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Effect of Fixed Orthodontic Appliances and Filgrastim on Body Weight: A Murine Model

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Abstract

Aim: The aim was to assess how orthodontic treatment and Filgrastim injections affect body weight in Wistar albino rats during and after treatment.**Materials and Methods:** Orthodontic tooth movement (OTM) was induced using the appliances, followed by a 14-day healing period after their removal. This study adopted a "split-mouth design," with OTM occurring exclusively on the left side of the mouths of 55 male Wistar albino rats, divided into five groups: A (no Filgrastim during active OTM, n = 12), B (Filgrastim during active OTM, n = 11), C (no Filgrastim during active OTM and healing, n = 11), D (Filgrastim during active OTM and healing, n = 11), and E (no Filgrastim during active OTM and healing, n = 10). Filgrastim (10 µg/kg) was administered intraperitoneally three times weekly throughout the trial. A force of 100 grams was applied mesially to the first molar using a fixed orthodontic device.**Results**: Rats exhibited significant body weight reduction ($p < 0.05$) after the first week, followed by gradual, slight weight recovery post-appliance removal. However, their final weight did not return to baseline.**Conclusion**: Orthodontic intervention might reduce the rat body weight, with Filgrastim showing minimal to no effect.

Keywords: Filgrastim, Fixed orthodontic appliance, Weight, Rat.

Introduction

Orthodontic treatments to straighten teeth can benefit patients suffering from malocclusion. Similar dental aesthetic treatments aid in concealing unattractive occlusal contours. During orthodontic operations, it might be difficult to see the interior condition of the alveolar bone. Previous studies have indicated that orthodontic methods promote permanent root resorption that will initiate root shortening, and eventually lead to an increase in the crown-root ratio and cause the teeth to become loose. There is a correlation between orthodontic therapy and bone resorption and tooth root loss. **(Bhardwaj et al., 2016, Eissa et al., 2018, Hyder and Shahzeb 2019).**

In vertebrates, the granulocyte colony-stimulating factor (G-CSF) cytokine regulates the production of neutrophils. **(Panopoulos and Watowich 2008).**

Granulocyte colony-stimulating factor (G-CSF) is a glycoprotein that is employed in human therapies because it is a member of the Class-1 superfamily of cytokine receptors and can mobilise medullary hematopoietic stem cells in systemic circulation. Both vascular stem cells **(Minana, Carbonell-Uberos and Mirabet 2008)** and mesenchymal stem cells **(Levesque et al., 2007; Zhdanov et al., 2007 and Tatsumi et al., 2008)** play a role in the healing of skeletal tissue. Granulocyte colony-stimulating factor (G-CSF) is known to mobilise both these types of stem cells. For instance, five successive doses of G-CSF have been shown to significantly increase CD34+ progenitor cells in rats (Herrmann et al., 2018). Additionally, these cells can give rise to osteogenic and vascular lineages (Sidney et al., 2014; Yi et al., 2019 Casado et al., 2020). Filgrastim is the brand name for the recombinant human granulocyte colony stimulating factor (rhG-CSF). It also increases vascularization and innervation, promotes the differentiation of mesenchymal bone marrow stem cells, and acts as an anti-inflammatory agent. The latter results from the fact that it increases the number of circulating hematopoietic stem cells available to the brain, heart, and bone (Sidney et al., 2014; Yi et al., 2019; Casado et al., 2020; Machla et al., 2022). Mesial movements have been tested in several studies on living tissues with murine models since they can be easily stored for an extended period at an affordable cost and deliver large sample sizes (Rygh et al., 1986; King et al., 1991; Hussein and Saloom, 2020). Furthermore, most of the antibodies employed in cellular and molecular biology experiments are rodent-specific **(Ren et al., 2004)** and the histological preparation of murine materials is simpler than that of other animals **(Aljuboury and Al-Shawi, 2022).**

With regard to the use of an orthodontic appliance on an animal model and its effect on its weight, it was discovered that the weight of the animal decreased after it had been fitted with an orthodontic device and during orthodontic tooth movement (OTM) **(Al-Najar, 2017; Hussein and Saloom, 2020).**

There is a great demand to identify the impact of drug therapy on weight changes. The purpose of the current was to quantify weight changes among experimental rats due to prescribed drugs and/or in relation to orthodontic appliance. The findings of the current study will provide pivotal information to healthcare professionals to individualize treatment decisions. Moreover, these results will aid health authorities in formulating treatment

guidelines along with the management of drug therapy-related adverse events.

