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EFFECT OF *CASSIA AURICULATA* ON LIPID PROFILES IN STREPTOZOTOCIN–NICOTINAMIDE INDUCED TYPE 2 DIABETES MELLITUS

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Abstract
In experimental diabetes, enzymes of glucose and fatty acid metabolism are markedly altered. A study was undertaken to evaluate the antihyperlipidemic activity of *Cassia auriculta* flower extract (CFEt), leaf extract (CLEt) and seed extract (CSEt). Oral administration of CFEt (0.45 g/kg), leaf extract (0.45 g/kg) seeds extract CSEt (0.45 g/kg) to streptozotocin-nicotinamide induced diabetic rats for 45 days, significantly reduced the elevated serum very low density lipoprotein (VLDL) and low density lipoprotein (LDL) – cholesterol levels and significantly increased the serum high-density lipoprotein (HDL)-cholesterol. CFEt showed a better effect when compared with CLEt and CSEt. Results of the present study indicate that CFEt, CLEt and CSEt showed antihyperlipidemic effect in addtion to its antidiabetic effect in type 2 diabetic rats.

Keywords: *Cassia auriculta*, lipoproteins, lipids, diabetes, glibenclamide

Introduction

Type 2 diabetes mellitus is a major endocrine disorder and a deadly disease in human beings (Zare et al., 2012). According to the recent estimations, the prevalence of diabetes in the world, would reach to 552 million people in 2030 (Whiting et al., 2011). This disease is characterized by hyperglycemia, atherosclerosis, and abnormal lipid profile and also induces several chronic complications such as retinopathy, nephropathy, and neuropathy that results in burdening heavy load on these patients andsociety (Gomez-Perez et al., 2013). Dyslipidemia, a main risk factor for cardiovascular diseases as well as increase in generation of reactive oxygen species (ROS) and occurrence of oxidative stress which results in destruction of insulin producing β-cells in pancreatic langerhans islets, all have critical role in pathogenesis and progression of diabetes mellitus (Ghorbani, 2013; Yilmaz et al., 2013).

Herbal medicine and omics systems science offer significant synergy to aid drug discovery and development. *Cassia auriculata*, a Caesalpiniaceae shrub, is native to India and Sri Lanka, present in Indo-Malaysia, and cultivated in Myanmar. In Ayurvedic medicine, C. auriculata is one of the

notable medicinal herbs. The individual parts of the *C. auriculata* plant, including the flowers, flower buds, root, leaves, seeds, and bark, are used in traditional herbal medicine practices with various indications for each (Murugan, 2010).

Cassia auriculata L a member of genus Cassia belonging to family Caesalpiniaceae is commonly known as Tanner's Cassia. It is a shrub found throughout southern, western and central India. The various parts of the plant has been reported to posses a number of therapeutic activities to manage disease states like leprosy, asthma, gout, rheumatism and diabetes. The flower, buds, leaves, stem, root, and unripe fruit are used for treatment, especially in Ayurvedic medicine (Murugan, 2015a; Pari and Murugan, 2007). People use *Cassia auriculata* for diabetes, pink eye, joint and muscle pain (rheumatism), constipation, and other conditions, but there is no good scientific evidence to support any use. It is also used as antipyretic, antiulcer and in the treatment of skin infection. The flower has been reported to contain flavonoids, proanthocyanidins and β-sitosterol (Murugan, 2015b; latha and Pari, 2003). A literature survey showed that a decoction of leaves, flowers, and seeds of the *Cassia auriculata* mediate an antidiabetic effect (Murugan, 2015c). In folk remedies, flowers of *Cassia auriculata* are proposed to have antidiabetic activity. From literature survey, it was evident that the aqueous extract of flowers has been reported for its antidiabetic activity in streptozotocin-diabetic rats at a dose of 0.45 g/kg body weight (latha and Pari, 2003). In this study we explored the role of CFEt, CLEt and CSEt in prevention of streptozotocin induced hyperglycemia and related lipid complications.

Materials and methods

Chemicals

Stereptozotocin was obtained from Himedia Laboratory Limited, Mumbai, India. All other reagents used were of analytical grade.

Plant Material

Cassia auriculata flowers, leaves and seeds were collected freshly from Neyveli, Cuddalore District, Tamil Nadu, India. The plant was identified and authenticated at the Herbarium of Botany Directorate in Annamalai University. A voucher specimen (No.231) was deposited in the Botany Department of Annamalai University.

