Bulletin of Environment, Pharmacology and Life Sciences
Bull. Env.Pharmacol. Life Sci., Vol 4 [6] May 2015: 127-132

Online ISSN 2277-1808
Journal's URL:http://www.bepls.com
CODEN: BEPLAD
Global Impact Factor 0.533
Universal Impact Factor 0.9804

ORIGINAL ARTICLE

Effect of Fabaceae(*Galegaofficinalis L.*) consumption on levels of blood glucose, lipids and Lipoproteins in Streptozotocin-induced diabetic rats

Mehrdad Pashazadeh[1*],Jafar Mirzazadeh[2],Ali Alizadeh[2]
[1.] Young Researchers and Elite Club, Ahar Branch, Islamic Azad University, Ahar, Iran.
[2.] Student of MS Biology, Ahar Branch, Islamic Azad University, Ahar, Iran
mehrdad_pashazadeh@yahoo.com

ABSTRACT
Diabetes is the most common endocrine disease which blood sugar and fat increases followed. Research has shown that some plant extracts have anti-diabetic and so people with diabetes to lower blood sugar, be used therefore, in this study it was decided to establish an experimental diabetic rats, the same situation occurs with type 1diabetes in rats. The aim of present study was to assay ethnopharmacological effects of Galegaofficinalis consumption on levels of blood glucose and lipids in streptozotocin -induced diabetic rats. 40 male Wistar rats, weighing 200±20 g and 9 to 10 weeks old, were obtained from the animal breeding center of Islamic Azad University. The rats were randomly divided into 4 equal groups of 10 animals including: 1- normal control, 2- normal rats treated with extract, 3- diabetic control, and 4- diabetics treated with extract. For induction of diabetes, after 15 h fasting, the rats were intraperitoneally injected with streptozotocin at a dose of 60 mg/kg body weight (bw), freshly dissolved in distilled water (5%). Animals with fasting blood glucose of 120 to 250 mg/dl were considered diabetic. Results showed a significant difference among animals of groups 3 and 4 with control group during 3rd week. Results showed that blood glucose level on weeks 3 and 6 in groups 3 and 4 was higher than control group significantly. Increased cholesterol level in group 3 was observed on weeks 3 and 6 compared with prior the study. A significant increase in serum triglycerides was observed on weeks 3 and 6 in group 3 compared with prior the study. Measurement of HDL has revealed that this parameter in rats of group 3 decreased significantly in compared with prior the study. Results showed that LDL levels were increased in rats of group 3 in compared with control group.
Keywords: *Galega officinalis, blood glucose, cholesterol, LDL, HDL, triglycerides, streptozotocin, rats.*

Received 02.04.2015 Revised 21.04.2015 Accepted 01.05. 2015

INTRODUCTION

Diabetes is the most Prevalent endocrine diseases that its characteristics are the glycerin Levels increase in blood (Hyperglycemia) and Carbohydrates, Lipid and protein metabolism disturbance. Diabetes mellitus is a metabolic disorder as old as mankind and its incidence (4 to 5%) is considered to be high all over the world [1, 2]. This endocrine disorder results from abnormal metabolism of carbohydrates, fats and proteins and causes the increase in blood glucose values. Hepatic and renal failure is the main cause of death in diabetic patients [3, 4]. This disease emerges in the effects of Insulin secretion, its operation disturbance or both of them. In the duration of disease involve all of body systems and organs. With notice to side effects and expensiveness of chemical drugs for reduce glycerin levels; access to compounds that reduce the side effects and reduce the levels of glycerin is essential. We must notice this disease because it has various effects and diabetes is increasing in the World [5, 6]. As Herbal drugs used for treatment of diseases from the past and used the served herbs and its extractions too and had miraculous patterns some of these herbs, we can see the increasing usage of these herbs for treatments [7]. Diabetes is induced in mice by using streptozotocin (STZ), a compound that has a preferential toxicity toward pancreatic β cells. We evaluated nude male mice from various sources for their sensitivity to a single high dose (160 to 240 mg/kg) of STZ. Diabetes was induced in male mice (age: median, 12 wk.interquartile range, 11 to 14 wk. body weight, about 30 g) from Taconic Farms (TAC), Jackson Laboratories (JAX), and Charles River Laboratories (CRL). Mice were monitored for 30 d for adverse side effects, blood glucose, and insulin requirements. In CRL mice given 240 mg/kg STZ, more than 95% developed diabetes within 4

