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BENEFICIAL EFFECT OF METFORMIN AND NEBIVOLOL IN HIGH FAT DIET INDUCED OBESITY IN WISTAR RATS

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ABSTRACT

This study evaluated the combined effects of metformin and nebivolol on high-fat diet (HFD)-induced obesity in Wistar rats, compared to the standard drug orlistat. Obesity, a chronic disease driven by genetic, environmental, and psychological factors, leads to significant health risks. HFD-fed rats showed increased body weight, glucose, triglycerides, and cholesterol levels, indicative of obesity and dyslipidemia. Metformin, an AMPK activator, and nebivolol, an antihypertensive agent, were administered individually and in combination from the fourth to the eighth week of HFD treatment. The combination therapy significantly reduced obesity markers such as body weight, BMI, Lee index, and feed intake more effectively than individual high-dose treatments. Additionally, it improved lipid profiles, decreasing LDL, triglycerides, and total cholesterol while increasing HDL levels. The study demonstrated that the combination of metformin and nebivolol is more effective in managing obesity than either drug alone, likely due to a synergistic effect on reducing feed intake and improving metabolic parameters. This provides a pharmacological basis for using a combination of metformin and nebivolol in treating obesity, highlighting their potential role in reducing body adiposity and improving overall metabolic health in obesity management.

Keywords HFD-fed, Obesity, *Arctium lappa*, Metformin, etc.

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INTRODUCTION

Obesity is the major health burden in the western world, in terms of increased risk of diabetes (type2), cardiovascular morbidity, cancer and also in economic costs to healthcare providers. It is characterized with accumulation of excess fat in body causing adverse effects on health. Obesity occurs when the balance between food intake and energy expenditure is disrupted, i.e., more food is consumed than utilized, leading to excess fat stores being laid down. There are many environmental factors that predispose individuals to gain weight, e.g., freely available high-calorie food and sedentary life style. Genetic factors also contribute to this imbalance. In the severely obese, surgical intervention may be necessary. An alternative approach is to develop therapeutic agents that can either reduce food consumption or increase energy utilization [1,2].

Nebivolol is a highly selective and long acting third generation β_1 adrenoceptors (AR) blocker. Nebivolol a third generation beta blocker, is used for the treatment of hypertension and it lowers blood pressure by reducing peripheral vascular resistance, Shows lipolytic action on human visceral adipocytes in recent ex-vivo studies. It has been observed that Nebivolol also has β_3 agonist effect and activates UCP1 (uncoupling proteins) which further induces gene expression in human visceral adipocytes, a pathway responsible for thermogenesis and weight loss [3]. Many studies based on the use of drugs activating β_3 adrenoceptors and other adrenoceptors confirmed that the sympathetic nervous system was the main trigger of UCP1 activation and induction. Nebivolol upregulate expression of Uncoupling protein-1, increases thermogenesis and is a fat burning compound. The lower dysmetabolic effects of nebivolol may depend on its ß3 agonist activity on human visceral adipose tissue. Metformin an antidiabetic drug also has lipid lowering potential. Several reports revealed that metformin modulate the AMPK and thus it may have antiobesity potential [4,5]. Therefore, the present study is designed to investigate the combined effects of nebivolol and metformin in High Fat Diet induced obesity in Wistar rats.

MATERIALS AND METHODS

ssessment of anthropometric parameters

The raise in the body weight as compared to age matched normal rats is regard as obesity. Body Mass Index (BMI) i.e. weight (g)/ height (cm) 2 (Novelli et al., 2007), Lee index i.e. (Body Wt)1/3 / ano-nasallength (cm) x 1000 (Bernardis and Bellinger, 1982) was calculated after the treatment as an index of obesity and compared with normal rats. Body weight was measured weekly. Food intake measurements for individual rats were recorded biweekly. To

evaluate the effect of high calorie diet and drug interventions, fat depots (Epididymal, retroperitoneal and mesenteric fat depots) was isolated, remove from nearby tissues, and weigh individually and after that total weight was calculated [5].

