

Effect of soybean seeds alone or in combination with insulin or glibenclamide on serum lipid profiles in alloxan-induced diabetic rats

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(Received August 12, 2008; Accepted March 25, 2009)

Abstract

The present study was conducted to evaluate the effect of soybean seeds administration alone or combined with either insulin or glibenclamide on serum lipid profiles in diabetic rats. Male Wister rats were induced diabetes by a single subcutaneous injection of alloxan 100 mg/kg.b.w. The rats randomly divided into six groups (eight rats in each group): The first group served as a control, the second group was administered soybean seed 400 mg/kg.b.w orally as suspension, the third group injected insulin 10 I.U/kg.b.w, subcutaneously, the fifth group administered glibenclamide 5 mg/kg.b.w. orally. Accompaniment of soybean seeds with either of insulin or glibenclamide given the fourth and sixth groups, using the same routes and doses in the individual groups. All treatments were once daily for two weeks. Soybean seeds treatment alone resulted in an improvement of body weight and decrease of triglyceride and total lipids levels. While treatment with insulin or glibenclamide as alone or combination with soybean seeds showed a significant reduction in the levels of total cholesterol, triglyceride, low density lipoprotein, and total lipids, beside a significant increase in both body weight and high density lipoprotein. Beneficial effects were seen when soybean seeds combined with either of insulin or glibenclamide treatment. These results indicate the usefulness of soybean seeds in the management of diabetes through the hypolipidemic effects of soybean seeds in diabetic rats.

Keyword: Soybean, Diabetes, Alloxan, Insulin, Glibenclamide, Lipid profile.

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تأثير بذور فول الصويا لوحدها أو بالترافق مع الأنسولين أو الداونيل على مستويات شحوم الدم في الجرذان المصابة بداء السكر المحدث الالوكسان

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الخلاصة

صممت تجارب هذه الدراسة لتقييم تأثير بذور فول الصويا لوحدها أو بالترافق مع الأنسولين أو الداونيل في مستويات شحوم مصل التي استحدثت بها داء السكر عن طريق حقنها بمادة Wister الدم في الجرذان المصابة بداء السكر. استخدمت ذكور جرذان من نوع الالوكسان تحت الجلد بجرعة 100 ملغم/كغم من وزن الجسم. قسمت الجرذان عشوائياً إلى ستة مجاميع (كل مجموعة ثمانية جرذان): المجموعة الأولى: تركت دون معاملة كمجموعة سيطرة، المجموعة الثانية: تم إعطائها بذور فول الصويا 400 ملغم/كغم من وزن الجسم عن طريق الفم بشكل معلق، المجموعة الثالثة: حقنت تحت الجلد بالأنسولين 10 وحدة دولية/كغم من وزن الجسم، المجموعة الخامسة: أعطيت الداونيل 5 ملغم/كغم من وزن الجسم عن طريق الفم، الترافق بين بذور فول الصويا والأنسولين أو الداونيل أعطيت للمجموعة الرابعة والسادسة على الترتيب باستخدام نفس الطريقة والجرعة المستخدمة في المعاملات الانفرادية. كل المعاملات كانت

لمرة واحدة باليوم ولمدة أسبوعان. أظهرت نتائج المعاملة الانفرادية ببذور فول الصويا تحسناً في وزن الجسم مع انخفاض في مستوى الكليسيريدات الثلاثية ومستوى الشحوم الكلية، بينما المعاملة الانفرادية لكل من الأنسولين والداونيل أو بالتزامن مع بذور فول الصويا فقد أظهرت انخفاض معنوي في مستويات: الكولسترول، الكليسيريدات الثلاثية، الشحوم البروتينية ذات الكثافة الواطئة، والشحوم الكلية، إلى جانب زيادة معنوية في وزن الجسم ومستوى الشحوم البروتينية ذات الكثافة العالية. التأثير الأفضل لوحظ عند استخدام بذور فول الصويا بالتزامن مع الأنسولين أو الداونيل. تشير نتائج الدراسة الحالية إلى أن بذور فول الصويا تمتلك دوراً في السيطرة على داء السكر من خلال التأثير المخفض لمستويات شحوم الدم في الجرذان المصابة بداء السكر.

