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Research Article

COMPARISON OF EFFECTS OF SITAGLIPTIN AND A COMBINATION OF NALTREXONE AND BUPROPION IN HIGH FAT DIET-INDUCED OBESITY MODEL IN RATS

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ABSTRACT

Objective: The study aimed to compare the anti-obesity effect of Sitagliptin and a combination of Naltrexone and Bupropion in high-fat diet-induced obesity model in animals.

Methods: This study was a prospective study of 17 weeks duration. Obesity was induced in rats by feeding them a high-fat diet over a period of 17 weeks. Sitagliptin and a combination of Naltrexone and Bupropion were administered to two groups for 5 weeks and various parameters such as body weight, blood glucose, food intake, and BMI were measured and analyzed over a period of 5 weeks.

Results: In this study, on administration of Sitagliptin and a combination of Naltrexone and Bupropion, there was a gradual weight loss in rats. The combination of Naltrexone and Bupropion showed a significant ($p<0.05$) effect on reduction in body weight, reduction in food intake, and reduction of BMI in obese rats, whereas Sitagliptin showed a significant reduction in blood glucose in rats.

Conclusion: In the present study combination of Naltrexone and Bupropion stood most effective in reducing weight, food intake, as well as BMI.

Keywords: Anti-obesity drugs, High-fat diet, Weight loss agents, Naltrexone, Bupropion, Sitagliptin.

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INTRODUCTION

Obesity is a troublesome disorder affecting developed and developing worlds alike. The major shift occurring in our lifestyle in recent few decades has led to a more sedentary lifestyle and drastic changes in our eating habits. This has led to a habit of consuming food rich in calories and a decrease in manual labor as humans are more dependent on processed food now. The World Health Organization lists obesity as one of the eight principal causes of preventable chronic disease worldwide [1]. The major burden on health-care resources in many countries is due to increases in the prevalence of obesity. The worldwide cases are estimated at 1.5 billion people who are overweight and another 500 million obese [2]. Obesity is also becoming a major health problem in India with individuals developing obesity reaching 5% of the country's population [3]. In India, a study conducted by ICMR-INDIAB in 2015, the prevalence rate of obesity was found to be in the range from 11.8% to 31.3% [4]. Obesity often leads to the risk of developing cardiovascular disease, peripheral vascular disease stroke, renal failure, and Type 2 diabetes mellitus [3,5]. The pathophysiology of obesity is still not very well understood. It has been observed that a modest decrease in body weight eventually leads to a fall in blood pressure and improved blood glucose control in patients with diabetes [6]. At present, bariatric surgery, lifestyle modification, and pharmacotherapy are the mainstays in the management of obesity and its squeals [7]. The history of drug treatment of obesity is not a success story in itself, as many anti-obesity drugs were developed, marketed, and later withdrawn due to their adverse effects profile. Neuronal pathways are known to play a role in the short-term regulation of appetite and satiety, while long-term weight control is more or less governed by hormonal stimuli released by the gastrointestinal tract and adipose tissue [8]. The effect of anorexigenic GLP-1 analogs such as Exenatide has shown significant weight loss in studies [9]. Dipeptidyl-peptidase 4 (DPP-4)-mediated degradation resistant GLP-1 analogs like Sitagliptin are currently under investigation for possible anti-obesity effects. Within the hypothalamus, there are two groups of neurons involved in the regulation of food intake, the appetite inhibiting pro-opiomelanocortin (POMC) neurons, and appetite-stimulating neuropeptide Y (NPY) [10]. Regulation of body weight is through these brain circuits and these are responsive to endocrine and metabolic signals in the body. Targeting these circuits with novel pharmaceutical drugs can lead to the development of new drugs for obesity. Endogenous opiates take part in this regulation of appetite and satiety. Opiates increase food intake and this intake may be blocked by opiate antagonists. At present, drug therapies based on this are being investigated such as the role of Naltrexone and its combination with bupropion in reducing appetite and weight loss [11]. With this background, this study was planned with an aim to study the effects of Sitagliptin and a combination of naltrexone and bupropion on high fat diet-induced obesity in rats and to compare the effect produced by them.