Materials And Methods

Preparing rhG-CSF recombinant human granulocyte colony-stimulating factor

The drug was prepared from Sidco Company, Egypt, at a concentration of 10 µg/kg in an intraperitoneal injection once every two days (Guang-Yin et al., 2016). The brand name is Filgrastim.

Preparation of murine model

According to the recommended criteria of the previous study, 55 Wistar male albino rats aged 10 to 12 weeks, weighing 150-300 gm, were included and housed in the animal housing at the Iraqi Centre of Cancer and Genetic Research of Al-Mustansiriya University. The rats were acclimatized for two weeks before the tests. Each rat was put in its cage and tagged with a unique number according to the stratification of samples. All animals were subjected to a continuous cycle of 12 hours of dark followed by 12 hours of light. Environmental conditions inside the dwelling unit were a temperature of 25°c and relative humidity of 30 to 50% (Hudson et al., 2012 Yadav et al., 2016). The animals had free access to water and were fed on a staple diet of laboratory pellets. Monitoring the weight and health of rats was carried out using a Camry® four-digit electronic price-computing scale (China) every week. Ethical approval was received from the Scientific Research and Ethical Committee at the College of Dentistry, University of Baghdad (Ref. 623. in 2022).

Trial design:

The total number of male Wistar albino rats for the experiment is 55, which are further divided into five groups: group A without Filgrastim injections during active OTM (n = 12), group B with Filgrastim injections from the first day of OTM actively present (n = 11), group C without Filgrastim injections during active OTM and healing phase (n = 11), group D with Filgrastim injections from the first day of OTM during both active OTM and healing phases of treatment (n = 11) and group E without Filgrastim injections during the active OTM and healing phase (n = 10).

The animals received intraperitoneal injections of a therapeutic dose of Filgrastim (10 micg/km) every two days throughout the trial period **(Nair and Jacob, 2016; Shen, Park and Song, 2016). (**Figure 1)

The animals in groups A and B were weighed once a week up to the day of scarification while the animals in groups C, D, and E were weighed after 28 days.

Before any procedures were done on the rats, the rats were put under general anaesthesia via an intramuscular injection of a combination of 40 to 75 mg/kg of body weight of ketamine and 5 to 12 mg/kg of body weight xylazine **(Plumb, 2018).**

Placement of orthodontic appliance

An altered orthodontic device, as suggested by prior research, was affixed with the incisors acting as the point of support. Simultaneously, a compressed coil spring exerted a force of 100 grams to displace the first molar in a mesial direction. The cervical surfaces of both the maxillary incisors were grooved using an

angled handpiece and an inverted cone bur.

A stainless steel ligature wire with a diameter of 0.009 inches is wrapped around the cervical region of the first maxillary molar and then positioned between the teeth in the interdental space. A 6-mm closed coil spring was affixed to the wire and its unfettered end was meticulously bent towards the buccal surface of the tooth to avoid mechanical disruption to the adjacent oral tissue and coil slippage. Subsequently, the wire was securely ligated to achieve optimal stabilization. A second little pre-made stainless-steel wire, with a diameter of 0.009 inches, was wrapped around the grooves in the incisors of the rats. This was done to provide mechanical support and accommodate the tapered shape of the incisors. The pressure gauge measured that the closed coil spring of the fixed orthodontic device applied a total force of 100 grams to move the maxillary first molar towards the front. A ligature wire was used to secure the opposite end of the closed coil spring. Following a 60-second exposure to a 37% ortho-phosphoric acid etching solution, the enamel was promptly rinsed with water. Following the etching and drying of the teeth using cotton rolls and an air bulb, a minimally cured filling composite material was implanted. The etched and dried teeth surfaces, along with the grooves created by the ligature wire, were covered with a thin layer of a partially cured bonding compound using a disposable brush. Prior to a 20-second light exposure, the labial and palatal wires were fully immersed in the filling material within the grooves. The filling was applied with a single-use spatula. Every week, the appliance was examined for any indications of loosening or harm, utilizing the incisors as anchors, resulting in the forward movement of the first molar in response to orthodontic stress. (**Figure 1).**

This in vivo experimental study on orthodontic force was performed on rats, where a heavy force of 100 gm was applied to the upper left first molar to move it mesially similar in previous study **(Krishnan and Davidovitch, 2006; Meikle, 2006; Gonzales et al., 2008; Alnajar and Al Groosh, 2020).** Using a nickel-titanium (NiTi) closed coil spring with an active length of 6 mm.