Introduction

Diabetes mellitus is a chronic metabolic disorder caused by insulin deficiency, often combined with insulin resistance, and it is a major cause of disability and hospitalization and it results in significant financial burden (1). WHO indicates that diabetes mellitus is one of the major killers of humans in our time (2). Management of diabetes without any side effect is still a challenge to the medical system, this has led to an increasing demand for natural products with antidiabetic activity and fewer side effects (3). Millions of people throughout the world consume soy products, in Asian countries; soybean has been stable for 5000 years (4). Soybean is unique foods because of their rich nutrient content, their complex carbohydrate and dietary fiber content contribute to their low glycemic indexes which benefit diabetic individuals and reduce the risk of developing diabetes (5). Previous study indicates that protein of soybean decreased cholesterol level in rats consume a diet rich in cholesterol (6). Soybean protein administration reduced cholesterol, triglyceride, and Low density lipoprotein levels in healthy persons (7) as well as in diabetic patients (8), similar effect was also noticed in rats (9). The present study was designed to investigate the effect of soybean seeds alone or in combination with insulin or glibenclamide as hypolipidemic agent in experimentally diabetic rats.

Materials and methods

Animals: Fifty six male albino rats, 3-4 months old and 167-218 gm body weight were housed in hanging cages and maintained under laboratory controlled of temperature (25 ±2) and light (14 hour light and 10 hour dark), pelleted food and tap water were given.

Induction of diabetes: Rats were fasted for 48 hour before inducing diabetes with alloxan (sigma chemical co., st, Louis, Mo. USA). Diabetes was induced by a single subcutaneous injection of alloxan in a dose of 100 mg/kg of body weight (10). Rats were allowed to drink 5% glucose solution overnight to prevent drug induced hypoglycemia (11). Alloxan treated animals were monitored by periodic testing for glucosuria using Lilly Test Tap and ketonuria

using chemstrip MH 5000/k (Boehringer, Mannheim, Germany) for four weeks before treatments.

Experimental design: Soybean seeds were purchased from the local market in Mosul, and it's identify was confirmed biochemically in the Research Center of the college of Science, university of Mosul. The seeds were powdered before uses. After four weeks of induced diabetes the animals were divided into six groups each of 6-8 rats. Group 1: Diabetic control rats; Group 2: diabetic rats were treated with soybean seeds at a dose of 400 mg/kg of body weight (12); Group 3: diabetic rats were treated subcutaneously with insulin (Actrapid, Novo Nordisk, Denmark) at a dose of 10 I.U. /kg of body weight (13); Group 5: diabetic rats were treated with glibenclamide (Medochemic LTD-Cyprus) at a dose of 5 mg tablet (5 mg /kg of body weight) (14), Group 4 and 6; diabetic rats were given soybean coadminstrated with insulin and glibenclamide, respectively at same routes and dose as in individual treatment. Soybean seeds and glibenclamide were given as a suspension orally by gavage needle. All treatment was once daily and lasted for two weeks.

Samples collection: Blood samples were collected from the orbital plexus of vein into clean dry centrifuge tubes allowed to clot, serum was separated after centrifugation at 1500 rpm for 15 minutes (15), serum lipid profiles were measured using colorimetric assay kits (Bicon, Diagnostic GmbH, Burbach, Germany).

Statistical analysis: All data were analyzed by one-way analysis of variance, the specific group differences were determined using Duncan multiple range test (16). The accepted level of significant was $P < 0.05$.

Results

Induced diabetes by alloxan lead after four weeks to a significant increase in levels of cholesterol, triglyceride, low density lipoprotein, and total lipids, with decreased significantly both body weight and high density lipoprotein (Table 1). Administration of soybean seeds as alone resulted in a significant increase in body weight with a reduction in levels of, triglyceride, low density lipoprotein in second week and total lipids in both two weeks (Table 2). When rats treated with insulin as alone or when accompanied with soybean seeds resulted a significant increase in body weight with a reduction in levels of low density lipoprotein, and total lipids in both two weeks, with significant reduction of levels of cholesterol, triglyceride

and increased level of high density lipoprotein in the second week only. Glibenclamide alone significantly increased body weight and high density lipoprotein levels in second week, while causes reduction in cholesterol, triglyceride, low density lipoprotein, and total lipids levels in both two weeks (Table 2).

Combination of soybean seeds with glibenclamide resulted an elevation of body weight with reduction of triglyceride level in the second week, and increase level of high density lipoprotein, with reduction levels of cholesterol, low density lipoprotein, and total lipids in both two weeks (Table 2).

Table 1: Comparison between normal and diabetic rats in body weight and lipid profiles.

