were purchased from Pavo Research Solution, Baroda. The experimental protocol was approved by the Institutional Animal Ethical Committee (IAEC) of Anand Pharmacy College as per the guidance of the committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA), Ministry of Social Justice and Empowerment, Government of India (Protocol no. APC/2021-IAEC/2107) (Annexure I). The animal housing facility of APC is registered under Rule 5(a) for the Breeding and Experiments on Animal control and supervision rules 1998 (Registration no. 277/CPCSEA24th 2000).

2.5. Induction of Obesity

Obesity was induced in rats by giving a high-fat diet for the duration of 8 weeks (56 days). The composition of high-fat diet was selected from Sharma et al.[10]

2.6. Experiment design

The experimental animals were randomized based on body weight into five groups with n=6 animals in each group: Group I Normal Control: Received vehicle (0.5% CMC) orally and regular rat chow diet & R.O water *ad libitum*. Group II Model Control: Received HFD + R.O water for duration of 8 weeks. Group III Standard Control: Received orlistat (10 mg/kg p.o.) along with HFD + R.O water for duration of 8 weeks. Group IV Treatment group -I: Received bromhexine (5 mg/kg p.o.) along with HFD + R.O water for duration of 8 weeks. Group V Treatment group – II: Received bromhexine (10 mg/kg p.o.) along with HFD + R.O water for duration of 8 weeks.

2.7. Anthropometric parameter

The body weight of each animal was measured start of the experiments and end of the experiments. Food intake and water intake was recorded daily for all animals per cage and the average was calculated. Abdominal circumference (immediately anterior to the forefoot) and nasal to anus ratio (nose-to-anus or nose–anus length) was measured start of the experiments and end of the experiments by standard measuring tape. Using abdominal circumference and nose to anus length, BMI and Lee's Index was calculated.[11]

Body mass index (BMI) = body weight (g)/length^2 (cm^2)

Lee's index = cube root of body weight (g) /nose-to-anus length (cm)

2.8. Evaluation of biochemical parameters

The rats were anesthetized and blood samples were collected from the eye through retro-orbital plexus muscle using ketamine (50mg/kg) and xylazine (10mg/kg) i.p. as an anesthetic agent. Blood collection was done on 56th day.Glucose, HDL, LDL, VLDL, Cholesterol, Triglycerides, was measured in serum samples by was performed in serum samples using commercially available standard enzymatic kits (Span Diagnostics Pvt. Ltd., India).

2.9. Isolated Perfused Heart Preparation Langendorff[12]

Perfusion of isolated hearts was performed according to the Langendorff technique. The hearts were excised after thoracotomy and tied to the aortic cannula. Hearts were perfused with modified Krebs-Henseleit buffer (composition: CaCl2 (1.5 mM), KCl (4.7 mM), KH2PO4 (1.18 mM), MgSO4 (1.66 mM), NaCl (118 mM), NaHCO3 (24.88 mM), glucose (5.55 mM), Na-pyruvate (2 mM), and bovine albumin (0.1%w/v)). The buffer was filtered by 0.45 µm membrane filter before use. The cannulated heart was rapidly connected with the Langendorff perfusion apparatus (flow rate of buffer: 9.7 ± 0.5 ml/min; carbogen (95% O2 and 5% CO2), and temperature: 37℃). A latex balloon filled with 50% methanol was tied to the end of a polyethylene tube

connected to the pressure transducer and was inserted into a left ventricle of the isolated heart. The diastolic pressure of 5 to 6 mmHg was adjusted and after 30 minutes, various parameters were measured. Parameters measured included dP/dtmax (rate of maximum LV pressure rise); dP/dtmin (rate of minimum LV pressure fall) and left ventricular end-diastolic pressure (LVEDP) as measurements of relaxation. The data was recorded by physiological recording system and Biopac recording device (MP-36 Biopac Systems,Inc., USA).

2.10. Oxidative stress marker parameters (Liver Tissue)

Liver tissue was excised immediately and rinsed in ice-chilled normal saline and the whole liver was weighed. A known weight of liver tissue was taken and homogenized (EIE Instruments Pvt Ltd, 0603121) in 5.0 mL 0.1 M Tris- HCl buffer (pH 7.4). The homogenate was centrifuged at 10,000 rpm for 15 min using the Remi C-24 (high-speed cooling) centrifuge and the supernatant was used for estimation of indicator of lipid peroxidation (MDA assay)[13], Superoxide dismutase (SOD)[14], Catalase[15] and glutathione(GSH)[16].

2.11. Histopathology

Adipose tissue and liver were fixed in 10 % neutral buffered formalin and were embedded in paraffin wax. These tissues were sliced in thin sections (5 mm) and stained with haematoxylin and eosin (H & E) for the determination of morphological change.

