Anti-Obesity, Fat Lowering and Liver Steatosis Protective Effects of Ferula asafoetida Gum in Type 2 Diabetic Rats: Possible Involvement of Leptin

Hossain Azizian[1], Mohammad Ebrahim Rezvani[2 *], Mansour Esmaeilidehaj[2], Seyyed Majid Bagheri[1]

1. Msc of physiology, Department of physiology, school of medicine, Shahid Sadoughi University of medical sciences, Yazd, Iran
2. Assistant professor of physiology, Department of physiology, school of medicine, Shahid Sadoughi University of medical sciences, Yazd, Iran

*Correspondence:
Mohammad Ebrahim Rezvani, Assistant professor of physiology, School of medicine, Shahid Sadoughi University of medical sciences, Yazd, Iran
Tel: + (98)9131566295
Email: erezvani@yahoo.com

Received: 4 July 2012
Accepted: 10 September 2012

Abstract

Objective: Control of weight gain is an important strategy in reducing the diabetes incidence. Recently, herbal drugs have been used as a complementary and alternative medicinal care. This study was conducted to determine the effect of Ferula asafoetida on weight gain, fat accumulation, liver steatosis and leptin level.

Materials and Methods: All rats of control and treatment groups received daily tap water (P.O) as vehicle mixed with fructose 10%. Two treatment groups received FAF oleo-gum resin at doses of 25 or 50 mg/kg (P.O). Normal rats received only tap water and standard chow food. Body weights, abdominal fat, size of epididymal adipocyte and serum leptin were recorded.

Result: Administration of Ferula asafoetida significantly decreased body weights, abdominal fat and size of epididymal adipocyte compared to untreated rats ($P<0.05$). Levels of serum leptin were significantly decreased in treated rats ($P<0.05$).

Conclusion: This study showed that Ferula asafoetida extract has anti-obesity, fat lowering effects and can prevent liver steatosis in type 2 diabetic rats. Reduction of serum leptin is associated with protective effects of Ferula asafoetida in obese diabetic rats.

Keywords: Ferula asafoetida, Diabetes mellitus, Obesity, Adipocyte, Liver, Rat

Introduction

Both overweight and obesity are health problems that increase mortality and morbidity rates. WHO recently reported that up to 2015, near 2.3 billion people will be overweighed and about 700 billion will be obese (1). Extensive researches are required to prevent this alarming increase in weight abnormality. The most consequences of the overweight and obesity are cardiovascular and endocrine diseases, especially type 2 diabetes (2, 3).

Control of weight gain is an important strategy in reducing diabetes incidence. Recently, herbal drugs have been used as a complementary and alternative medicinal care. Utility of anti-obesity remedies such as Ilex paraguariensis (4), galegine originally derived from galega officinalis (5), Allium cepa Linn.

(6), Gymnema sylvestre (7) and others that recently reviewed (8), reported in the litrature.
Ferula asafoetida (FAF) is native plant of Iran that grows up to the height of 2 meters. Its usage part is oleo-gum-resin that is acquired by incision of its root or stem (9-11). Among other compounds in the dried gum can be pointed to the following: coumarin derivatives, assafeotidnol A and B, sesquiterpene, arabinose, rhamnose and ferulic acid (12-14). Oleo-gum-resin of FAF has already been traditionally used in Asia for treatment of epilepsy, bronchitis, wood coughing, amenorrhea, and as a contraceptive remedy (15). Experimental and clinical studies also reported its anticarcinogenic, hypotensive, antiviral, antidiabetic, antioxidant, contraceptive, antifungal, antithepatotoxicity and anticoagulant properties (16). In spite of some studies reported usage of this herbal medicine for treatment of type 1 diabetes, few studies have been conducted to clarify its anti-obesity effect and underlying mechanisms. Additionally, in a pilot study, we noted a significant weight loss in diabetic rats treated with FAF oleo-gum-resin. Based on these evidences and given to extensive use of this remedy in Asia, we conducted this study to determine its anti-obesity effect and possible involvement of leptin.

Materials and Methods

Animals

Male Wistar rats, weighing 285-300 g were used in this study. Rats were maintained under standard conditions (12/12 light-dark cycle, 20-24°C, 55% humidity) without limitations of food and drink in animal house of Shahid Sadoughi University of Medical Sciences, Faulty of medicine, Iran. All efforts were considered to minimize the number of treated rats and their suffering. All experimental procedures and treatments were in accordance with the Shahid Sadoughi University guidelines for laboratory animal car and use.

