Academic Sciences

Asian Journal of Pharmaceutical and Clinical Research

Vol. 4, Issue 4, 2011 ISSN - 0974-2441

Research Article

BENEFICIAL EFFECT OF COMBINATION OF METFORMIN AND TELMISARTAN ON HIGH FAT DIET-INDUCED OBESITY IN WISTAR RATS

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Received: 2 Sep 2011, Revised and Accepted: 21 Sep 2011

ABSTRACT

Objectives Metformin is reported to promote weight loss and reduce appetite, and Telmisartan increases energy expenditure, improve lipid metabolism and reduce visceral fat accumulation. The present study was designed to investigate the, *per se* effect of metformin and telmisartan and their combination on high fat diet induced obesity in rat.

Methods Male Wistar albino rats (170-210 g) (n=6) were employed. Experimental obesity was induced by feeding high fat diet (HFD) for 10 weeks. Body weight, body mass index, Lee index, weight of epididymal, retroperitoneal and mesenteric fat depots and serum glucose, cholesterol, HDL, LDL, VLDL and TG were assessed as an index of obesity.

Key findings HFD treatment caused significant increase in body weight, body mass index, Lee index; weights of different fat depots; serum glucose, cholesterol, TG, LDL, VLDL; feed intake (Kcal); and decrease in serum HDL and feed intake (g) in HFD control group. Metformin, telmisartan, and combination, in low and high doses, significantly attenuated the effects of HFD treatment.

Conclusions It may be concluded that the treatment with metformin, telmisartan and their combination significantly prevented the HFD-induced obesity. This provides a rational pharmacological basis for combined clinical use of these drugs in obesity.

Keywords: Obesity; Metformin; Telmisartan; High-fat diet; Combination

INTRODUCTION

Obesity is the major health burden in the western world, in terms of increased risk of diabetes (type 2), cardiovascular morbidity, cancer and also in economic costs to healthcare providers.[1] It is characterized with accumulation of excess fat in body causing adverse affect on health.[2,3] Several therapeutic strategies for obesity are: medication, behavioral strategies and bariatric surgery.[4] The medications include orlistat, which is the only approved drug by FDA for long term use in obesity.

Metformin a widely used anti-diabetic agent displays the unique characteristic of promoting weight loss and reducing appetite.[5,6,7] It inhibits hepatic glucose production, intestinal absorption and enhances sensitivity of insulin on glucose uptake in skeletal muscles and adipocytes.[8] Metformin targets AMP-activated protein kinase (AMPK), which is also activated by leptin, and AMPK activation appears to be necessary for leptin's effect on acetyl-Co-A carboxylase (ACC) activity and thereby fatty acid oxidation in muscle takes place.[9,10,11]

Telmisartan is an angiotensin II receptor blocker reported to increase energy expenditure and improve glucose and lipid metabolism. It has been reported to reduce visceral fat accumulation in patients with metabolic syndrome, at least in part by increasing muscle fatty acid oxidation through activation PPARγ.[12]

Keeping this in view it may be hypothesized that the use of metformin (an AMPK activator), by increasing leptin sensitivity; and partial activation of PPARγ through telmisartan, alone and in combination may produce the beneficial effects in obesity. The purpose of present study was to investigate the, *per se,* and the combined effect of metformin and telmisartan on high fat diet-induced obesity in the Wistar rat.

MATERIALS AND METHODS

Chemicals and Reagents

Casein was purchased from Modern Diary, New Karnal, India; cholesterol from Thomas Baker, Mumbai; metformin from USV limited, Baddi H.P, India; telmisartan from Macleods pharmaceutical ltd, Mumbai were used. The biochemical enzymatic kits purchased from Coral diagnostics ltd., Mumbai, India. All other chemicals used were of analytical grade and freshly prepared.