ASSESSMENT OF BIOCHEMICAL PARAMETERS

The hyperlipidemia was assessed by estimating the levels of Total cholesterol, High Density Lipoprotein (HDL), Low Density Lipoprotein (LDL), Very Low Density Lipoprotein (VLDL) and Triglycerides in blood serum using commercially available kits. Values were expressed in mg/dl. Additionally glucose level in serum was also estimated by using commercially available kits. Values are expressed in mg/dl.

Estimation of serum total cholesterol

The total cholesterol was estimated by cholesterol oxidase peroxidase CHOD-POD method [6] using commercially available kit (ADI Diagnostics [P] Ltd., Hyderabad, India). 1000 µl of cholesterol reagent was added to 10 µl of serum, 10 µl of standard cholesterol (200 mg/dl) and 10 µl of purified water to prepare test, standard and blank, respectively. All the test tubes were incubated at room temperature for 20 mins. The absorbances of test and standard samples were noted against blank at 520 nm spectophotometrically

$$\text{Cholesterol ester} + H_2O \xrightarrow{\text{CHE}} \text{Cholesterol} + \text{Free Fatty acids}$$

$$\text{Cholesterol} + O_2 \xrightarrow{\text{CHOD}} \text{Cholest} - 4\text{ene } 3 - \text{one} + H_2O_2$$

$$H_2O_2 + \text{Phenol} + 4 - \text{Aminoantip yrine} \xrightarrow{\text{POD}} \text{Red quinoneimi ne Complex} + H_2O_2$$

Cholesterol is determined after enzymatic hydrolysis and oxidation. Cholesterol esters are hydrolysed by the enzyme cholesterol esterase (CHE) to give free cholesterol and free fatty molecules. This free cholesterol gets oxidized in the presence of cholesterol oxidase (CHOD) to liberate Cholest-4ene 3-one and H_2O_2. The indicator quinoneimine is formed from hydrogen peroxide and 4-Aminoantipyrine in the presence of phenol and peroxides (POD). The intensity of the colour complex is directly proportional to the cholesterol concentration present in sample.

The serum total cholesterol was calculated using the following formula:

$$\text{Total cholesterol (mg/dl)} = \frac{\text{Absorbance of Test}}{\text{Absorbance of Standard}} \times 200$$

Estimation of serum triglycerides

The serum triglyceride was estimated by glycerol phosphate oxidase peroxidase GPO-PAP method [7] using commercially available kit (ADI Diagnostics [P] Ltd., Hyderabad, India). 1 ml of Triglyceride reagent was added to 10 µl of serum and 10 µl of standard (200 mg/dl) and

10 µl of purified water to prepared test, standard and blank respectively. All the test tubes were incubated at room temperature for 20 min. The absorbances of test and standard samples were noted against blank at 520 nm spectrophotometrically.

$$\text{Triglycerides} + H_2O \xrightarrow{\text{Lipase}} \text{Glycerol} + \text{Fatty acids}$$

$$\text{Glycerol} + \text{ATP} \xrightarrow{\text{GK}} \text{Glycerol} - 1 - \text{Phosphate (G} - 3 - \text{P)} + \text{ADP}$$

$$\text{G} - 1 - \text{P} + O_2 \xrightarrow{\text{GPO}} H_2O_2 + \text{Dihydroxyacetone phosphate}$$

$$H_2O_2 + 4\,\text{AAP} + 4 - \text{Chlorophenol} \xrightarrow{\text{POD}} \text{Purplish brown quinoneimine complex} + H_2O + HCl$$

Serum triglycerides are hydrolysed to glycerol and free fatty acids by lipase. In the presence of ATP and glycerol kinase (GK), glycerol is converted to glycerol-1-phosphate which is then oxidized by glycerol-1-phosphate oxidase (GPO) to yield H_2O_2. The H_2O_2, thus formed reacts with 4-aminoantipyrine (4-AAP) and 4-Chlorophenol in presence of enzyme peroxidase (POD) to form purplish brown quinoneimine complex, which is measured spectophotometrically.