METHODS

Drugs that were used in the study:

1. Test drug – Sitagliptin
2. Test drug – Combination of Naltrexone and Bupropion

Experimental animals

Male and female albino rats of Wistar strain weighing between 90 and 110 g were used in the study. Animals were kept in the animal house and were housed in a group of three animals per polypropylene cage at 22±2°a C with 12-h light/12-h dark cycle. They had access to water and standard laboratory chowad libitum. The animals were acclimatized to laboratory conditions for a period of 7 days before the commencement of experiment. The experimental protocol was duly submitted and approved by the Institutional Animal Ethics Committee (IAEC) as per the CPCSEA guidelines.

High fat diet

Obesity was induced in 50 rats over a period of 12 weeks using high-fat diet [12] consisting of gram flour (40%), saturated fat (25%), dried coconut (10%), cheese (5%), condensed milk (15%), peanuts (5%), and multivitamin powder. The ingredients were then mixed and made in to the form of small balls and this was given to rats, in addition to free access to standard laboratory chow and water. At the end of 12 weeks,

animals weighing between 130 and 200 grams were included in the study and were divided into below-mentioned Group I to Group IV.

Grouping of animals

For the study, rats were sorted into four groups with six rats in each group (n=6).

- Group I: This group served as non-obese control (NOC). Obesity was neither induced in this group nor did it receive any treatment for obesity. This group was provided with the standard laboratory chow and water.
- Group II: This group served as obese control (OC) in which obesity was induced by high-fat diet over a period of 17 weeks. This group received no treatment for obesity.
- Group III: This group served as obese Naltrexone plus bupropion (ONB) treated group in which obesity was induced over a period of 17 weeks. This group received a combination of Naltrexone (3 mg/kg body weight/day) and Bupropion (33 mg/kg body weight/day) dissolved in distilled water and given orally with the help of a rat feeding needle from week 13 to week 17 for a period of 5 weeks.
- Group IV: This group served as the obese Sitagliptin (OS) treated group in which obesity was induced over a period of 17 weeks. This group received Sitagliptin (9 mg/kg body weight/day) dissolved in distilled water and given orally with the help of a rat feeding needle from week 13 to week 17 for a period of 5 weeks.

Weight recording

Weekly weight recording of each rat was done from 0 to 17 weeks using the weighing machine. Weights were expressed in grams.

Food intake measurement

The food was given daily to the rats and the remaining food in the cage was then collected the next day. The total remaining food for the whole week was weighed at the end of the week. The net food intake of each group for each week was calculated by measuring the difference between the food given throughout the week and the food remaining at the end of the week and was expressed as g/week/group.

Blood glucose estimation

Blood glucose was measured at 0, 4, 8, 12, 13, 14, 15, 16, and 17 weeks as mg/dl (milligram per deciliter) after overnight fasting and was estimated using the tail clip method for blood collection.

Measurement of body mass index (BMI)

At the end of week 17, animals from group I to Group IV were subjected to BMI estimation. It was calculated by measuring weight and measuring the length from mouth to anus [13] with the help of Vernier Caliper. The BMI was expressed as g/cm^2.

Statistical analysis

Body weight and blood glucose values were reported as mean±standard deviation, food intake was expressed in absolute values and BMI was expressed as mean. Statistical analysis was done using "R" software as data analysis tool. Data were analyzed using analysis of variance (ANOVA) to determine differences among groups. $p \leq 0.05$ was accepted as significant.