Parameters	Groups		Normal Rats	Diabetic Rats
Body Weight (gm)	Before Treatment		201 ± 4.2	187 ± 4.5*
		1	204 ± 5.8	176 ± 5.3*
	After Treatment (weeks)	2	217 ± 8.2	168 ± 5.8*
Serum Cholesterol level (mg/dl)	Before Treatment		77.2 ± 2.4	121.1 ± 3.4*
		1	78.4 ± 3.5	122.5 ± 5.5*
	After Treatment (weeks)	2	77.0 ± 3.5	121.6 ± 5.1*
Serum Triglyceride level (mg/dl)	Before Treatment		58.8 ± 2.4	84.9 ± 4.3*
		1	58.5 ± 3.9	84.6 ± 3.8*
	After Treatment (weeks)	2	59.9 ± 4.7	85.1 ± 3.2*
Serum HDL level (mg/dl)	Before Treatment		36.5 ± 2.4	69.8 ± 3.7*
		1	38.9 ± 2.7	72.6 ± 1.9*
	After Treatment (weeks)	2	37.0 ± 3.1	74.5 ± 1.7*
Serum LDL level (mg/dl)	Before Treatment		28.9 ± 2.5	34.3 ± 4.1*
		1	26.7 ± 2.9	32.9 ± 1.4*
	After Treatment (weeks)	2	28.0 ± 2.3	30.1 ± 3.3*
Serum Total lipids level (mg/dl)	Before Treatment		441 ± 12	863 ± 12*
		1	447 ± 12	875 ± 18*
	After Treatment (weeks)	2	443 ± 12	871 ± 19*

No. of rats (6-8) in each group, Data is the mean ± SEM, * Significant with normal rats at P < 0.05.

Discussion

In the present study, we have demonstrated that diabetes induced experimentally by alloxan produced significantly decreased in body weight, this result agrees with results of (17) in rats. The reduction of body weight in diabetic rats is due to dehydration and catabolism of fats and proteins (18), increased catabolic reaction leading to muscle wasting can be the cause of the reduced body weight gain in diabetic rats (19). Also the glucose can not enter the cells when there is lack of insulin lead to deficiency of energy for cells (20). Induced diabetes lead to increase level of cholesterol, (21) reported similar results in rats. The increase of cholesterol level occurs when there is an absence of insulin cause decrease level of Apo-mRNA (22), also deficiency of insulin lead to increase cholesterol- acyl- transferase that absorbs cholesterol from intestine, leading to increase level of cholesterol (23). Triglyceride levels also increased when diabetes induced by alloxan, this result agree with result of (24) in rats. This may be due to deficiency of insulin causing decrease activity of lipoprotein lipase that convert triglyceride to glycerol and fatty acids (25). Low density lipoprotein levels also increased, similar result reported by (26) in rats, this is due to increase synthesis from chylomicron and very low density lipoproteins (27).

Alloxan induced diabetes lead to increase levels of total lipids, this result agrees with result of (28) in rats, increase synthesis of very low density lipoproteins and chylomicron or decrease removal it from blood may be the causes of increase of total lipid levels (29).

Significant weight gain was observed in soybean treatment and these results are consistent with those of previous study (30) in mice, but our result not agree with result of (31) in rats. The possible mechanisms include, that soybean contain high percentage of protein, isoflavone, or fiber that increase metabolic processes in body (32). Insulin treatment showed an increase of body weight, similar observation has been reported in diabetic rats (33), but disagree with results of (34) in diabetic rats. This effect is due to its metabolic stimulative effects that decrease lipolysis, increase fatty acid synthesis, increase amino acid uptake by the tissue, and increase protein synthesis (35) also insulin stimulate DNA replication, modulation of various enzyme activities, through increase translation of mRNA, and stimulate ribosome to produce protein (36), all this processes lead to improvement of body weight. Treatment with glibenclamide leads to improvement of body weight, (37) reported similar effects in diabetic rabbit, and this result disagree to result of (38) in type 2 diabetic patient.

Table 2: Effect of soybean seeds, insulin, and glibenclamide as alone and combination of soybean with either insulin, or glibenclamide on body weight, and serum lipid profiles.