to 5 d, and loss of body weight was relatively low (mean, 0.4 g). In comparison, both TAC and JAX mice were more sensitive to STZ, as evidenced by faster development of diabetes (even at a lower STZ dose), greater need for insulin after STZ, greater body weight loss (mean: TAC, 3.5 g; JAX, 3.7 g), and greater mortality. We recommend conducting exploratory safety assessments when selecting a nude mouse source, with the aim of limiting morbidity and mortality to less than 10%.Galegaofficinalis has been known since the middle Ages for relieving the symptoms of diabetes mellitus. Upon analysis, it turned out to contain compounds related to guanidine, a substance that decreases blood sugar by mechanisms including a decrease in insulin resistance, but was too toxic for human use. Georges Tanret identified an alkaloid from this plant, galegine, that was less toxic, and this was evaluated in unsuccessful clinical trials in patients with diabetes in the 1920s and 1930s.[9]. Other related compounds were being investigated clinically at this time, including biguanide derivatives. This work led ultimately to the discovery of metformin (Glucophage), currently recommended in international guidelines for diabetes management as the first choice for antidiabetic pharmacotherapy alongside diet and exercise [9] and the older agent phenformin, which has been withdrawn in most countries due to an unacceptable risk of lactic acidosis (the risk of lactic acidosis with metformin is no higher than with other antidiabetic therapies when it is prescribed according to its label)[5]. The study of galegine and related molecules in the first half of the 20th century is regarded as an important milestone in the development of oral antidiabetic pharmacotherapy[6].Galega officinalis (galega, Goat's Rue, French Lilac) is well known for its hypoglycaemic action and has been used as part of a plant mixture in the treatment of diabetes mellitus. During pharmacological investigations of an ethanolic extract of a powdered mixture of equal proportions of G. officinalis, Cressacretica, Mangiferaindica and Syzygiumjambolanum, a weight reducing effect of galega was discovered. In this study we have investigated the novel weight reducing effect of galega in mice. Galega herb (10% w/w in the diet) caused a significant reduction in body weight in both normal and genetically obese animals treated for 28 days when compared with respective controls ($P < 0.01$). In normal mice, the weight loss was reversible and initially associated with a transient reduction in food intake but was then maintained even in the presence of increased eating above the control level. Pair-fed normal mice receiving galega for seven days also showed significant weight loss ($P < 0.01$, compared with the control) in the presence of increasing food intake. In sharp contrast, weight loss in galega-treated mice was accompanied by a persistent reduction in food intake over the 28-day treatment period. Post-mortem examinations of all galega-treated mice revealed a striking absence of body fat. Serum glucose was significantly reduced in both strains of mice receiving galega for 28 days ($P < 0.01$), whereas serum insulin was significantly reduced only in obese mice ($P < 0.01$). In summary, together with its established hypoglycemic effects, galega has a novel weight reducing action that, in normal mice, is largely independent of a reduction in food intake. The mechanism of the weight reducing action of galega is unclear but involves loss of body fat[10]. The leaves and stems are very hairy with non-stinging hairs and also bear many stinging hairs (trichomes), whose tips come off when touched, transforming the hair into a needle that will inject several chemicals: acetylcholine, histamine, 5-HT (serotonin), moroidin leukotrienes and possibly formic acid [11]. This mixture of chemical compounds cause a painful sting or paresthesia from which the species derives its common name, as well as the colloquial names burn Fabaceae, burn weed, burn hazel. Fabaceae is an herb that has a long tradition of use as an adjuvant remedy in the treatment of arthritis in Germany. Fabaceae extract contains active compounds that reduce TNF-α and other inflammatory cytokines [12]. It has been demonstrated that Fabaceae lowers TNF-α levels by potently inhibiting the genetic transcription factor that activates TNF-α and IL-1B in the synovial tissue that lines the joint [13].Symptoms compared to placebo both by themselves and when combined with other herbal medicines [14].Because it contains 3, 4-divanillyltetrahydrofuran, certain extracts of the Fabaceae are used by bodybuilders in an effort to increase free testosterone by occupying sex-hormone binding globulin [15].A fraction from crude extract of Galegaofficinalis L. was purified by gel filtration on Sephadex G-25, Sepharose 4B, and ion-exchange chromatography on diethylaminoethyl (DEAE)-cellulose[9].The in vitro inhibiting and disaggregating effect on platelet aggregation of a gel-fractionated herbal extract from Galegaofficinalis L. is examined. The obtained Sephadex G-25 filtered fraction was 35-36 times more active than the crude extract. The threshold concentration at which this fraction inhibits platelet aggregation (5-10% inhibition) by 50 microM adenosine 5'-diphosphate (ADP) is 4.5-5 microg per 1 ml platelet-rich plasma (PRP). At a concentration of35 microg/ml PRP the fraction inhibits 50% of aggregation by ADP and at a concentration of 125 microg/ml PRP fully inhibits the aggregation of PRP by ADP. At a concentration of40 microg/ml PRP the fraction inhibits initiation of platelet aggregation by 0.18 mg/ml collagen and at 50 microg/ml PRP inhibits the initiation of aggregation by 0.7 units/ml thrombin. The G-25 filtered fraction shows a strong disaggregating effect on aggregated PRP. At a concentration of 65-75 microg/ml PRP, the fraction is able to disaggregate the 50-53% of aggregated platelet-rich plasma by 50 microM ADP, and 25% of aggregated PRP by 0.18 mg/ml