2.12. Statistical Analysis

All the data are presented as mean ± SEM. Statistical analysis of biochemical parameters, LV functions parameters and oxidative stress marker parameters were carried out using one-way analysis of variance (ANOVA) followed by Dunnett's post hoc test. (Prism, GraphPad version 6.01, GraphPad Software, Inc.). Data were considered statistically significant at P≤0.05.

3.0. RESULT

3.1. Effect of bromhexine on anthropometric parameters in HFD induced obesity in rats:

3.1.1. Effect of bromhexine on % increase in body weight in HFD induced obesity in rats:

The high fat induced Obesity in rats showed significant rise (62.67±0.1146) in % increase in body weight as compared to normal control group. While the treatment animal receiving Bromhexine (5mg/kg) with high fat diet showed significant decline (42.18±0.09452) in % increase in body weight and Bromhexine (10 mg/kg) with high fat diet showed significant decline (18.36±0.2301) in % increase in body weight as compared to model control group

.

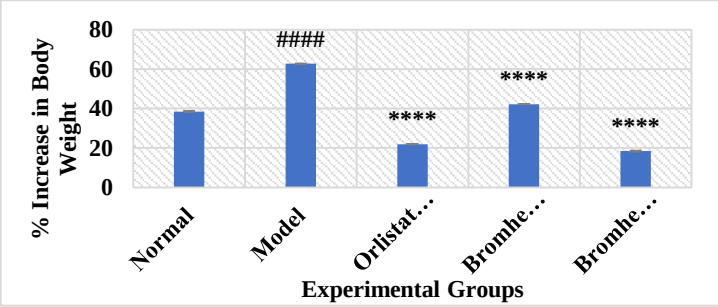


Figure 1 Effect of Bromhexine on % increase in body weight in HFD induced Obesity in rats

Results are represented as Mean ± SEM with n-6 animals in each group. One way ANOVA was used to determine the significant statistical difference between the groups by using graph pad prism 6.01. #### indicates significant difference from normal control P≤0.0001, * indicates significant difference from model control P≤0.05, ** indicates significant difference from model control P≤0.01, *** indicates significant difference from model control P≤0.001, **** indicates significant difference from model control P≤0.0001.

3.1.2. Effect of bromhexine on Body Mass Index(BMI) in HFD induced obesity in rats:

HFD fed rats showed significant rise (0.62±0.022) in BMI as compared to normal control group. While the Bromhexine (5 mg/kg) treated animal showed significant decline (0.5154±0.03263) in BMI and Bromhexine (10 mg/kg) treated animal showed significant decline (0.4392±0.008797) in BMI compared to model control group.

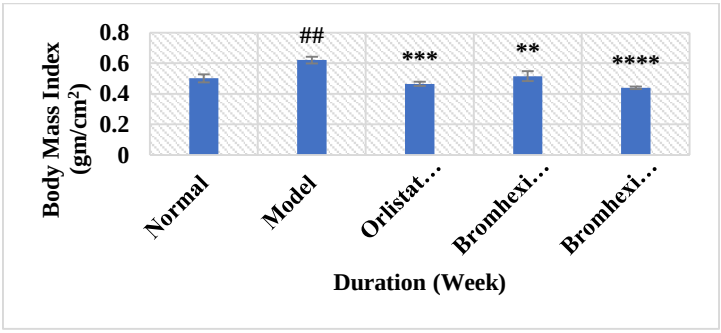


Figure 2: Effect of Bromhexine on BMI (gm/cm^2)in HFD induced Obesity in rats

Results are represented as Mean ± SEM with n-6 animals in each group. One way ANOVA was used to determine the significant statistical difference between the groups by using graph pad prism 6.01. ## indicates significant difference from normal control P≤0.01, ** indicates significant difference from model control P≤0.01, *** indicates significant difference from model control P≤0.001, **** indicates significant difference from model control P≤0.0001.

3.1.3. Effect of bromhexine on weight of white adipose tissue, brown adipose tissue, perirenal fat pad, epididymal fat pad, subcutaneous fat pad in HFD induced obesity in rats:

Then abdominal fat pad was weight which was found in the mesenteric area. In model there was significant (1.8± 0.2345) rise in weight as compared to normal control group, while in treatment control group bromhexine (5mg) there was significant (1.02±0.1158) decline in the weight as compared to model control group, while in treatment control group bromhexine (10 mg) there was significant (0.65±0.1285) decline in the weight as compared to model control group. The perirenal fat pad was significantly (1.92±0.1293) increased in weight of fat pad of model control group as compared to that of normal control group, while there was significant decline (1.1±0.05477) and (0.6667±0.1308) in the weight of fat pad in the treatment control group bromhexine (5mg) and bromhexine (10mg) as compared to model control group. Subcutaneous fat pad is collected and it is seen that model control group is having more amount of subcutaneous fat pad as compared to that of normal control group, while there is decrease in weight of fat pad in treatment control group as compared to model control group. Epididymal fat pad was collected near the region of epididymis and it is seen that, model control group is having significant (4.4±1.073) high amount of fat as compared to that of normal control group, while is treatment control group bromhexine (5mg) & bromhexine (10mg) significant (2.0±0.3286) & (1.283±0.2509) decline in the weight of fat pad as compared to that of model control group.