Groups	Weight Body g			Serum Cholesterol level (mg/dl)			Serum Triglyceride level (mg/dl)		
	Before	After (weeks)		Before	After (weeks)		Before	After (weeks)	
	0	1	2	0	1	2	0	1	2
Control diabetic	E – F	FG	G	B – F	C – F	C – F	CD	CD	CD
	187 ± 4	176 ± 5	168 ± 5	121.1 ± 3.4	122.5 ± 5	121.6 ± 5.1	84.9 ± 4.3	84.6 ± 3.8	85.1 ± 3.2
Soybean seeds 400 mg/kg.b.w.	EFG	D – G	B – F	EF	B – F	A – F	BCD	BCD	AB
	181 ± 4	183 ± 7	192 ± 4	124.6 ± 5.2	120.4 ± 6.6	112.7 ± 5.4	79.9 ± 5	80.9 ± 2.7	69.2 ± 2.8
Insulin 10 I.U./Kg.b.w	A – E	A – E	ABC	EF	A – E	A	CD	BC	A
	195 ± 5	198 ± 6	202 ± 6	125.5 ± 2.4	112.8 ± 3.2	102 ± 4.8	86.1 ± 2.4	75.3 ± 2.2	56.6 ± 2.6
Soybean seeds 400mg/kg.b.w + Insulin 10 I.U./Kg.b.w.	D – G	A – D	AB	B – F	ABC	AB	BCD	BC	A
	184 ± 4	200 ± 6	208 ± 4	121.1 ± 2.3	109.3 ± 1.9	107.7 ± 3.7	81.7 ± 2.9	75.6 ± 2.4	54.3 ± 4.5
Glibenclamide 5mg/kg.b.w	D – G	C – F	B – F	DEF	AB	A	D	BC	BC
	184 ± 4	189 ± 6	192 ± 4	123.5 ± 2.9	108.3 ± 3.6	106.5 ± 3.8	91.1 ± 3.9	78.1 ± 4.7	71.5 ± 3.4
Glibenclamide 5mg/kg.b.w + Soybean seeds 400 mg/kg.b.w	B – F	C – F	A	F	A – D	A	CD	BCD	B
	191 ± 5	190 ± 5	210 ± 4	125.9 ± 2.6	110.7 ± 2.6	105.3 ± 3.7	83.5 ± 3.8	80.5 ± 3.6	74.3 ± 2.5

Groups	Serum HDL level (mg/dl)			Serum LDL level (mg/dl)			Serum Total lipids level (mg/dl)		
	Before	After (weeks)		Before	After (weeks)		Before	After (weeks)	
	0	1	2	0	1	2	0	1	2
Control diabetic	CDE	CDE	BCD	EFG	EF	DE	F	F	F
	69.8 ± 3	72.6 ± 1.9	74.5 ± 1.7	34.3 ± 4.1	32.9 ± 1.4	30.1 ± 3.3	863 ± 12	875 ± 18	871 ± 19
Soybean seeds 400 mg/kg.b.w .	DE	E	CDE	FG	FG	CD	F	E	D
	66.5 ± 2.8	63.6 ± 3.2	69.8 ± 2.9	42.1 ± 3.4	40.6 ± 2.4	25 ± 1.9	856 ± 16	796 ± 15	701 ± 17
Insulin 10 I.U./Kg.b.w	DE	CDE	AB	FG	BCD	AB	F	E	D
	67.4 ± 4	72.4 ± 3.6	81.6 ± 3.2	40.9 ± 6	22.3 ± 2.8	14 ± 0.5	859 ± 17	775 ± 16	659 ± 11
Soybean seeds 400mg/kg.b.w + Insulin 10 I.U./Kg.b.w .	CDE	ABC	AB	EFG	BC	A	F	D	A
	68.2 ± 2.8	77 ± 2.9	83.2 ± 2.1	36.5 ± 3.1	20 ± 3.5	4.9 ± 0.7	867 ± 29	604 ± 12	382 ± 21
Glibenclamide 5mg/kg.b.w	E	CDE	AB	G	BCD	A	F	E	E
	63.8 ± 2.5	71.5 ± 3	80.5 ± 2.9	43 ± 3	21.1 ± 1.6	9.7 ± 1.7	854 ± 18	788 ± 12	776 ± 11
Glibenclamide 5mg/kg.b.w + Soybean seeds 400 mg/kg.b.w	E	BCD	A	G	B	A	F	D	B
	64.7 ± 3.3	74.7 ± 1.8	84.6 ± 2.5	42.9 ± 4.6	18.3 ± 3.1	5.9 ± 0.8	867 ± 16	679 ± 12	433 ± 13

No. of rats (6-8) in each group, Data is the mean ± SEM,

Different letters indicate significant differences between groups horizontally and vertically at P < 0.05.

Glibenclamide have an action like insulin so increasing the glucose translator and increase metabolism of it (39).