Sample grouping:

Group A: These rats served as the control group. As such, they were subjected to a heavy orthodontic force of 100g to the upper right first molar for 14 days, after which the appliance was removed and the animals were scarified on Day 14.

Group B: They were given a systemic intraperitoneal injection of 10 µg/kg of Filgrastim every second day for 14 days, after which, on the 14th day, the appliance was removed and the animals were sacrificed.

Group C: Their first molar on the left upper side received a heavy orthodontic force of 100 g for 14 days, whereupon, after removal of the appliance, the rats were given another 14 days to allow routine healing. The total trial period of this group was hence 28 days, and the animals were scarified on Day 28.

Group D: These rats received 10 µg/kg of Filgrastim systemically intraperitoneally every other day for 14 days. At the same time, they underwent active OTM followed by removal of the appliance and were allowed another 14 days for routine healing.

Therefore, the total trial period for this group was 28 days and the animals were scarified on Day 28.

All the rats were fed a soft standard diet after orthodontic appliance insertion to make it easier for them to swallow and to keep the orthodontic devices from loosening. At the end of the trial period, the rats in each treatment group were weighed before they were humanely slaughtered under general anaesthetic, after which the fixed orthodontic appliance was removed.

Statistical analyses

The IBM® Statistical Package for the Social Sciences (SPSS) was used to compute the mean and standard deviation for each group. The mean differences between 7, 14, and 28 days were analysed using an F-test while the variance in means of the five groups post- 28 days was assessed using a greenhouse test. Lastly, the Bonferroni test was used to examine the statistical significance between the means of the groups at each point in time, with $p \leq 0.05$ considered statistically significant.

Results

Data analysis

Statisticians use the Shapiro-Wilk test to determine whether data are normally distributed. This test assumes a normally distributed population as its null hypothesis. If the p value is greater than the planned alpha (α) level (0.05), then it will not be possible to reject the null hypothesis and the data originated from a normally distributed population as showen in **Table 1.**

Since the data were normally distributed for all the groups, the parametric tests could be used. The analysis of variance (ANOVA) test was used to determine whether the groups were different or not, while Duncan's multiple range test (DMRT) was employed to make a pairwise comparison between the groups.

Table 2 compares the average body weights of groups A, B, C, D, and E at different times. In all the groups, the average body weight was different on different days. In general, the average body weight decreased on Days 7, 14, and 28. This was illustrated by the least significant difference (LSD) of the ANOVA test, where all the p-values were significant, thus indicating that there was a significant difference between the groups. The pairwise comparisons between the groups could be seen from the DMRT. **Figure 2** show that there was a decrease in average body weight on Days 7, 14, and 28.

Effect of Fixed Orthodontic Appliances and Filgrastim on the Body Weight of the Experimental Rats

Tables 3 and 4 compare the average body weights of the rats in all the groups at different times. The LSD of the ANOVA test was used to check the differences between the groups. At different times, the average body weight was different in the various groups since all the p-values were significant. Also, the DMRT revealed the pairwise comparison between the groups. Group E had the highest weight, as denoted by the letter "a", while Group B had the lowest weight, as denoted by the letter "b". **Figure 3** illustrate that the average decrease in body weight on Days 7, 14, and 28.

Discussion

The present study chose to work with rats for biological and practical reasons. More specifically, they are low cost, readily possess most of the antibodies

required for cellular and molecular biology procedures, and changes in their tissues post-OTM are comparable to that of humans and appear more quickly **(Ren et al., 2004; Ibrahim, Gudhimella and Pandruvada, 2017).** Rats were chosen over mice as mice are too tiny to properly insert an orthodontic device.

The G-CSF cytokine was chosen as it is widely believed to regulate the generation of neutrophilic granulocytes **(Basu et al., 2002).** It is also highly effective at improving vascularisation and innervation as well as exhibits anti-inflammatory properties and increase the number of circulating hematopoietic stem cells that can be directed to the brain, heart, and bone **(Walasek et al., 2012; Czekanska et al., 2014 and Machla et al., 2022).** Furthermore, G-CSF injections significantly accelerate the healing of early femur fractures. As such, they can, potentially, be used in human therapeutic settings for osteotomy and fracture planning **(Moukoko et al., 2018).** This present study used filgrastim, which is the brand name for rhG-CSF, to lessen the severity of bone resorption and root resorption, both of which are unavoidable side effects of orthodontic therapy.