OBSERVATIONS AND RESULTS

Body weight

Table 1 shows the mean weight (in g)±SD of all groups of rats over a period of 17 weeks. Administration of high-fat diet led to increasing in weight in Groups II, III, and IV (Table 1). The mean weight of rats in Group III ONB and Group IV OS gradually increased till week 12. In Group III ONB after starting Naltrexone plus Bupropion (Naltrexone 3 mg/kg/day and Bupropion 33 mg/kg/day), the mean weight gradually started declining and was 140.6±14.78 g at the end of the week 17 and in Group IV OS after starting Sitagliptin (9 mg/kg/day), the mean weight started to decline and was 125.0±9.44 g at the end of week 17. Group II did not receive any treatment and it showed a further increase in body weight and was 169.1±7.90 g at the end of week 17.

In Table 2, normalization of mean weight at week 13 was done for all groups and the percentage change in weight was calculate dover a period of 5 weeks during which drugs were administered. It shows a decline in weight in Group III ONB and Group IV OS.

Food intake

Food intake (Table 3) of Group I NOC and Group II OC increased gradually over a period of 17 weeks. In Group III ONB after starting Naltrexone plus Bupropion, the food intake started to decline from week 13 onward and continued to decline till week 17 while in Group IV OS after starting Sitagliptin, the food intake almost remained the same from week 13 onward.

Table 1: Body weight of groups (mean±SD in g)

Weeks	Group I NOC	Group II OC	Group III ONB	Group IV OS
0	98.5±5.37	105.0±4.38	102.7±7.36	100.4±6.04
1	99.9±4.64	107.9±3.53	105.8±6.91	102.8±7.68
2	100.2±4.07	110.9±4.56	112.8±7.79	106.3±9.13
3	102.4±3.94	114.8±4.99	117.8±7.00	110.6±10.08
4	105.0±3.38	121.4±5.76	122.7±8.13	112.5±9.68
5	107.7±2.69	125.4±5.35	126.5±9.95	116.0±9.34
6	109.4±2.94	129.9±5.26	131.7±11.01	120.4±7.40
7	110.7±4.23	133.9±3.94	136.2±11.31	122.1±8.82
8	111.8±3.35	137.5±3.14	139.2±12.14	125.1±8.33
9	113.5±3.02	141.6±1.99	142.9±12.73	128.5±7.35
10	115.9±3.23	144.0±2.94	146.5±12.00	131.4±6.75
11	118.1±3.49	146.7±3.99	152.2±12.18	133.9±6.87
12	119.9±3.97	149.8±5.89	155.3±13.24	137.2±6.17
13	121.8±4.42	153.0±6.51	154.1±12.70	135.9±5.71
14	124.7±4.99	156.2±7.67	152.1±12.97	133.7±6.82
15	127.6±5.66	160.3±7.57	148.3±13.80	131.5±7.46
16	129.0±4.61	162.4±7.05	143.6±14.24	128.3±8.61
17	132.1±4.30	169.1±7.90	140.6±14.78	125.0±9.44

NOC: Non-obese control, OC: Obese control, ONB: Obese naltrexone plus bupropion, OS: Obese Sitagliptin

Table 2: Percentage change in body weight during treatment

Weeks	Group I NOC	Group II OC	Group III ONB	Group IV OS
13	100%	100%	100%	100%
14	102%	102%	99%	98%
15	105%	105%	96%	97%
16	106%	106%	93%	94%
17	108%	111%	91%	92%

NOC: Non-obese control, OC: Obese control, ONB: Obese naltrexone plus bupropion, OS: Obese Sitagliptin

Table 3: Food intake of groups (g/week)

Weeks	Group I NOC	Group II OC	Group III ONB	Group IV OS
1	122.9	147	138	127.45
2	133.8	164.2	144.52	136.7
3	144.87	172	151.22	148.98
4	149.7	183.2	170.55	170.35
5	155.32	196.99	230.32	184.54
6	150.33	211.73	225.55	210.32
7	160.45	230.67	240.48	236.78
8	157.89	245.87	279.75	240.22
9	161.32	267.22	275.54	237.9
10	160.43	270.89	270.81	256.87
11	154.32	263.44	281.21	252.81
12	158.94	261.43	272.24	259
13	167.89	268.84	265.32	260.91
14	167.58	269.62	260.56	262.54
15	168.97	270.32	258.39	261.87
16	170.43	271.38	252.56	263.32
17	169.51	270.43	247.73	265.82