The serum triglyceride was calculated using the following formula:

$$\text{Triglycerides (mg/dl)} = \frac{\text{Absorbance of Test}}{\text{Absorbance of Standard}} \text{ X } 200$$

Estimation of high density lipoprotein (HDL)

The HDL was estimated by cholesterol oxidase peroxidase CHOD-POD method [8] using commercially available kit (ADI Diagnostics [P] Ltd., Hyderabad, Indi Step1. 200 µl of serum and 200 µl of precipitating reagent were taken into the centrifuge tube, mixed well and were incubated at room temperature for 5 min and then centrifuged at 3000 rpm for 10 min to get clear supernatant. Step 2- 1000 µl of cholesterol reagent was added to 100 µl of supernatant (from step1), 100 µl of HDL cholesterol standard (50 mg/dl) and 100 µl of purified water to prepare test, standard and blank, respectively. All the test tubes were incubated at room temperature for 10 min. The absorbances of test and standard samples were noted against blank at 505 nm spectophotometrically. On addition of the precipitating reagent to the serum, followed by centrifugation, HDL fraction remains in the supernatant while the lipoprotein precipitate out.

$$\text{Cholesterol ester} + H_2O \xrightarrow{\text{CHE}} \text{Cholesterol} + \text{Free Fatty acids}$$

$$\text{Cholesterol} + O_2 \xrightarrow{\text{CHOD}} \text{Cholest} - 4\text{ene } 3 - \text{one} + H_2O_2$$

$$H_2O_2 + \text{Phenol} + 4 - \text{Aminoantip yrine} \xrightarrow{\text{POD}} \text{Red quinoneimi ne Complex} + H_2O_2$$

Cholesterol is determined after enzymatic hydrolysis and oxidation. Cholesterol esters are hydrolysed by the enzyme cholesterol esterase (CHE) to give free cholesterol and free fatty molecules. This free cholesterol gets oxidized in the presence of cholesterol oxidase (CHOD) to liberate Cholest-4ene 3-one and H_2O_2. The indicator quinoneimine is formed from hydrogen peroxide and 4-Aminoantipyrine in the presence of phenol and peroxides (POD). The intensity of the colour complex is directly proportional to the cholesterol concentration present in sample. The serum High density lipo protein cholesterol was calculated using the following formula:

$$\text{HDL cholesterol (mg/dl)} = \frac{\text{Absorbance of Test}}{\text{Absorbance of Standard}} \text{ X } 50$$

Estimation of Very Low Density Lipoprotein (VLDL) and Low Density Lipoprotein (LDL) Level

VLDL and LDL concentrations were calculated from the Friedewald equation [9].

VLDL Level

Serum VLDL levels (mg/dl) = Triglyceride level/ 5, and

LDL Level

Serum LDL levels (mg/dl) = Total cholesterol-(HDL level + VLDL level)

Estimation of serum glucose

At the end of the experimental protocol, the blood samples were collected and serum was separated. The serum samples were frozen until analyzing the biochemical parameters. The glucose concentration was estimated by glucose oxidase peroxidase GOD-POD method [10] using commercially available kit (Reckon Diagnostics [P] Ltd., Vadodara, India). 1000 µl of working glucose reagent was added to 10 µl of serum, 10 µl of standard glucose (100 mg/dl) and 10 µl of purified water to prepare test, standard and blank, respectively. All the test tubes were incubated at room temperature for 20 min. The absorbances of test and standard samples were noted against blank at 520 nm spectophotometrically.

$$\text{Glucose} + O_2 + H_2O \longrightarrow \text{Gluconate} + H_2O_2$$

$$H_2O_2 + \text{4 Aminoantipyrine} + \text{Phenol} \xrightarrow{\text{Peroxidase}} \text{Red Quinoneimine dye} + H_2O$$

Glucose is oxidized to gluconic acid and hydrogen peroxide in presence of glucose oxidase. Hydrogen peroxide further reacts with phenol and 4 aminoantipyrine by the catalytic action

of peroxidase to form a red colour quinoneimine dye complex. The intensity of the colour formed is directly proportional to the amount of glucose present in the sample. The serum glucose was calculated using the following formula:

$$\text{The concentration of glucose (mg/dl)} = \frac{\text{Absorbance of Test}}{\text{Absorbance of Standard}} \text{ X } 100$$