Furthermore, when a drug is used, its effects and side effects on the general state of the body should be explored to determine their potential influence on the outcome of overall intervention. As filgrastim has been found to negatively affect body weight (, it was routinely investigated in this present study.

As the data revealed the animals who were given filgrastim in the first week of the trial period lost significantly more weight than their counterparts, who were only given Filgrastim after their orthodontic appliances had been removed, during the healing phase in Group E. A gradual increase in body weight was observed as an adaptive response after day 7 until the end of the trial as illustrated in figure 1 and analysed in **Table 2.**

Therefore, orthodontic treatments significantly contribute to weight loss, particularly during the initial phase or first month of an intervention. This is due to the presence of a fixed orthodontic appliance in the oral cavity, which may impede eating and swallowing, and ultimately restrict the intake of food. The reduced masticatory ability and difficulty in chewing and swallowing hard food were particularly evident in the animals following the placement of a fixed appliance. Additionally, a soft food regimen in postoperative care could have additive weight-loss consequences with all the classes. This is also in comparison with the previous studies observed in (Sandeep et al., 2016; Hussein and Saloom, 2020; Khamees and Al-Groosh, 2023; Mahdi et al., 2023). G-CSF, being a glycoprotein, is utilized in human therapeutic applications since it can trigger the medullary hematopoietic stem cells and can circulate through the bloodstream. G-CSF was reported to promote skeletal tissue regeneration by mobilization of vascular stem cells (Minamino et al. 2005) and mesenchymal stem cells (Levesque et al. 2007; Zhdanov et al. 2007; Tatsumi et al. 2008). Results reported by Herrmann et al., 2018, indicated a substantial rise in CD34+ progenitor cells after five daily administrations of G-CSF and did not alter the total body weight. Similar results were reported by Sidney et al., 2014; Yi et al., 2019; and Casado et al., 2020. In addition, the above cells have shown the ability to differentiate into osteogenic and vasculogenic lineages. Filgrastim does not

affect apatite and metabolic activity, especially if ingested in the short term. This present study found that the weights remained steady or increased after removing the orthodontic appliance on Day 14 of the trial, as illustrated in Figure 2 and Table 3.

. However, the weight did not return to the original body weight at the end of the trial (table 4) and these findings are in consistent with the results of previous studies that revealed the effect of rh-GCSF on body weight had no effect **(Hussein and Saloom, 2020; Alnajar and Al Groosh, 2020; Khamees et al., 2023**). Investigations on the effects of rh-GCSF on both BMI and peripheral blood stem cells (PBSCs) yield a very similar result in that larger-sized donors have a more vigorous response to mobilization with CD34+ progenitor cells per kilogram of body weight (Bensinger et al., 2009 and Han et al., 2018). Since total blood volume is directly proportional to body mass, it is obvious then that bigger-sized donors will yield a greater volume of blood.

Therefore, even a small amount of blood from a larger donor will contain more PBSCs **(Minana, Carbonell-Uberos and Mirabet, 2008).**

Farhadfar et al., 2020 examined the average daily G-CSF dosage per kilogram of donor weight and found no significant variations across the BMI of the groups which is coincide with the finding of this study. They also discovered that donors who had a higher BMI may need less G-CSF per kilogram of body weight than the donors with a lower BMI. As in this study the frequent injection of drug had non-significant effect on body weight especially after removal of orthodontic appliance; the finding of this study also matches the result of the previous studies on filgrastim tested on human smilar to the findings of **Bellows et al., 2011; Blogowski et al., 2012 and Do Carmo et al., 2013 and Ziru et al., 2023.**

Recently, it has been proved that recombinant humen granulocyte colony-stimulating factor (filgrastem) is well established in regenerative medicine for its potential effect in mobilization of hematopoietic stem cells and mesenchymal stem cells to the defect site and consequently enhance healing.

In current study; the data revealed that fligrastem may be promised option for therapeutic treatment in orthodontic field with out significant effect on normal metabolic activity regarding the body weight. However, further studies are required to analyze the impact of different body weight ranges on the therapeutic effect of fligrastem.

Furthermore, as experimental rats have a high death rate and the study used permanent orthodontic devices, the effects of the intervention could only be observed over a short 28-day period. Therefore, further studies are required to better evaluate the long-term effects of Filgrastim on body weight in terms of gender, age, and level of activity.