Earlier study has shown that cholesterol level was decreased in mice treated with soybean seeds by (40), and this has not confirmed in present study. Therefore, we believed that 15 days would not provide adequate exposure to have an effect. Treatment with insulin significantly decreased cholesterol level, previous studies by (41) in diabetic rats, showed same results. Alternatively, inhibition of acylcoenzyme of cholesterol acyl transferase in intestine that's absorbing cholesterol from intestine may play a role in observed lowering of serum cholesterol level (23), also insulin increase or stimulate synthesis of ApoE mRNA that leads to decrease level of cholesterol (42). Glibenclamide significantly decreased cholesterol levels, (43) reported similar result in type 2 diabetic patient, but not similar to result of (44) in type 2 diabetic patient, the glibenclamide reduce lipolysis that stimulated by isoproterenol lead to decrease liberate free fatty acids and cholesterol in blood (45), also decrease of free fatty acids leads to decrease cholesterol level (46).

From the results soybean seeds reduced triglyceride level significantly, this result is in agreement with studies of (47) in diabetic rats, while (48) reported different result on postmenopausal women. Possible explanations for lowering triglyceride level include that soybean's content of isoflavones serve as a natural selective estrogen receptor modulator that exert an effect on lipid metabolism through their biological similarities to estrogen-receptor-dependent gene expressions. (49). or isoflavones affect in cellular lipid homeostasis by the down-regulation of sterol-regulatory-element-binding-protein (SREBP) and its target genes in the liver which are involved in the synthesis of triglyceride (50). Insulin treatment decreased triglyceride levels, a similar result reported by (36) in alloxan diabetic rats, insulin stimulate lipogenesis so increases level of triglyceride in fatty tissues and decreases its level in serum (51). Treatment with glibenclamide decrease triglyceride level, this result agree with (52) in type 2 diabetic patient, while (53) reported different result in type 2 diabetic patient. The reduction of triglyceride may be due to that glibenclamide is capable of exerting direct insulin like effect (54).

High density lipoprotein increased significantly in insulin treated rats; these result agreements with results of (55) in streptozotocin-induced diabetes rats, the insulin stimulate production and secret high density lipoprotein from intestine (56). Glibenclamide increase high density lipoprotein level, because it can act as insulin like effect as describe above.

Our study showed that rats treated with soybean resulted in reduction in low density lipoprotein levels; similar decreases was described previously in rats and hamster (57), our results disagreement with (58) in rabbit. This decrease duo to the fatty acids content of soybean

increase activity of receptors of low density lipoproteins on adipose cells (25). Insulin treatment produced significant decrease in low density lipoprotein levels; similar observation was reported by (24) in diabetic rats. The increase number of receptors on monocyte cells by insulin leading to increase removal of low density lipoprotein from blood (59). Treatment of rats with glibenclamide produce a significant decrease of low density lipoprotein levels, also (55) reported similar results in type 2 diabetic patients, but disagreement with (60), in type 2 patient.

Total lipids levels decreased when rats treated with either of soybean, or insulin, or glibenclamide, as alone, This may be due to reduction in levels of cholesterol, triglyceride, low density lipoprotein, leading to decrease levels of total lipids because they are the total amount of lipids in blood at constant percentage (27).

When there are a combination treatment of soybean with either insulin or glibenclamide the improvement of body weight and increase of high density lipoprotein level, also reduction of cholesterol, triglyceride, low density lipoprotein and total lipids levels become better than rats treated with soybean, insulin, or glibenclamide as alone. This due to synergism of activity of soybean with activity of either insulin or glibenclamide.

The results of the present investigation clearly indicate that soybean seeds in a dose of 400 mg/kg of body weight found to be effective as antidiabetic through managing the complications associated with diabetes such as body weight maintenance and hyperlipidaemia, also our results suggested that combinations of soybean seeds with either insulin or glibenclamide increased antidiabetic activity

References

1. Vats V, Grover JK, Rathi SS. Evaluation of anti-hyperglycaemic and hypoglycaemic effect of *Trigonella foenum-graecum* Linn., *Ocimum sanctum* Linn and *Pterocarpus marsupium* Linn in normal and alloxanised diabetic rats. *J Ethnopharmacol* 2002; 79: 95-00.
2. Ashok KT, Madhusudana RJ. Diabetes mellitus and multiple therapeutic approaches of phytochemicals: present status and future prospects *Current Science* 2002; 83(1):30-38.
3. Galletto R, Siqueira VL, Ferreira EB, Oliveira AJ, Bazotti RB. Absence of Antidiabetic and hypolipidemic effect of *Gymnema sylvestre* in non diabetic and alloxan diabetic rats. *Brazilian Archives of Biology and Technology* 2004; 47: 545-551.
4. Complementary & Integrative Therapies Soy (*Glycin max*). Natural Standard Resarch Collaboration. New York. USA. 2003.
5. Salmeron J, Manson JE, Stampfer MJ, Colditz G, Wing AL, Willett WC. Dietary fiber, glycemic load, and risk of non-insulin-dependent diabetes mellitus in women. *JAMA* 1997; 91:828-835.
6. Koba K, Lin JW, Bobik E, Mill DE, Sugano M, Huang YS. Effect of phytate in soy protein on the serum and liver cholesterol levels and liver fatty acid profile in rats. *Biosci Biotechnol Biochem* 2003; 7 (1): 15-22.
7. Demonty I, Lamarche B, Jones PT. Role of isoflavones in the hypocholesterolemic effect of soy. *Nutr Rev* 2003; 61 (6 pt 1): 189-203.
8. Azadbakht L, Shakerhosseini R, Atabak S, Jamshidian M, Mehrabi Y, Esmail-Zadeh A. Beneficiary effect of dietary soy protein on

