

Feed consumption and weight gaining behavior of zucker fatty (fa/fa) rats with single and in combination treatment of clonidine and metformin

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ABSTRACT

Background: Diabetes is metabolic disorder with high blood glucose level. Type-2 diabetes is associated with obesity, insulin resistance, and chronic hyperglycemia. So agents that efficiently reduces weight gain along with diabetic control may play beneficial role in obese diabetic subjects.

Methods: Zucker Fatty Rats were used for the study. Animals were divided in four groups, (control group, Clonidine group, metformin group and combination group). Normal weight gain and feed consumption data were obtained up to 9 weeks of age there after animals were started treatment according to above mentioned groups. During treatment again feed consumption and weight gain data were generated. The experimental results are expressed as the Mean \pm SEM in each group. Statistical analysis has been performed by one way analysis of variance (ANOVA).

Results: The data suggest that there is decrease in body weight gain during the treatment with clonidine and in combination of clonidine and metformin. If we consider weight gain of control as 100 % weekly than clonidine group shows 10.45 % weight gain while metformin shows 96.5 % weight gain and in combination group it shows 33.63 % weight gain. So, there is significant reduction in weight gain of the group treated with clonidine and combination of clonidine and metformin. Same time there is reduction in feed consumption of animals.

Conclusions: The centrally acting drug Clonidine affects the feed consuming behavior and weight gain of animals. It shows hypophagic effect and reduces weight gain in single as well as in combination with metformin.

Keywords: Zucker fatty rats, body weight, feed consumption

INTRODUCTION

Diabetes is metabolic disorder with high blood glucose level. India leads the world with largest number of diabetic subjects.¹ Since diabetes and hypertension frequently co-exists it is helpful to have a drug which shows beneficial effects in both diseases. Clonidine is centrally acting agent with guanabenz, guanfacine, and methyldopa. They lower BP primarily by stimulating α -adrenergic receptors in the brain.² Chronic treatment with clonidine produces a number of beneficial effects such as prevention of hyperlipidaemia and hypothyroidism and improvement in cardiomyopathy and glycaemic control in diabetic and diabetic hypertensive rats.³ Clonidine prevented the hypertension, tachycardia, and insulin resistance associated with feeding dogs the high fat diet

but did not affect weight gain. The study suggests that the central sympathetic nervous system plays a critical role in the development of both insulin resistance and hypertension associated with feeding dogs a high fat diet.⁴ Metformin is indicated in the treatment of patients with type 2 diabetes who do not respond adequately to a program of dietary modification and exercise. In contrast to sulfonylureas, metformin exerts its glucose-lowering action independent of any effects on insulin secretion. Indeed, serum insulin concentrations are unchanged or decreased by metformin.⁵ In addition to its antidiabetic actions, metformin causes weight loss in obese diabetic patients and may be useful in managing associated lipid disorders.⁶

Zucker fatty rats are generally hyper phagic in nature. Obesity is evident at 5 weeks and may be noted as early as 3 weeks of age. By 14 weeks of age, their body composition is over 40 percent lipid and at 40 weeks of age, *fa/fa* rats weigh almost double as much as normal *fa/+* littermates.⁷

METHODS

The study was conducted after getting permission from Institutional Animal Ethics Committee (IAEC) at Zydus Research Center, Ahmedabad. Zucker Fatty (*fa/fa*) rats were used for the study. The animals were housed in clean, sterilized cages under standard laboratory condition. Temperature: 22 ± 3 °C, Humidity: 30-70 %, Light/dark cycle: 12/12 hours, Feed and water has been provided ad libitum.

Total 24 animals were used for the study. After weaning animals were kept in separate cages and divided in four groups each having six animals. Feed consumption and body weight of animals were taken before treatment period. There after animals were treated with the different treatment as below mentioned groups orally. Treatment was given for 28 days during which body weight as well as feed consumption data was generated weekly.

Treatments: group I for control [Vehicle], group II for clonidine [0.15^{8,9} mg/kg], group III for metformin [200¹⁰ mg/kg] and group IV for combination of both drugs.