collagen(2)An agent thus used is known as a rubefacient (something that causes redness). This is done as a folk remedy for rheumatism, providing temporary relief from pain. The counter-irritant action to which this is often attributed can be preserved by the preparation of an alcoholic tincture which can be applied as part of a topical preparation, but not as an infusion, which drastically reduces the irritant action. The aim of present study was to assay ethnopharmacological effects of Galegaofficinalis consumption on levels of blood glucose and lipids in streptozotocin-induced diabetic rats.

MATERIALS AND METHODS

Experimental plan

This experimental study was carried out in Islamic Azad University Research Center. All procedures were conducted under supervision of Animal Rights Monitoring Committee of Islamic Azad University Research Center.

Tree preparation and maintenance

Fabaceae was collected from Azerbaijan Province in North of Iran, during April 2012. The plant was identified by Pharmacognosy Department of Islamic Azad University. Fresh roots were cut and their content extracted three times with ethanol. The extracted solutions were filtered and dried using a rotary evaporator under reduced pressure.

The ethanolic extract yields after vacuum evaporation was 10.6 g per 100 g of fresh root material. Dried extract was kept in the refrigerator at 4°C.

Chemicals

All chemicals used in this study were of analytical grade and obtained from Nanjing Jiancheng Bioengineering Institute, Nanjing, China and ZiestChemi Co., Iran.

Animals

40 male Wistar rats, weighing 200±20 g and 9 to 10 weeks old, were obtained from the animal breeding center of Islamic Azad University. The rats were randomly divided into 4 equal groups of 10 animals including: 1- normal control, 2- normal rats treated with extract, 3- diabetic control, and 4- diabetics treated with extract. Management and husbandry conditions were identical in all groups with 12/12 h light/dark cycle at 21±2°C. Food and water were provided *ad libitum*. At the beginning of study, prior to induction of diabetes, blood glucose was measured in all experimental rats after 12 h of fasting.

For induction of diabetes, after 15 h fasting, the rats were intraperitoneally injected with streptozotocin at a dose of 60 mg/kg body weight (bw), freshly dissolved in distilled water (5%). Animals with fasting blood glucose of 120 to 250 mg/dl were considered diabetic [16]. Blood glucose was estimated by commercially available glucose kit (ZiestChemi Co., Iran) based on glucose oxidase method. Thereafter, TREE (200 mg/kg in 10 ml/kg normal saline) was gavaged for eight consecutive weeks to groups 2 and 4 [17]. Simultaneously, groups 1 and 3 were gavaged with similar volume of normal saline solution.

At the end of the experiment, blood samples were collected from the retro-orbital plexus and the sera prepared through centrifuging at 2500 × g for 15 min at 30°C. After 12 h fasting, blood glucose and serum lipid profiles were measured using commercially available kits.

Statistical analysis

The statistical package for social sciences (SPSS Inc., Chicago, IL, USA), version 13.0, was used for statistical analysis. All data are presented as mean ± SEM. Before statistical analysis, all variables were checked for normality and homogeneity of variance by using the Kolmogorov-Smirnoff and Levene tests, respectively. The data obtained were tested by ANOVA followed by Tukey's post-hoc multiple comparison test. $P<0.05$ was considered statistically significant.

RESULTS

Results showed a significant difference among animals of groups 3 and 4 with control group during 3rd week ($P<0.05$). This weight loss also was significant on week 6 ($P<0.01$). On the other hands, the difference between groups 3 and 4 was not significant however, the weight average in group 4 was higher than group 3. Also, this evidence was observed among groups 1 and 2.