Table 1 Effect of bromhexine on weight of white adipose tissue, brown adipose tissue, perirenal fat pad, epididymal fat pad, subcutaneous fat pad in HFD induced obesity in rats

	Normal	Model	Orlistat (10mg)	Bromhexine (5mg)	Bromhexine (10mg)
White Adipose	0.4 ± 0.057	1.8 ± 0.234 ###	0.9667 ± 0.160 **	1.02 ± 0.115 *	0.65 ± 0.128 ***
Brown Adipose	0.9667 ± 0.088	0.525 ± 0.075	0.6 ± 0.146	0.56 ± 0.067	0.6667 ± 0.145
Perirenal	0.5333 ± 0.145	1.92 ± 0.129 ###	0.9 ± 0.222 ***	1.1 ± 0.054 **	0.6667 ± 0.130 ***
Epididymal	1.767 ± 0.176	4.4 ± 1.073 #	2.25 ± 0.348 *	2.0 ± 0.328 *	1.283 ± 0.250 ***
Subcutaneous	2.333 ± 0.554	3.475 ± 0.746	2.183 ± 0.313	2.52 ± 0.452	1.883 ± 0.212

Results were represented as Mean±SEM with n=6 animals in each group. One way ANOVA was used to determine the significant statistical difference between the groups by using graph pad prism 6.01. # Indicates significant difference from normal control group p≤0.05, ### Indicates significant difference from normal control group p≤0.001, * indicates significant difference from model control group P≤ 0.05, ** indicates significant difference from model control group P≤ 0.01, *** indicates significant difference from model control group P≤ 0.001.

3.1.4. Effect of bromhexine on Adiposity Index, Lee's Index and conicity index in HFD induced obesity in rats:

Model control rats showed rise in adiposity index, lee's index and conicity index as compared to normal control group. While the animal receiving Bromhexine treatment (5mg/kg) with high fat diet showed significant decline in adiposity index, lee's index and Conicity index in Bromhexine (10 mg/kg) with high fat diet showed significant decline in Conicity index as compared to model control group.

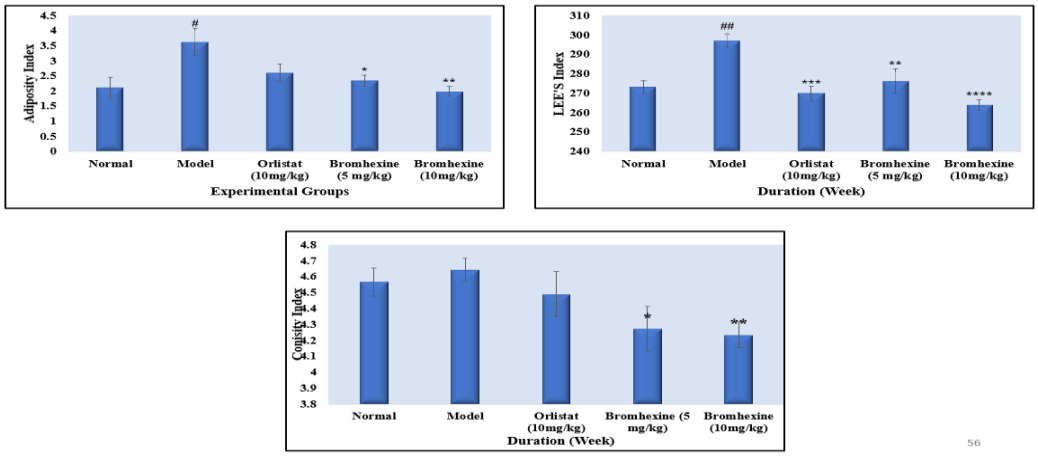


Figure 3: Effect of Bromhexine on Conicity Index in HFD induced Obesity in rats

Results were represented as Mean ± SEM with n-6 animals in each group. One way ANOVA was used to determine the significant statistical difference between the groups by using graph pad prism 6.01. * Indicates significant difference from model control P≤0.05, ** indicates significant difference from model control P≤0.01.

On 16th week i.e., 112th day the serum glucose level was measure, it was shown that significant rise (168.3±4.715) in glucose level as compared to that of normal control groups. While the serum glucose level of group receiving high fat diet along with the treatment Bromhexine (5mg/kg), and Bromhexine (10mg/kg) has shown decline in serum glucose level as compared to model control group.