Preparation of drug formulation: Clonidine and Metformin both APIs were provided by Zydus Cadila. The formulations were prepared in 0.5 % w/v sodium carboxy-methyl cellulose (Na-CMC) in the following strength; Metformin: 200 mg/ml, Clonidine: 0.15 mg/ml. Formulation were prepared daily.

Statistical analysis:

The experimental results are expressed as the Mean \pm SEM in each group. Statistical analysis has been performed by one way analysis of variance (ANOVA) followed by Dunnett's Multiple Comparison Test.

RESULTS

Body weight of animals:

Body weights of animals are given in table 1. Table 1 (a) shows the weight of animals before starting the treatment. There is increase in body weight of all animals every week.

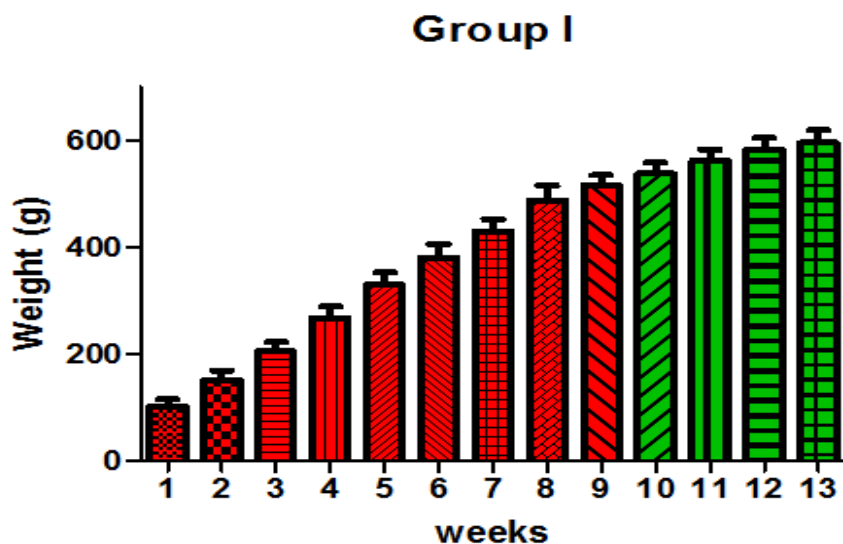


Figure 1 (a): Body weight of animals for control group; n=6.

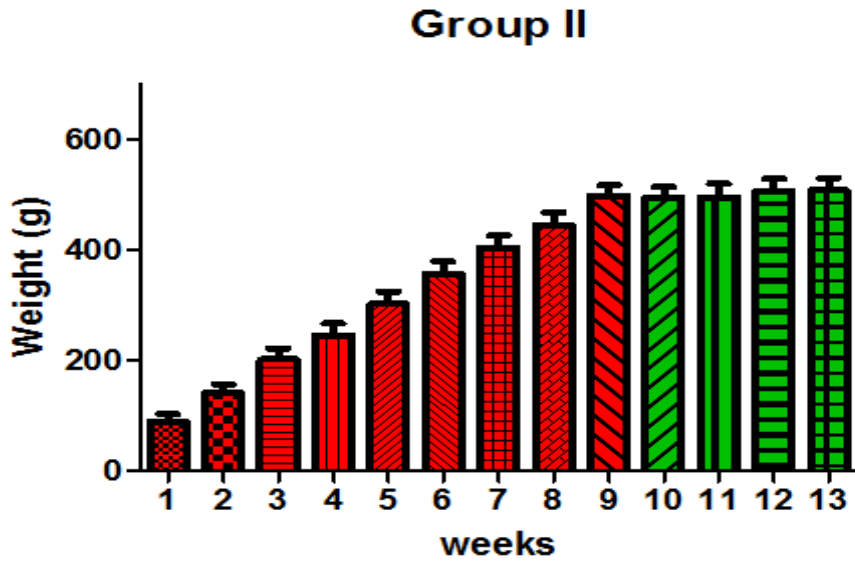


Figure 1 (b): Body weight of clonidine treated animals; n=6.