Animals

Male, Wistar albino rats (170-210 g) were employed. They were procured from Animal house, ISF College of Pharmacy, Moga, Punjab and maintained on normal chow diet (Ashirwad Industries Private Ltd., Ropar, Punjab, India) and water *ad libitum*; at 12-12h light/dark cycles; temperature 25±2 °C and relative humidity 55±5 %. The experimental protocol was duly been approved by Institutional Animal Ethics Committee (IAEC) and performed under Committee for the Purpose of Control and Supervision on Experiments on Animals (CPCSEA) guidelines (No. 816/04/c/CPCSEA, dated: 20.02.2010).

High fat diet-induced obesity

Experimental obesity was developed by feeding high fat diet (HFD) (Powdered normal chow, 365 g; lard, 310 g; casein, 250 g; cholesterol, 10 g; vitamin and mineral mix, 60 g; *dl*-methionine, 03 g; yeast powder, 01 g; and NaCl, 01 g were mixed for 1.0 kg of diet),[13] for 10 weeks. The high fat diet contained 5.33 kcal/g while the normal chow contained 3.80 kcal/g.

Experimental protocol

Animals were divided into different groups each comprising six animals (n=6) (Table. 1). The drugs were administered by oral route. The anthropometric parameters were assessed. Animals were anaesthetized, blood collected and serum separated for biochemical estimations. Animals were sacrificed; different fat depots were surgically dissected out and weighed.

Pharmacological Assessment

Anthropometric parameters

The body mass index (BMI) [weight (g) / height $(cm)^2$],[14] and Lee index [$(body\ wt)^{1/3}$/ano-nasal length (cm)x1000][15] were assessed before and after the drug treatment as an index of obesity. Body weight and food intake were also assessed weekly. Weight of different fat depots: epididymal, retroperitoneal and mesenteric and total fats were estimated.[16]

Assessment of biochemical parameters

The estimation of serum glucose, total cholesterol, high density lipoprotein (HDL), low density lipoprotein (LDL), very low density lipoprotein (VLDL) and triglyceride (TG) were done spectrophotometrically using biochemical enzymatic kits.

Table 1: Experimental Protocol

S. No.	Groups	Treatment
1.	Normal control (NC)	Normal chow diet for 10 Wks
2.	NC + Telmi (7.5) *per se*	Normal chow diet for 6 Wks + Telmisartan (7.5mg/kg/day)
3.	NC + Met (350) *per se*	Normal chow diet for 6 Wks + Metformin (350mg/kg/day) for 4Wks
4.	NC + Met (225) *per se* + Telmi (7.5) *per se*	Normal chow diet for 6 Wks + Metformin (225mg/kg/day) + Telmisartan (5mg/kg/day) for 4 Wks
5.	HFD control	High fat diet for 10 Wks
6.	HFD + Telmi- 5	HFD for 6 Wks + Telmisartan (5mg/kg/day)
7.	HFD + Telmi- 7.5	HFD for 6Wks + Telmisartan (7.5mg/kg/day)for 4 Wks
8.	HFD + Met- 225	HFD for 6 Wks + Metformin (225mg/kg/day) for 4 Wks
9.	HFD + Met- 350	HFD for 6 Wks + Metformin (350mg/kg/day) for 4 Wks
10.	HFD +Met- 225 + Telmi- 5	HFD for 6 Wks + Metformin (225mg/kg/day) + Telmisartan (5mg/kg/day) for 4Wks
11.	HFD + Met- 350 + Telmi-7.5	HFD for 6 Wks + Metformin (350mg/kg/day) + Telmisartan (7.5mg/kg/day) for 4Wks

Table 2: Effect of various pharmacological interventions on the body weight, body mass index, lee index, feed intake (g) and feed intake (Kcal)

