ملخص البحث

التأثيرات الخافضة لسكر الدم والمضادة للإلتهابات لنبات البرسيم الحجازى فى الفئران

أجريت هذه الدراسة لتحقيق الدور الوظيفي لبذور وبراعم البرسيم في السيطرة على مستويات السكر فى الدم وعلاج الالتهابات فى الفئران المصابه بمرض السكر . تم تقسيم (ن = ٥٦) من ذكور الفئران البيضاء الى مجموعتين رئيسيتين. المجموعة الأولى (ن = ٦) تغذت على النظام الغذائي الأساسى خلال الفترة التجريبية كمجموعة ضابطة سالبة ، والمجموعة الثانية (ن = ٥٠) تم حقنها بمادة الآلوكسان (١٢٠ ملجم \ كجم من وزن الجسم) لإحداث مرض السكر وتم تقسيمها الى خمسة مجموعات فرعية ، ثم تم تغذيتهم على نظام غذائى مدعم بمستويين من بذور وبراعم البرسيم (٥٪ و ١٠٪) لمدة أربعة أسابيع. فى نهاية التجربه تم حساب نسبة الجلوكوز والأنسولين وإنزيمات الكبد و جليكوجين الكبد فى سيرم الدم كما تم حساب الهيموجلوبين السكري. وتم إصابة الفئران بالالتهابات عن طريق حقنها بالفورمالين فى مخالبها . حدث إنخفاض فى نسبة الجلوكوز وإنزيمات الكبد واليوريا والكرياتينين فى سيرم الدم وايضا الهيموجلوبين السكرى بشكل ملحوظ فى المجموعات التى تغذت على الغذاء الأساسى المدعم ببذور وبراعم البرسيم . كما أشارت النتائج الى حدوث زيادة كبيرة في نسبة الإنسولين و الجليكوجين فى السيرم بالمقارنة بالمجموعة المصابة بالسكر و لم يتم علاجها. أظهرت التحاليل النسيجية للكبد والكلى تحسنا في خلايا الكبد والأنابيب الكلوية مع العلاج ببذور البرسيم و براعمه . وكانت براعم البرسيم أكثر فاعلية من البذور فى علاج السكر والإلتهابات فى الفئران المصابة . ولذلك، ينبغي تشجيع برامج التثقيف الغذائي لمعرفة الجمهور بقيمة بذور البرسيم وبراعمه ودوره في السيطرة على إرتفاع السكر في الدم وعلاج الإلتهابات . وتشجيع اجراء البحوث فى هذا المجال .

كلمات البحث:

بذور البرسيم الحجازى– براعم البرسيم الحجازى – النوع الثانى من مرض السكر – الالتهابات – الفئران

Abstract

The Hypoglycemic and Anti-inflammatory Effects of Alfalfa (*Medicago sativa L.)* Plant in Rats

This study was carried out to investigate the functional role of alfalfa seeds and its sprouts in controlling diabetes mellitus and treating inflammation in rats. Male albino rats (n= 56) were divided into two main groups. The first group (n=6) was fed on basal diet during the experimental period as -ve control, the second group (n=50) was injected with alloxan (120 mg\kg) for diabetes induction and was divided into five subgroups then fed on diets with alfalfa seeds and sprouts at two levels of intake (5% and 10%) for four weeks. Serum glucose, insulin, liver function enzymes serum liver glycogen and glycated hemoglobin were determined. Feeding of diabetic rats on diets supplemented with alfalfa seeds or sprouts decreased blood glucose levels significantly. Results showed that liver function enzymes and glycated hemoglobin levels were significantly increased. Also, insulin as well as glycogen level were increased significantly compared to diabetic untreated group. Alfalfa seeds and its sprouts helped in healing inflammation compared with the untreated diabetic ones. The histological studies showed improved in hepatocytes and renal tubules with alfalfa seeds and sprouts supplementation. Alfalfa sprouts were more effective in controlling diabetes mellitus and inflammation than alfalfa seeds. Therefore, nutrition education programs should be encouraged to inform the public about the hypoglycemic and anti-inflammatory effects of alfalfa seeds and sprouts .Further research is required.

Keywords: alfalfa seeds-alfalfa sprouts-diabetes mellitus- inflammation- rats.