Results showed that blood glucose level on weeks 3 and 6 in groups 3 and 4 was higher than control group significantly ($P<0.001$) and group 2 showed no significant difference from control group. Also, treatment with Fabaceae in diabetic rats (group 4) made no significant blood glucose lowering effects compared with control group (table 1).

Increased cholesterol level in group 3 was observed on weeks 3 and 6 compared with prior the study ($P<0.05$, $P<0.01$). Also, total cholesterol level in group 4 on weeks 3 and 6 was significantly lower than group 3 ($P<0.05$). Also, treatment with extract yields to decrease in total cholesterol level in group 2 in compared with prior the study (figure 1).

A significant increase in serum triglycerides was observed on weeks 3 and 6 in group 3 compared with prior the study (P<0.05). On the other hands, the difference between groups 3 and 4 on weeks 3 and specially 6 was significant (P<0.05, P<0.01). Also, this change was not observed in group 2 (figure 2).
Measurement of HDL has revealed that this parameter in rats of group 3 decreased significantly in compared with prior the study (P<0.01) and treatment with extract resulted in significant increase in HDL in compared with group 3 (P<0.05, figure 3).
Results showed that LDL levels were increased in rats of group 3 in compared with control group (P<0.01) and treatment with extract decreased its levels (P<0.01, figure 4).

Table 1: effect of extract on blood glucose level (mg/dl) in animals

Time of the study / Groups	0	3	6
1 (control)	126.1±5.4	111.7±6.9	123.6±8.6
2 (control+extract)	107.5±8.2	123.4±5.2	92.5±1.4
3 (diabetics)	110.3±12.4	398.7±18.4	408.8±12.9
4 (diabetics+extract)	80.4±9.4	364.6±12.9	345.6±16.1

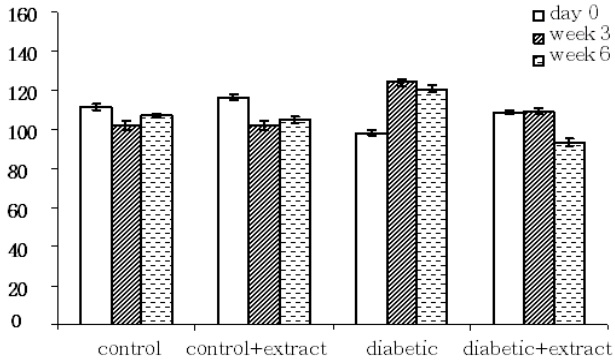

Figure 1: comparison of serum values of triglycerides in groups.

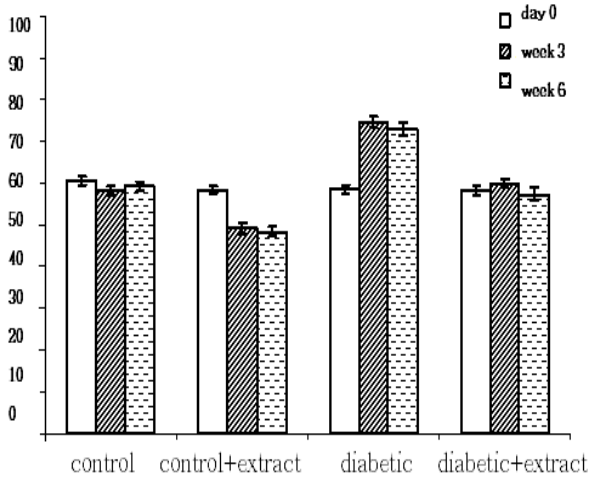

Figure 2: comparison of serum values of cholesterol in groups.

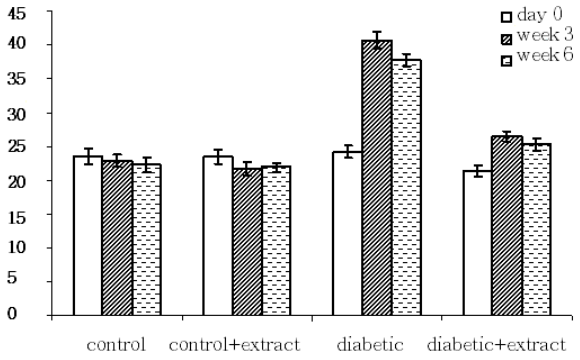

Figure 3: comparison of serum values of LDL in groups.