- lowering plasma levels of lipid and improving kidney function in type II diabetes with nephropathy. *Eur J Clin Nutr* 2003; 57 (10): 1292-1294.
9. Ali AA, Velasquez MT, Hansen CT, Mohamad AI, Bhatena SJ. Effects of soybean isoflavones, probiotics and their interaction on lipid metabolism and endocrine system in an animal model of obesity and diabetes. *J Nutr Biochem* 2004; 15(10): 585-590.
 10. Katsumata K, Katsumata Y. The potentiating effect of the simultaneous administration of tolbutamide, glibenclamide, and gliclazide on the development alloxan - induced diabetes in rats. *Hum Metab Res* 1990; 22:51-52.
 11. Szkudelski T. The mechanism of alloxan and streptozotocin action in β cells of the rat's pancreas. *Physiol Res* 2001; 50: 536-546.
 12. Aziz OH. The Effect of Soybean Seeds on Some Biochemical Parameters in Normal and Alloxan -Induced Diabetic Rats. (M.Sc. thesis) College of veterinary medicine, university of Mosul Iraq 2005.
 13. Wohaieb SA, Godin DV. Alteration in free radical tissue defense mechanism in streptozotocin-induced diabetes in the rat: effect of insulin treatment. *Diabetes* 1987; 36:1014-1018.
 14. Mahomed IM, Ojewole JA. Hypoglycemic effect of Hypoxis hemerocallidea corm (African potato) aqueous extract in rats. *Methods Find Exp Clin Pharmacol* 2003; 25(8):617-623.
 15. Fox JG, Cohen BJ, Loew FM. *Laboratory Animal Medicine*. Academic press London, U.K. 1984: 19-120.
 16. Bruning JL, Kintz BL. *Computational Handbook of Statistics*. 2nd ed. Scott Foresman and Co. Glenview, Illinois USA 1977: 75-80, 102-138.
 17. Abed MA. Effect of Coriander plant on Some Biochemical Parameters in Normal and Alloxan-Induced Diabetic Rats. (M.Sc. thesis). College of veterinary medicine, university of Mosul Iraq 2002.
 18. Hakim ZS, Patel BK, Goyal RK. Effect of chronic ramipril treatment in streptozotocin-induced diabetic rats. *Indian J Physiol Pharmacol* 1997; 41:353-60.
 19. Rajagopal K, Sasikala K. Antihyperglycaemic and antihyperlipidaemic effects of *Nymphaea stellata* in alloxan-induced diabetic rats. *Singapore Med J Original Article* 2008; 49 (2): 137.
 20. Holm B. Diabetes mellitus in the dog. (Part 1). *Eur J comp anim Pract* 1997; 7: 61-66.
 21. Kheder E. The effect of dexamethasone on some biochemical parameters of normal and alloxan-induced diabetic rats. (M.Sc. thesis). College of veterinary medicine, university of Mosul Iraq 2007.
 22. Lenich AC, Hobanian AV, Brecher P, Zannis VI. Effect of dietary cholesterol and alloxan diabetes on tissue cholesterol and apo lipoprotein E mRNA levels in the rabbit. *J Lipids Res* 1991; 32 (3): 432-438.
 23. Hori M, Satoh M, Furukawa K, Sakamoto Y, Hakamoto H, Komahara Y. Acyl-Co-A: cholesterol acyl transferase-2 (ACAT-2) is responsible for elevated intestinal ACTA activity in diabetic rats. *Arterioscler Thromb Vasc Biol* 2004; 24:1968-1695.
 24. Kaleem M, Sarmade H, Bano B. Protective effect of *Piper nigrum* and *Vincarooea* in alloxan induced diabetic rats. *Indian J Physiol Pharmacol* 2005; 49(1): 65-71.
 25. Nelson DL, Cox MM. *Lehninger Principles of Biochemistry*. 4th ed. Worth Publishers. USA 2005: Pp., 790-885.
 26. Yin XZ, Quan JS, Takemichi K, Mukoto T. Antiatherosclerotic effect of soybean isoflavones and soyasaponins in diabetic rats. *Zhonghua Yu Fang Yi Xue Za Zhi* 2004; 35(1): 26-28.
 27. Murray RK, Granner DK, Mayes DA. Rod well VW. *Harper's Illustrated Biochemistry*. 26th ed. Appeton and lange. USA 2003: 180,223-352.
 28. Prince DS, Michael RU, Margaretha H, Allison W. Metformin improves vascular function in insulin-resisitant rats. *Hypertension* 2004; 35: 108-117.
 29. Ayoub RS, Yousif WH, Aziz BN. Serum glucose, cholesterol and total lipids levels and tissue lipid peroxidation in alloxan diabetic rats treated with aqueous extract of *Nigella sativa* seeds. *Iraqi J Vet Sci* 2000; 12(1):44-48.
 30. Kim S, Sohn I, Lee YS, Lee YS. Hepatic gene expression profiles are altered by genistein supplementation in mice with diet-induced obesity *J Nutr* 2005; 135 (1): 33-41.