3.2. Biochemical Parameter:

3.2.1. Effect of bromhexine on glucose in HFD induced obesity in rats:

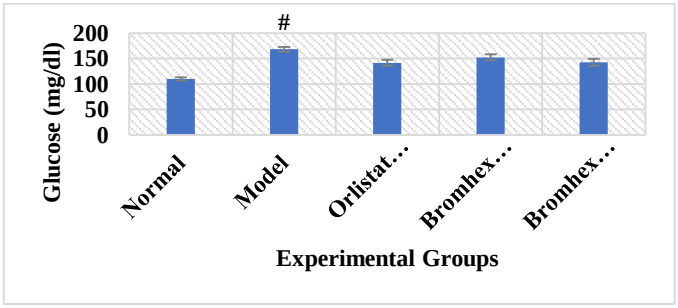
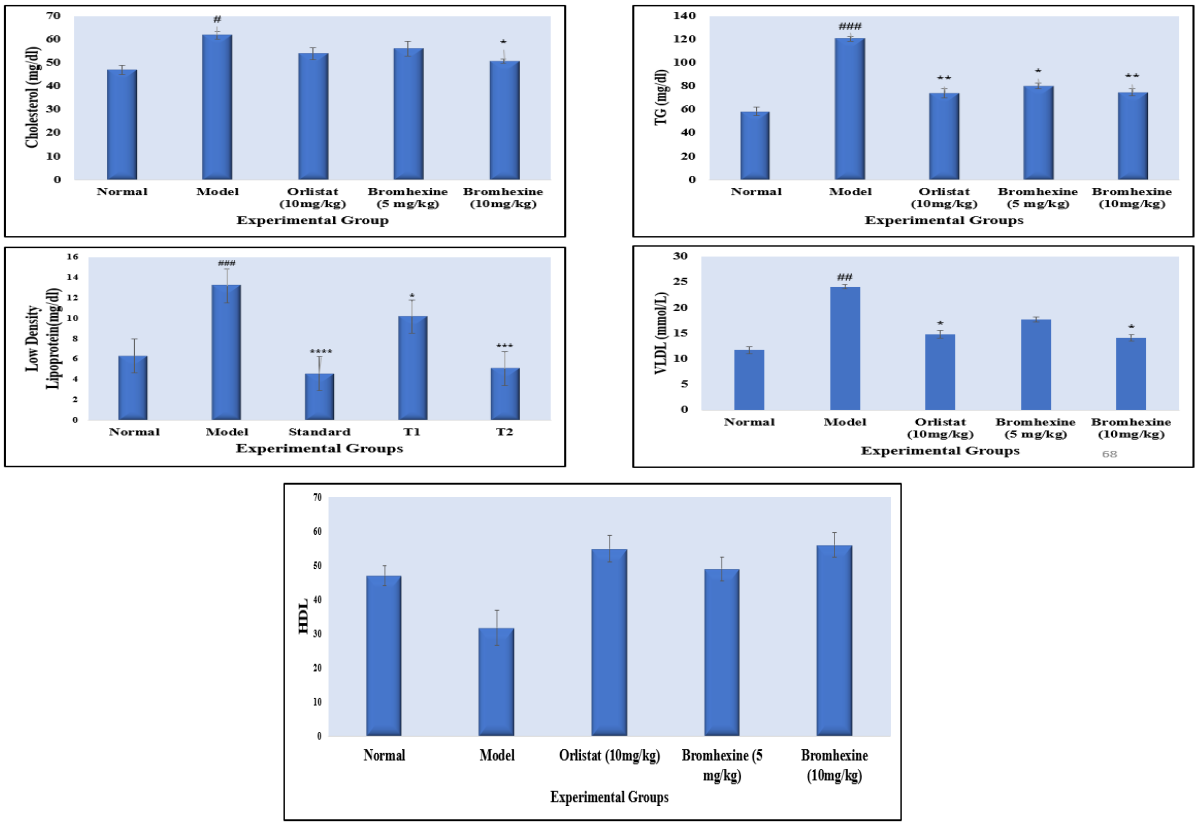


Figure 4: Effect of Bromhexine on glucose in HFD induced Obesity in rats

Results were represented as Mean ± SEM with n-6 animals in each group. One way ANOVA was used to determine the significant statistical difference between the groups by using graph pad prism 6.01. # Indicates significant difference from normal control P≤0.05.

3.2.2. Effect of bromhexine on cholesterol in HFD induced obesity in rats:



3.2.3. Effect of bromhexine on pancreatic lipase in HFD induced obesity in rats:

Pancreatic lipase activity is measured to identify the inhibitory activity of the treatment that is bromhexine 5mg/kg and 10mg/kg. It was found that model control group receiving HFD diet showing higher amount of lipase enzyme as compared to normal control group, while in treatment control group i.e., Bromhexine (5mg/kg) has lower amount lipase enzyme as compared to that of model control group. Bromhexine (10mg/kg) has significant (9.14E+19 ± 4.91E+19) lower amount lipase enzyme as compared to that of model control group

.