Siguel Agno lMD , Zuanazzi JAS (2012) : *In vivo* and *in vitro* anti-inflammatory activity of red clover *Trifoliumpratense* dry extract, *Curitiba* , 22 (1) .
Srinivasan M, Sudheer AR and MenonVP(2007) : Ferulic acid: therapeutic potential through its antioxidant property. *J .ClinBiochemNutr* ,**40**:92-100
Srinivasan S ,Muthukumaran J, Muruganathan U , Venkatesan RS and Jalaludeen AM(2014): Antihyperglycemic effect of syringic acid on attenuating the key enzymes of carbohydrate metabolism in experimental diabetic rats**.** *J .Biomedicine and Preventive Nutrition* , 4(4) : 595-602.
Temple C, clark P and Hales N (1992): Measurement of insulin secretion in type 2 diabetes: problems and pitfalls. *Diabetic medicine*, 9: 503- 512.
Trinder P (1969) : Enzymatic method of glucose estimation. *Ann ClinBiochem* , 6: 24-33.
TrivelliLA ,Ranney PH and Lai HT (1971):*New Eng .J. Med* 284-353 .
Tsai SJ , Huang CS ,Mong M C,Kam W Y , Huang HY and Yin MC (2012) : Anti-inflammatory and antifibrotic effects of naringenin in diabetic mice , *J. Agric. Food Chem*, *60* (1) : 514–521.
Vasco C, Ruales J , Kamal-Eldin A, (2008): Total phenolic compounds and antioxidant capacities of major fruits from Ecuador. *Food Chemistry* 111 (4):816-823.
Venkatesh S, Thilagavathi J and Sundar D (2008) :Antidiabetic activity of flowers of hibiscus rosasinensis, *Firoterapia,* 79: 79- 81.
Vuksan V , Rogovik AL, Jovanovski E, Jenkins AL (2009): Fiber facts: benefits and recommendations for individuals with type 2 diabetes, *currdiab rep*, 9(5):405-411.
WHO (2012): The World health statistics report , puts the spotlight on the growing problem of the noncommunicable diseases burden.
WHO (2013): The burdenof diabetes is increasing globally, particularly in developing countries.
WHO(2014) : Diabetes Programme.
Winiarska H , Dworacka M , Borowska M ,Kozłowska TB (Teresa Bobkiewicz-Kozłowska), Gorecki P , Mścisz A ,and Mrozikiewicz P M (2007) : The effects of plant extracts of *Medicago sativa and Trigonellafoenum-graecum* on postprandial glucose levels in type 2 diabetic rats. *Research Institute of Medicinal Plants*.53 (4) .
Yoon SA, Kang SI, Shin HS, Kang SW, Kim JH, Ko HC and Kim SJ (2013): p-Coumaric acid modulates glucose and lipid metabolism via AMP-activated protein kinase in L6 skeletal muscle cells. *Biochem and Biophys. Res. Commun*. 432 :553–557.
Zinca G and Vizireanu C (2013) : Impact of germination on phenolic compounds content and antioxidant activity of alfalfa seeds (*Medicago sativa* L.) ,*J. Agroalimentary Processes and Technologies* , 19(1), 105-110.