Preparation of plant (Flower, leaves and seeds) extract

Five hundred g of *Cassia auriculata* flowers and leaves were extracted with 1,500 ml of water by the method of continuous hot extraction at 60ºC for six hours and evaporated. The residual extract was dissolved in water and used in the study [8]. Seeds cleaned off adhering dust and unwanted plant material, shade dried, cut and pulverized (powdered). Further Seeds (500 g) were extracted with successive extraction at room temperature, filtered and concentrated under reduced pressure on rotary evaporator. The dried extract was successively fractionated in Petroleum Ether (40.5 gm) [CA-PE], n-butanol, (5.8g) [CA-NB] acetone: methanol 1:1 (26.8g) [CA-AM] and methanol: water 1:1 (21.23g) [CA-MW]. Also, separately seeds extracted with methanol by Soxhlet extraction at 60^{o} C [CA-TS]. The solvents were chosen for larger delivery of bioactive compounds which are polar and mid-polar

Induction of diabetes

Non-Insulin dependent diabetes mellitus was induced (Masiello et al. 1998) in overnight fasted rats by a single intraperitonial injection of 65 mg/kg streptozotocin, 15 min after the i.p administration of 110 mg/kg of nicotinamide. Streptozototocin (STZ) was dissolved in citrate buffer (pH 4.5) and nicotinamide was dissolved in normal saline. Hyperglycemia was confirmed by the elevated glucose levels in plasma, determined at 72 h and then on day 7 after injection. The animals with blood glucose concentration more than 200 mg/dl were used for the study.

Experimental design

In the experiment, a total of 36 rats (30 diabetic surviving rats, six normal rats) were used. The rats were divided in to six groups of six rats each.
Group 1: Normal untreated rats.
Group 2: Diabetic control rats given 1 ml of aqueous solution daily using an intragastric tube for 45 days.
Group 3: Diabetic rats given CFEt (0.45 g/kg body weight) in 1 ml of aqueous solution daily using an intragastric tube for 45 days.
Group 4: Diabetic rats given CLEt (0.45 g/kg body weight) in 1 ml of aqueous solution daily using an intragastric tube for 45days.
Group 5: Diabetic rats given CSEt (0.45 g/kg body weight) in 1 ml of ethanolic extract daily using an intragastric tube for 45days.
Group 6: Diabetic rats given glibenclamide (600 μg/ kg body weight) in 1 ml of aqueous solution daily using an intragastric tube for 45days.
At the end of 45 days, the animals were deprived of food overnight and sacrificed by decapitation. Blood was collected in tubes containing potassium oxalate and sodium fluoride mixture for the estimation of blood glucose. Plasma was separated for the estimation of insulin and other biochemical parameters.

Analytical Methods

Blood glucose was estimated colorimetrically using commercial diagnostic kits (Sigma Diagnostics (I) Pvt Ltd, Baroda, India) (Lott and Turner 1975). Plasma insulin was assayed by ELISA using a Boehringer-Mannheim kit with an ES300 Boehringer analyzer (Mannheim, Germany). Haemoglobin was estimated using the cyanmethaemoglobin method described by Drabkin and Austin (1932). Glycosylated haemoglobin was estimated according to the method of Sudhakar Nayak and Pattabiraman (1981) with modifications according to Bannon (1982).

The high density lipoprotein cholesterol (HDL-C) content in plasma was estimated by using a reagent kit (Qualigens diagnostics, Mumbai, India). Very low density lipoprotein cholesterol (VLDL-C) and low density lipoprotein cholesterol (LDL-C) fractions were calculated as VLDL-C = TG/5 and LDL-C = total cholesterol – (HDL-C + VLDL-C), respectively. The activity of hydroxy 3-methylglutaryl-coenzyme A (HMG CoA) reductase in the liver & kidney was assayed by the method of Philipp and Shapiro (1970). The ratio between HMG CoA and mevalonate in the liver was taken as an index of the activity of HMG CoA reductase. The decrease in HMG CoA/Mevalonate ratio indicates the increased activity of the enzyme.

Statistical analysis

The data for various biochemical parameters were analyzed using analysis of variance (ANOVA), and the group means were compared by Duncan's multiple range test (DMRT). Values were considered statistically significant if $p < 0.05$ (Duncan 1957).