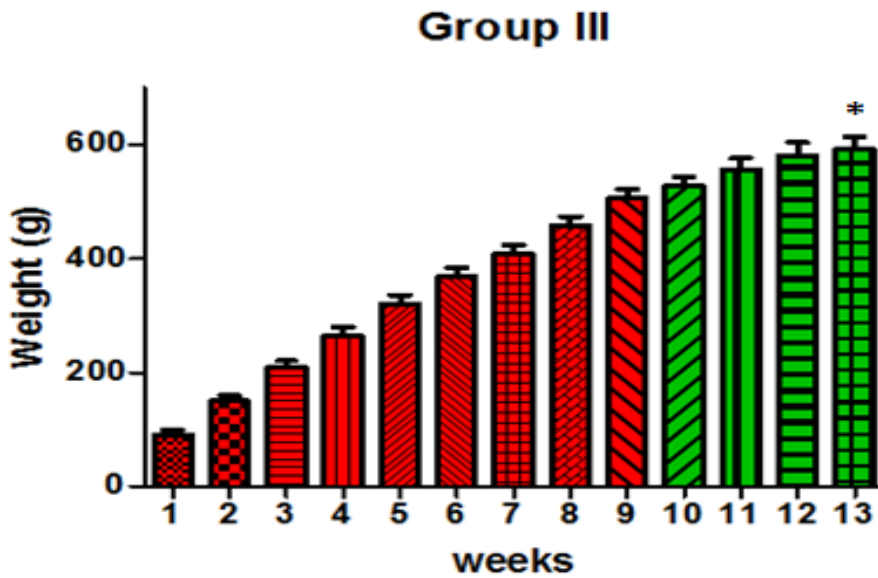


Figure 1 (c): Body weight of metformin treated animals; n=6.

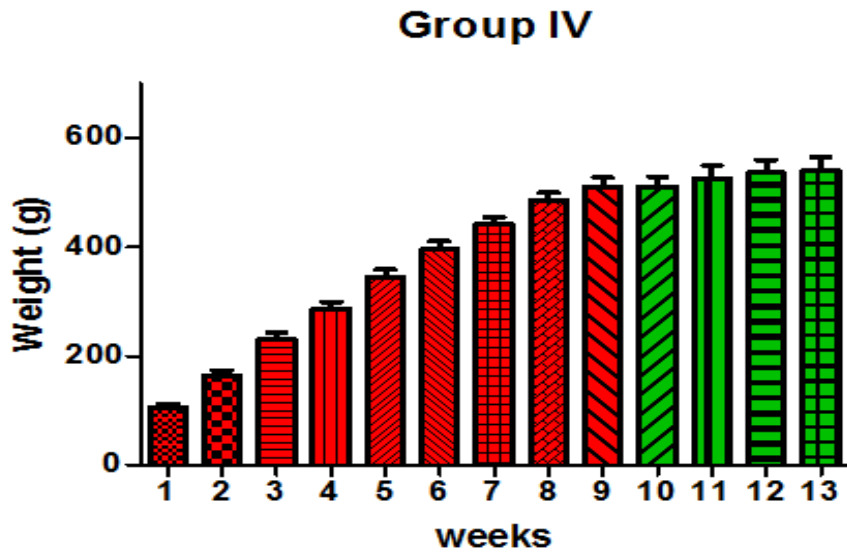


Figure 1 (d): Body weight of clonidine and metformin in combination treated animals; n=6. [ANOVA followed by dunnett's test was applied to compare 9th week of data for weight with remaining data of weight during treatment. There was non-significant result observed in all the groups except in group III at 13th week (p<0.05)].