Parameters	Initial body weight (g)	Final body weight (g)	BMI (g/cm^2)	Lee index (gm/cm)	Feed intake (g)	Feed intake (Kcal)
Normal diet treatment						
Normal control	197.3 ± 25.96	273.16 ± 33.01	0.580 ± 0.03	293.52 ± 5.81	26.13 ± 1.36	94.09 ± 4.9
Telmisartan *per se* (high)	198.25 ± 27.79	222.12 ± 37.55	0.52 ± 0.05	290.3 ± 8.13	22.93 ± 3.29	82.58 ± 11.86
Metformin *per se* (high)	195.33 ± 37.38	238.5 ± 33.83	0.54 ± 0.05	291.6 ± 6.39	21.74 ± 2.2	70.88 ± 7.19
Telmi+met *per se* (low)	215.3 ± 41.25	262.83 ± 38.17	0.58 ± 0.05	296.4 ± 8.01	21.93 ± 2.14	78.98 ± 7.7
High fat diet treatment						
HFD control	181.66 ± 13.27	354.66 ± 16.07[a]	0.766 ± 0.06[a]	322.77 ± 10.74[a]	20.54 ± 0.85[a]	109.30 ± 4.5[a]
HFD + Telmisartan high	190.33 ± 28.11	287.5 ± 30.70[b]	0.62 ± 0.03[b]	302.09 ± 3.03[b]	16.51 ± 1.21[b]	87.86 ± 6.44[b]
HFD + Telmisartan low	191.66 ± 36.56	301.5 ± 44.22[b]	0.66 ± 0.06[b]	310.44 ± 6.54[b]	15.86 ± 1.31[b]	85.60 ± 5.84[b]
HFD + Metformin High	181.66 ± 26.39	288.66 ± 21.55[b]	0.64 ± 0.06[b]	305.27 ± 10.92[b]	15.81± 1.85[b]	84.15 ± 9.8[b]
HFD + Metformin low	173.33 ± 31.25	293.83± 27.88[b]	0.66 ± 0.04[b]	310.44 ± 6.54[b]	15.86 ± 1.31[b]	84.39 ± 7[b]
HFD + Telmi +Met (high)	199.66 ± 20.63	277.5 ± 22.47[b]	0.76 ± 0.05[b]	322.7 ± 10.74[b]	15.90 ± 2.5[b]	84.63 ± 13.39[b]
HFD + Telmi +Met (low)	170.83 ± 19.69	267.33 ± 14.08[b]	0.60 ± 0.04[b]	299.93 ± 7.60[b]	16.78 ± 3.03[b]	89.31 ± 16.12[b]

Results: mean ± SD; a=p<0.05 vs Normal control, b=p<0.05 vs HFD control.

Table 3: Effect of various pharmacological interventions on the serum glucose and serum 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 treatment						
Normal control	93.6 ± 13.5	54.4 ± 6.5	78.9 ± 11.7	33.1 ± 5.5	15.7 ± 2.3	5.46 ± 9.7
Telmisartan *per se* (high)	96.5 ± 12.05	51.4 ± 2.03	66.3 ± 7.81	33.2 ± 3.54	13.27 ± 3.71	4.9 ± 1.56
Metformin *per se* (high)	94 ± 6.17	54.8 ± 4.38	76.7 ± 9.67	31.5 ± 3.20	15.3 ± 5.00	7.9 ± 1.93
Telmi+met *per se* (low)	93.7 ± 6.03	56.7 ± 4.94	76.4 ± 9.65	30.3 ± 2.73	15.2 ± 6.94	11.1 ± 1.93
High fat diet treatment						
High fat diet (HFD)	155.41 ± 3.93[a]	130.8 ± 8.00[a]	145.39 ± 6.67[a]	23.39 ± 3.05[a]	29.07 ± 1.33[a]	78.38 ± 9.6[a]
HFD + Telmisartan high	134.4 ± 7.76[b]	75.9 ± 5.59[b]	118.3 ± 11.12[b]	32.8 ± 2.13[b]	23.6 ± 4.57[b]	19.35 ± 2.22[b]
HFD + Telmisartan low	138.1 ± 8.07[b]	78.6 ± 4.50[b]	126 ± 9.61[b]	26.6 ± 3.29	25.2 ± 4.82[b]	26.7 ± 1.92[b]
HFD + Metformin High	129.8 ± 10.60[b]	76.6 ± 5.40[b]	119.8 ± 9.13[b]	27.1 ± 2.93	23.9 ± 5.81[b]	25.4 ± 1.82[b]
HFD + Metformin low	138.9 ± 8.08[b]	88.2 ± 9.12[b]	126 ± 6.80[b]	23.5 ± 2.97	25.2 ± 10.46[b]	39.4 ± 1.36[b]
HFD + Telmi +Met (high)	124.7 ± 3.41[b]	71.61 ± 7.37[b]	114.83 ±7.62[b]	33.62 ± 3.91[b]	15.02 ± 5.12[b]	22.96 ± 1.52[b]
HFD + Telmi +Met (low)	134.37 ± 3.43[b]	77.63 ± 5.69[b]	118.16 ± 6.96[b]	30.93 ± 3.25[b]	23.06 ± 6.77[b]	23.63 ± 1.39[b]