Histopathological study

The high fat diet induced change in size of body fat depots were assessed histologically for fat depots [11]. During dissections, standard necropsy procedure was maintained between animals to minimize any variance in tissue collection. A wet-tissue weight is taken immediately after removal using an analytical balance. Special care was taken to remove any non adipose-associated tissues from the depot including glands and lymph nodes. Once weighed, 100–500 mg of the adipose tissue of interest was placed in a sealable tube and covered by a ratio of >10 mL of 10% formalin per gram of WAT. Covered tissue is placed at 4 °C for 72 h, then switched to 70% ethanol (equal volume as was used for formalin) for 48 h. A standard procedure was strictly followed for WAT fixing and dehydration.

1. Adipose tissue depot of interest was identified
2. Carefully dissect out adipose depot removing non adipose-associated tissue (e.g., glands, lymph nodes)
3. Adipose tissue depot was weighed
4. Cover 100–500 mg of tissue with >10-fold volume (mL) of 10% formalin to tissue (g) and let sit at 4 °C for a minimum of 72 h
5. Remove tissue and place in 70% ethanol for 48 h

Tissue embedding, Preserved tissues were then placed in labeled histology cassettes and paraffin processed below are the steps used for paraffin processing adipose tissue.

1. 1 h in 70% ethanol
2. 1 h in 80% ethanol
3. 1 h in 95% ethanol (×2)
4. 1 h in 100% ethanol (×2)
5. 1 h in xylene (×2)
6. 1 h in 60 °C paraffin (×3)

Once the sample was processed, the tissue was embedded into a paraffin block and stored at 4 °C. Time and care should be taken in the embedding process to ensure the tissue is placed into the center of the block. The fat depots was excised, weighed and instantly engross in

10% formalin. The fat depots were dehydrated in various percentages of alcohol, engrossed in xylene and then fixed in paraffin. From the paraffin blocks, sections of 5µm thickness were made and stained with hematoxylin and eosin to measure changes in size of fat depots using light microscopy (400 X).

Experimental Obesity;

High fat diet-induced obesity

Experimental obesity was produced by feeding high fat diet (containing; Powdered Normal chow, 365g; Lard, 310g; Casein, 250g; Cholesterol, 10g; Vitamin mix and mineral mix, 60g; dl-Methionine, 03g; Yeast powder, 01g; Nacl, 01g were added to make 1.0 kg of diet) (Srinivasan *et al.,* 2005), to rats for a period of 8 weeks. Mineral mix was composed of NaCl, 5.57g; KCl, 32mg; $MgSO_4$, 2.29g; $FeSO_4.7H_2O$, 108g; $CaHPO_4$, 70mg; $CuSO_4.5H_2O$, 0.1mg; $MnSO_4.H_2O$, 0.01mg; $ZnSO_4.H_2O$, 28.7mg; KI, 0.025mg; $COCl_2.6H_2O$, 9mg and MgO, 0.15mg. The vitamin mix contained Retinol acetate, 5000 IU; cholecalciferol, 400 IU; 7-hydrochloride, dehydrocholesterol, 2-nicotinamide, 45mg; D-panthenol, 5mg; pyridoxine 2mg; ascorbic acid, 75mg; folic acid, 1000µg and cyanocobalamin, 5µg. The High Fat Diet contained 5.33 Kcal/gm while the normal chow contains 3.80 Kcal/gm.

Experimental design

Six groups of male wistar rats will be employed in the present study. All animals will randomly divided into different groups. Each group was comprised of six animals.

Group I: {Normal Control} Normal Rats will be maintained on standard chow diet and water *ad libitum.* No treatment will be given to these rats.

