Journal of Research in Medical and Dental Science
2020, Volume 8, Issue 2, Page No: 42-48

Available Online at: www.jrmds.in
eISSN No.2347-2367: pISSN No.2347-2545

Impact of Fixed Orthodontic Appliance with Diabetes Mellitus and Curcumin on the Body Weight of Experimental Rat

Zahra Kased Hussein*, Hayder Fadhil Saloom

Department of Orthodontic, College of Dentistry, University of Baghdad, Iraq

ABSTRACT

Background: This study aims to investigate the effect of diabetes mellitus, fixed orthodontic appliance and curcumin on the weight of experimental rats.
Materials and Methods: Orthodontic tooth movement (OTM) take place in one side of a 'Split- mouth design study in 40 male Wistar albino rats, divided into four groups: non-diabetic group (n=10), non-diabetic and curcumin group (n=10), induced diabetes group (n=10) and induced diabetes and curcumin group (n=10). Diabetes type1 was induced and insulin was given throughout the experiments for controlling high blood glucose levels. Fixed orthodontic appliance with a closing-coil spring delivering 30 gm of force was used to move the first molar mesially while the incisors serving as an anchor unit. The weight of the animals was measured once a week till the day of scarifying the animal after 3weeks.
Results: the results showed that all groups suffered from an intense weight loss during the third week and concluded that fixed orthodontic appliance, diabetes mellitus and curcumin can be considered as causative factors.
Conclusion: the study concluded that diabetes mellitus, orthodontic treatment and curcumin cause reduction of body weight in rats.

Key words: Curcumin, Rat, Fixed orthodontic appliance, Diabetes mellitus, Weight

HOW TO CITE THIS ARTICLE: Zahra Kased Hussein, Hayder Fadhil Saloom, Impact of Fixed Orthodontic Appliance with Diabetes Mellitus and Curcumin on the Body Weight of Experimental Rat, J Res Med Dent Sci, 2020, 8(2): 42-48

Corresponding author: Zahra Kased Hussein
e-mail ✉ : ali.mario28@yahoo.com
Received: 16/01/2020
Accepted: 02/04/2020

INTRODUCTION

Animals were used to develop a better understanding of animal and human anatomy, physiology, pathology and pharmacology. To be used as a model, animal species must meet specific criteria in line with the final goal of the research [1].

Although, rat teeth are very tiny (a rat molar is approximately 50 times smaller than a human molar) which complicates the design of an efficient orthodontic appliance that is suited to produce a constant and continuous force with an acceptable force range. Rats are one of the most popular animal models to study OTM, they are relatively inexpensive to allow the use of large samples and can be housed for a long period of time. Additionally, the histological preparation of rat material is easier than, for example, dog material and most antibodies required for cellular and molecular biological techniques are only available for mice and rats [2].

Diabetes mellitus (DM) is accompanied with increased glycogenolysis, lipolysis, gluconeogenesis and these biochemical activities result in muscles wasting and loss of tissue protein, thus reducing the body weight [3,4]. DM has been successfully induced in a variety of animal species; rabbits, mice, rats, monkeys, cats and dog [5,6]. Alloxan is one of the most common diabetogenic agents, it is used in experimental animals and was first reported by Dunn and McLetchie in their study in which they successfully induced diabetes in experimental rabbits [7].

Various injected or ingested materials can also effects on body weight of healthy and diabetic rats. Curcumin (diferuloylmethane), a natural compound and a novel therapeutic ingredient, is the most active polyphenolic ingredient responsible for the biologic activity of turmeric. The plant curcuma contains 60-70% carbohydrate, 6-8% protein, 5-10% fat, 2-7% fibre, 1-6% curcuminoids (50-70% curcumin) and up to 3-7% essential oils and resins [8-11].

Curcumin is effective at attenuating adipose tissue growth without effect on tissue proteins that can be lost due to DM. It significantly decreased body mass index (BMI), weight, waist circumference, and leptin, and significantly increased adiponectin levels. Moreover, bioavailable form of curcumin resulted in improved weight management in overweight subjects [12,13].