Mendel B , Kemp A and Myers DK (1954) :A colorimetric micro-method for the determination of glucose , *Biochem.J* . 56-639 .
Moharib SA and El-Batran SA (2008): Hypoglycemic effect of dietary fibre in diabetic rats .*J.Agriculture and Bio Sci* , 4(5): 455-461.
Narasimhan , AChinnaiyanM and KarundeviB (2015) : Ferulic acid exerts its antidiabetic effect by modulating insulin signalling molecules in the liver of high fat diet and fructose - induced type-2 diabetic adult male rat , *Applied Physiology, Nutrition, and Metabolism*, 10.1139/apnm- 0002.
NarenjkarJ ,Roghani M , Alambeygi H , Sedaghati F (2011) : The Effect of the flavonoid quercetin on pain sensation in
diabetic rats, *Basic and Clinical Neur Science* , 2 (3)
Navarro C ,Montilla P, Martin A, Jimenez J and Utrilla P(1993): *Plant Medicine***,** 59:312- 314.
NeeliGS ,Girase GS, Kute SH , Shaikh MI (2007):Antidiabetic activity of herb of *Cynodondactylon Linn*. inalloxan induced diabetic rats and in euglycemic rats. *Indian Drugs* ,44: 602–605.
Nishi ,Ahad A and Kumar P (2013) : Protective Effect Of chlorogenic acid against diabetic nephropathy in high fat diet/streptozotocin induced type-2 diabetic rats . *Int J Pharm Sci,* 5(2) :489-495 .
Northover B and Subramanian G (1962): Pedal inflammation induced by chemical agents. *Br. J. Pharmacol*, 18:346-349.
OyedemiSO ,Adewusi EA , Aiyegoro OA and Akinpelu DA (2011): Antidiabetic and haematological effect of aqueous extract of stem bark of afzeliaafricana (smith) on streptozotocin-induced diabetic wistar rats. *Asian Pac.J.Trop.Biomed.* 1(5):353-58.
Patel, Prasad, Kumar and Hemalatha(2012) : An overview on antidiabetic medicinal plants having insulin mimetic property. *Asian Pac J Trop Biomed* , 320–330.
Penarrieta JM, Alvarado J A, Akesson B and Bergenstahl B (2008) : Total antioxidant capacity and content of flavonoids and other phenolic compounds in canihua (Chenopodiumpallidicaule): an Andean pseudocereal, *Molecular Nutrition and Food Research*, 52 (6) : 708–717.
Qiu L, Chen T, Zhong F , Hong Y,Chen,Land Yeh O (2012) : Red clover extract exerts antidiabetic and hypolipidemic effects in db/db mice , *ExpTher Med.* 4(4): 699–704.
Ramos G P, Apel MA, Morais CBD, Ceolato PC, SchapovalEE
Reeves G, Nielsen H and Fahey C (1993): AIN-93 purified diets for laboratory rodents: final report of the American institute of nutrition ad hoc writing committee on the reformulation of the AIN-76A rodent diet. *J. Nutr*. 123:1939–1951.
Reitman S and Frankel S (1957): A colorimetric method for the determination of serum Reitman glutamic oxaloacetic and glutamic pyruvic transaminase. *Am. J. Clin. Path* , 28:56-63.
Robert E, Post MD, MS, Arch G. Mainous III, PhD, Dana E. King, MD, MS, and Kit N. Simpson, DrP H (2012):Dietary Fiber for the Treatment of Type 2 Diabetes Mellitus: A Meta-Analysis. *JABFM* 25 (1).

El Gohary O A, Shoman A A and Samea T A (2013) : Protective effect of quercetin on liver damage in streptozotocin- induced diabetic rats.
Elseweidy M M, AtefE ,Abd El-Baky and Mahmoud MF(2008): Green teacatechins as hypolipemic agents-probable mode of action. *J.ASMR*, 3(2): 95-101.
Gupta SC ,Patchva S , Koh W and Aggarwal BB (2012) : Discovery of curcumin, a component of the golden spice, and its miraculous biological activities, *Clin. Exp. Pharmacol. Physiol* ,39(3): 283-99.
Hamden K, Ayadi F, Jamoussi K, Masmoudi H and Elfeki A (2008) : Therapeutic effect of phytoecdysteroids rich extract from Ajugaiva on alloxan induced diabetic rats liver, kidney and pancreas. *Bio Factors*, 33:165–175**.**
Harini R and Pugalendi KV (2010) : Antihyperglycemic effect of protocatechuic acid on streptozotocin diabetic rats , *Freund Publishing House Ltd*, *608 -002* .
Hegsted D, Mills R ,Elvehjen C and Hrat E (1941): Salt mixture. *J. Biol. Chem* , 138-149.
Hong YH, Chao WW, Chen ML and Lin BF (2009$_a$): Ethyl acetate extracts of alfalfa (Medicago sativa L.) sprouts inhibit lipopolysaccharide-induced inflammation in vitro and in vivo . *J Biomed Sci* (1)16-64.
Hong YH, Huang CJ, Wang SC and Lin BF(2009$_b$): The ethyl acetate extract of alfalfa sprouts ameliorates autoimmune-prone disease of MRL-lpr/lpr mice.*Lupus* , 18:206-215.
IgweOU andOkwu DE (2013) : Phytochemical composition and anti-inflammatory activities of *Brachystegiaeurycoma*seeds and stem bark, *Der PharmaChemica* , 5(1):224-228
Joseph S ,Sabulal B, George V, Smina TP and Janardhanan KK (2009): Antioxidative and anti-inflammatory activities of the chloroform extract of Ganodermalucidum found in south india. Sci. Pharm, 77: 111–121.
Kumar PA, Haseeb A, Suryanarayana P, Ehtesham NZ, Reddy GB (2005) : Elevated expression of alpha- and alphaB-crystallins in streptozotocin-induced diabetic rat. *Arch BiochemBiophys* , 444: 77-83.
Kundan SB and Anupam S (2011): Evaluation of antioxidant and cerebroprotective effect of *Medicago sativa Linn*. againest ischemia and reperfusion insult. *Evid Based Complement Alternat. Med*. , 1-9.
Liang B, Guo Z, Xie F and Zhao A (2013): Antihyperglycemic and antihyperlipidemic activities of aqueous extract of *Hericiumerinaceus* in experimental diabetic rats. *BMC Complementary and Alternative Medicine* , 13-253 .
Malini P, Kanchana G and Rajadurai M (2011) : Antibiabetic efficacy of ellagic acid in streptozotocininduced diabetes mellitus in albino wistar rats , *Asian J Pharm Clin Res*,4(3): 124-128.
Mbaka GO, Ogbonnia S O, Oyeniran KJ and Awopetu PI (2012): Effect of *raphiahookeri*seed extract on blood glucose, glycosylated haemoglobin and lipid profile of alloxan induced diabetic rats *British J. Medicine & Medical Research* 2(4): 621-635