Results: mean ± SD; [a]=p<0.05 vs Normal control, [b]=p<0.05 vs HFD control.

Table 4: Effect of various pharmacological interventions on the different fat depots

Parameters	Epididymal fat	Mesentric fat	Retroperitoneal fat	Total fat
Normal diet treatment				
Normal diet control	1.9 ± 0.10	2.13 ± 0.42	1.8 ± 0.17	5.83 ± 0.61
Telmisartan *per se* (high)	1.82 ± 0.53	1.67 ± 0.50	1.12 ± 0.55	4.62 ± 1.22
Metformin *per se* (high)	1.98 ± 0.13	1.8 ± 0.38	1.36 ± 0.49	5.15 ± 0.89
Telmi + Met *per se* (low)	1.7 ± 0.20	1.66 ± 0.19	1.4 ± 0.21	4.76 ± 0.58
High fat diet treatment				
High fat diet (HFD)	7.16 ± 0.413[a]	7.5 ± 0.48[a]	7.36 ± 0.52[a]	22.03 ± 0.77[a]
HFD + Telmisartan (high)	3.83 ± 0.15[b]	3.66 ± 0.24[b]	3.66 ± 0.27[b]	11.16 ± 0.61[b]
HFD + Telmisartan (low)	3.8 ± 0.53[b]	3.73 ± 0.45[b]	3.76 ± 0.51[b]	11.3 ± 1.41[b]
HFD + Metformin (high)	3.66 ± 0.35[b]	3.45 ± 0.38[b]	3.9 ± 0.16[b]	11.01 ± 0.47[b]
HFD + Metformin (low)	3.73 ± 0.37[b]	3.68 ± 0.46[b]	3.4 ± 0.53[b]	10.81 ± 1.22[b]
HFD + Telmi + Met (high)	3.1 ± 0.20[b]	3.1 ± 0.10[b]	3.06 ± 0.20[b]	9.2 ± 0.35[b]
HFD + Telmi + Met (low)	3.56 ± 0.34[b]	3.5 ± 0.41[b]	3.66 ± 0.39[b]	10.73 ± 0.99[b]

Results: mean ± SD; [a]=p<0.05 vs Normal control, [b]=p<0.05 vs HFD control.

Histopathological study

The liver and fat depots were excised out and preserved in 10% formalin solution. The histological study was carried out after staining with hematoxylin and eosin, and observed under binocular microscope (10x) to assess changes in liver tissue and size of fat depots.

Statistical analysis

The results were expressed as mean ± standard deviation (SD) analyzed by one-way and two-way ANOVAs followed by Tukey's multiple comparison test as post hoc analysis. p<0.05 was considered to be statistically significant.