Conclusion:

As such, this present study found that the significant cause of weight loss was related to orthodontic device while the frequent dose of the G-CSF drug with in limited duration may has no discernible impact on body weight. Nevertheless, the ability of this dosage technique to lessen discomfort and decrease toxicity warrants further investigation.

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Table 1: Testing the normality of data distribution

Groups	Time	Shapiro-Wilk	p-value
A	1_day	0.980	0.322
	7_days	0.926	0.218
	14_days	0.970	0.328
B	1_day	0.861	0.328
	7_days	0.944	0.238
	14_days	0.834	0.338
C	1_day	0.892	0.293
	7_days	0.936	0.344
	14_days	0.933	0.212
	28_days	0.854	0.223
D	1_day	0.841	0.934
	7_days	0.948	0.312
	14_days	0.881	0.145
	28_days	0.959	0.723
E	1_day	0.949	0.623
	7_days	0.938	0.723
	14_days	0.849	0.829
	28_days	0.885	0.059

Table 2. Comparison between body average weight for different time in diffrent groups (A, B, C, D, and E).

Group	**Time**				**ANOVA (LSD) F**	**P value**
	1_day (Mean±SD)	**7_days** (Mean±SD)	**14_days** (Mean±SD)	**28_days** (Mean±SD)		
A	267.75±31.88 **A**	238.33±44.12 **B**	222.33±43.68 **C**	____	3.562	0.002*
B	245.27±37.26 **a**	211.00±46.66 **B**	191.54±53.19 **C**	____	4.563	0.001*
C	277.18±61.65 **A**	250.81±61.52 **B**	248.81±57.44 **B**	245.90±57.52 **B**	3.263	0.023*
D	258.18±45.70 **A**	224.36±42.61 **B**	218.18±42.91 **C**	211.09±43.63 **D**	3.369	0.037*
E	313.70±70.06 **A**	263.40±51.10 **B**	262.30±51.27 **B**	248.80±35.82 **C**	3.169	0.039*

***Significant difference between groups p value ≤ 0.05**
.Different letters means significant difference between groups.
.Mean: Body average weight in grams.
.SD: Standard deviation
.ANOVA: Analysis of Variance
.LSD: Least squared different

Table 3. Comparison between body average weight for different groups for different times (1 day, 7 days, 14 days, and 28 days).

Time	Groups					ANOVA (LSD) F	P value
	A (Mean± SD)	**B** (Mean± SD)	**C** (Mean± SD)	**D** (Mean± SD)	**E** (Mean± SD)		
1_day	267.75± 31.88 **c**	245.27± 37.26 **e**	277.18 ±61.65 **b**	258.18± 45.70 **d**	313.70± 70.06**a**	3.562	0.002**
7_days	238.33± 44.12 **c**	211.00± 46.66 **e**	250.81 ±61.52 **b**	224.36± 42.61 **d**	263.40± 51.10**a**	4.563	0.001**
14_days	222.33± 43.68 **c**	191.54± 53.19 **e**	248.81 ±57.44 **b**	218.18± 42.91 **d**	262.30± 51.27 **a**	3.263	0.023*
28_days	____	____	245.90 ±57.52 **b**	211.09± 43.63**d**	248.80± 35.82 **a**	3.369	0.037*

***Significant difference between groups at (0.05 level)**

.Different letters means significant difference between groups.

.Mean: Body average weight

.SD: Standard deviation

.ANOVA: Analysis of Variance

.LSD: Least squared different

Table 4. Comparision between average body weight in different time interval for each group

Groups	**Time**	Mean ± S.D.	LSD	P-value
A	**1_day**	267.75±31.88**a**	3.562	0.002*
	7_days	238.33±44.12**b**		
	14_days	222.33±43.68**c**		
	28_days	____		
B	**1_day**	245.27±37.26 **a**	4.563	0.001*
	7_days	211.00±46.66**b**		
	14_days	191.54±53.19**c**		
	28_days	____		
C	**1_day**	277.18±61.65**a**	3.263	0.023*

	7_days	250.81±61.52**b**		
	14_days	248.81±57.44**b**		
	28_days	245.90±57.52**b**		
D	**1_day**	258.18±45.70**a**	3.369	0.037*
	7_days	224.36±42.61**b**		
	14_days	218.18±42.91**c**		
	28_days	211.09±43.63**d**		
E	**1_day**	313.70±70.06**a**	3.169	0.039*
	7_days	263.40±51.10**b**		
	14_days	262.30±51.27**b**		
	28_days	248.80±35.82**c**		