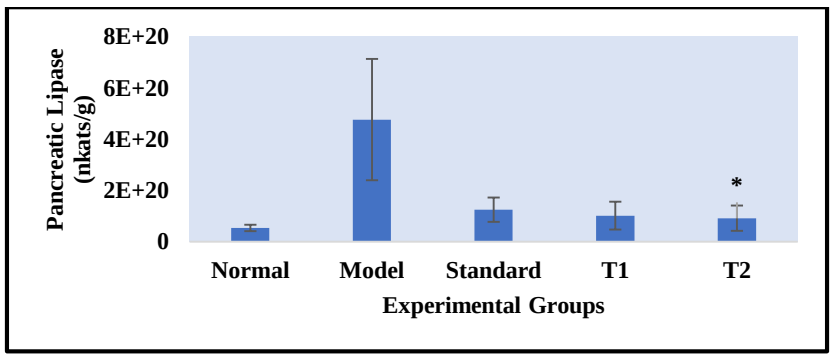


Figure 7 Effect of Bromhexine on Pancreatic lipase in HFD induced Obesity in rats

Results were represented as Mean ± SEM with n-6 animals in each group. One way ANOVA was used to determine the Significant statistical difference between the groups by using graph pad prism 6.01. * Indicates significant difference from model control P≤0.05.

3.2.4. Effect of bromhexine on antioxidant in HFD induced obesity in rats:

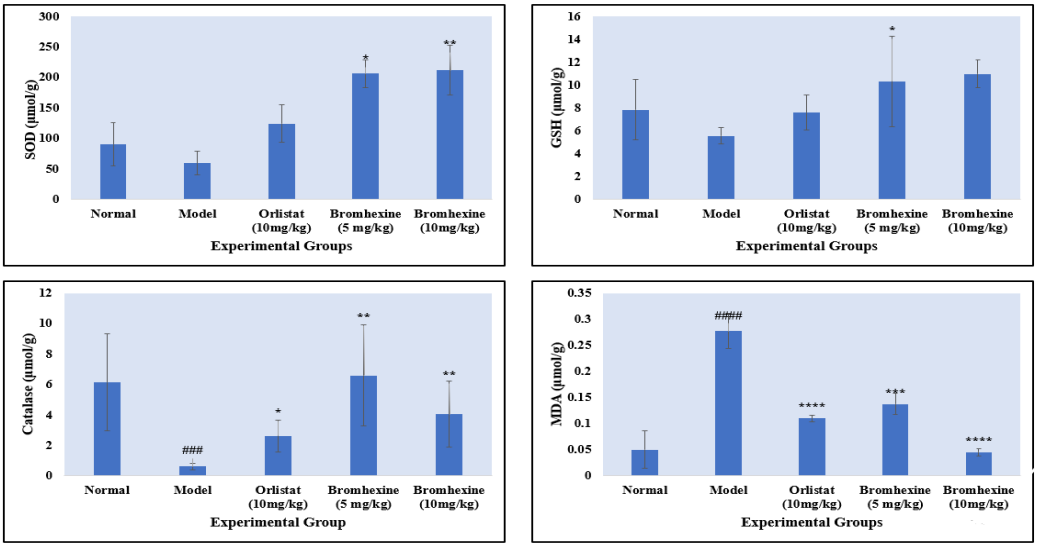


Figure 8 Effect of Bromhexine on antioxidant in HFD induced Obesity in rats

Results were represented as Mean ± SEM with n-6 animals in each group. One way ANOVA was used to determine the significant statistical difference between the groups by using graph pad prism 6.01. * Indicates significant difference from model control P≤0.05.

3.2.5. Effect of bromhexine on histopathology in HFD induced obesity in rats:

There was more accumulation of white adipose tissue and more dense area was seen as compared to that of normal control group and treatment control group of bromhexine 5mg/kg and bromhexine 10mg/kg. Brown adipose tissue model control group appears to have abnormality in brown adipose tissue as compared to that of normal control group. The treatment group both bromhexine 5mg/kg and 10mg/kg has not shown any abnormality. Liver of model control group has shown macrovascular steatosis which is seen during non-alcoholic fatty liver diseases. While there all the liver cell appeared to be normal in normal control group.