Plant gum extract

Ferula asafoetida gum was collected from specimen number was kept in record (O 2343) at the Medicine Research Center of Shahid Sadoughi University of Medical Sciences, Yazd, Iran. The powdered dried gum (10 g) was soaked in distilled water (100 ml) at room temperature and filtered for daily use.

Diabetes induction and treatment protocol

All stages of this research were done in deptment of physiology, faculty of medicine, Shaid Sadoughi University of medical sciences as a MSc student thesis (thesis no. 82401). Twenty-one rats were randomly allocated in three groups. All groups were treated daily with fresh fructose 10% in drinking water, for eight weeks. Rats of control group received daily tape water (P.O.) as vehicle, and two treatment groups received FAF oleo-gum resin at doses of 25 or 50 mg/kg (P.O.). Treatment was started at the beginning of fructose feeding. Induction of diabetes was authenticated by fasting blood glucose (FBS) and intraperitoneal (I.P.) glucose tolerance tests (IPGT). Control rats were fasted overnight and injected 2 g/kg glucose (I.P.). After 30 minutes, blood sample obtained from tail vein and level of plasma glucose was measured. Plasma glucose levels greater than 140 mg/dl were considered as diabetes induction (17).

Measurements of body, abdominal fat and liver weights

Body weights were measured every day during the fructose 10% feeding. At the end of experiments, rats were weighted and deeply anesthetized with sodium thiopental (Nani pharmaceuticals Co, England). Liver and abdominal fat mass were exactly removed and weighted.

Histological assessment of epididymal and liver fat

Samples of epididymal fat and liver tissues were frozen at −70 ºC for histological analysis. Frozen tissues were sliced (50 mm) and fixed in 10% buffered formalin. All slices were embedded in paraffin. Then, the slices were again cut into small size at a thickness of 4 µm for staining. Hematoxylin-Eosin and Oil red O

staining methods were used. Adipocyte sizes were measured in randomly chosen microscopic areas from independent animals using a Zeiss microscope system, and average adipocyte size was determined using Photoshop software, Adobe Systems, Mountain View, CS5, CA and calculated as number of pixel per adipocyte cell in the scope. Also, adipocyte depots in liver tissue were determined (18).

Assessment of leptin level

Level of serum leptin was measured at the end of experiments. Serum leptin level of normal and treated rats were measured using the Rat Leptin RIA-Kit (DRG Instruments GmbH, Marburg, Germany). RIA assay of leptin level in serum was accorded to kit instruction.

Acute toxicity study

The rats were overnight fasted for FAF treatment. Extract was administered orally to FAF treated groups in ascending manner doses 50 and 100 mg/kg respectively. For the signs of toxicity, rats were monitored 4 times per day for 2 days. Additionally, the obtained data were the base and rationale for the doses that selected in this study.

Statistical Analysis

All data were presented as mean ± SEM. Data were analyzed using one-way ANOVA with GraphPad Prism (San Diego, CA). Post-tests were conducted with Dennett's test. $P<0.05$ were considered statistically significant.

Result

Diabetes induction

As shown in figure 1, control rats treated with fructose 10% for 8 weeks indicated significant increase in FBS ($P<0.05$) and IPGT ($P<0.01$). Thus, diabetes development was confirmed in this study.

Effects of FAF extract on body, abdominal fat and liver weights

Body weight was measured every day for 2 weeks. As appeared in fig. 2, FAF extract at doses 25 and 50 mg/kg decreased body weight ($P<0.01$), abdominal fat and liver weight

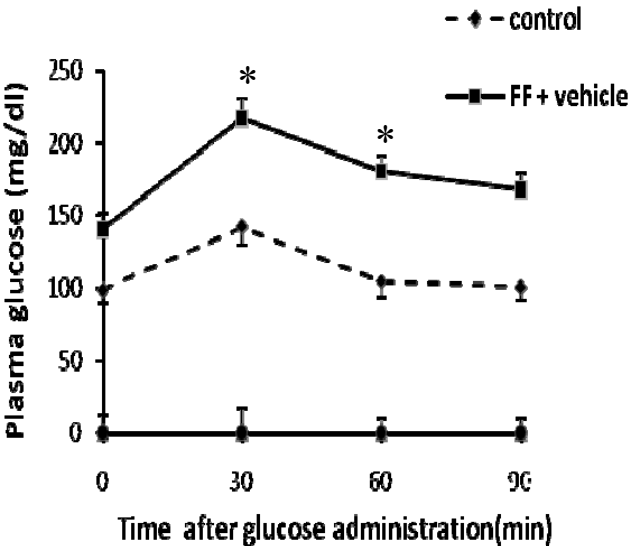

Figure 1: Chronic oral administration of fructose 10% induced intraperitoneal glucose intolerance compared to control group. Also, level of fasting blood glucose (at time 0 of glucose administration) in fructose fed (FF) rats was significantly higher than that in controls. Values are mean ± SEM, (eight animals per group). One-way repeated measure ANOVA followed by Duncan post test: *P < 0.05 is the significant levels.