From the previous literatures, it is clear that weight loss in experimental animals is a multifactorial; however, to our knowledge no previous study investigated and compared

the effect of these factors on weight loss. The aim of this study is to assess the effect of orthodontic tooth movement using fixed appliance, DM and curcumin on body weight of experimental rat.

MATERIALS AND METHODS

Laboratory animals

Forty, 10-week old male Wistar albino rats weighing 250 to 350 gm were included in this study, blood glucose test was performed to ensure that rats are non-diabetic, the animals was kept in temperature controlled cages, exposed to 24 hours light-dark cycle of equal time, and had free access to water and food [14]. Rats were divided into 4 groups: Non-diabetic (non-DM; n= 10); Non-diabetic and curcumin treatment (non-DM+ CUR; n=10); Induced DM and insulin treatment (DM+INS; n=10); and Induced DM and curcumin/insulin treatment (DM+CURC +INS; n=10). All procedures on animals in this study were carried out under general anaesthesia using intramuscular injections of a mixture of ketamine (40-75 mg/kg body weight) and xylazine (5-12mg/kg body weight) [15].

Induction of experimental diabetes

The diabetic groups were injected with alloxan, before injecting the alloxan, the rats are rechecked to ensure they are undiebetic healthy animals. Then injecting alloxan as 150 mg/kg, after 2 hours of alloxan injection, blood glucose concentration was measured every 8 hours or at least twice a day for the first 2 days.

Dosage and administration of insulin

A stock solution of insulin diluted in distilled water was prepared according to blood glucose and average body weight of animal. When blood glucose of animal was 200 mg/dl, the insulin dose administered subcutaneously was 1 IU/kg [16]. Since the average body weight of animal in this study was 300gm, then the dose of injected insulin was 0.3 IU. When blood glucose was 201-250mg/dl then 0.45 IU of insulin was injected. The same dilution formula is implemented for various blood glucose readings. Blood glucose was measured twice daily, and weight of the animal was checked once a week. The injected dose of insulin was changed, and another stock solution was prepared if the weight of animal was altered.

Placement of orthodontic appliance

Firstly, each rat was inspected for a complete and intact set of teeth. The OTM movement has been done using a fixed orthodontic appliance [2]. Angled hand piece with an inverted-cone bur was used to make grooves cervically on the labial and distal surfaces of both maxillary incisors. Stainless steel ligature-wire with a diameter of 0.009" inserted interdentally between the 1st and 2nd maxillary molars which twisted around the cervical part of the 1st molar. It was ligated tightly to ensure maximum stabilization of the wire to which a closing-coil spring 8 mm in length was attached and the end of the wire was curved carefully toward the buccal surface of the tooth to avoid any mechanical disturbance to the surrounding oral tissues and the slippage of the coil. Another preformed short stainless steel ligature wire, with a diameter of 0.009", was twisted around the grooves that have been made on both incisors as a mechanical retention to compensate for the conical shape of the rats' incisors and subsequently inhibit the slippage of the wire as well as the appliance. The ligature wire ligated tightly to which the other end of the closing-coil spring was attached, so that the closing-coil spring of fixed orthodontic appliance delivers a total orthodontic force of 30 gm for mesial traction of maxillary 1st molar which was measured by pressure-gauge. Enamel was etched by etching solution (ortho-phosphoric acid 37%) for 60 seconds, then rinsed with a jet of water. Dryness was achieved with cotton rolls and air bulb for effective placement of light-cured filling composite material, a thin layer of light-cure bonding agent was applied to both etched and dried teeth surfaces and on the grooves with ligature wire, using disposable brush, followed by light-curing for 20 seconds. A light-cure composite filling material was applied using disposable spatula until the labial and palatal wires in the grooves and its end were completely embedded with the filling material, after which it was light cured for 20 seconds. The closing-coil spring was kept free from filling material at the distal end of the maxillary incisor. The appliance was checked weekly to ensure any loose or damage. Consequently, a medially directed orthodontic force to the maxillary first molar with the incisors served as anchored teeth resulted in mesial traction of the 1st molar and space conception between the 1st and 2nd molar teeth.