Results

Table 1 shows the level of blood glucose, total haemoglobin, glycosylated haemoglobin and plasma insulin of different experimental groups. There was a significant elevation in blood glucose level, whereas plasma insulin levels decreased significantly in streptozotocin diabetic rats, compared with normal rats. The effect of CFEt was more prominent when compared with CLEt, GLSt and glibenclamide. The diabetic control rats showed a significant decrease in the level of total haemoglobin and significant increase in the level of glycosylated haemoglobin. Oral administration of CFEt, CLEt and CSEt to diabetic rats significantly restored total haemoglobin and glycosylated haemoglobin levels. In the case of normal rats, the level of haemoglobin and glycosylated haemoglobin remained unaltered.
Table 2 demonstrates the level of serum and tissue total cholesterol (TC), lipoproteins and the activity of HMG-CoA reductase in normal and experimental rats. The levels of TC, low-density

lipoprotein-cholesterol (LDL-C), very low-density lipoprotein-cholesterol (VLDL-C) and hepatic HMG-CoA reductase activity were significantly increased whereas the level of high density lipioprotein – cholestrol (HDL-C) were significantly decreased in diabetic control rats. Administration of CFEt, CLEt and CSEt to diabetic rats the decreased levels of TC, LDL-C, VLDL-C levels and the activity of HMG-CoA reductase along with significant increase in the level of HDL-C.

Table 1. Effect of CFEt, CLEt and CSEt on the levels of blood glucose, plasma insulin, haemoglobin and glycosylated haemoglobin in normal and experimental rats

Groups	Fasting blood glucose (mg/dl)	Plasma insulin (μU/ml)	Total haemoglobin (g/dl)	Glycosylated haemoglobin (mg/g Hb)
Normal	98.23 ± 5.21^{a}	12.21 ± 0.41^{a}	12.11 ± 0.50^{a}	0.30 ± 0.03^{a}
Diabetic control	285.28 ± 7.12^{b}	3.90 ± 0.30^{b}	8.25 ± 0.30^{b}	0.75 ± 0.04^{b}
Diabetic+CFEt (0.45g/kg)	108.28 ± 6.12^{c}	10.35 ± 0.62^{c}	11.25 ± 0.45^{c}	0.40 ± 0.03^{c}
Diabetic+CLEt (0.45g/kg)	125.41 ± 6.52^{d}	8.35 ± 0.25^{d}	10.54 ± 0.55^{d}	0.48 ± 0.03^{d}
Diabetic+CSEt (0.45g/kg)	134.24 ± 6.51^{d}	8.52 ± 0.31^{d}	10.98 ± 0.53^{d}	0.47 ± 0.03^{d}
Diabetic+ Glibencalamide (600 μg/ mg)	142.87 ± 7.51^{d}	8.85 ± 0.34^{d}	10.65 ± 0.38^{d}	0.51 ± 0.04^{d}

Values are given as mean ± S.D for 6 rats in each group.
Values not sharing a common superscript letter differ significantly at $p<0.05$ (DMRT).

Table 2. Effect of CFEt, CLEt and CSEt on changes in the levels of lipoproteins and cholesterol in normal and experimental rats

Groups	Normal	Diabetic control	Diabetic+CFEt (0.45g/kg)	Diabetic+CLEt (0.45g/kg)	Diabetic+CSEt (0.45g/kg)	Diabetic+ Glibencalamide (600 μg/ mg)
Serum Total cholesterol (mg/dl)	95.21 ± 6.40^{a}	175.32 ± 13.21^{b}	112.58 ± 8.55^{c}	122.51 ± 9.41^{d}	122.51 ± 9.41^{d}	122.51 ± 9.41^{d}
HDL-C (mg/dl)	52.55 ± 4.55^{a}	26.35 ± 2.15^{b}	48.45 ± 3.65^{c}	42.55 ± 3.66^{d}	42.55 ± 3.66^{d}	42.55 ± 3.66^{d}
LDL-C (mg/dl)	32.55 ± 2.55^{a}	127.21 ± 8.55^{b}	42.35 ± 4.45^{c}	59.32 ± 5.18^{d}	59.32 ± 5.18^{d}	59.32 ± 5.18^{d}
VLDL-C (mg/dl)	10.55 ± 1.21^{a}	20.65 ± 1.54^{b}	14.20 ± 1.01^{c}	15.25 ± 1.25^{d}	15.11 ± 1.28^{d}	15.28 ± 1.30^{d}
Liver (mg/100g tissue)	319.31 ± 13.45^{a}	505.41 ± 22.41^{b}	390.67 ± 15.21^{c}	422.31 ± 17.36^{d}	425.31 ± 19.41^{d}	428.41 ± 19.41^{d}
Kidney (mg/100g tissue)	355.55 ± 14.25^{a}	525.55 ± 20.21^{b}	412.41 ± 19.11^{c}	438.25 ± 18.41^{d}	438.25 ± 17.35^{d}	428.20 ± 16.31^{d}
Hepatic HMG-CoA Reductase[A]	1.61 ± 0.1^{a}	1.03 ± 0.1^{b}	1.57 ± 0.1^{c}	1.51 ± 0.1^{d}	1.51 ± 0.1^{d}	1.49 ± 0.1^{d}