Reference

AbouZaid OAR , El-Sonbaty SM and Hamam N M A (2014) : Biochemical role of ferulic acid in modulation and treatment of hyperglycemiaassociated with hyperlipidemia , *J,Benha Veterinary Medical* , 27 (2):375-381.

Al sadek HA (2013) : Nutrition , technological and microbiological studies of alfalfa seeds .

Amalan and vijayakumar (2015) : Antihyperglycemic effect of p-coumaric acid on Streptozotocin induced Diabetic Rats, *J. Indian of applied research* , 5 (1) 2249-555X

American Diabetes Association (2014) :Diagnosis and classification of diabetes mellitus. *Diabetes Care* 37 (1) 81- 90.

Armitage P and Berry G (1987): Statistical Methods in Medical Research. *Blackwell, Oxford*, 93 – 213.

Bancroft J D , Cook H C , Stirling RW and Turner DR (1994): Manual of histological techniques and their diagnostic applications, 2nd ed. *Churchill alaivingstone, Edinburgh, London.*

BaxiDB , Singh PK , Doshi AA , Arya S, Mukherjee R and Ramachandran AV (2010) :*Medicago Sativa* leaf extract supplementation corrects diabetes induced dyslipidemia, oxidative stress and hepatic renal functions and exerts antihyperglycaemic action as effective as Metformin, *Annals of Biological Research* , 1(3) : 107-119.

Bhardwaj P, Khanna D, Balakumar P (2014) :Catechin averts experimental diabetes mellitus-induced vascular endothelial structural and functional abnormalities . *Cardiovascular Toxicology* ,14(1), 41-51

BirosovaL ,Mikulasova M and Vaverkova S (2007): Phenolic acids from plant foods can increase or decrease the mutation frequency to antibiotic resistance, *J .Agricultural and Food Chemistry*, 55 (25) : 10183–10186.

Campbell J (1963): Methodology of protein evaluation PG. Nutri. Document R101 Add. *Meeting, New York.*

Caunii A , Pribac G , Grozea I , Gaitin D and Samfira I (2012) :Design of optimal solvent for extraction of bio–active ingredients from six varieties of *Medicago sativa*. *J ,Chemistry Central*, 6:123.

Chapman D, Gastilla C and Campbell J (1959): Evaluation of protein in food .I.A. method for the determination of protein efficiency ratio. *Can. J. Biochem.Pysiol*, 37:679-686.

Choi R, Kim BH, Naowaboot J , Lee MY, Hyun M R , Cho EJ , Lee EY, YangYC and ChungCH(2011) : Effects of ferulic acid on diabetic nephropathy in a rat model of type 2 diabetes, *Experimental & Molecular Medicine* 43: 676-683 .

Colodny LR, Montgomery A and Houston M (2001) : The role of esterin processed alfalfa saponins in reducing cholesterol. *J. Am NutraceuticalAssoc* , 3:1-10.

Daisy P and Rajathi M (2009) : Hypoglycemic effects of *Clitoriaternatea*linn. *(fabaceae*) in alloxan-induced diabetes in rats , *Trop J .Pharm Res* , 8 (5): 393-398.