RESULTS

Effect of various pharmacological interventions on anthropometric parameters

A significant (p<0.05) increase in body weight, BMI, Lee index, feed consumption (Kcal); and decrease in feed consumption (g) were observed in rats fed over high fat diet, as compared to age matched normal rats fed on standard diet. Treatment with metformin, telmisartan, and combined administrations in high and low doses produced significant (p<0.05) decrease in body weight, BMI and Lee index as compared to HFD control group. (Table. 2)

Effect of various pharmacological interventions on serum biochemical parameters

A significant (p<0.05) increase in serum glucose, cholesterol, TG, LDL, VLDL and decrease in HDL levels were observed in HFD control group after 10 weeks as compared to NC. Treatment with metfromin, telmisartan and combination of metformin and telmisartan in low and high doses caused significant (p<0.05) attenuation of HFD induced changes in serum biochemical parameters, as compared to HFD control. (Table. 3)

Effect of various pharmacological interventions on different fat depots

HFD treatment for 10 weeks caused significant (p<0.05) increase in weight of epididymal, retroperitoneal, mesenteric and total fats. Treatment with metformin, telmisartan and combined administration produced significant (p<0.05) decrease in weight of fat depots, in comparison to HFD control. (Table. 4)

Effect of various pharmacological interventions on histopathology

The high fat diet treatment for 10 weeks produced microvesicular and macrovesicular steatosis in liver, as compared to normal histology of rat liver. Treatment with metformin and telmisartan alone and combination, at high and low doses significantly prevented these changes in liver (figure. 1). HFD treatment produced significant increase in size of adipocytes (epididymal fat depot) as compared to rats fed on normal diet. Treatment with metformin and telmisartan each and in combination at both doses produced significant decrease in size of adipocytes. (Figure. 2)