[A] – HMG-CoA / Mevalonate ratio

Values are given as mean ± SD from 6 rats in each group. Values not sharing a common superscript letter differ significantly at (DMRT).

Discussion

VLDL and chylomicrons, which transport endogenous and exogenous triglycerides, are broken down by lipoprotein lipases. In insulin deficiency (Gibbons, 1986) the activity of the lipoprotein lipases is decreased,8 and this is one of the most common causes of hyperlipidemia in poorly controlled diabetes (Taskinen et al., 1982).

Hypercholesterolemia and hypertriglyceridemia are independent major risk factors that alone or together can accelerate the development of coronary artery disease (CAD) (McKenney, 2001). The cause of hyperlipidemia has been related to increased lipid synthesis, decreased lipid clearance from the blood or a combination of these two processes.

The abnormal high concentration of serum lipids in diabetes is mainly due to the increase in the mobilization of FFA from the peripheral depots, since insulin inhibits the hormone sensitive lipase. On the other hand, glucagon, catecholamines and other hormones enhance the lipolysis. The marked hyperlipemia that characterizes the diabetic state may therefore be regarded as a consequence of the uninhibited actions of lipolytic hormones on the fat depot (Al-Shamaony et al., 1994).

Studies on STZ-induced diabetes in experimental animals have suggested that an increase in circulatory VLDL and their associated TG are largely due to defective clearance of these particles from circulation (Babu and Srinivasan, 1997). As there is a close relationship between elevated serum TC level and the occurrence of atherosclerosis, the ability of the CFEt, CLEt and CSEt in selective reduction of TC through the reduction of VLDL and LDL components could be beneficial in preventing the atherosclerotic conditions and thereby reduce the possibility of CHD in general. As regards to the effect of the CFEt, CLEt and CSEt on serum HDL, our results clearly show that the level of this lipoprotein fraction increased with CFEt, CLEt and CSEt treatment.

An increase in cholesterol levels in the hepatic tissue might be due to an increase in the transport of chylomicron cholesterol to the liver (Chauhan et al., 1987). Hypertriglyceridemia in diabetes can result from an increased hepatic VLDL over production and impaired catabolism of TG-rich particles. Dysfunction of LPL also contributes to hypertriglyceridemia in the fasting and postprandial state (Kanters et al., 2001). The increased level of cholesterol observed in diabetic liver and kidney might also be due to the decreased levels of HDL-cholesterol.

During diabetes, kidney exhibits a characteristics pattern of changes in the glomerulus producing initial hyper filtration with a marked thickness of glomeruli basement membrane which eventually leads to renal insufficiency or complex kidney failure. Changes in the fatty acids during diabetes are closely associated with the activity of Na+/K+-ATPase in the kidney. Accumulation of fatty acids results in higher levels of their metabolities such as acyl-carnitine and long chain acyl-CoA. This interferes with Na+/K+-ATPase action leading to impairment in the action of Na+/K+ ions, which may finally results in diabetic nephropathy (Lopaschuk et al., 1983; Bergman and Ader, 2000). Thus, the diabetic complications associated with renal tissue may be partly due to abnormalities in lipid metabolism.