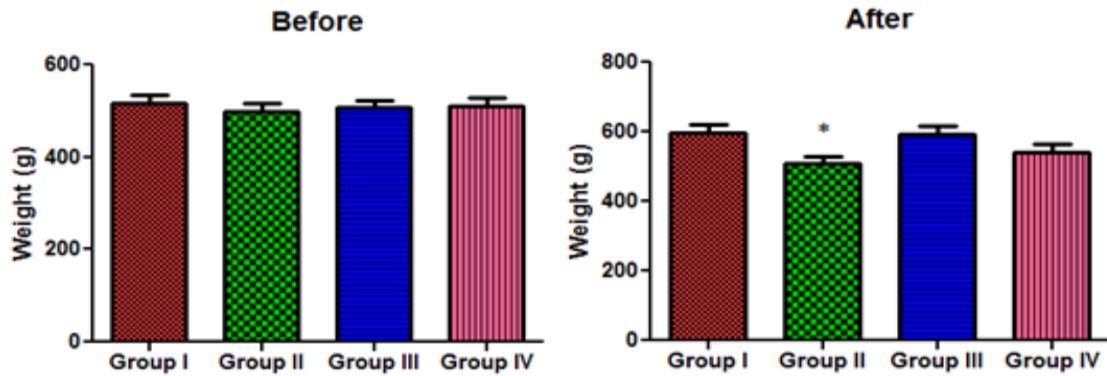


Figure 2: Body weights of animals before treatment (9th week) and after treatment (13th week); [ANOVA followed by dunnett's test shows that there is non-significant result in 9th week data when compare to control group while there is significant result in group II when compared to control group at 13th week of treatment. (*: P <0.05)].

Table 1: Body weight of animals (A) Before treatment (B) After treatment**(A) Before treatment**

| Weeks | Group I (g) | Group II (g) | Group III (g) | Group IV (g) |
|-----------------------|--------------------|---------------------|----------------------|---------------------|
| | Mean ± SEM | Mean ± SEM | Mean ± SEM | Mean ± SEM |
| 1st | 102.2 ± 13.42 | 88.75 ± 13.25 | 90.67 ± 7.93 | 106 ± 6.04 |
| 2nd | 152.4 ± 17.69 | 140.6 ± 15.98 | 149.7 ± 10.52 | 164.9 ± 8.37 |
| 3rd | 205.6 ± 17.47 | 201.8 ± 20.41 | 208.6 ± 11.54 | 231.3 ± 11.22 |
| 4th | 268.6 ± 20.92 | 245.3 ± 21.33 | 265.4 ± 14.05 | 287.1 ± 12.56 |
| 5th | 329.8 ± 23.46 | 301.9 ± 22.03 | 321 ± 14.59 | 344.3 ± 14.19 |
| 6th | 381.3 ± 24.76 | 355.9 ± 22.81 | 369.2 ± 15.25 | 395.8 ± 14.41 |
| 7th | 430.3 ± 23.24 | 404.4 ± 21.9 | 409.2 ± 14.85 | 442.3 ± 12.17 |
| 8th | 488.4 ± 27.09 | 445.3 ± 22.09 | 458.1 ± 15.79 | 485.8 ± 14.21 |
| 9th | 516.2 ± 19 | 498.5 ± 18.75 | 506.6 ± 15.26 | 511.2 ± 16.74 |

(B) During treatment

| Weeks | Group I (g) | Group II (g) | Group III (g) | Group IV (g) |
|------------------------|--------------------|---------------------|----------------------|---------------------|
| | Mean ± SEM | Mean ± SEM | Mean ± SEM | Mean ± SEM |
| 10th | 538.1 ± 20.38 | 494.2 ± 19.51 | 529.5 ± 15.31 | 511.2 ± 18.46 |
| 11th | 561.6 ± 21.44 | 495.3 ± 24.47 | 557.4 ± 19.63 | 526.2 ± 23.82 |
| 12th | 582.9 ± 22.45 | 506.3 ± 22.52 | 581.7 ± 22.37 | 536.6 ± 23.52 |
| 13th | 595.7 ± 24.09 | 507.7 ± 22.94 | 591.5 ± 23.51 | 540.8 ± 24.03 |

Table 2: Feed consumption (A) Before and (B) During treatment.**(A) Before treatment**

| Weeks | Group I | Group II | Group III | Group IV |
|-----------------------|-------------------|-------------------|-------------------|-------------------|
| | Mean ± SEM | Mean ± SEM | Mean ± SEM | Mean ± SEM |
| 1st | - | - | - | - |
| 2nd | 127.9 ± 12.35 | 116 ± 10.6 | 119.9 ± 8.55 | 133.3 ± 6.37 |
| 3rd | 151.8 ± 10.94 | 152.8 ± 12.1 | 147.9 ± 7.40 | 169.5 ± 8.09 |
| 4th | 171.7 ± 8.45 | 159.1 ± 8.25 | 164.2 ± 5.38 | 172 ± 7.89 |
| 5th | 181.8 ± 8.74 | 174 ± 8.59 | 172.1 ± 4.68 | 188.9 ± 7.88 |
| 6th | 190.2 ± 9.45 | 181.8 ± 8.33 | 175.8 ± 4.59 | 194 ± 5.95 |
| 7th | 189.2 ± 8.19 | 189.3 ± 5.62 | 182.9 ± 2.63 | 193.5 ± 4.15 |
| 8th | 205.2 ± 8.73 | 189.8 ± 6.23 | 188.2 ± 5.03 | 200.6 ± 4.25 |
| 9th | 192 ± 9.71 | 187.2 ± 4.39 | 183.4 ± 4.24 | 196.4 ± 3.79 |