NOC: Non-obese control, OC: Obese control, ONB: Obese naltrexone plus bupropion, OS: Obese Sitagliptin

Blood glucose

Blood glucose measured every 4 weeks showed that there was a gradual reduction in blood glucose (Table 4) in Group III ONB and Group IV OS after starting drug therapy. The maximum reduction in blood glucose was found in Group IV OS.

BMI

Table 5 shows the mean BMI (in g/cm^2) of each animal from Groups I–IV. It is evident that Group III ONB has the lowest BMI and Group II OC has the highest BMI.

DISCUSSION

The growing pandemic of obesity needs to be addressed as it presents a glaring problem in today's modern world. It is an important risk factor for cardiovascular and metabolic syndromes, leading to adverse effects on health and life expectancy [14]. Lifestyle modifications including diet and exercise are essential for both the prevention and management of obesity but the role of pharmacotherapy can be considered if the interventions fail. Many anti-obesity drugs that were approved previously and were sold have now been withdrawn due to their serious adverse effects. Recently, there have been many drugs in development for the effective treatment of obesity; therefore, we need to evaluate the efficacy of the new drugs for potential anti-obesity effects. The present study was undertaken to get a comparison between Sitagliptin and a combination of Naltrexone and Bupropion. Although the efficacy of these drugs has been studied individually, the effect of their combination can be explored for a better understanding of the treatment of obesity. In this study, animal model of obesity is been used to emulate human obesity like conditions. Obesity induction in animals can be done by various methods, for example, hypothalamic obesity, monosodium glutamate-induced obesity, and obesity induction by high-fat diet. We have preferred using high-fat diet model to induce obesity as it very closely resembles human obesity. During the study, it was observed that gain in weight with high-fat diet is a long and gradual process. The features of this model were a progressive increase in total body weight, progressive impairment of blood glucose and a significant increase in BMI. The methodology of obesity induction used in our study has been used previously in other studies [15-17].

Table 4: Blood glucose of groups (mean±SD in mg/dl)

Week	Group I NOC	Group II OC	Group III ONB	Group IV OS
0	98.50±9.61	98.67±10.41	104.50±17.44	97.33±9.27
4	104.67±4.08	116.83±11.07	129.17±13.82	130.33±8.36
8	103.83±8.70	129.50±5.32	125.33±23.02	139.33±13.22
12	107.83±6.08	136.00±5.51	144.83±9.45	153.50±9.63
13	111.67±5.79	141.50±6.72	146.53±7.68	151.83±10.38
14	113.83±5.12	143.67±7.63	139.67±5.20	142.83±9.45
15	117.50±3.73	147.33±9.03	137.00±7.56	134.33±10.37
16	118.17±2.14	153.17±7.49	126.83±6.88	126.17±6.01
17	120.83±2.23	157.50±8.55	124.50±4.23	104.83±15.84

NOC: Non-obese control, OC: Obese control, ONB: Obese naltrexone plus bupropion, OS: Obese Sitagliptin, SD: Standard deviation

Table 5: Mean BMI (g/cm^2)

Animal No.	Group I NOC	Group II OC	Group III ONB	Group IV OS
A	0.49	0.44	0.51	0.47
B	0.52	0.61	0.57	0.5
C	0.59	0.52	0.56	0.55
D	0.53	0.6	0.41	0.57
E	0.49	0.66	0.54	0.59
F	0.57	0.61	0.56	0.57
Mean	0.53	0.57	0.52	0.54