($P<0.05$). Weight of control but not FAF-treated rats was increased during 2 weeks of fructose administration.

Effect of FAF extract on epididymal fat and liver histological changes

Liver fat density was studied using histological staining. Lipid dense droplet is an index of hepatic steatosis. FAF treated rats indicated low hepatic steatosis when compared to control group ($P<0.05$). Also, size of abdominal adipocytes in FAF treated group was significantly lesser than that of control fructose fed rats ($P<0.05$) (Fig. 3).

Effects of FAF extract on leptin level

FAF at doses 25 and 50 mg/kg reduced the serum level of leptin. The leptin levels of the FAF treated groups at both doses were significantly lower ($P<0.05$) compared to that of the control group (Fig. 4).

Acute toxicity of FAF extract

Forty-eight hours following administration of 50 and 100 mg/kg of FAF extract no sign of toxicity or incidence of death was observed. Hence, it was argued to be safe at these doses. Thus, we selected doses of 25 and 50 mg/kg because they are safe.

Discussion

The acute toxicity study revealed that the doses of drugs in this experiment are safe and did not cause toxic signs or behavioral deficits. Data of this study showed that FAF extract elicit significant anti-obesity, fat lowering and

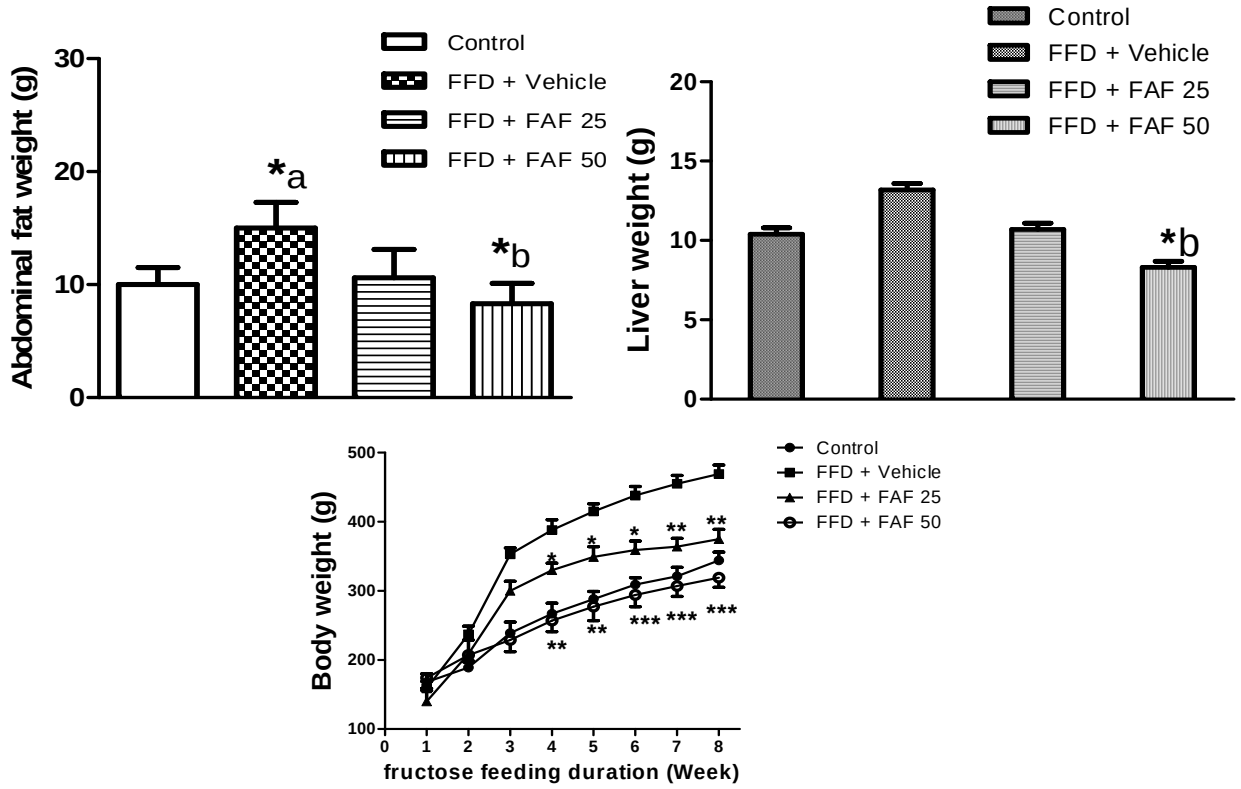