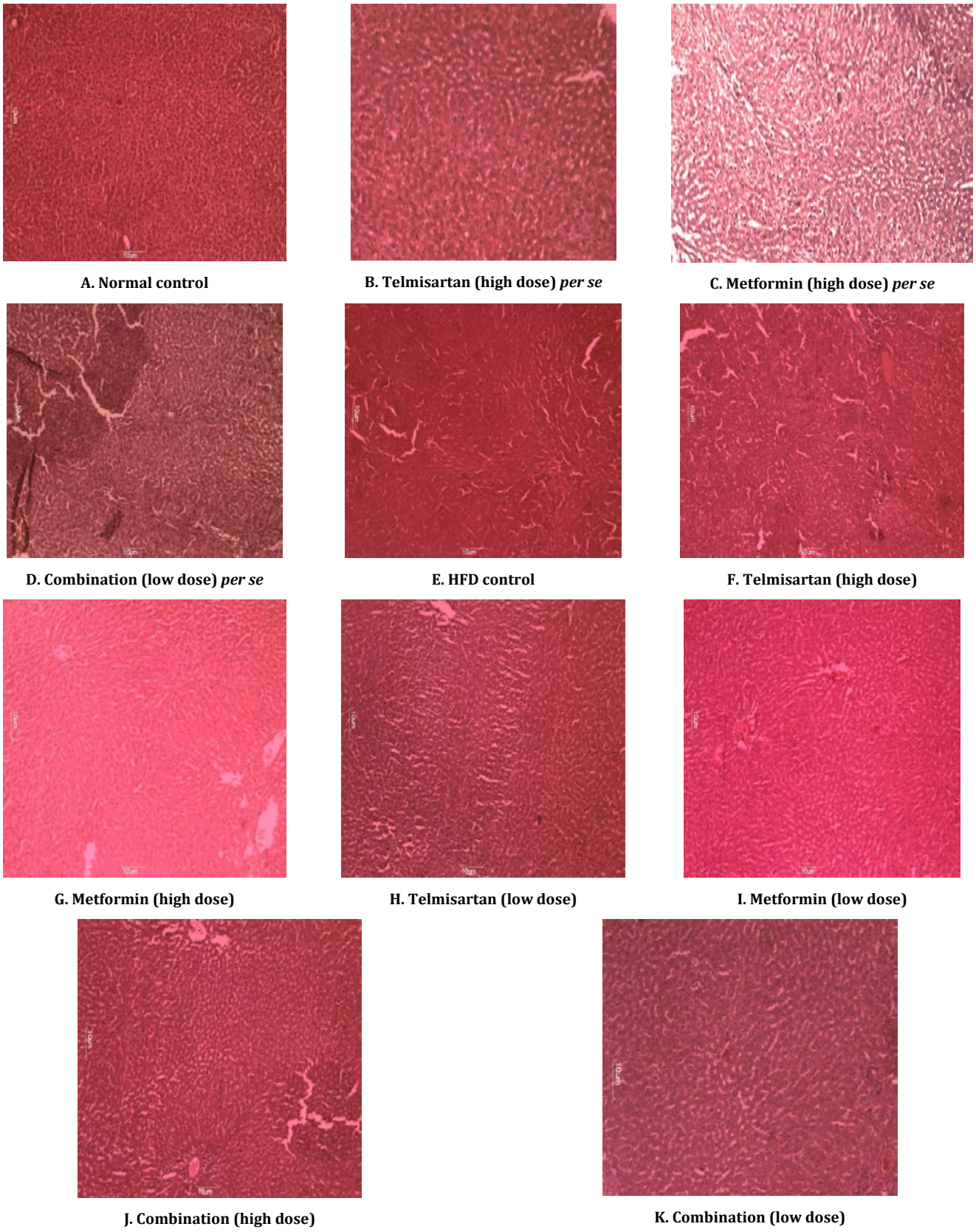
DISCUSSION

The present study demonstrated the beneficial effect of metformin, telmisartan and their combination on high fat diet induced obesity in Wistar rats.

High fat diet treatment has been widely used model to develop experimental obesity characterized with dyslipidemia and insulin resistance in rodents leading to certain metabolic deformations.[17] Induction with HFD for 10 weeks caused significant gain in body weight, increased feed intake (Kcal), BMI, Lee index and decreased feed intake (g), and confirmed the characteristics of obesity and dyslipidemia from earlier literatures.[18] This effect may due to increased fat accumulation and impaired glucose metabolism. Treatment with metformin, telmisartan and their combination attenuated the effects of HFD treatment. The decrease in body weight by metformin may due to activation of AMP-activated protein kinase (AMPK), which is activated by leptin.[9,10,11] The above findings imply that a more delicate interaction takes place between metformin and leptin. Therefore, metformin increases leptin sensitivity and hence may result anorexic and leptin-reducing effects. Telmisartan is reported to increase the expression of PPARγ target genes,[19] increasing energy expenditure and thus may decrease body weight.[20] In present study, treatment with low dose combination of metformin and telmisartan showed an additive effect in lowering body weight, in comparison to low doses of metformin and telmisartan in HFD control rats. This indicates that the synergistic effect of the combination of metformin and telmisartan may be due to their different mechanism of action.

The lipogenesis was upregulated by HFD leads to elevation of plasma lipid levels which is characterized by elevated TG, LDL-C and decreased serum HDL-C in obese rats.[21,22,17] Further, feeding with high fat diet caused hyperglycemia in rats.[23] Therefore, the serum lipid profile (cholesterol, LDL, VLDL, HDL and TGs) and glucose were altered in present study and thus developed hyperlipidemia and hyperglycemia. Treatment with metformin, telmisartan and their combination significantly attenuated these metabolic changes of HFD in serum. Metformin decreased glucose level, lipid level, and liver steatosis in the present study and this may be due to the phosphorylation and inactivation of ACC, as a result of AMPK activation, that inhibited the proximal and rate-limiting step of lipogenesis. Reduced synthesis of ACC product, malonyl CoA, is also predicted to relieve inhibition of CPT-1, resulting in increased fatty acid oxidation. These effects are likely to contribute to metformin *in-vivo* ability to lower triglycerides and VLDL. AMPK mediates decrease in SREBP-1 mRNA and protein expression. Known target genes for SREBP-1, which include FAS and S14, are also down regulated in liver, further contribute to effects of metformin to modulate circulating lipids, reduce hepatic lipid synthesis and fatty liver. Moreover, metformin-mediated effects on hepatic glucose production contribute to its glucose-lowering efficacy. AMPK activation is implicated as a mechanism for the induction of skeletal