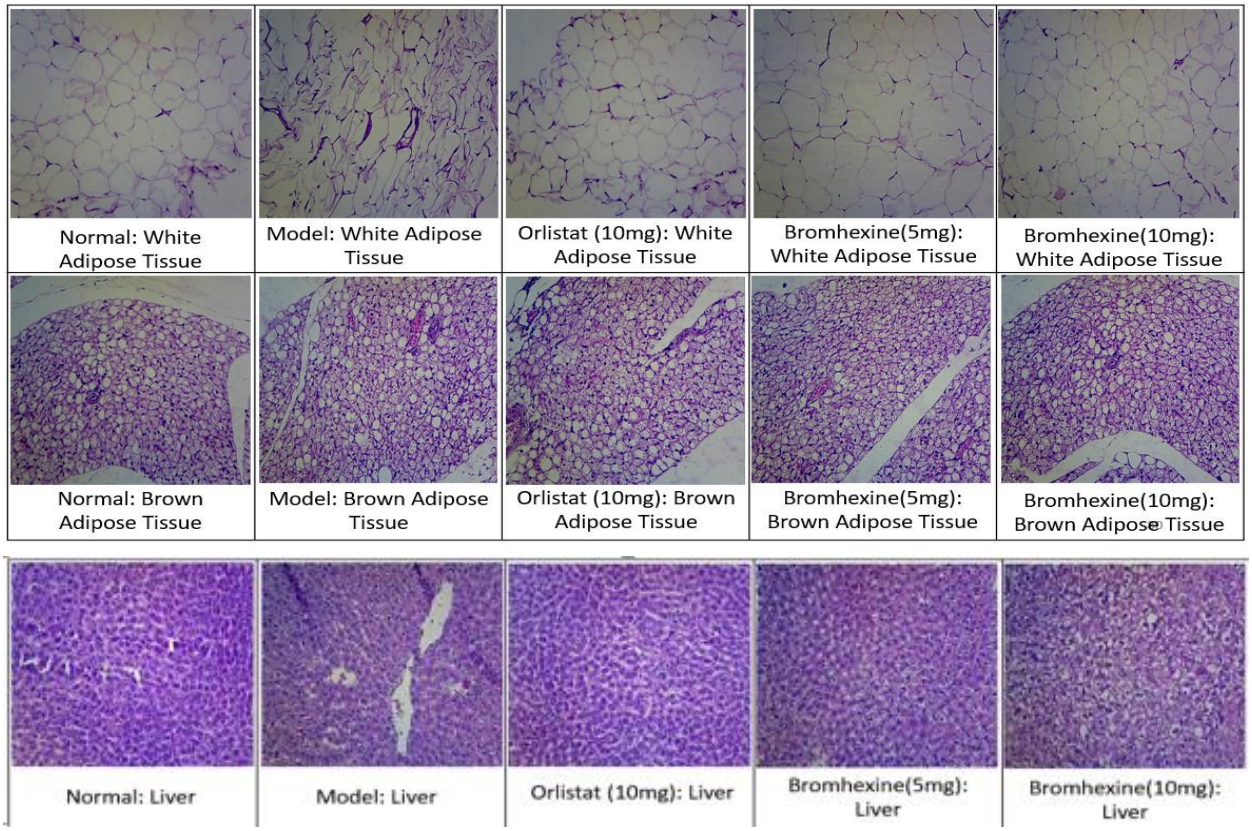


Figure 9 Effect of Bromhexine on histopathology in HFD induced obesity in rats

4.0. DISCUSSION

Obesity is a long-term problem that has persisted for many years. Obesity not only results in aesthetic problems but also causes abnormal physiological metabolism, which causes a series of physiological, psychological and social problems. Diseases such as coronary heart disease, hypertension, osteoarthritis and gout increase the risk of obesity, and there are some reproduction effects as well.[5] The incidence of systemic and gastrointestinal tumours, such as endometrial cancer, breast cancer, colon cancer, polycystic ovary syndrome, infertility, is also significantly elevated in obese patients.[17] Therefore, prevention and treatment of obesity itself is the key to reducing the prevalence and mortality of chronic metabolic diseases. An adequate animal model that replicates all of these symptoms of human obesity is required to evaluate potential pharmacological therapeutics to treat organ failure. So, high fat diet suits perfect to test the effect of drug in obesity as diet includes Sucrose, dalda ghee, normal rat chow pallets and butter. The relationship between sugar preference, sugar-induced hyperphagia and obesity is unresolved. Excess amount of sucrose helps to development of obesity by gained more total body mass (TBM), had greater fat mass, and displayed impaired glucose tolerance. Sucrose intake promotes obesity by increasing serum insulin levels and tissue lipid accumulation.[18] Dalda ghee used in high fat diet can lead to the higher cholesterol level which can also cause obesity. Dalda reduce the level of high-density lipoprotein (HDL), which is also term good cholesterol. It increases the overall cholesterol level, which significantly affect the functioning of the heart and might leads to cardiovascular disease. Adding butter in to the high fat diet causes significant rises in obesity as well as triglyceride content and cholesterol. Butter consumption for long period of time is associated with type 2 diabetes and also in CVD. Butter is a high-fat dairy product that is likely to raise cholesterol levels. Dairy fat includes a lot of long-chain SFAs including myristic and palmitic acids, which are known to raise LDL cholesterol levels in the blood.[19] Sucrose and fat feeding caused dyslipidaemia in experimental animals, which was also observed in the model. High sugar intake also promotes inflammation, as demonstrated by augmented peritoneal macrophage IL-6 production.[20] Dyslipidaemia in these rats was defined by an increase in plasma concentrations of triglycerides, total cholesterol. Lipase inhibitor have become potential research hotspot. Both chemically synthesized lipase inhibitor and plant derived lipase inhibitor are available. Several animal and clinical studies have demonstrated that lipase inhibitors improve lipid metabolism in obese persons. The quantity of LDL in the blood is lowered while the level of HDL is elevated by blocking fatty acid absorption and so decreasing fatty acid build-up in the body.[21] By interacting with the active lipase component of the stomach and small intestine, lipase inhibitors affect the conformation of the stomach/trypsin, decrease catalytic activity, and thereby lower lipids such as triglycerides. The hydrolysis reduces fat digestion and absorption in meals, as well as adipose tissue build-up, and has implications for obesity management and treatment. A number of clinical trials have confirmed that orlistat as a pancreatic lipase inhibitor can reduce obesity caused by a high-fat diet, and that taking orlistat can lead to oily stool. Orlistat has been widely used in clinical practice as an inhibitor on pancreatic triacylglycerol lipase and gastric triacylglycerol lipase.