Group II: {High Fat Diet} High Fat Diet will be administered to rats for 10 weeks

Group III: {Orlistat 350 mg/kg/day p.o 4 weeks} Orlistat (350mg/kg/day p.o, * 4 weeks) will be administered to rats on high fat diet at the end of sixth week continued up to the end of the tenth week.

Group IV: {Nebivolol 1mg/kg/day p.o, 4 weeks} Nebivolol (1mg/kg/day p.o, * 4 weeks) will be administered to rats on high fat diet at the end of sixth week and continued up to the end of the tenth week.

Group V: {metformin 500mg/kg/day p.o, 4 weeks} Metformin (500mg/kg/day p.o, * 4 weeks) will be administered to rats on high fat diet at the end of sixth week and continued up to the end of the tenth week.

Group VI: {Nebivolol .5mg/kg/day p.o and Metformin 300mg/kg/day p.o, 10 weeks} Nebivolol (0.5mg/kg/day p.o, *) and Metformin (300mg/kg/day p.o) will be administered to rats on high fat diet for 10 weeks.

Statistical analysis

All values are express as mean ± S.D. The data obtain from various groups were statistically analyze using ANOVA followed by Tukey's Post hoc method. The p value ≤0.05 is considered to be statistically significant.

RESULTS AND DISCUSSION

. Effect of various pharmacological interventions on body weight and feed intak

In High fat diet model, a significant ($p<0.05$) increase in body weight, feed intake (in kilocalories) (Kcal) and decrease in feed intake (in grams) was observed in rats fed on high fat diet as compared to the normal rats fed on standard diet. Orlistat which was positive control in the present study decreases body weight and feed intake (in Kcal). Oral supplementation of metformin 500mg/kg and nebivolol 1mg/kg and their combination at lower doses i.e. metformin 300mg/kg with nebivolol 0.5 mg/kg produced significant ($p<0.05$) reduction in body weight and feed intake (in Kcal) as compared to the HFD fed group and the result was very near to the positive control group i.e. HFD + Orlistat (Table 1).

Effect of various pharmacological interventions on Lee's index and body mass Index

Administration of high fat diet (HFD) for 8 weeks cause a significant ($p<0.05$) increase in Lee's index and Body Mass Index (BMI) as compared to age matched normal rats fed on standard diet. Administration of metformin 500 mg/kg and nebivolol 1mg/kg and their combination at lower doses i.e.metformin 300mg/kg with nebivolol 0.5 mg/kg produced significant ($p<0.05$) reduction in BMI and Lee's Index when compared to the high fat diet fed group and the result was very near to the positive control group i.e. HFD + Orlistat (Table 1).

Effect of various pharmacological interventions in different fat depots

Administration of high fat diet (HFD) for 8 weeks cause a significant ($p<0.05$) increase in body fat depots :epididymal, retroperitoneal and mesentric fat depots and total fat. Treatment with metformin and nebivolol administration in combination at lower doses (300mg/kg and 0.5 mg/kg respectively) produced more significant ($p<0.05$) decrease in body fat depots :epididymal, retroperitoneal and mesentric fat and total fat in comparison to HFD control and their administration at higher doses (metformin: 500mg/kg and nebivolol: 0.5 mg/kg) (Table 3).

Effect of various pharmacological interventions on serum biochemical parameters

High fat diet induces significant ($p<0.05$) elevation in serum total cholesterol (TC), triglycerides (TG), LDL and VLDL and decrease in HDL in HFD group as compared to age

matched normal rats fed on standard diet and Orlistat which was positive control in present study decreases all the biochemical parameters of obesity. Treatment of high fed diet fed rats with metformin 500mg/kg and nebivolol 1mg/kg and their combination at lower dosesi.e. metformin 3000mg/kg and nebivolol 0.5mg/kg produced significant($p<0.05$) reduction in serum total cholesterol (TC), triglycerides (TG), LDL and VLDL of HFD fed rats and significant increase in the level of HDL as compared to HFD control group and the result was very near to the positive control group i.e. HFD + Orlistat (Table 2).