muscle glucose uptake[24] as also observed in present study. Earlier study reported that thiazolidine, a PPARγ agonist, reduced hepatic TG in Zucker fatty rats associated with a reduction of hepatic lipogenic enzyme and showed insulin-sensitizing effects through PPARγ.[25,26,27] The telmisartan induced activation of PPARγ would be expected to improve insulin resistance in obese animal models.



A. Normal control **B. Telmisartan (high dose) *per se*** **C. Metformin (high dose) *per se***

D. Combination (low dose) *per se* **E. HFD control** **F. Telmisartan (high dose)**

G. Metformin (high dose) **H. Telmisartan (low dose)** **I. Metformin (low dose)**

J. Combination (high dose) **K. Combination (low dose)**

Fig. 1: Effect of various pharmacological interventions on histological characteristics of liver (10x)

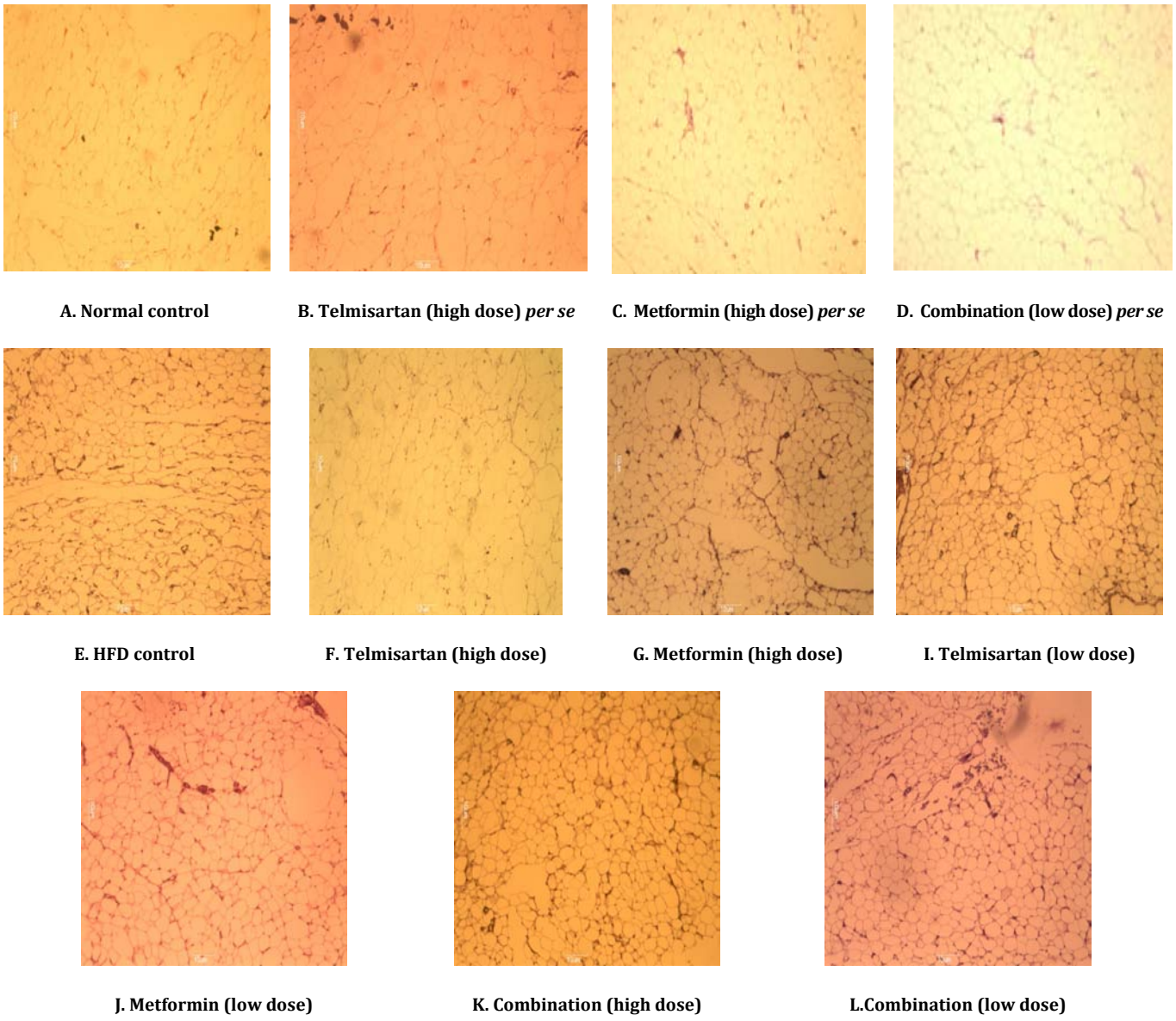


Fig. 2: Effect of various pharmacological interventions on histological characteristics of different fat depots (10x)

The weight of the adipose tissues: epididymal, perirenal, mesenteric fat depots increased progressively due to the *ad libitum* HFD feeding[28,29] as also observed in present study due to adiposity. Metformin, telmisartan and their combination reversed the effect of HFD on adipose tissues. Telmisartan decreases adipose mass in the present study by directly stimulation of PPARγ.[20,30] Telmisartan treatment *in vitro* has been shown to augment the expression of PPARγ as well as target genes, including adipocyte fatty acid-binding protein (aP2), adiponectin, and ACC in murine and human adipocytes.[20] Liver steatosis and size of adipocyte are increased during HFD treatment[31] as also evidenced in present study. These pathological changes were prevented by treatment with metformin, telmisartan and their combination effectively. Our results raise a question regarding the differential effects of