[21] Bromhexine has been shown to lipase inhibitory activity by inhibit the activity of lipase to convert triglycerides to adipose tissue. These benefits of Bromhexine are comparable to those of other well-studied lipase inhibitors such as Orlistat. The BMI of the model control group was seen significantly higher as due to consumption of high calorie diet and diet containing dense calorie. These leads to accumulation of visceral body fat which leads to increases in waist circumference then body height. But the BMI of bromhexine treated group was significantly due to lower weight as due to lower accumulation of fat. Lipase transforms triglycerides to monoglycerides and free fatty acids due to over secretion of the enzyme. The free fatty acid was then transformed to white adipose tissue, which is unhealthy. These were built up around the waist, causing an increase in waist circumference while maintaining the same height. The animal's weight and stomach circumference eventually rise, but not its height. As a result, animals eating a high-fat diet was accumulate visceral fat and have a higher BMI. By using lipase inhibitor such as bromhexine is stops the conversion of triglycerides to adipose tissue which prevents accumulation of visceral fat. Adiposity index was higher in animal receiving high fat diet as there was increases in activity of lipase which was lead to increase in accumulation of fat and causes fat storage in the body and leads to formation of white adipose tissue. Bromhexine as a lipase inhibitor can blocks the formation of triglycerides and can cause stops the formation of white adipose tissue, hence the adiposity index was lower in the treatment group. Conicity index is used to measure the fat accumulation in the body. The conicity index was found to be higher in model control group as they consume high fat diet containing butter and vegetable oil, in these the lipase activity is higher due uncontrolled lipase activity can cause accumulation of fat deposition in the body especially in the visceral region. In treatment control group the conicity index of animal receiving bromhexine 5mg/kg was higher as compared to that of bromhexine 10mg/kg, from these it can be seen that there is dose dependent inhibition of lipase by bromhexine. Lee's index was high in animal receiving high fat diet. Because bromhexine is a lipase inhibitor, the lees index was lowered in the treatment group. Lipase inhibitors stop triglycerides from becoming free fatty acids and monoglycerides. It were prevent atherogenic dyslipidaemia and protect the heart by lowering triglyceride levels. The pancreas weight was higher in both treatment control group as they control the pancreatic lipase activity and controls the accumulation of white adipose tissue. As

described in previous study bromhexine has inhibitory effect of pancreatic lipase enzyme, it was seen in the study that by giving Bromhexine (5mg/kg and 10mg/kg) has shown dose depended inhibition.[22] 10mg/kg dose has shown significant inhibition on the pancreatic lipase as compared to 5mg/kg dose. So due to the inhibition there is less or negligible conversion of triglyceride in to monoglyceride and free fatty acid. Which ultimately leads decrease in fat deposition and decrease in obesity. Cholesterol was found to be higher in model control group as the diet consist of butter which is responsible for increase in cholesterol and VLDL in the serum. Higher level of cholesterol is responsible for the thrombosis and atherogenic dyslipidaemia situation. The cholesterol level was lower in both treatment group as due to lipase inhibitory activity of bromhexine 5mg/kg and 10mg/kg. Bromhexine (10 mg/kg is effective in treating obesity as compared to bromhexine (5 mg/kg). As these has shown multiple effect and has shown to protect multiple organs so it can be repurposed in obesity. Multiple drugs are to be used in the market but bromhexine (5mg/kg and 10mg/kg) has proven to full fill all the criteria of the treatment of obesity as given similar effect as compared to standard drug orlistat.

REFERENCES

I. Lee A, Cardel M, Donahoo WT. Social and Environmental Factors Influencing Obesity. In: Feingold KR, Anawalt B, Blackman MR, Boyce A, Chrousos G, Corpas E, et al., editors. Endotext. South Dartmouth (MA): MDText.com, Inc.Copyright © 2000-2024, MDText.com, Inc.; 2000.

II. Lin X, Li H. Obesity: Epidemiology, Pathophysiology, and Therapeutics. Frontiers in endocrinology. 2021;12:706978 DOI: 10.3389/fendo.2021.706978.