Curcumin administration

In order to improve the oral bioavailability and solubility of curcumin, a submicron emulsion called nano-emulsions (NE) was prepared [17-19]. Since the minimal effective dose of curcumin is 20mg/kg [20], the average weight of the animals had been considered in experiment is 300gm. According to (C1W1=C2W2) formula the needed concentration of the drug would be 6mg.

Nanoemulsion-curcumin (NE-Cur) formulation was prepared by dissolving 6mg of curcumin in a solvent mixture consisting of 200µl of polyethylene gycole 400 (PEG 400), 100 µl of propylene glycol, 50µl of ethanol and 10 µl of tween 80. The mixture was placed in a magnetic stirrer with heat. The volume of the solution after evaporation was around 0.3 ml. Gelatin solution of 10% weight/volume (W/V) was made by dissolving 0.01 mg of gelatin powder in 0.1ml of double-distilled water. Gelatine hydrogel is then added to curcumin solution and mixed thoroughly in a magnetic stirrer with heat of 80C for 10 minutes then it was sonicated carefully with a probe ultrasonic sonicator at room temperature for approximately 1 hour, an emulsion was formed as a fine dispersion. The material was injected by insulin syringe into the buccal vestibular mucosa next to the mesial root of the left first molar with a volume of 0.4 ml of curcumin-gelatine hydrogel. Each rat was fed a soft diet

during the study to prevent detaching orthodontic appliances and ease swallowing. After 21 days, all the rats were weighed and then sacrificed by inhalation chloroform in a saturated desiccator.

Statistical analysis

Statistical analyses were performed; means and standard deviations of the mean were calculated for each group. F-test for difference in mean changes after 1, 2, 3 weeks was performed. Tukey's HSD test was used for the difference in mean changes between the 3 study groups after 3 weeks. Bonferoni test was used for comparison between difference in mean of groups at different time points. A value of $P \leq 0.05$ was considered significant, $P<0.001$was considered high significant (HS) and $P>0.05$ is non-significant (NS).

RESULTS

Comparing the body weight among the four studied groups, F- test showed that there was no significant difference between the four tested groups during the first week (P=0.480), the body weight was reduced during the second week, but the reduction was not statistically significant (P=0.161). However, the body weight was significantly reduced in all groups at the third week (P=0.001) (Table 1). This significant difference was furtherly statistically analyzed using Tukey's HSD test, which showed significant difference in body weight between non-diabetic group and diabetic group; between non-diabetic group and diabetic/curcumin group; and between non-diabetic/curcumin group and diabetic/curcumin group (P=0.041, P=0.002 and P=0.004 respectively), (Table 2, Figure 1).

In comparison of body weight among the three weeks of study period, F-test showed significant difference in body weight among all groups (Table 3). Bonferroni test compare body weight at different time in each group, showed a non-significant difference (P=0.078) in body weight of non-diabetic/curcumin group during the 2nd and 3rd weeks; however, there was significant differences among the other groups (Table 4, Figure 2).

Table 1: Descriptive statistics and comparison of the weight of rats among different groups (G1=Non-diabetic, G2=Non-diabetic and curcumin, G3=Induced diabetes, and G4=Induced diabetes and curcumin) at each week.

Periods	Groups	Descriptive statistics					Groups difference (d.f.=39)	
		N	Mean	S.D.	Min.	Max.	F-test	p-value
1 Week	G1	10	300.9	29.426	266	350	0.842	0.480 (NS)
	G2	10	314.2	27.84	266	350		
	G3	10	301.6	33.364	252	350		
	G4	10	317.6	27.208	277	350		
2 Weeks	G1	10	293.5	29.587	260	345	1.822	0.161 (NS)
	G2	10	300.9	28.781	251	341		
	G3	10	276.7	36.703	229	340		
	G4	10	275.6	19.912	250	302		
3 Weeks	G1	10	282.5	30.7	250	339	7.32	0.001 (HS)
	G2	10	278.6	38.552	200	322		
	G3	10	242.1	36.562	198	310		
	G4	10	225.6	21.655	201	255		

Table 2: Multiple comparisons using Tukey's HSD test (G1=Non-diabetic, G2=Non-diabetic and curcumin, G3=Induced diabetes, and G4=Induced diabetes and curcumin).