*Statistically significant difference p value < 0.05

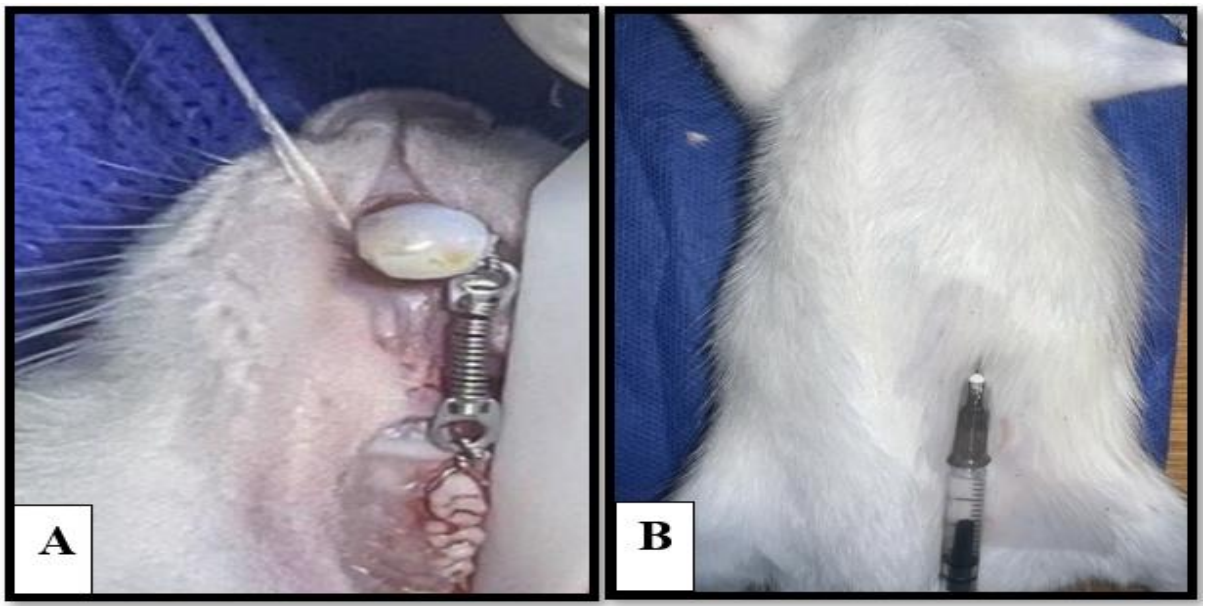

Figure 1: A. Orthodontic applianse bonding. B. Intraperitoneal injection of a therapeutic dose of Filgrastim