III. Masood B, Moorthy M. Causes of obesity: a review. Clinical medicine (London, England). 2023;23(4):284-91 DOI: 10.7861/clinmed.2023-0168.

IV. Khanna D, Khanna S, Khanna P, Kahar P, Patel BM. Obesity: A Chronic Low-Grade Inflammation and Its Markers. Cureus. 2022;14(2):e22711 DOI: 10.7759/cureus.22711.

V. Liu TT, Liu XT, Chen QX, Shi Y. Lipase Inhibitors for Obesity: A Review. Biomedicine & pharmacotherapy = Biomedecine & pharmacotherapie. 2020;128:110314 DOI: 10.1016/j.biopha.2020.110314.

VI. Birari RB, Bhutani KK. Pancreatic lipase inhibitors from natural sources: unexplored potential. Drug Discovery Today. 2007;12(19):879-89 DOI: https://doi.org/10.1016/j.drudis.2007.07.024.

VII. Gholami A, Minai-Tehrani D. Discovery of novel inhibitors for Pseudomonas aeruginosa lipase enzyme from in silico and in vitro studies. 2024;42(5):2197-210 DOI: 10.1080/07391102.2023.2203258.

VIII. Fu Q, Zheng X, Zhou Y, Tang L, Chen Z, Ni S. Re-recognizing bromhexine hydrochloride: pharmaceutical properties and its possible role in treating pediatric COVID-19. European Journal of Clinical Pharmacology. 2021;77(2):261-3 DOI: 10.1007/s00228-020-02971-4.

IX. Othman ZA, Zakaria Z. Anti-Atherogenic Effects of Orlistat on Obesity-Induced Vascular Oxidative Stress Rat Model. 2021;10(2) DOI: 10.3390/antiox10020251.

X. Sharma G, Ashhar MU, Aeri V, Katare DP. Development and characterization of late-stage diabetes mellitus and -associated vascular complications. Life sciences. 2019;216:295-304 DOI: 10.1016/j.lfs.2018.11.005.

XI. Bastías-Pérez M, Serra D, Herrero L. Dietary Options for Rodents in the Study of Obesity. Nutrients. 2020;12(11) DOI: 10.3390/nu12113234.

XII. Skrzypiec-Spring M, Grotthus B, Szelag A, Schulz R. Isolated heart perfusion according to Langendorff---still viable in the new millennium. Journal of pharmacological and toxicological methods. 2007;55(2):113-26 DOI: 10.1016/j.vascn.2006.05.006.

XIII. Slater TF, Sawyer BC. The stimulatory effects of carbon tetrachloride and other halogenoalkanes on peroxidative reactions in rat liver fractions in vitro. General features of the systems used. The Biochemical journal. 1971;123(5):805-14 DOI: 10.1042/bj1230805.

XIV. Misra HP, Fridovich I. The role of superoxide anion in the autoxidation of epinephrine and a simple assay for superoxide dismutase. The Journal of biological chemistry. 1972;247(10):3170-5.

XV. Aebi H. Catalase in vitro. Methods in enzymology. 1984;105:121-6 DOI: 10.1016/s0076-6879(84)05016-3.

XVI. Moron MS, Depierre JW, Mannervik B. Levels of glutathione, glutathione reductase and glutathione S-transferase activities in rat lung and liver. Biochimica et biophysica acta. 1979;582(1):67-78 DOI: 10.1016/0304-4165(79)90289-7.

XVII. Ignatov A, Ortmann O. Endocrine Risk Factors of Endometrial Cancer: Polycystic Ovary Syndrome, Oral Contraceptives, Infertility, Tamoxifen. Cancers. 2020;12(7) DOI: 10.3390/cancers12071766.

XVIII. Burke SJ, Batdorf HM, Martin TM, Burk DH, Noland RC, Cooley CR, et al. Liquid Sucrose Consumption Promotes Obesity and Impairs Glucose Tolerance Without Altering Circulating Insulin Levels. 2018;26(7):1188-96

DOI: 10.1002/oby.22217.

XIX. Zabetakis I. Foods (Basel, Switzerland). DOI: 10.3390/foods7030029.

XX. Masi LN, Martins AR, Crisma AR, do Amaral CL, Davanso MR, Serdan TDA, et al. Combination of a high-fat diet with sweetened condensed milk exacerbates inflammation and insulin resistance induced by each separately in mice. Scientific reports. 2017;7(1):3937 DOI: 10.1038/s41598-017-04308-1.

XXI. Lunagariya NA, Patel NK, Jagtap SC, Bhutani KK. Inhibitors of pancreatic lipase: state of the art and clinical perspectives. EXCLI journal. 2014;13:897-921.

XXII. Gholami A, Minai-Tehrani D. Bromhexine and its inhibitory effect on lipase - kinetics and structural study. 2022;128(6):1687-92 DOI: 10.1080/13813455.2020.178860