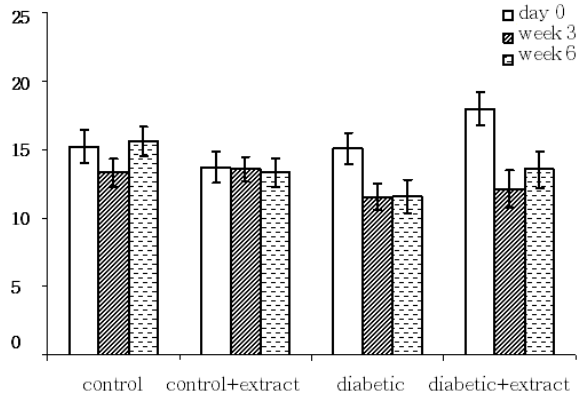

Figure 4: comparison of serum values of HDL in groups.

DISCUSSION AND CONCLUSION

Type 2diabetes is a multi-factorial disease, frequently associated with a cluster of pathologies including obesity, hypertriglyceridemia, impaired glucose tolerance, and insulin resistance. Fructose intake may be associated with increased risk of type 2 diabetes through several biological mechanisms [18]. A higher fructose intake may play a role in an increase in body weight due to the positive energy balance. Positive energy balance leads to obesity that is associated with a higher concentration of nonesterified fatty acids, which may reduce insulin sensitivity, increase hepatic glucose production, and have a deleterious effect on the beta cell function [8].

Galega officinalis has been used as antihypertensive, antihyperlipidemic and antidiabetic herbal medicine. In one study by Ahangarpour et al., [19] it has been shown that compared to control group, daily administration of fructose was associated with significant increase in FIRI, blood glucose and insulin, significant decrease in lepin, and no significant change in TG, HDL, LDL, LDL/HDL ratio, VLDL, ALT, and ALP. The extract significantly decreased serum glucose, insulin, LDL and leptin, and LDL/HDL ratio and FIRI. It also significantly increased serum TG, VLDL, and AST, but did not change serum ALP. They concluded that Galega officinalis extract, by decreasing serum glucose, and FIRI, may be useful to improve type 2 diabetes mellitus. Also, by positive effect on lipid profile and by decreasing effect on leptin, it may improve metabolic syndrome.

Bnouham et al.,[3] demonstrated that i.p. administration of the water extract (WE) of Ap and Ud (150 mg/kg) 30 minutes before the glucose overload (2 g/kg) showed a significant reduction glycemia, respectively of 36 % at 60 min ($p<0.05$) and 50 % at 180 min ($p<0.05$) after glucose overload compared with controls. In contrast, the effect of WE of Au and Th (150 mg/kg, i.p.) was not significant. The in vitro study of glucose utilization by isolated rat hemidiaphragm suggests that these extracts in combination with insulin potentiate its activity and enhance the utilization of glucose. They concluded that these plants possess antidiabetic activity. The structure and function of these glucose-conducting pores are discussed herein.Their study showed the protective administration of hydroalcholic extract of Galegaofficinalis has hypoglycemic effect and protective activity of beta-cells of Langerhans in hyperglycemic rats.

Cholesterol is one of the body fats and is an important building block in the structure of biological membranes, and used in the biosynthesis of steroid hormones, bile acids and vitamin D. Moreover, the

high cholesterol concentration in the blood increases the risk of developing atherosclerosis and related cardiovascular diseases [5,20]. Low-density lipoprotein takes the cholesterol from liver to tissues, whereas high-density lipoprotein facilitates the translocation of cholesterol from the peripheral tissues to liver for catabolism. Therefore, HDL has a useful effect in reducing serum cholesterol and the increase of its level in serum is suggested [21,22]. The LDL/HDL ratio is an important predictor of coronary heart disease risk. Therefore, this dose of extract had more efficacies to decrease liver damage.