(B) During treatment

| Weeks | Group I Mean ± SEM | Group II Mean ± SEM | Group III Mean ± SEM | Group IV Mean ± SEM |
|------------------|-----------------------|------------------------|-------------------------|------------------------|
| 10 th | 160.8 ± 7.93 | 120.9 ± 11.27 | 147 ± 6.77 | 136.8 ± 12.4 |
| 11 th | 160.2 ± 9.54 | 119.7 ± 12.72 | 157.1 ± 10.52 | 129.7 ± 9.9 |
| 12 th | 149.7 ± 10.45 | 125.1 ± 11.01 | 157.6 ± 11.85 | 131 ± 9.17 |
| 13 th | 149.6 ± 8.00 | 126 ± 11.34 | 147.1 ± 12.73 | 132.7 ± 8.18 |

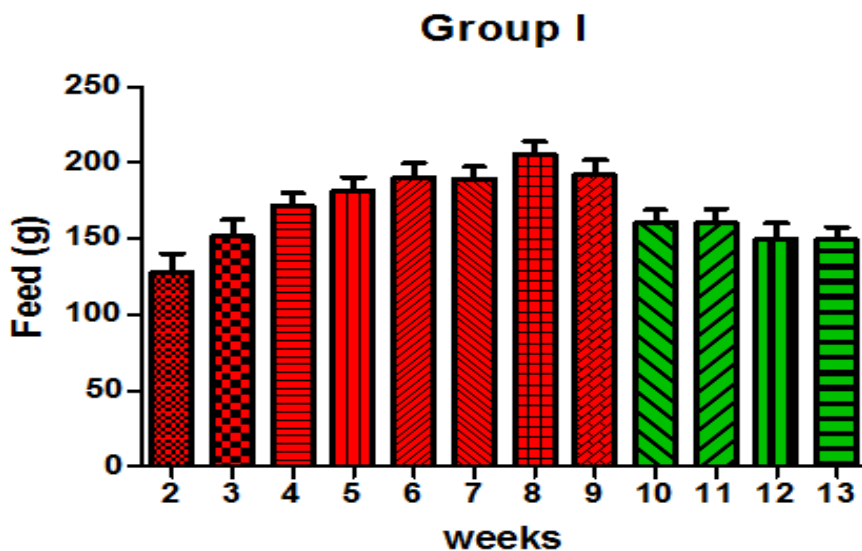


Figure 3 (a): Feed consumption of animals of control group; (n=6).

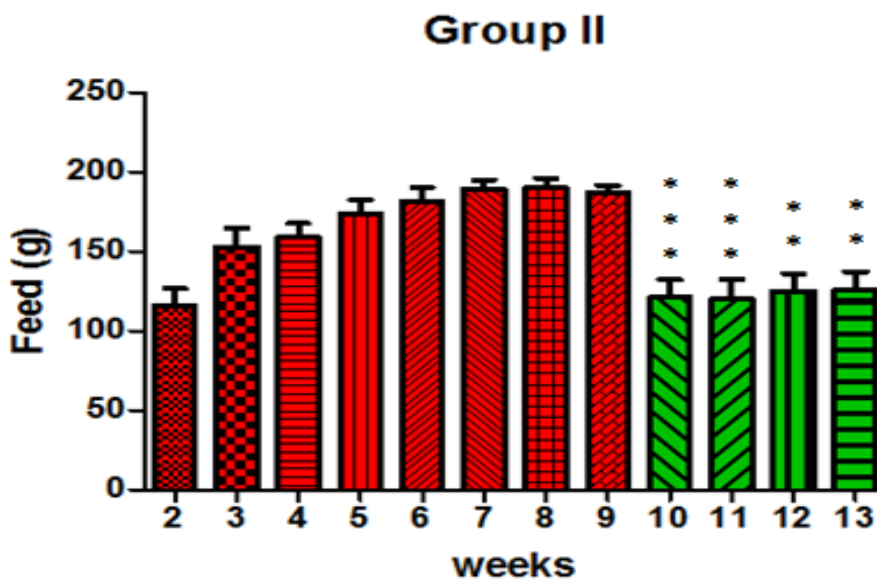


Figure 3 (b): Feed consumption of clonidine treated animals; (n=6).