Periods	Groups		Mean difference	p-value
3 weeks	G1	G2	3.9	0.993 (NS)
		G3	40.4	0.041 (S)
		G4	56.9	0.002 (HS)
	G2	G3	36.5	0.076 (NS)
		G4	53	0.004 (HS)

G3	G4	16.5	0.671 (NS)

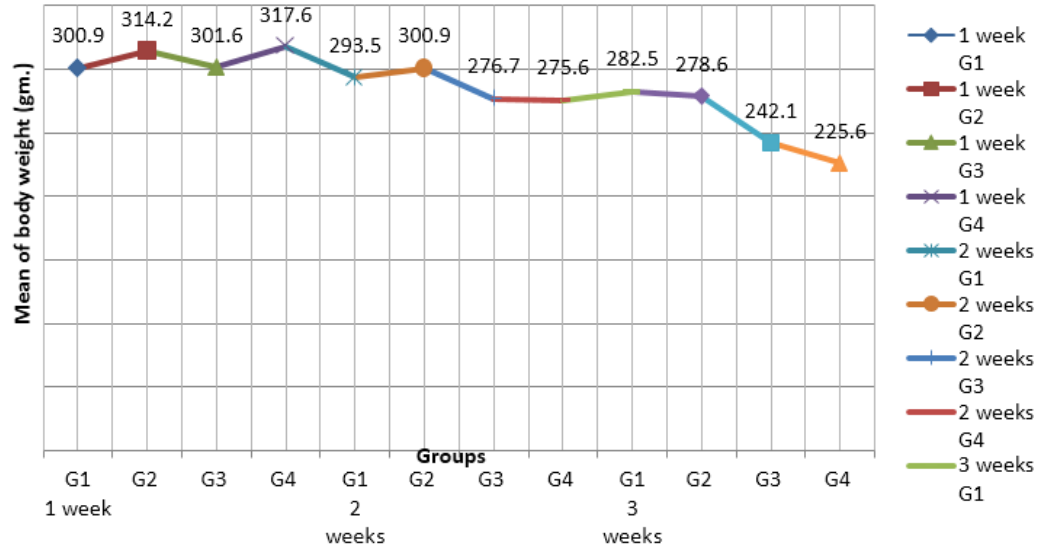

Figure 1: Linear chart showing the mean changes in animal body weight of the experimental groups (G1=Non-diabetic, G2=Non-diabetic and curcumin, G3=Induced diabetes, and G4= induced diabetes and curcumin) during the experimental period.

Table 3: Descriptive statistics and comparison of the weight of rats among different periods in each group (G1= Non-diabetic, G2= Non-diabetic and curcumin, G3=Induced diabetes, and G4=Induced diabetes and curcumin).

Groups	Periods	Descriptive statistics					Period difference (d.f.=29)	
		N	Mean	S.D.	Min.	Max.	F-test	p-value
G1	One week	10	300.9	29.426	266	350	21.032	0.000 (HS)
	Two weeks	10	293.5	29.587	260	345		
	Three weeks	10	282.5	30.7	250	339		
G2	One week	10	314.2	27.84	266	350	13.72	.004 (HS)
	Two weeks	10	300.9	28.781	251	341		
	Three weeks	10	278.6	38.552	200	322		
G3	One week	10	301.6	33.364	252	350	42.497	0.000 (HS)
	Two weeks	10	276.7	36.703	229	340		
	Three weeks	10	242.1	36.562	198	310		
G4	One week	10	317.6	27.208	277	350	81.243	0.000 (HS)
	Two weeks	10	275.6	19.912	250	302		
	Three weeks	10	225.6	21.655	201	255		

Table 4: Multiple comparisons using Bonferroni test (G1=Non-diabetic, G2=Non-diabetic and curcumin, G3=Induced diabetes, and G4=Induced diabetes and curcumin).