 31. Davis J, Steinle J, Higginbotham DA, Oitker J, Perterson RG, Banz WJ. Soy protein influences insulin sensitivity and cardiovascular risk in male lean SHHF rats. *Horm Metab Res* 2005; 37(5):309-315.
 32. Hermansen K, Ndergaard M, Lars H, Carstensen M, Brock B. Beneficial effects of a soy based dietary supplement on lipid levels and subjects. *Diabetes Care*. 2001; 24: 228-233.
 33. Kakil SJ. Effect of thyme extract alone and in combination with insulin on some biochemical parameters in normal and alloxan-induced diabetic rats. (M.Sc thesis). College of veterinary medicine, university of Mosul Iraq 2005.
 34. Abdul-Rhmane SY. Effect of hungry and induced diabetes on levels of glutathione and lipid rancidity in rat tissue. (PhD thesis). University of Mosul Iraq 1995.
 35. Chatterjea MN, Shinde R. *Textbook of Medical Biochemistry*. 6th ed. Jaypee Brothers. India 2005: Pp., 511-513.
 36. Guton AC, Hall JE. *Textbook of Medical Physiology*. 11th ed. Elsevier Saunders Press, Philadelphia, USA. 2006: 963-969.
 37. Annamala PT, Augusti KT. Studies on the biochemical effects of glibenclamide on alloxan diabetic rabbits. *Cellular and Molecular Life Sciences* 1980; 36(4):383-384.
 38. Charles A, Charles RG, Rodney FC, Charles AS, Charles RG, Rodney FC. Effect of adding a sulfonylurea in patient with non-insulin-dependent diabetes mellitus previously well controlled with insulin. *Endocrine Practice* 1997; 3 (6): 344 - 348.
 39. Tayek JA. Low-dose oral glyburide reduces fasting blood glucose by decreasing hepatic glucose production in healthy volunteers without increasing carbohydrate oxidation. *Am J Med Sci* 1995; 309(3): 134-139.
 40. Kirk EA, Sutherland P, Wang SA, Chait A, Boen C. Dietary isoflavones reduce plasma cholesterol and atherosclerosis in (57BL/6) mice but not LDL receptor deficient mice. *J Nutr* 1998; 128 (6): 954-959.
 41. Mohamed IS. Effect of insulin, paracetamol, and oxytetracycline on Some Biochemical Parameters in Normal and Alloxan -Induced Diabetic Rats. (M.Sc thesis) College of veterinary medicine, university of Mosul Iraq 1998.
 42. Lenich CM, Chobanian AV, Brecher P, Zannis VI. Effect of dietary cholesterol and alloxan-diabetes on tissue levels in the rabbit. *J Lipid Res* 1991; 32(3): 432-438.
 43. Aleksandra SK, Jura G, Marta W, Marta K, Marcin S, Robert P, Wtadysaw G, Krzysztof S. Glucose and insulin profiles in type 2 diabetic patients treated with gliclazide MR and glibemipiride: an 8-week, randomised, single-centre, open-label, controlled, cross-over study. *Diabetologia Dowiadczenia i Kliniczna* 2007; 7(1).
 44. Waysbort J, Regitz G, Chaimowitz D, Tuval M, Nakash I, Brunner D. Effects of glibenclamide on serum lipids, lipoproteins, thromboxane, beta-thromboglobulin, and prostacyclin in non-insulin-dependent diabetes mellitus. *Clin Ther* 1988; 10(4):358-371.
 45. Suh KI, Murata C, Song YM, Goyce M, Gumbiner B, Ditzler TM, Henry RR. Intracellular glucose metabolism after long term metabolic control with glyburide: improved glucose oxidation with unchanged glycogen synthase activity. *J Clin Endocrinol Metab* 1993; 77(2): 464-470.
 46. Avogaro A, Valerio A, Gnudi L, Maran A, Zolli M, Duner E, Riccio A, Del PS, Tiengo A, Nosadini R. Ketone body metabolism in NIDDM effect of sulfonylurea treatment. *Diabetes* 1992; 41(8): 968-974.
 47. Kawakami Y, Tsurugasaki W, Nakamura S, Osada K. Comparison of regulative functions between dietary soy isoflavones aglycone and glucoside on lipid metabolism in rats fed cholesterol. *J Nutr Biochem*. 2005; 16(4): 205-212.
 48. Gruen DG, Silverstein DK. Usual dietary isoflavones intake is associated with cardiovascular disease risk factors in postmenopausal women. *J Nutr* 2001; 131: 1202-1206.
 49. Burger HG, Teede HJ. Endocrine changes in the perimenopause. In: Lobo RA, ed. *Treatment of the postmenopausal women*. 2nd ed. London: Lippencott Ravenwood, 1999:52-68.
 50. Shukla A, Brandsch C, Bettzieche A, Hirche F, Stangl GI, Eder K. Isoflavone-poor soy protein alters the lipid metabolism of rats by