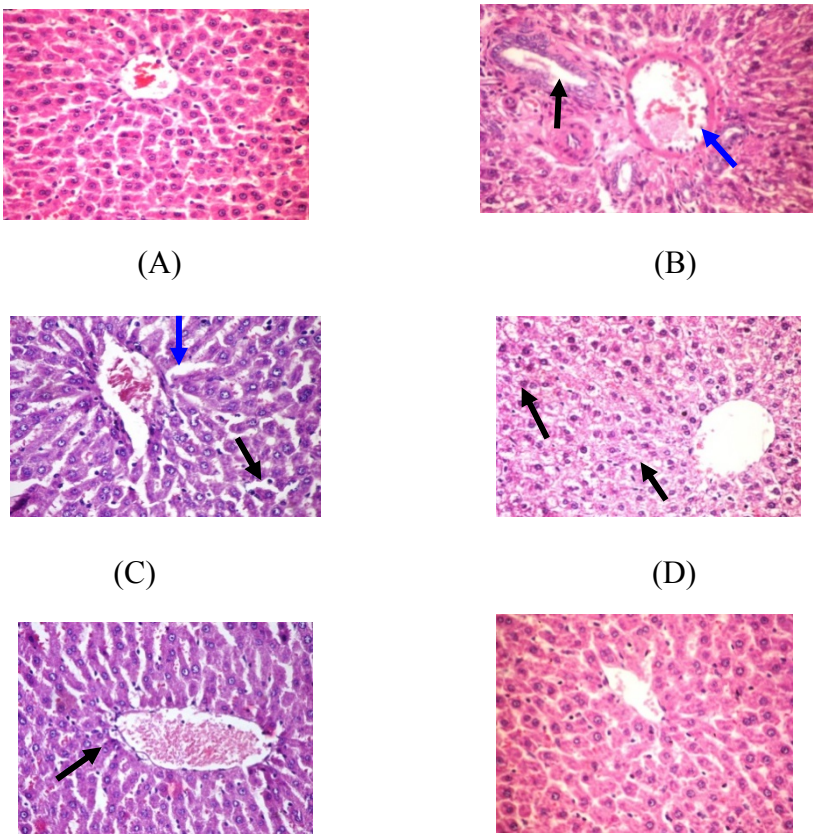

Figure (1) Effect of Diet Supplementation with Alfalfa Seeds and Its Sprouts on the Histology of Liver Tissues of Rats.

(A) Normal control diet (B) positive control groups
(C) Basal diet supplemented with 5% alfalfa seeds powder.
(D) Basal diet supplemented with 10% alfalfa seeds powder.
(E) Basal diet supplemented with 5% alfalfa sprouts powder.
(F) Basal diet supplemented with 10% alfalfa sprouts powder.
(H and EX 200)

Table (6): Effect of Diet Supplementation with Alfalfa Seeds and Its Sprouts on Serum Liver Enzymes Concentration of Rats.

Variables / Groups	ALT u/l	AST u/l
Control (-)	26.58 ± 1.29^{e}	48.43± 1.39^{d}
Control (+)	51.87 ± 2.26^{a}	95.50 ± 5.41^{a}
5% Alfalfa Seeds	44.77 ±1.38^{b}	82.70± 2.09^{b}
10% Alfalfa Seeds	40.17±1.94^{c}	67.92± 3.71^{c}
5 % Alfalfa Sprouts	32.28 ±2.51^{d}	64.58 ±3.49^{c}
10 %Alfalfa Sprouts	28.78 ±1.48^{e}	52.12 ±2.21^{d}

Mean values are expressed as means ± SD.
Means with different superscript letters in the column are significantly different at P≤ 0.05.
Control (-) = Normal rats fed basal diet. Control (+) = Diabetic rats fed basal diet.
ALT : Alanine Aminotransferase.AST **:** Aspartate Aminotransferase

Table (7): Effect of Diet Supplementation with Alfalfa Seeds and Their Sprouts on the Paw's Thickness of Rats after Inflammation Induction.