Table 1. Effect of normal diet, HFD and HFD + Orlistat on the body weight, body mass index, Lee index and Feed intake in gram and feed intake in Kcal:

Parameter	Normal control	High fat diet	HFD + Orlistat
Initial Body weight (gm)	208.33 ± 14.7	216.66 ± 20.4	210.7 ± 11.6
Final Body weight (gm)	264.16 ± 8.61	360.5 ± 22.6[a]	250.35 ± 12.39[b]
Body mass index (g/cm^2)	0.79 ± 0.04	1.19 ± 0.04[a]	0.82 ± 0.08[b]
Lee index ($gm^{1/3}/cm*1000$)	352.0.58 ± 8.56	409.86 ± 11.05[a]	362 ± 14.20[b]
Feed intake (gm)	25.33 ± 3.50	24.9 ± 2.01	18.75 ± 3.41[b]
Feed intake (Kcal)	96.26 ± 13.30	116.72 ± 11.99[a]	85.66 ± 3.12[b]

All values are expressed as Mean ± S.D; [a] = $p< 0.05$ vs normal diet control, [b] = $p< 0.05$ vs. High fat Diet.

Table 2.Effect of normal diet, HFD and HFD + Orlistat on the serum glucose, serum lipid profile:

Parameter	Normal diet control	High fat diet	HFD + Orlistat
GLU (mg/dl)	93.32 ± 3.08	159.87 ± 2.56[a]	101.54 ± 1.06[b]
TC (mg/dl)	99.63 ± 0.82	158.42 ± 4.03[a]	108.30± 2.15[b]
TG (mg/dl)	68.35 ± 3.57	149.29 ± 1.85[a]	76.55 ± 1.22[b]
HDL (mg/dl)	32.27±1.66	21.29 ± 0.60[a]	33.29 ± 0.82[b]
VLDL (mg/dl)	13.64 ± 0.73	29.85 ± 0.37[a]	15.31 ± 0.24[b]
LDL (mg/dl)	53.70 ± 1.90	107.27 ± 3.66[a]	59.69 ± 2.40[b]

All values are represented as Mean ± S.D; [a] = $p < 0.05$ vs normal diet control, [b] = $p < 0.05$ vs. High fat Diet.

Table 3. Effect of normal diet, HFD and HFD + Orlistat on the various fat pads:

Parameter	Normal diet control	High fat diet	HFD + Orlistat
MES (gm)	2.74 ± 0.29	7.91 ± 0.38[a]	2.56 ± 0.54[b]
RET (gm)	1.58 ± 0.31	7.51 ± 0.45[a]	2.49 ± 0.39[b]
EPI(gm)	1.92 ± 0.07	4.89 ± 0.56[a]	2.79 ± 0.31[b]
TF (gm)	6.24 ± 0.48	20.31 ± 1.33[a]	7.84 ± 0.12[b]

All values are expressed as Mean ± S.D; [a] = $p < 0.05$ vs. normal diet control, [b] = $p < 0.05$ vs. High fat Diet.

Table 4: Effect of various pharmacological interventions on the body weight, body mass index, lee index, feed intake in gram and feed intake in Kcal:

Parameters	Initial Body weight (gm)	Final Body Weight (gm)	Body mass Index (gm/cm^2)	Lee index ($gm^{1/3}/cm$ *1000)	Feed intake (gm)	Feed Intake (Kcal)
Normal diet control	208.33 ± 14.7	264.16 ± 8.61	0.79 ± 0.04	352.05 ± 8.56	25.33 ± 3.50	96.26 ± 13.30
High fat diet	216.66 ± 20.4	360.5 ± 22.6[a]	1.19 ± 0.04[a]	409.86 ± 11.05[a]	24.9 ± 2.01	116.72 ± 11.99[a]
HFD + Orlistat	210.7 ± 11.6	250.35 ± 12.39[b]	0.82 ± 0.08[b]	362 ± 14.20[b]	18.75 ± 3.41[b]	85.66 ± 1.13[b]
HFD + Nebivolol 1 mg	210.73 ± 17.55	265.5 ± 13.02[b]	0.861 ± 0.04[b]	379.78 ± 7.6[b]	17.68 ± 1.17[b]	90.78 ± 6.26[b]
HFD + Metformin 500 mg	206.66 ± 10.3	258.33 ± 11.2[b]	0.855 ± 0.04[b]	367.20 ± 8.81[b]	17.38 ± 0.72[b]	87.79 ± 3.85[b]
HFD + Nebivolol 0.5 & Met 300	217.5 ± 6.12	256.33 ± 7.8[b]	0.867 ± 0.07[b]	365.22 ± 14.44[b]	16.08 ± 0.36[b]	86.97 ± 1.67[b]