- SREBP-mediated down-regulation of hepatic genes. *J Nutr Biochem* 2007; 18(5):313-21.
51. Swenson TL. The role of cholesteryl estertransfer protein in lipoprotein metabolism. *Diabetes Metab Rev* 1991; 7: 139-153.
 52. Angela AR, Lidia P, Geremia R, Francesca I, Lucrezia D, Giovanni A, Mario I, Gustavo AC, Gabriele R. Effect of Insulin and Sulfonylurea Therapy, at the Same Level of Blood Glucose Control, on Low Density Lipoprotein Subfractions in Type 2 Diabetic Patients. *J Endocrinology & Metabolism* 2000; 85(1): 4188-4192.
 53. Mori Y, Itoh Y, Obata T, Tajima N. Effects of pioglitazone vs glibenclamide on postprandial increases in glucose and triglyceride levels and on oxidative stress in Japanese patients with type 2 diabetes. *Endocrine* 2006; 29(1):143-8.
 54. Altan N, Ongun CO, Hasanoglu E, Engin A, Tuncer C, Sindel S. Effect of the sulfonylurea glyburide on superoxide dismutase activity in alloxan-induced diabetic rat hepatocytes. *Diabetes Res Clin Pract* 1994; 22(2-3): 95-98.
 55. David JS. Microsomal triglyceride transfer protein. *J Current opinion of lipodology* 1997; 8: 131.
 56. Tamai T, Nakai T, Yamada S, Kobayashi T, Hayashi T, Kutsumi Y, Oida K, Takeda R. The effect of glibenclamide and insulin on plasma high density lipoprotein in diabetics. *Artery* 1981; 9(6): 477-493.
 57. Balmir F, Staack R, Jeffrey E, Jimenez MD, Wang L, Potter SM. An extract of soy flour influences serum cholesterol and thyroid hormones in rats and hamsters. *J Nutr* 1996; 126(12): 3046-3053.
 58. Yousef MI, Esmail AM, Baghdadi HH. Effect of isoflavones on reproduction performance, testosterone levels, lipid peroxidation and seminal plasma biochemistry of male rabbits. *J Environ Sc Health B* 2004; 39 (5-6): 819-833.
 59. Duvillard L, Florentin E, Lizard G, Petit J, Galland F, Monier S, Gambert P, Verges B. Cell surface expression of LDL receptor is decreased in type 2 diabetic patients and is normalized by insulin therapy. *Diabetes Care* 2003; 26: 1540-1544.
 60. Skrapari I, Perrea D, Ioannidis I, Karabina SA, Elisaf M, Tselepis AD, Karagiannacos P, Katsilambros N. Glibenclamide improves postprandial hypertriglyceridaemia in type 2 diabetic patients by reducing chylomicrons but not the very low-density lipoprotein subfraction levels. *Diabet Med* 2001; 18(10):781-5.