Groups	Periods		Mean difference	p-value
G1	One week	2 weeks	7.4	0.000 (HS)
		3 weeks	18.4	0.000 (HS)
	2 weeks	3 weeks	11	0.000 (HS)
G2	One week	2 weeks	13.3	0.000 (HS)
		3 weeks	35.6	0.006 (HS)

	2 weeks	3 weeks	22.3	0.078 (NS)
G3	One week	2 weeks	24.9	0.001 (HS)
		3 weeks	59.5	0.000 (HS)
	2 weeks	3 weeks	34.6	0.002 (HS)
G4	One week	2 weeks	42	0.000 (HS)
		3 weeks	92	0.000 (HS)
	2 weeks	3 weeks	50	0.000 (HS)

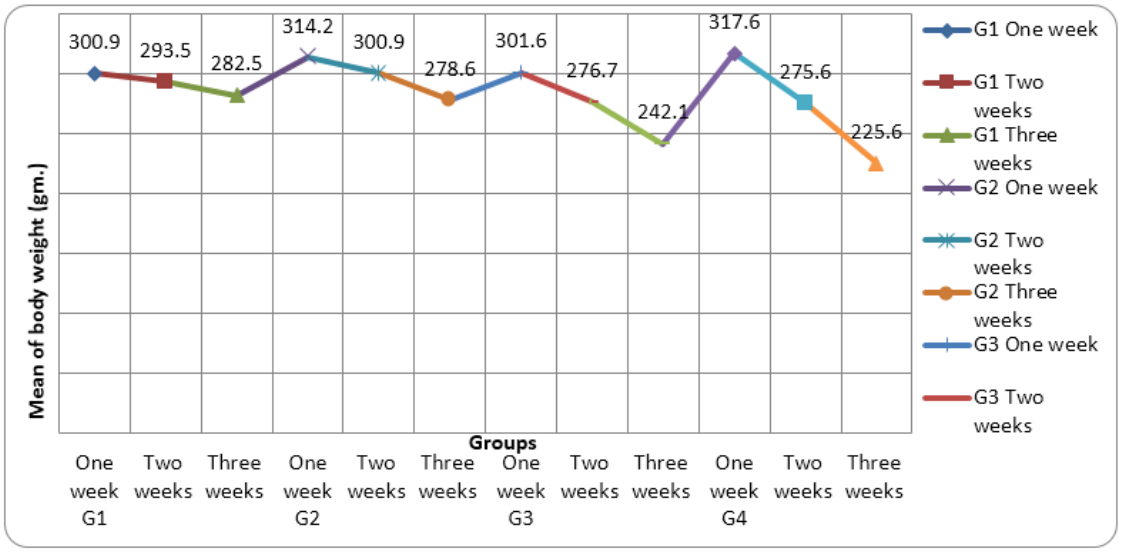

Figure 2: Descriptive statistics and comparison of the weight of rats among different periods in each group (G1=Non-diabetic, G2=Non-diabetic and curcumin, G3=Induced diabetes, and G4=Induced diabetes and curcumin).

DISCUSSION

Rats were used as animal model for several biological and practical advantages, for example, animal is relatively inexpensive, tissue changes incident to OTM are similar in rats and humans, however, these changes are faster in rats than in humans and most antibodies required for cellular and molecular biological techniques are available for rat and mice [2,21]. As mice are too small to place an effective orthodontic appliance; it is obvious that rats are the first choice in this field.

DM is an increasingly common metabolic disease affecting different age groups; therefore, a mandatory demand is required to perform a controlled orthodontic treatment protocol and to have knowledge on disease side effects. In this study, in order to induce DM in the experimental rats, alloxan was used. The mechanism of action of alloxan basically involves the production of reactive oxygen species in the β cells of the pancreas, resulting in partial degradation of β cells and subsequent compromise in the quality and quantity of insulin produced by these cells [16,22]. It causes a multiphasic blood glucose response when injected into experimental animals. Although alloxan is well known to cause hyperglycaemia, it was found that in the first 48 hours after its administration it can lead to marked hypoglycaemia, presumably due to release of preformed insulin from damaged β cells [16]. For this reason, we test blood glucose concentration every 8 hours or at least twice per day for the first 48 hour after alloxan administration and allow animals unlimited access to a tasty oral sugar-containing fluid; however, most of the injected animals experienced induced diabetic hyperglycaemia (blood glucose ≥ 200 mg/dl).