Time / Groups	Zero Time	3 hours		6 hours		First day		Second days		Fourth days		Sixth days	
		±	%	±	%	±	%	±	%	±	%	±	%
Control (+)	1.50 ± 0.20^{a}	5.50 ± 0.50^{a}	----	5.50 ± 0.50^{a}	----	5.83± 0.58^{a}	----	5.33 ± 0.58^{a}	----	4.67 ± 0.58^{a}	----	4.50± 0.50^{a}	----
5% Afalfa Seeds	1.53± 0.06^{a}	4.81 ± 0.29^{b,a}	13	4.83 ± 0.29^{a,b}	12	4.67 ± 0.58^{b}	20	4.50 ± 0.50^{a,b}	16	4.08 ± 0.38^{a,b}	13	3.42± 0.38^{b}	24
10% Alfalfa Seeds	1.57± 0.12^{a}	4.85 ± 0.32^{b,a}	12	4.67 ± 0.29^{a,b}	15	4.50 ± 0.50^{b}	23	4.17 ± 0.76^{b,c}	22	3.33 ± 0.76^{b,c}	29	3.13± 0.38^{b}	30
5 % Alfalfa Sprouts	1.50± 0.20^{a}	4.17 ± 0.29^{b}	24	4.33 ± 0.58^{b}	21	4.17± 0.76^{b}	28	3.50 ± 0.50^{b,c}	34	2.83 ± 0.76^{b}	39	2.00± 0.50^{c}	56
10% Alfalfa Sprouts	1.63± 0.15^{a}	3.33 ± 0.58^{c}	39	3.83 ± 0.76^{b}	30	3.67± 0.58^{b}	37	3.33 ± 0.58^{c}	38	2.67 ± 0.58^{b}	43	1.83± 0.29^{c}	59

Mean values are expressed as means ± SD.
Means with different superscript letters in the column are significantly different at P≤ 0.05. Control (+) = Diabetic rats fed basal diet % : Inhibition

Table (4): Effect of Diet Supplementation with Alfalfa Seeds and Its Sprouts on Feed Intake (FI), Body Weight Gain (BWG) and Feed Efficiency Ratio (FER) of Rats.

Variables / Groups	Feed Intake g/day	BWG g	FER
Control (-)	15.12± 1.05 ^{d}	40.17± 2.64 ^{a}	0.095 ± 0.008 ^{a}
Control (+)	20.17± 0.93^{a}	17.00 ± 2.61^{d}	0.030 ± 0.005 ^{f}
5%Alfalfa Seeds	17.75± 0.88 ^{b}	21.33± 1.21^{c}	0.043± 0.004 ^{e}
10%Alfalfa Seed	17.00± 0.63^{b,c}	23.67± 2.16 ^{c}	0.050 ± 0.006^{d}
5%Alfalfa Sprouts	16.25± 1.08^{c,d}	26.42 ± 1.36^{b}	0.058± 0.004 ^{c}
10%Alfalfa Sprouts	15.33± 0.98 ^{d}	28.00 ± 1.67 ^{b}	0.065± 0.003^{b}

Mean values are expressed as means ± SD.
Means with different superscript letters in the column are significantly different at P≤ 0.05.
Control (-) = Normal rats fed basal diet.Control (+) = Diabetic rats fed basal diet.

Table (5): Effect of Diet Supplementation with Alfalfa Seeds and Its Sprouts on some glycemic parameters of Rats.

Variables / Groups	Insulin µ/ml	Glucose mg/dl	Glycogen ng/L	HbA_{1c} %
Control (-)	13.94± 1.80^{a}	92.17± 9.13^{d}	175.62± 2.60^{a}	5.53 ± 0.94^{e}
Control (+)	7.24± 0.69^{c}	235.83± 22.72^{a}	65.78± 4.91^{e}	13.58 ± 1.48^{a}
5% Alfalfa Seeds	11.23 ± 1.57^{b}	151.00 ±12.43^{b}	110.00± 5.50^{d}	11.75 ± 0.91^{b}
10% Alfalfa Seeds	11.66 ± 1.78^{b}	149.33± 15.58^{b}	117.85± 9.88^{c}	10.65 ± 1.04^{b,c}
5% Alfalfa Sprouts	11.42± 1.73^{b}	140.00± 9.55^{b}	120.02± 6.34^{c}	9.90± 1.64^{c}
10 % Alfalfa Sprouts	12.65 ± 1.41^{a,b}	121.50± 12.72^{c}	154.51± 2.85^{b}	7.98± 0.83^{d}

Mean values are expressed as means ± SD. HbA_{1c} :Glycated hemoglobin
Means with different superscript letters in the column are significantly different at P≤ 0.05.
Control (-) = Normal rats fed basal diet. Control (+) = Diabetic rats fed basal diet.

Table (1) : Chemical Composition of Alfalfa Seeds and Its Sprouts (Dry Weight %).

Type / Constituent	Alfalfa Seeds %	Alfalfa Sprouts %
Protein	40.274	41.622
Fat	19.158	13.215
Total Carbohydrate	29.823	31.352
Crude Fiber	15.450	15.180
Moisture	07.245	10.273
Ash	03.498	03.536

Table (2) : Active Flavonoid Compounds Content in Alfalfa seeds and Its sprouts (ppm).