Figure 2: Effects of FAF gum at doses of 25 and 50mg/kg on body weight alterations in high fructose fed rats. Values are mean ± SEM, (eight animals per group). One-way ANOVA followed by Dennett's post test: *P<0.05 ** P<0.01 and ***P<0.001 are the significant levels. *a* and *b* represent when compared to control and FFD + Vehicle groups respectively. All data are presented as mean ± SEM, (n=8). FFD: fructose fed diet.

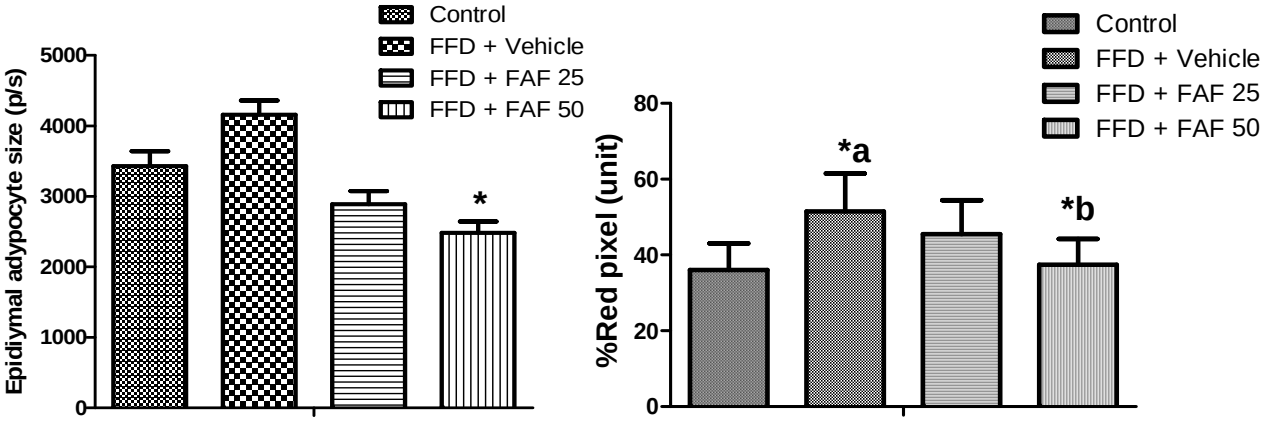

Figure 3: Effects of FAF gum extract on histological adipocyte size and liver fat depots in obese rats fed with fructose. Histological analysis showed that the adipocyte size changed less in the groups treated with FAF gum. Percent of fat depots were significantly accentuated in fructose fed rats and FAF gum at doses 25 and 50 mg/kg can reversed this elevating in density of depots. One-way ANOVA followed by Bonferroni post test were applied. *P < 0.05 is the significant levels. *a* and *b* represent when compared to control and FFD + Vehicle groups respectively. FFD: fructose fed diet. All data are presented as mean ± SEM, (n=3).

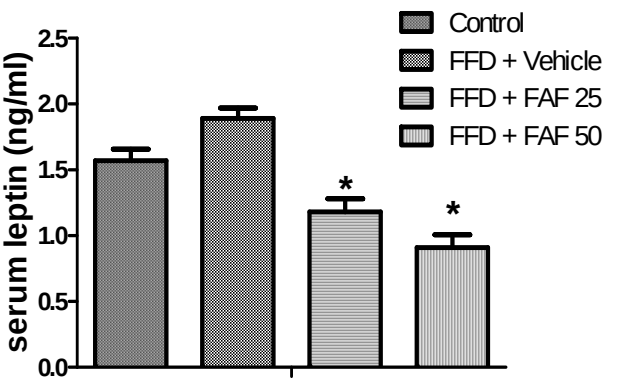

Figure 4: Effects of FAF gum Extract on the serum leptin level in fructose fed rats. This study indicate that FAF treatment can significantly reduce serum level of leptin probably through the increase in leptin sensitivity (reduce leptin resistance). ANOVA were done and followed by Dennett's post-test. *P<0.05 represents the significant levels when compared to FFD + Vehicle group (n=6).