NOC: Non-obese control, OC: Obese control, ONB: Obese naltrexone plus bupropion, OS: Obese Sitagliptin, BMI: Body mass index

Body weight

In our study of comparison of the anti-obesity effect of Sitagliptin and a combination of Naltrexone and Bupropion, weight changes were observed in all the groups. Group I NOC due to normal growth of the rats showed gradual weight gain whereas much higher weight gain was found in rats of Group II OC to Group IV OS from week 1 onward due to the presence of high fat in their diet. Group I NOC and Group II OC did not receive any drug treatment for obesity throughout the study period. In Group III ONB combination of the drug Naltrexone (3 mg/kg body weight/day) and Bupropion (33 mg/kg body weight/day) was started at the beginning of week 13 and was continued till week 17. There was a significant decline in weight ($p<0.05$) in Group III ONB rats after drug administration (Tables 1 and 6). As evidenced by other studies, neurotransmitter effects of Bupropion cause weak inhibition of the synaptic reuptake of norepinephrine and dopamine, in turn, causing increase in dopamine activity and stimulating pro-opiomelanocortin (POMC) neurons leading to their activation, this leads to a reduction in appetite and increase in energy expenditure [18]. Naltrexone, an opioid receptor antagonist blocks opioid receptors on the POMC neurons, preventing feedback inhibition and thereby increasing POMC activity. Increased POMC signaling has been associated with decreased appetite, increased metabolism, and weight loss, whereas decreased POMC signaling is associated with hyperphagia and energy conservation [19]. From this, it can be correlated that the weight loss encountered in rats in Group III ONB can be the result of the anorectic effect of the combination of Naltrexone and Bupropion. In Group IV OS, Sitagliptin (9 mg/kg body weight/day per orally) was started at the beginning of week 13 and was continued till week 17. There was a significant decline in weight ($P<0.05$) in rats after Sitagliptin administration (Tables 1 and 6). Sitagliptin is a Dipeptidyl-peptidase 4 (DPP-4) inhibitor which regulates body adiposity and energy expenditure through GLP-1 pathways by inhibiting the breakdown of glucagon-like peptide-1 (GLP-1) [20,21]. GLP-1 has a regulatory effect on the hypothalamus thus modulating hunger and satiety which might lead to weight loss. The same pattern of weight loss was found in our study. Sitagliptin caused significant weight loss in obese rats. In our study, weight loss was found in both groups which were given drug treatment (Group III ONB – 9% and Group IV OS – 8%) (Table 2) and it was comparable. Tukey's multiple comparisons test showed that there was no significant difference ($p=0.0656$) in means of Group III ONB and Group IV OS when compared together.

Food intake

Group I NOC which was given normal laboratory chow showed an only a modest increase in food intake throughout the study till the end of week 17 (Table 2). In Group II OC, food intake was higher (Table 2) because it was observed that rats preferred high-fat diet as compared to laboratory chow. In Group III ONB, food intake started to decline from week 13 onward after starting a combination of naltrexone and bupropion. There was a significant ($p<0.05$) reduction in food intake in this group. This can be due to anorectic effect of Naltrexone and Bupropion [22]. Different subtypes of adrenoceptor and dopamine receptors mediate bupropion's effects on the size of meals and on satiety [23]. As discussed above, combination of Naltrexone and Bupropion causes a decrease in appetite, an increase in energy expenditure, and a reduction in food intake by regulating POMC activity. The acute anorexic effects of Naltrexone and Bupropion in obese rats are also supported by previously published data [24]. From the findings of Group III ONB, we can say that a combination of

Table 6: Repeated measures ANOVA summary

ANOVA summary	Body weight	Food intake	Blood glucose
F	9.536	13.28	4.785
P value	<0.0001	<0.0001	0.0073
P value summary	****	****	**
Significant diff. among means ($p<0.05$)?	Yes	Yes	Yes
R^2	0.2961	0.3836	0.3097

naltrexone and bupropion has an anorectic effect in obese rats thereby leading to a decrease in food intake and subsequently causing weight loss. In Group IV OS, Sitagliptin administration did not lead to any significant increase/decrease in food intake. It has also been reported that Sitagliptin causes attenuation in body adiposity, without affecting food intake [21].