The results of this study had shown a non-significant weight loss during the first and second weeks; however, a noticeable weight loss had taken place during the experimental third week except for non-diabetic/ curcumin group. The reduction of body weight in diabetic groups is evident, this could be due to inability to metabolize carbohydrate preventing the body from getting glucose from the blood into the cells, with increase time insulin deficiency shifts energy sources to fatty acids and proteins. These findings come in line with the results of previous studies which reported that DM cause glycogenolysis and lipolysis resulting in loss of tissue fats and proteins, respectively [23]. Figlewicz, et al. demonstrated a suppression of sucrose intake in rats and suppression of dopamine regulation of appetite via insulin action at hypothalamus [24]. Additionally, Ewenighi, et al. found that Alloxan induced diabetes significantly decreases body weight of the diabetic untreated rat as the study duration increase compared with the diabetic treated and normal control rats [4].

Without doubt, orthodontic treatment is a significant cause of weight loss especially in the initial period (first month) of orthodontic treatment because of the presence of fixed orthodontic appliance in animal mouth that could interfere with eating and swallowing resulting in limited amount of food intake, since chewing and swallowing hard food can be difficult and the masticatory ability is reduced after insertion of fixed appliance. Moreover, changing the type of diet to soft food only may have synergistic on weight loss in all studied groups, this is in agree with previous study of [25].

Curcumin, on the other hand is effective at attenuating adipose tissue growth without effect on tissue proteins that can be lost due to DM. It significantly decreased BMI, weight, waist circumference, and leptin, and significantly increased adiponectin levels, hence, curcumin intake among patients with metabolic syndrome cause consumption of body fat sources for energy and adding another factor of weight loss, this is marked in diabetic/ curcumin group which suffered from reduction in body weight in comparison with non-diabetic/curcumin group that had a non-significant weight loss during 2nd and 3rd weeks of experiment. These findings are in consistent with the results of previous studies that revealed the effect of curcumin on reduction of body weight [12,13].

The main limitation of the current study is poor control on animal life span in particular with the induction of DM and the presence of fixed orthodontic appliance; therefore, the duration of study was limited to 3 weeks. However, the remarkable reduction of body weight take place with time. In addition, body weight was inconsistent for all rats; thus, nearly individualized doses of insulin were needed toward the end of the study.

CONCLUSION

The study concluded that diabetes mellitus, orthodontic treatment and curcumin collectively or individually cause reduction of body weight in rats.

ACKNOWLEDGMENTS

Special gratitude to our colleagues at orthodontic department/ college of dentistry/ university of Baghdad Dr. Harra'a S. Al-Shaibany and Dr. Hadeel Adel for their practical involvement and assistance during this study, as well as, Dr. Mohammed Nahidh for performing the statistical analyses.

CONFLICT OF INTEREST

The authors declare no potential conflicts of interest with respect to the authorship and/or publication of this article.