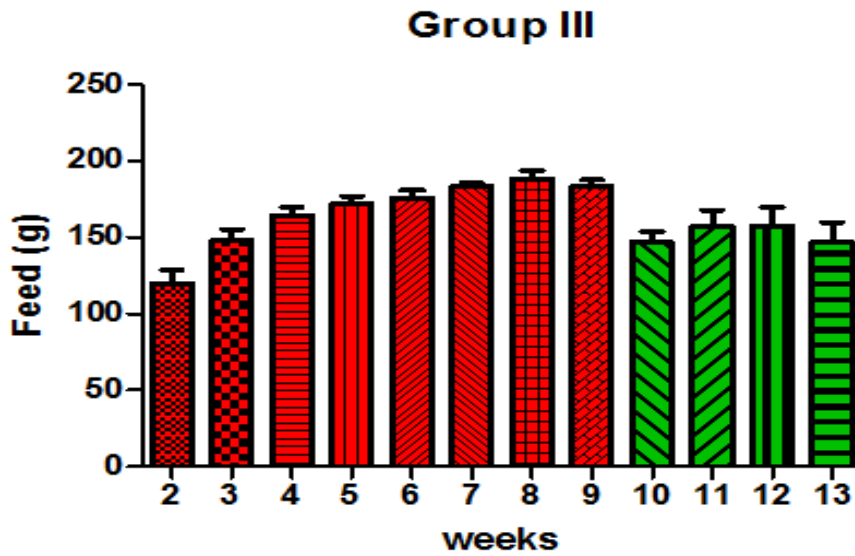


Figure 3 (c): Feed consumption of metformin treated animals; (n=6).

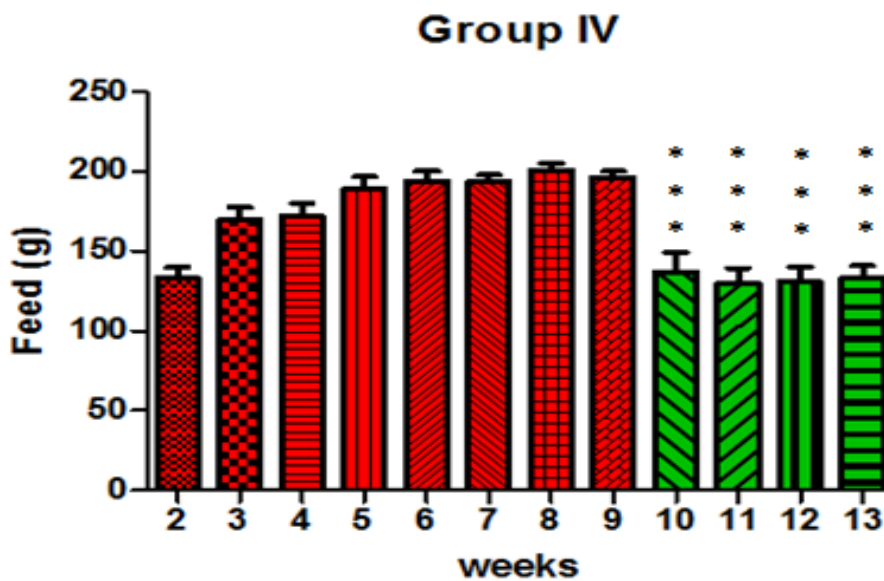


Figure 3 (d): Feed consumption of clonidine and metformin combination treated animals; (n=6).

[ANOVA followed by dunnett's test was applied to compare 9th week of feed consumption data with the feed consumption data during treatment. There was significant result observed in group II and group IV which is shown by * mark in figure. (***: $p < 0.001$, **: $p = 0.001$ to 0.01 , and *: $p < 0.05$).