liver steatosis protective effects in type 2 diabetic rats. Additionally, along with increase in body weight in type 2 diabetes, accumulation of fat in mesenteric and epididymal area was enhanced. Also, the level of serum leptin was associated with body weight and fat deposition; so that high level of serum leptin in fructose-fed rats that did not receive FAF treatment can be attributed to these factors. Fructose supplementation of food leads to positive energy balance and elevated nonestrified fatty acids in serum. Positive energy balance and hyperlipidemia lead to obesity and hepatosteatosis (23). However, increase in fat tissue of fructose fed rats induced high level of serum leptin. FAF inhibited the obesity and high fat tissue caused by fructose treatment in rats. Also, FAF reduced the level of leptin appropriate to its fat lowering effect. Hepatosteatosis was significantly ameliorated in obese type 2 diabetic rats treated with FAF at doses 25 and 50 mg/kg.

In animal studies, there are considerable evidences indicate that high fructose diet induces diabetes and obesity (19). Type 2 diabetes, obesity, decrease in insulin sensitivity and cardiovascular disease occur concurrently (20). Being obese is one of the main causes of diabetes and its complications. Despite the large data regarding control of obesity, it is hard to recommend protective pharmaceutical or nutraceutical regimens that have no complications especially in diabetic patients. Recently, use of traditional remedies such as Ilex paraguariensis (4), galega officinalis (5), Allium cepa Linn. (6), Gymnema sylvestre (7) and Corni fructus (8) for control of diabetes and obesity have been grown up. Anti-diabetic and hypoglycemic effects of FAF extract were observed in type 1 diabetes (21). The main objective of this study was to determine the role of FAF extract in weight control and decrease of fat accumulation in type 2 diabetic rats.

Leptin is a peptide that is produced mainly by fat tissue and rate of leptin production is depending on triglyceride content of adipocytes (22). Because leptin concurrently reduces food intake and body weight, elevation in leptin level with in obese subjects is attributed to the phenomenon named leptin resistance which leads to increased food intake and becoming more overweight and obese (23). Our data indicated that high fructose diet can elicit fat accumulation, obesity and increased level of leptin in diabetic rats. In our study, the high level of leptin in the obese diabetic rats may be related to leptin resistance. Indeed, the obesity induced by high fructose diet in diabetic rats activates cellular processes lead to leptin resistance (18).

Control of diabetes type 2, as a chronic disorder, needs to pharmacotherapy for long time periods. Indeed, metabolic diseases and diabetes are becoming more common as the population ages and there will be more need for drugs that reduce risk factors of the diseases without complications. Obesity is one of the risk factors for the disease and main goal of this study was reduction of the risk factors through weight management. FAF could decrease adipocyte proliferation in fat tissue such as abdominal area and reduce obesity. Previous studies revealed that FAF administration at dose of 50 mg/kg shown

anti-hyperglycemic but not anti-hyperlipidemic effects in streptozotocin-diabetic rats (24). Since antioxidative agents are involved in attenuating the diabetes symptoms, the anti-diabetic, anti-obesity and liver seatosis preventing effects may be partly mediated by the phenolic acids such as ferula, tannins and umbelliprenin that present in the ferula gum.

In conclusion, these results revealed that Ferula asafoetida gum has potent anti-obesity activities. Also, this traditional remedy protects diabetic rats from liver steatosis. Additionally, this study proposes a mechanism for the anti-obesity and liver protective effects of ferula through the leptin levels. Presently, ferula gum can be a good candidate for the treatment of type 2 diabetes-induced obesity and hepatosteatosis.

Acknowledgments

This work was supported by vice chancellor of research, Shahid sadoughi University of medical sciences, Yazd, Iran through a grant to Hossain Azizian (grant no. 82401), Msc student of physiology. We thank technicians of physiology and biochemistry laboratories for their assistance.