telmisartan and thiazolidine (PPARγ agonists). In present study, telmisartan was found to be effective in reducing body weight and adiposity. However, numerous previous studies showed the thiazolidine treatment failed to reduce body weight and adiposity and frequently increased body weight and/or adiposity.[32] The selective PPAR modulator activity of telmisartan could retain the metabolic efficacy of PPARγ activation while reducing adverse effects by concurrently blocking AngII type1 receptor activation.[19] Thus, telmisartan may reduce the weight-promoting effects of PPARγ activation yet retain PPAR-mediated metabolic efficacy. These observations suggest that the effect of low dose combination of metformin and telmisartan is more effective as compared when used alone and this effect is similar to the effects observed with their higher doses when used alone.

To sum up the findings that the metformin leads to decrease in food intake, body weight and hyperlipidemia by activation of AMPK, and telmisartan significantly reduced HFD induced obesity and adipogenesis by activation of PPARγ. Moreover, the combination of metformin and telmisartan has potent synergistic activity in attenuating the HFD-induced obesity by modulation of AMPK and PPARγ signaling cascades. The synergistic effect of this combination may thus be due to their different mechanism of action.

CONCLUSION

On the basis of the result obtained in this study, it may be concluded that the treatment with metformin, telmisartan and their combination significantly prevented the HFD-induced obesity. This study has provided a rational pharmacological basis for the combined use of low doses of telmisartan and metformin in treatment of obesity in man.

Declarations

Conflict of interest

The Authors declare that they have no conflicts of interest to disclose.

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