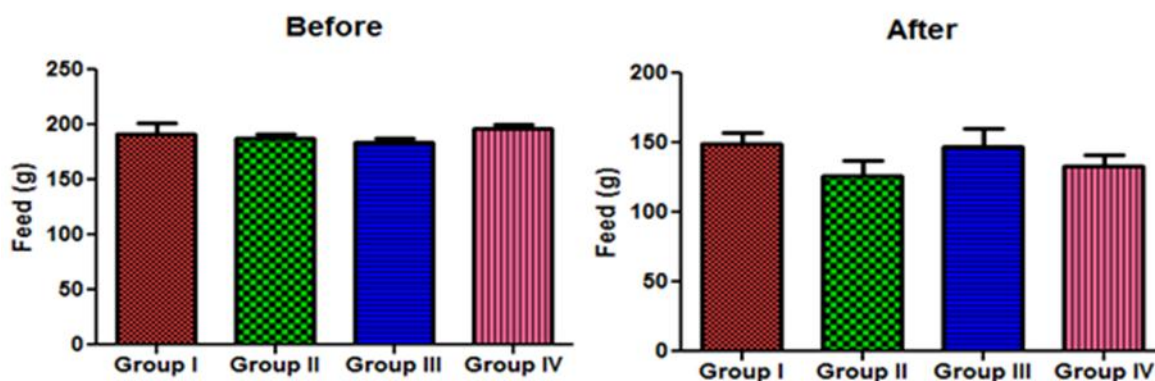


Figure 4: Comparison of feed consumption before treatment (9th week) and after treatment (13th week); [ANOVA followed by dunnett's test showed non-significant result in both before and after treatment when compared to control group].

Now when ANOVA followed by Dunnett's test is applied to compare body weight of animals on 9th week and remaining weeks of same treatment, There is significant ($p < 0.05$) result was observed in group III while remaining were non-significant which is shown in figure 1 (a), (b), (c) and (d).

There is non-significant result was observed when we compare body weight with control group just before starting treatment means at the 9th week of age which is shown in figure 2.

Table 1 (b) shows the weight of animals during treatment. This data suggest that there is reduction in weight gain in group II and group IV when compare to other groups during treatment. When ANOVA followed by dunnett's test was applied after treatment there is significant result ($p < 0.05$) was observed in group II compare to control group while remaining groups shows non-significant change which is shown in figure 2.

Feed consumption before and during treatment:

Table 2 shows the data for feed consumption weekly. Here in table 2 (a) feed consumption of animals before treatment is given. Data suggest that there is increase in feed consumption at the age of 5 week there after there is no drastic increase in feed consumption.

Table 2 (b) shows feed consumption during treatment. It shows that there is reduction in feed consumption in all the groups. Now to compare feed consumption before (9th week of age) and after treatment in particular group ANOVA followed by dunnett's test was applied. There is significant result was observed in group II and group IV while other groups shows non-significant result which is shown in figure 3 (a), (b), (c) and (d). When the feed consumption of 9th week and 13th week was compared to control group there is non-significant result was observed which is shown in figure 4.

DISCUSSION

Zucker fatty rats are normally obese in nature which is evident from 3 week of age. The data suggest that there is decrease in body weight gain during the treatment with clonidine and in combination of clonidine and metformin. If we consider weight gain of control group as 100 % during the treatment than clonidine group shows 10.45 % weight gain while metformin shows 96.5 % weight gain and in combination group it shows 33.63 % weight gain. This data shows that there is significant reduction in weight gain of the group treated with clonidine and combination of clonidine and metformin. So, the centrally acting drug clonidine affects the weight gain of the animals. Metformin group does not affect the weight of animals this group shows almost similar weight gaining behavior as control group.

Normally zucker fatty rats are hyper phagic in nature. Feed consumption data shows that there is change in feed taking behavior in animals treated with clonidine as well as in combination. Data shows that there is significant alteration in feed consumption in clonidine group and in combination group. The group treated with metformin does not show significant changes in feed consumption. So, the centrally acting drug Clonidine affects the feed consuming behavior of animals. It shows hypophagic effect in single as well as in combination with metformin.

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