Type / Flavonoids	Alfalfa Seeds (ppm)	Alfalfa Sprouts (ppm)
Romarinic	5.38	10.27
Quercetin	2.39	36.79
Narenginin	0.15	8.53
Kampferol	0.36	3.44
Luteolin	33.07	57.86
Rutin	78.43	20.70

Table (3): Active Phenolic Compounds Content in Alfalfa Seeds and Its Sprouts (ppm).

Type / Phenolic Compounds	Alfalfa seeds (ppm)	Alfalfa sprouts (ppm)
Syringic	1582.19	45351.08
Gallic	10.80	9.40
Protocatechuic	33.42	233.06
Catechein	107.32	839.47
Chlorogenic	82.07	471.75
Epicatechen	10.72	59.88
Caffeic	1.24	16.82
P-Coumaric	7.14	5.24
Ferulic	7.05	39.97
Iso-ferulic	4.32	13.26
Ellagic	59.06	10.05

(**Navarro *et al*., 1993**) which gives an indication on the hepatotoxic effect of alloxan. The present results showed that treatment of diabetic rats by alfalfa seeds and sprouts significantly lowered ALT and AST concentrations in diabetic animals compared with untreated diabetic ones. The ability of alfalfa seeds (15% of diet) to cause significant reduction in serum ALT and AST were confirmed by **Al sadek , (2013)** in hyperlipedemic rats .These improvements may be related to the effect of some constituents such as phytochemicals in alfalfa seeds and sprouts which function as antioxidant and in turn may improve the function of hepatocytes. The sprouts however showed more effective decrease in ALT and AST may be due to its high content in flavonoids such as quercetin and phenolic compounds such as ferulic acid compared to alfalfa seeds. each compound had significant effect in decreasing ALT and AST(**El Gohary *et al* ., 2013** , **AbouZaid *et al* ., 2014).**

Formalin-induced paw edema is also one of the most suitable test procedures to screen chronic anti-inflammatory agents as it closely resembled human arthritis **(Joseph *et al* ., 2009).**Different phenolic acid compounds, such as gallic acid, vanillic acid, caffeic acid, coumaric acid, and epicatechin as antioxidants, have several biological activities to reduce lipid peroxyl radicals, inhibit lipid peroxidation, and protect our cells against oxidative damage **(Birošová *et al* ., 2007 , Penarrieta *et al* ., 2008).** Alfalfa seeds and its sprouts helped in inflammation healing compared with +ve control. This improvement may be due to the presence of active phenolic compounds
such as ferulic acid and flavonoid compounds such as naringenin and quercetin. The alfalfa sprouts however showed the highest improvement in inflammation healing may be due to that it contains a larger proportion of the active compounds. Flavonoids have been reported to have high anti-inflammatory properties **(Igwe and Okwu , 2013).** The present results was in the same line with (**Hong *et al* ., 2009$_b$**)that component of alfalfa sprout ethyl acetate extract exerted anti-inflammatory effect. Also , **Ramos *et al* ., (2012)**reported that the oral administration of the red clover dry extract inhibition of rat pew edema. Several studies revealed that ferulic acid (**Srinivasan *et al*., 2007)**, catechins (**Elseweidy*et al* ., 2008)**, naringenin (**Annadurai *et al* .,(2013))** andp-coumaric acid (**Yoon *et al* .,2013**) have antioxidant and anti-inflammatory effects.

Results of the histological examination of the liver of hyperglycemic rats fed on diet supplemented with alfalfa seeds and its sprouts revealed a dose dependent reduction of degenerative changes caused by alloxan. The histological findings are affirmed by the observed improvements in AST and ALT .The curative effect of alfalfa seeds and their sprouts may be related to the presence of certain constituents such as flavonoids and phenolic compounds.

,catechin(**Bhardwaj *et al*., 2014**), protocatechuic acid **(Harini and Pugalendi , 2010),** chlorogenic acid **(Nishi *et al* ., 2013)** ,ferulic acid **(Narasimhan *et al* ., 2015)**and p-coumaric acid**(Amalan and vijayakumar, 2015).**

Another possible mechanism by which alfalfa seeds and sprouts supplementation may bring their hypoglycemic action is attributed to their fiber content. In this context ,**Moharib and El-Batran , (2008), Robert *et al*., (2012)** reported that fiber supplementation for type 2 diabetes mellitus can reduce fasting blood glucose and HbA_{1c} in rats and human respectively. Possible mechanisms for metabolic improvements with dietary fiber include delay of glucose absorption from intestine, increase in pancreatic extraction of insulin and increased insulin sensitivity at the cellular level (**Vuksan *et al*., 2009**).