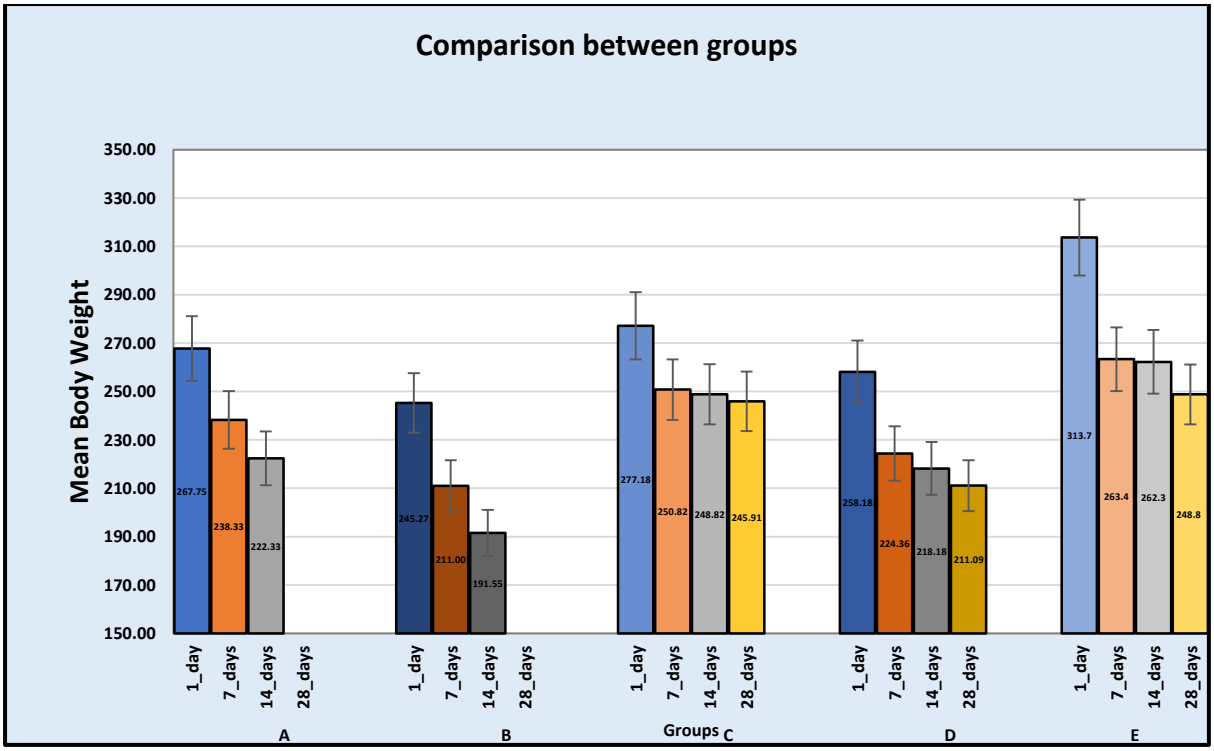

Figure 2. Comparison between body average weight for different time in diffrent groups (A, B, C, D, and E).

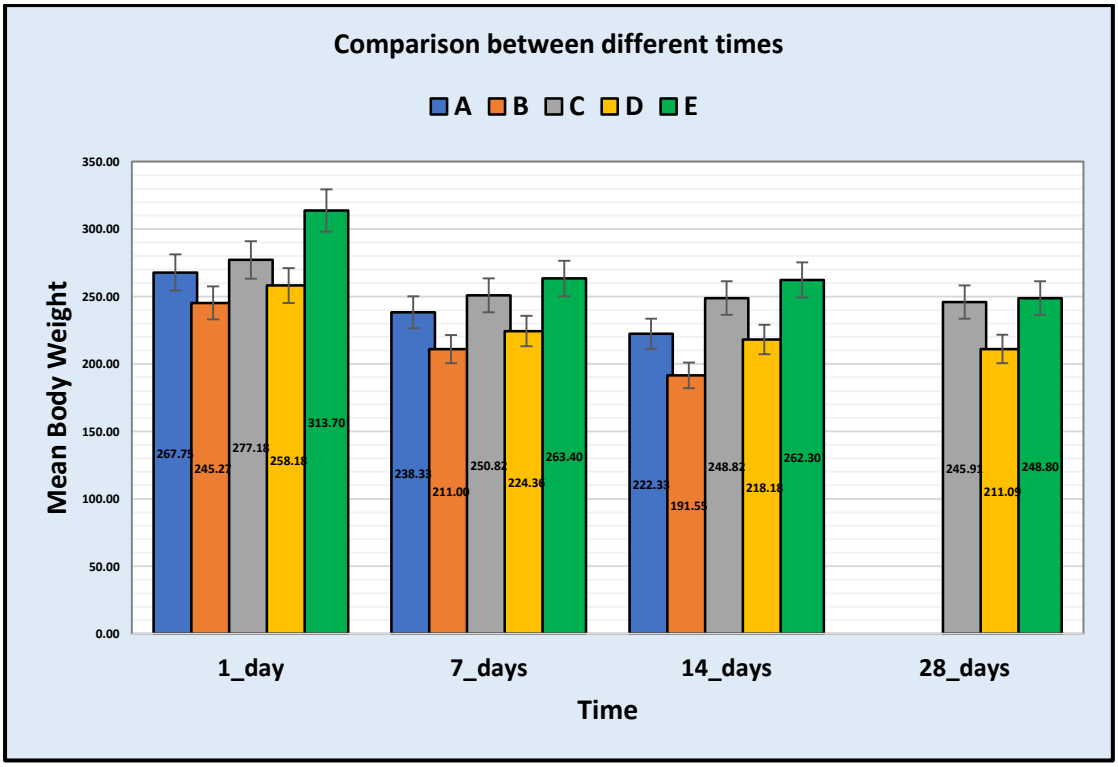

Figure 3. Comparison between average body weight for different groups in different times (1_day, 2_daya, 14-days, and 28_days).