Blood glucose

Blood glucose in Group I NOC was found within normal limits. The increase in blood sugar was more in Group II OC (Table 4). Due to the intake of calorie-rich diet, it is likely that the rats in that group have developed high blood glucose levels. The continuous intake of calorie and fat-rich diet lead to the development of obesity and high blood glucose in Group II OC rats. In Group III ONB, there was a decline in blood glucose after starting a combination of Naltrexone and Bupropion (Table 4). As mentioned earlier due to the anorectic effect of the combination of naltrexone and bupropion, there was weight loss and this reduction in weight loss could further have led to normalization of blood glucose levels. This change is many times observed in people with obesity who demonstrate improvement in blood glucose with progressive weight loss. Previously, published studies have concluded that insulin signaling in adipocytes could become increasingly impaired, eventually leading to massive adipocyte lipolysis, necrosis, and systemic insulin resistance [25]; therefore, we can assume that reduction in weight/adipose tissue often leads to a reduction in inflammation in adipose tissue thereby decreasing insulin resistance, which can be responsible for the improvement in blood glucose levels. Group IV OS showed a significant ($p<0.05$) reduction in blood glucose among all the groups (Table 4). Sitagliptin is a DPP-4 inhibitor is known to lower the activity of DPP-4. This causes an increase in plasma concentration of active GLP-1 and is associated with increased insulin secretion and improvement in hyperglycemia [26]. In our study, it was found that the most significant reduction in blood glucose was shown in Group IV OS followed by Group III ONB. Tukey's test result showed that the combination of naltrexone and bupropion and Sitagliptin caused a significant ($p<0.05$) reduction in blood glucose in obese rats in Groups III ONB and IV OS. However, there were no significant differences in the mean of Groups III ONB and IV OS when compared ($p>0.05$) together.

BMI

In this study, BMI of individual rats of all groups was measured at week 17. On comparing the respective BMI values, it was found that rats of Group II OC showed maximum BMI (Table 5) whereas rats of Group IV ONB had minimum BMI. Group IV OS showed BMI values more than Group I NOC and Group III ONB but less than Group II OC. Group III ONB showed a decrease in weight and a reduction in blood glucose, as well a decrease in food intake too. A literature review has shown that the risk of high blood glucose and subsequent diabetes diagnosis was more for individuals with higher BMI [27]. The rats in Group II OC were having high blood glucose and they also had the highest BMI among all the groups, whereas rats of Group III ONB were found to have low blood glucose as well as low BMI. Therefore, we can assume that a reduction in BMI leads to reduction in blood glucose levels in Group III ONB or it can be said that lower BMI leads to improvement in blood glucose levels.

CONCLUSION

In the present study, combination of Naltrexone and Bupropion stood most effective in reducing weight and food intake, as well as BMI. Similarly, Sitagliptin had a favorable effect on reducing body weight and was found most effective in reducing blood glucose levels. Although the study has given us a good insight into the metabolic effects of Naltrexone and Bupropion combination and Sitagliptin, nevertheless, the study has some limitations. The results would have been more elaborate if we would have done the study on a larger number of animals and for a longer duration of time. The present study can be further extended to include various other parameters such as an estimation of lipids, insulin level, and leptin level.

AUTHOR'S CONTRIBUTION

Dr. Mohit Kulmi: Concept design, defining intellectual content, data acquisition, and Manuscript preparation. Dr. Mohit Kulmi and Dr. Gaurav Saxena: Literature search, data analysis, data interpretation, Manuscript editing, review, and approval of final version of manuscript.

COMPETING INTERESTS

The authors declare that they have no conflicts of interest.

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