All values are expressed as Mean ± S.D; [a] = $p < 0.05$ vs. normal control,[b] = $p < 0.05$ vs. High fat Diet.

Table 5: Effect of various pharmacological interventions on the serum glucose and lipid profile:

Parameters	Serum glucose (mg/dl)	Serum total cholesterol (mg/dl)	Serum triglyceride (mg/dl)	Serum HDL (mg/dl)	Serum VLDL (mg/dl)	Serum LDL (mg/dl)
Normal diet control	93.32 ± 3.08	99.63 ± 0.82	68.24 ± 3.67	32.27 ± 1.66	13.64 ± 0.73	53.7 ± 1.90
High fat diet	159.87 ± 2.56[a]	158.42 ± 4.03[a]	149.29 ± 1.85[a]	21.29 ± 0.60[a]	29.85 ± 0.37[a]	107.27 ± 3.66[a]
HFD + Orlistat	101.54 ± 1.06[b]	108.30 ± 2.15[b]	76.55 ± 1.22[b]	33.29 ± 0.82[b]	15.31± 0.24[b]	59.69 ± 2.40[b]
HFD + Nebivolol 1 mg	100.10 ± 1.55[b]	109.37 ± 1.73[b]	77.70 ± 1.43[b]	30.01 ± 0.87[b]	16.08 ± 0.28[b]	57.81± 2.12[b]
HFD + Metformin 500 mg	107..34 ± 2.11[b]	99.90 ± 2.15[b]	74.50 ± 1.03[b]	29.07 ± 0.96[b]	16.22 ± 0.36[b]	58.33 ± 2.38[b]
HFD + Nebivolol 0.5 & Met 300	100.20 ± 1.22[b]	100.92 ± 0.86[b]	79.90 ± 1.58[b]	31.29 ± 1.40[b]	17.97 ± 0.31[b]	51.60 ± 1.64[b]

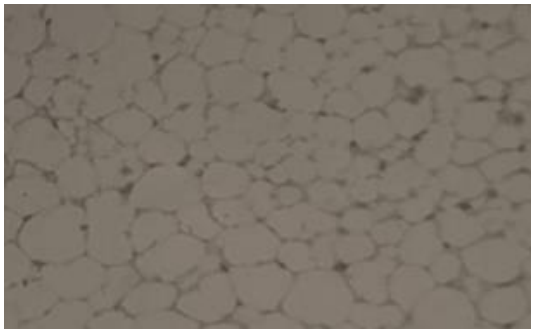
All values are expressed as Mean ± S.D; [a] = $p < 0.05$ vs. normal control, [b] =$p < 0.05$ vs. High fat Diet.

Table 6: Effect of various pharmacological interventions on the various fat pads:

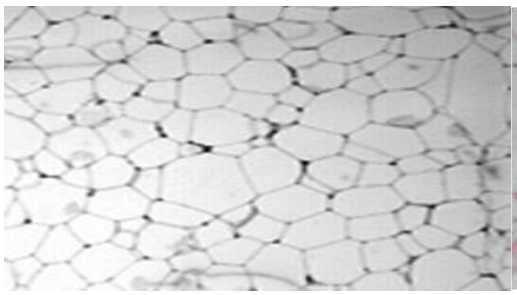
Parameters	MES	RET	EPI	TF
Normal diet control	2.74 ± 0.29	1.58 ± 0.31	1.92 ± 0.07	6.24 ± 0.4
High fat diet	6.91 ± 0.38[a]	5.51 ± 0.45[a]	5.89 ± 0.56[a]	18.31 ± 1.3[a]
HFD + Orlistat	2.56 ± 0.54[b]	2.49 ± 0.39[b]	2.79 ± 0.31[b]	7.84 ± 0.12[b]
HFD + Nebivolol 1 mg	4.07± 0.21[b]	4.0 ± 0.23[b]	3.96 ± 0.22[b]	12.02 ± 0.04[b]
HFD + Metformin 500 mg	4.08 ± 0.12[b]	3.99 ± 0.67[b]	3.96 ± 0.46[b]	12.04 ± 0.24[b]
HFD + Nebivolol 0.5 & Met 300	4.01 ± 0.40[b]	3.98 ± 0.45[b]	3.78 ± 0.24[b]	11.77 ± 0.41[b]

All values are expressed as Mean ± S.D; [a] = $p < 0.05$ vs. standard diet control. [b] = $p < 0.05$ vs. High fat Diet.

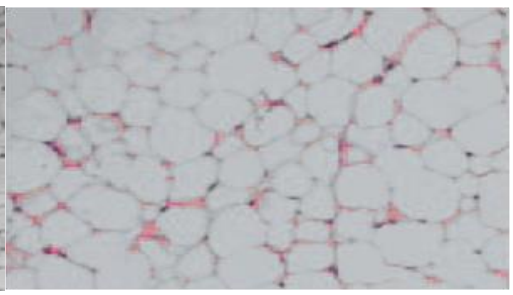
Normal Control



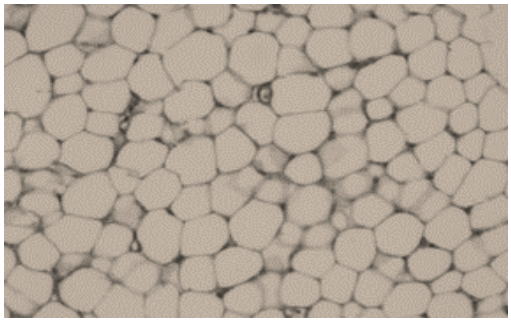
HFD Control



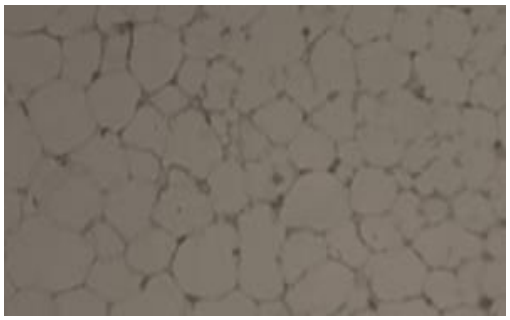
HFD + orlistat



HFD + Metformin 500



HFD + Nebivolol 1mg



HFD + Metformin 500 + Nebivolol 1mg

CONCLUSION

This study evaluated the combined effects of metformin and nebivolol on high-fat diet (HFD)-induced obesity in Wistar rats, compared to orlistat. Obesity, a chronic disease, stems from a complex interplay of genetic, environmental, and psychological factors, leading to excessive caloric intake over expenditure and resulting in significant health risks. The HFD model in rats led to increased body weight, glucose, triglycerides, and cholesterol levels. Metformin, an AMPK activator, reduces lipogenesis and promotes fatty acid oxidation, while nebivolol, with its antihypertensive properties, also aids in weight reduction. Rats were administered high-dose metformin (500mg/kg/day) and nebivolol (10mg/kg/day) individually, and a combined lower dose of both (250mg/kg/day metformin and 10mg/kg/day nebivolol) from the fourth to the eighth week of HFD treatment. Results showed significant reductions in obesity markers such as body weight, BMI, Lee index, and feed intake in the combination group compared to the HFD control and individual high-dose groups. The combination therapy also improved lipid profiles, decreasing LDL, triglycerides, and total cholesterol while increasing HDL. This study suggests that combined metformin and nebivolol therapy is more effective in managing obesity than either drug alone.

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