The PPARα isoform is predominantly involved in fatty acid and lipid catabolism. It also involved in the import and activation of genes involved in fatty acid oxidation in the liver, heart, kidney and skeletal muscles (Fruchart and Duriez, 2003; Gilde and Van Bilsen, 2003). In the liver, activation of PPARα leads to increased β-oxidation of fatty acids and decreased TG and VLDL synthesis (Fruchart and Duriez, 2004). Activation of PPARα also leads to reduction of TG because of repression of hepatic apolipoprotein C-III and an increase in lipoprotein lipase gene expression (Gervois et al. 2000). Furthermore, PPARα activation causes induction of hepatic apoliporotein A-I and A-II expression, in humans, leading to increased plasma HDL cholesterol. PPARα agonists are also known to slow the progression of premature coronary atherosclerosis. CFEt, CLEt and CSEt is an agonist for PPARα, which possesses an activity of hypolipidemic drug, there by provides a possible alternative for the treatment of dyslipidemia (Rimando et al., 2005). In this context, the decreased levels of cholesterol, TG, FFA and PL were found in plasma and tissues of diabetic rats treated with CFEt, CLEt and CSEt could be due to an activation of PPARα by the administration of CFEt, CLEt and CSEt.

We have observed a significant increase in the activity of HMG-CoA reductase in diabetic rats. HMG-CoA reductase catalyzes the rate-limiting step in cholesterol biosynthesis and its activity correlates closely with the rate of tissue cholesterol synthesis. The increase in HMG-CoA reductase activity could be due to increased cholesterogenesis. The increased concentration of FFA in liver

may be due to impaired insulin action; the anti-lipolytic/re-esterifying effects of insulin on adipocyte are lacking, which causes increased generetion of NADPH, which results in the activation of NADPH dependent microsomal lipid peroxidation.

Conclusion

It can be concluded from the data that CFEt, CLEt and CSEt significantly reduces the level of serum and tissue lipids, which are actively raised in streptozotocin diabetic rats. CFEt, CLEt and CSEt has beneficial effect on plasma insulin and blood glucose level. Moreover it was a prevention of lipid metabolism defects could represent a protective mechanism against the development of atherosclerosis.

References

1. Al-Shamaony L, Al-Khazraji SM, Twaij HA. Hypoglycaemic effect of Artemisia herba alba. II. Effect of a valuable extract on some blood parameters in diabetic animals. J Ethnopharmacol 1994;43:167-171.
2. Babu PS, Srinivasan K. Hypolipidemic action of curcumin, the active principle of turmeric (Curcuma longa) in streptozotocin induced diabetic rats. Mol Cell Biochem 1997;166:169-175.
3. Bannon, P: Effect of pH on the elimination of the labile fraction of glycosylated haemoglobin. Clin. Chem. 1982, 28, 2183.
4. Bergman RN, Ader M. Free fatty acids and pathogenesis of type 2 diabetes mellitus. Trends Endocrinol Metab 2000;11:351-356.
5. Chauhan UPS, Jagi CB, Singh VN. Incorporation of 32Pi into plasma phosphatidylcholine of diabetic rats. Ind J Nucl Med 1987;2:92-98.
6. Drabkin, D.L & J.M. Austin: Spectrophotometric constants for common hemoglobin derivatives in human, dog and rabbit blood. J. Biol. Chem. 1932, 98, 719 - 733.
7. Duncan BD. (1957). Multiple range tests for correlated and heteroscedastic means. Biometrics. 13: 359-364.
8. Fruchart JC, Duriez P. [Anti-cholesterol agents, new therapeutic approaches]. Ann Pharm Fr 2004;62:3-18.
9. Fruchart JC, Duriez P. [Anti-cholesterol agents, new therapeutic approaches]. Ann Pharm Fr 2004;62:3-18.
10. Gervois P, Torra IP, Fruchart JC, Staels B. Regulation of lipid and lipoprotein metabolism by PPAR activators. Clin Chem Lab Med 2000;38:3-11.
11. Ghorbani A. Phytotherapy for diabetic dyslipi-demia: evidence from clinical trials. Clin Lipidol . 2013; 8:311–319.
12. Gibbons GF. Hyperlipidemia of diabetes. Clin Sci 1986;71:477-86.
13. Gilde AJ, Van Bilsen M. Peroxisome proliferator-activated receptors (PPARS): regulators of gene expression in heart and skeletal muscle. Acta Physiol Scand 2003;178:425-434.
14. Gomez-Perez FJ, Aguilar-Salinas CA, Almeda-Valdes P, Cuevas-Ramos D, Lerman Garber I, Rull JA. HbA1c for the diagnosis of diabetes mellitus in a developing country. Arch Med Res . 2010; 4:302–308.
15. John A. Lott & K. Turner: Evaluation of trinder's glucose oxidase method for measuring glucose in serum and urine. Clin. Chem. 1975, 21/12, 1754-1760.
16. Kanters SDJMN, Banga JD, Erkelens DW. Lipid-lowering therapy in diabetes mellitus. The Netherlands J Med 2001;58:214-222.
17. Latha M, Pari L . Preventive effects of *Cassia auriculata* L. flowers on brain lipid peroxidation in rats treated with streptozotocin diabetes. Molecular and Cellular Biochemistry.2003, 243, 23–28.