References

1. Rigby N, Leach R, Lobstein T, Huxley R, Kumanyika S. Epidemiology and social impact of obesity. Obesity: Science to Practice 2009;21-41.
2. Bray GA. Medical consequences of obesity. Journal of Clinical Endocrinology & Metabolism 2004;89(6):2583-9.
3. Wyatt SB, Winters KP, Dubbert PM. Overweight and obesity: prevalence, consequences, and causes of a growing public health problem. The American journal of the medical sciences 2006;331(4):166-74.
4. Kang YR, Lee HY, Kim JH, Moon DI, Seo MY, Park SH, et al. Anti-obesity and anti-diabetic effects of Yerba Mate (Ilex paraguariensis) in C57BL/6J mice fed a high-fat diet. Laboratory Animal Research 2012;28(1):23-9.
5. Mooney MH, Fogarty S, Stevenson C, Gallagher AM, Palit P, Hawley SA, et al. Mechanisms underlying the metabolic actions of galegine that contribute to weight loss in mice. British journal of pharmacology 2009;153(8):1669-77.
6. Yoshinari O, Shiojima Y, Igarashi K. Anti-Obesity Effects of Onion Extract in Zucker Diabetic Fatty Rats. Nutrients 2012;4(10):1518-26.
7. Preuss HG, Bagchi D, Bagchi M, Rao CVS, Dey DK, Satyanarayana S. Effects of a natural extract of hydroxycitric acid (HCASX) and a combination of HCASX plus niacin bound chromium and Gymnema sylvestre extract on weight loss. Diabetes, Obesity and Metabolism 2004;6(3):171-80.
8. Hasani-Ranjbar S, Nayebi N, Larijani B ,Abdollahi M. A systematic review of the efficacy and safety of herbal medicines used in the treatment of obesity. World journal of gastroenterology: WJG 2009;15(25):3073.
9. Fatehi M, Farifteh F, Fatehi-Hassanabad Z. Antispasmodic and hypotensive effects of Ferula asafetida gum extract. Journal of ethnopharmacology 2004;91(2):321-4.
10. Rajanikanth B, Ravindranath B, Shankaranarayana ML. Volatile polysulphides of asafoetida. Phytochemistry 1984;23(4):899-900.
11. Srinivasan K. Spices as influencers of body metabolism: an overview of three decades of research. Food Research International 2005;38(1):77-86.
12. Takeoka G. Volatile constituents of asafoetida: ACS Publications 1999; 2001:33-44.
13. Noleau I, Richard H, Peyroux AS. Volatile compounds in leek and asafoetida. Journal of Essential Oil Research 1991;3(4):241-56.
14. Anis M, Iqbal M. Medicinal plantlore of Aligarh, India. Pharmaceutical Biology 1994;32(1):59-64.
15. Pullaiah T. Medicinal Plants in Andhra Pradesh, India. Daya Books, 2002.
16. Iranshahy M, Iranshahi M. Traditional uses, phytochemistry and pharmacology of asafoetida (Ferula assa-foetida oleo-gum-resin)—A review. Journal of ethnopharmacology 2011;134(1):1-10.
17. Elliott SS, Keim NL, Stern JS, Teff K, Havel PJ. Fructose, weight gain, and the insulin resistance syndrome. The American journal of clinical nutrition 2002;76(5):911-22.
18. Xiong Y, Shen L, Liu KJ, Tso P ,Xiong Y, Wang G, et al. Antiobesity and antihyperglycemic effects of ginsenoside Rb1 in rats. Diabetes 2010;59(10):2505-12.
19. Kanarek RB, Orthen-Gambill N. Differential effects of sucrose, fructose and glucose on carbohydrate-induced obesity in rats .The Journal of nutrition 1982;112(8):1546.

20. Weyer C, Funahashi T, Tanaka S, Hotta K, Matsuzawa Y, Pratley RE, et al. Hypoadiponectinemia in obesity and type 2 diabetes: close association with insulin resistance and hyperinsulinemia. Journal of Clinical Endocrinology & Metabolism 2001;86(5):1930-5.
21. Bu-Zaiton AS. Anti-diabetic activity of Ferula assafoetida extract in normal and alloxan-induced diabetic rats. Pak J BiolSci 2010;13:97-100.
22. Myers MG Jr, Munzberg H, Leinninger GM, Leshan RL. The geometry of leptin action in the brain:more complicated than a simple ARC. Cell Metab 2009;9:117–123.
23. Myers Jr MG, Leibel RL, Seeley RJ, Schwartz MW. Obesity and leptin resistance: distinguishing cause from effect. Trends in Endocrinology & Metabolism 2010;21(11):643-51.
24. Iranshahi M, Alizadeh M. Antihyperglycemic Effect of Asafoetida (Ferula assafoetida Oleo-Gum-Resin) in Streptozotocin-induced Diabetic Rats. World Applied Sciences Journal 2012;17(2):157-62.