REFERENCES

1. Phyllis A, James F. (2000) Prescription for Nutritional Healing, Avery Press; p. 104.
2. Safarinejad, MR. (2005) Galegaofficinalis for treatment of benign prostatic hyperplasia: A prospective, randomized, double-blind, placebo-controlled, crossover study. Journal of herbal pharmacotherapy; 5(4): 1–11.
3. Bnouham M, Merhfour FZ, Ziyyat A, Aziz M, Legssyer A, Mekhfi H. (2010)Antidiabetic effect of some medicinal plants of Oriental Morocco in neonatal non-insulin-dependent diabetes mellitus rats. Hum ExpToxicol; 29(10):865-71.
4. Obertreis B, Ruttkowski T, Teucher T, Behnke B, Schmitz H.(1996) Ex-vivo in-vitro inhibition of lipopolysaccharide stimulated tumor necrosis factor-alpha and interleukin-1 beta secretion in human whole blood by extractumurticaedioicaefoliorum. Arzneimittelforschung; Apr;46(4):389-94.
5. Avci G, Kupeli E, Eryavuz A, Yesilada E, Kucukkurt I. (2006)Antihypercholesterolaemic and antioxidant activity assessment of some plants used as remedy in Turkish folk medicine. J Ethnopharmacol; 107:418-423.
6. Kim YH, Kim YW, Oh YJ, Back NI, Chung SA, Chung HG, Jeong TS, Choi MS, Lee KT.(2006) Protective effect of the ethanol extract of the roots of Brassica rapa on cisplatin-induced nephrotoxicity in LLC-PK1 cells and rats. Biol. Pharm. Bull; 29(12): 2436-2441.
7. Mrozikiewicz A, Kiełczewska-Mrozikiewicz D, Lowicki Z, Chmara E, Korzeniowska K, Mrozikiewicz PM.(1994) Blood levels of alloxan in children with insulin-dependent diabetes mellitus. ActaDiabetol;31(4):236-7.
8. Bergman R, Ader M. Free fatty acids and pathogenesis of type 2 diabetes mellitus. (2000)Trends EndocrinolMetab. 11: 351-356.
9. Greenberg MI Occupational, industrial, and environmental toxicology. (2003) Elsevier Health Sciences; 2003. pp. 180.
10. Atanasov AT, Tchorbanov B. Anti-platelet fraction from Galegaofficinalis L. inhibits platelet aggregation. J Med Food. 2002 winter; 5(4):229-34.
11. Louis J. Casarett; Curtis D. Klaassen; John DoullCasarett and Doull's toxicology: the basic science of poisons. McGraw-Hill Professional.2008. pp. 1104.
12. Riehemann, K; Behnke, B; Schulze-Osthoff, K. Plant extracts from stinging nettle an antirheumatic remedy, inhibit the proinflammatory transcription factor NF-kappaB. FEBS letters 1999; 442 (1): 89–94.
13. Schöttner M, Gansser D, Spiteller G. Interaction of lignans with human sex hormone binding globulin (SHBG). Zeitschrift fur Naturforschung. C, Journal of biosciences 1997; 52 (11–12): 834–43.
14. Westfall R. Galactagogue herbs: a qualitative study and review. Canadian Journal of Midwifery Research and Practice2003; 2 (2): 22–27.
15. Atanasov AT, Spasov V. Inhibiting and disaggregating effect of gel-filtered Galegaofficinalis L. herbal extract on platelet aggregation. J Ethnopharmacol. 2000 Mar; 69(3):235-40.
16. Gupta RK, Kesari AN, Murthy PS, Chandra R, Tandon V, Watal G. (2005) Hypoglycemic and antidiabetic effect of ethanolic extract of leaves of Annona squamosa L. in experimental animals. J. Ethnopharmacol; 99(1): 75-81.
17. Brodal P.(2010) The Central Nervous System: Structure and Function. Oxford University Press US; p. 170.
18. Montonen J, Järvinen R, Knekt P, Heliövaara M, Reunanen A. (2007) Consumption of sweetened beverages and intakes of fructose and glucose predict type 2 diabetes occurrence. J Nutr; 137:1447-54.
19. Ahangarpour A, Mohammadian M, Dianat M.(2012) Antidiabetic effect of hydroalcholicurticadioica leaf extract in male rats with fructose-induced insulin resistance. Iran J Med Sci;37(3):181-186.
20. Lenzen, S.(2008) The mechanisms of alloxan- and streptozotocin-induced diabetes.Diabetologia 51, 216-226.
21. Nofer JR, Kehrel B, Fobker M, Levkau B, Assmann G, von Eckardstein A. (2002) HDL and arteriosclerosis: beyond reverse cholesterol transport. Atherosclerosis;161:1-16.
22. Keeler RF, Baker DC, Panter KE.(1992) Concentration of galegine in Verbesinaencelioides and Galegaoficinalis and the toxic and pathologic effects induced by the plants. J Environ PatholToxicolOncol. Mar-Apr;11(2):11-7.

CITATION OF THIS ARTICLE

Mehrdad P, Jafar M, Ali A. Effect of Fabaceae(*Galega officinalis L.*) consumption on levels of blood glucose, lipids and Lipoproteins in Streptozotocin-induced diabetic rats. Bull. Env.Pharmacol. Life Sci., Vol 4 [6] May 2015: 127-132