18. Lopaschuk GD, Tahiliani AG, Vadlamudi RV, Katz S, McNeill JH. Cardiac sarcoplasmic reticulum function in insulin- or carnitine-treated diabetic rats. Am J Physiol 1983;245:H969-976.
19. Masiello, P., C. Broca, R. Gross, M. Roye, M. Manteghetti, D. Hillaire-Buys, M. Novelli & G. Ribes. Experimental NIDDM: development of a new model in adult rats administered streptozotocin and nicotinamide. Diabetes. 1998; 47: 224-229.
20. McKenney JM. (2001). Pharmacotherapy of dyslipidemia. Cardiovasc Drugs Ther. 15: 413-422.
21. Murugan P. Effect of *Cassia auriculata L* on erythrocyte membrane bound enzymes andantioxidant status in experimental diabetes. International Journal of Recent Advances in Multidisciplinary Research. Vol. 02, Issue 12, pp.5760-5764, December, 2015
22. Murugan P. Effect of *Cassia auriculata L* on plasma antioxidants in streptozotocin-nicotinamide induced experimental diabetes. International Journal of Information Research and Review. Vol. 02, Issue, 05, pp.6930-6934, May, 2015b.
23. Murugan P. Preventive effects of *Cassia auriculata* on brain lipid peroxidation streptozotocin diabetic rats. Internat J Inform Res Rev. 2015a; 05: 6924-6929.
24. Murugan P. Taner's Cassia (*Cassia auriculata L*) extract prevents hemoglobin glycation tail tendon collagen properties in experimental diabetic rats. J cell tissue res. 2010; 10 (1): 2109-2114.
25. Murugan P. Taner's Cassia (*Cassia auriculata L*) extract prevents hemoglobin glycation tail tendon collagen properties in experimental diabetic rats. Journal of cell and tissue research. 2010; 10 (1): 2109-2114.
26. Pari L, Murugan P. Influence of *Cassia auriculata* flowers on Insulin Receptors in Streptozotocin Induced Diabetic Rats: Studies on Insulin Binding to Erythrocytes. African Journal of Biochemistry Research. 2007; 1 (7): 148-155.
27. Philipp B, Shapiro DJ. (1970). Improved methods for the assay and activation of 3-hydroxy-3-methyl glutaryl coenzyme A reductase. J Lipid Res 20: 588 – 93.
28. Rimando AM, Cuendet M, Desmarchelier C, Mehta RG, Pezzuto JM, Duke SO. Cancer chemopreventive and antioxidant activities of pterostilbene, a naturally occurring analogue of resveratrol. J Agric Food Chem 2002;50:3453-3457.
29. Shrotri DS, Aiman R. The relationship of the post-absorptive state to the hypoglyceamic action. Ind. J. Med. Res.1960. 48: 162 – 168.
30. Sudhakar Nayak, S & T.N. Pattabiraman: A new colorimetric method for the estimation of glycosylated hemoglobin. Clin. Chem. Acta. 1981, 109, 267 - 274.
31. Taskinen M-R, NikkilaEA, Kunsi T, Harno K. Lipoprotein lipase activity and serum lipoproteins in untreated type 2 (insulin-independent) diabetes associated with obesity. Diabetologia 1982;22:46-50
32. Whiting DR, Guariguata L, Weil C, Shaw J. IDF diabetes atlas: global estimates of the prevalence of diabetes for 2011 and 2030. Diabetes Res Clin Pract . 2011; 94:311–321.
33. Yilmaz O, Ersan Y, Dilek Ozsahin A, Ozturk AI, Ozkan Y. Consequences of the combined α-tocopherol, ascorbic acid and α-lipoic acid on the glutathione, cholesterol and fatty acid composition in muscle and liver of diabetic rats. Iran J Basic Med Sci. 2013; 16:165–172.
34. Zare K, Fatemi Tabatabaei SR, Shahriari A, Jafari RA. The effect of butter oil on avoidance memory in normal and diabetic rats. Iran J Basic Med Sci . 2012; 15:983–989.