REFERENCES

1. Anderesen LM, Winter FL. Animal models in biological and biomedical research: Experimental and ethical concerns. Anais Da Academia Brasileira de Ciências 2017; 91:1-14.
2. Ren YM, Maltha JC, Kuijpers-Jagtman AM. The rat as a model for orthodontic tooth movement: A critical review and a proposed solution. Eur J Orthodont 2004; 26:483-490.
3. Pournaghi P, Sadrkhanlou R, Hasanzadeh S, et al. An investigation on body weights, blood glucose levels and pituitary-gonadal axis hormones in diabetic and metformin-treated diabetic female rats. Veterinary Res Forum 2012; 3:79-84.
4. Ewenighi OC, Dimkpa U, Onyeanusi J, et al. Estimation of glucose level and body weight in alloxan induced diabetic rat treated with aqueous extract of garcinia kola see. Ulutas Med J 2015; 1:26-30.
5. Goldner MG, Gomori G. Studies on the mechanism of alloxan diabetes. Endocrinol 1944; 35:241-248.
6. Cruz AB, Amatuzio DS, Grande F, et al. Effect of intra-arterial insulin on tissue cholesterol and fatty acids in alloxan-diabetic dogs. Circulation Res 1961; 9:39-43.
7. Dunn JS, McLetchie NG. Experimental alloxan diabetes in the rat. Lancet 1943; 242:384-387.
8. Priyadarsini KI. The chemistry of curcumin: From extraction to therapeutic agent. Molecules 2014; 19:20091-20112.
9. Alok A. Curcumin pharmacological actions and its role in oral submucous fibrosis: A review. J Clin Diagn Res 2015; 9:1-3.
10. Rahimi RH, Nedaeinia R, Shamloo SA, et al. Novel delivery system for natural products: Nano-curcumin formulations. Avicenna J Phytomed 2016; 6:383-398.
11. Nelson KM, Dahlin JL, Bisson J, et al. The essential medicinal chemistry of curcumin. J Med Chem 2017; 60:1620-1637.
12. Di Pierro F, Bressan A, Ranaldi D, et al. Potential role of bioavailable curcumin in weight loss and omental adipose tissue decrease: Preliminary data of a randomized, controlled trial in overweight people with metabolic syndrome. Preliminary study. Eur Review Med Pharmacol Sci 2015; 19:4195-4202.
13. Akbari M, Lankarani BK, Tabrizi R, et al. The effects of curcumin on weight loss among patients with metabolic syndrome and related disorders: A systematic review and meta-analysis of randomized controlled trials. Frontiers Pharmacology 2019; 10:1-13.
14. Macedo CS, Capelletti SM, Mercadante MCS, et al. Role of metabolic control on diabetic nephorathy. Acta Cirurgica Brasileira 2002; 17:370-375.
15. Plumb CD. Plumb's veterinary drug handbook. 9th Edn Wiley-Blackwell 2018:656.
16. Ighodaro OM, Adeosun AM, Akinloye OA. Alloxan-induced diabetes, a common model for evaluating the glycemic-control potential of therapeutic compounds and plants extracts in experimental studies. Medicina 2017; 53:365-374.
17. Federiuk FI, Casey MH, Quinn JM, et al. Induction of type-1 diabetes mellitus in laboratory rats by use of

alloxan: Route of administration, pitfalls, and insulin treatment. Comparative Med 2004; 54:252-257.

18. Onoue S, Takahashi H, Kawabata Y, et al. Formulation design and photochemical studies on nanocrystal solid dispersion of curcumin with improved oral bioavailability. J Pharma Sci 2010; 99:1871-1881.
19. Asefi S, Seifi M, Fard HG, et al. Innovative evaluation of local injective gel of curcumin on the orthodontic tooth movement in rats. Dent Res J 2018; 15:40-49.
20. Mach CM, Mathew L, Mosley SA, et al. Determination of minimum effective dose and optimal dosing schedule for liposomal curcumin in a xenograft human pancreatic cancer model. Anticancer Res 2009; 29:1895-1899.
21. Ibrahim AY, Gudhimella S, Pandruvada NS, et al. Resolving differences between animal models for expedited orthodontic tooth movement. Orthodont Craniofacial Res 2017; 20:72–76.
22. Lenzen S. The mechanisms of alloxan- and streptozotocin-induced diabetes. Diabetologia 2008; 51:216-226.
23. Warne JP, Horneman HF, Wick EC, et al. Comparison of superior mesenteric versus jugular venous infusions of insulin in streptozotocin-diabetic rats on the choice of caloric intake, body weight and fat stores. Endocrinol 2005; 147:5443-5451.
24. Figlewicz DP, Benoit SC. Insulin, leptin, and food reward: Update 2008. Am J Physiol Regulatory Integrative Comparative Physiol 2009; 296:9-19.
25. Sandeep KS, Singaraju GS, Reddy VK, et al. Evaluation of body weight, body mass index, and body fat percentage changes in early stages of fixed orthodontic therapy. J Int Society Preventive Community Dent 2016; 6:349.