Liver glycogen content was significantly reduced in alloxan induced diabetic rats as compared to -ve control group. **Daisy and Rajathi ,(2009**) reported that the decrease in glycogen content of liver and skeletal muscle observed in diabetic rats is probably due to lack of insulin in the diabetic state.

In the present study, alfalfa seeds and sprouts supplementation restored the depressed glycogen levels in diabetic rats compared to untreated diabetic group possibly by increasing the level of insulin as a result of the presences of phytochemicals which may enhance insulin secretion or have insulin like effects. Alfalfa sprouts however, showed the highest improvement in liver glycogen content may be due to that it contain a larger proportion of the active compounds. **Malini *et al*., (2011)** stated that oral administration of ellagic acid to diabetic rats significantly increased liver and muscle glycogen content by stimulating the remnant β-cells to release insulin.

In accordance with the present results, **Kumar et *al*., (2005)** reported that in the diabetic group, HbA_{1C} level increased significantly suggesting glycosylation of hemoglobin in the presence of hyperglycaemia. In the alfalfa seeds and sprouts supplemented diabetic groups, marked decreases in HbA_{1C} concentration were observed when compared to that of diabetic untreated animals indicating decrease in blood glucose level and recovery to hemoglobin. The sprouts however, showed more effective decrease, confirming its anti-diabetic potency may be due to that alfalfa sprouts contain active compound such as syringic and chlorogenic acid in higher amounts than in alfalfa seeds. This effect seems to be a result of the noticed reduction of blood glucose level and the improvement in insulin secretion. Active constituents found in alfalfa seeds and sprouts may play a role in improving HbA_{1C} concentration of diabetic treated rats. Several studies concluded that administration of ellagic acid (**Malini *et al* ., 2011),**chlorogenic acid **(Nishi *et al* ., 2013),** syringic acid **(Srinivasan *et al* ., 2014)** andp-coumaric acid (**Amalan and vijayakumar , 2015)** significantly reduced levels of HbA1c in diabetic treated rats.

The activities of liver enzymes (AST and ALT) increased significantly in diabetic rats were in agreement with previous findings reported by **Hamden *et al*., (2008)** . The increased activities of serum AST and ALT may be due to the leakage of these enzymes from the liver cytosol into the blood stream

alfalfa seeds and sprouts treated diabetic groups, showed significant improvement in body weight gain and moderation in food intake compared to positive control group. This may be related to the protein content of alfalfa seeds and sprouts which may has protective effect in controlling muscle wasting, i.e. reversal of gluconeogenesis and may also be due to proper glycemic control. In addition, the presence of some active phenolic compounds such as p-coumaric , protocatechuic acid and ferulic acid in alfalfa seeds and sprouts may have indirect effect on body weight gain and feed intake through their effects on controlling serum glucose and improving serum insulin. Compared to diabetic rats fed on diet supplemented with alfalfa seeds, diabetic rats fed on diet supplemented with alfalfa sprouts showed better improvements in body weight gain and feed intake perhaps due to that alfalfa sprouts contain higher levels of many active compounds than alfalfa seeds .Several studies reported that protocatechuic acid(**Harini and Pugalendi, 2010)**, ferulic acid **(Choi *et al* ., 2011)** , p-coumaric acid **(Amalan and vijayakumar, 2015)**had the ability to increase body weight of the diabetic rats.

The results of the present study indicated that alfalfa seeds and its sprouts significantly reduced fasting serum glucose level and significantly improve serum insulin concentration in diabetic rats. In accordance with the present results, **Winiarsk*a et al*., (2007)** found that *Medicago sativa L.* extract modulate post-challenge carbohydrate metabolism in type 2 diabetes animal model by enhancing insulin secretion . On the same line ,**Baxi*et al*., (2010**) reported that *Medicago sativa L.* leaf extract was found to be very effective in lowering diabetic hyperglycaemia in four weeks by about 42%.while **Qiu*et al* ., (2012)** reported that red clover extract treatment attenuated hyperglycemia in type 2 diabetic animals.

The hypoglycemic effects alfalfa seeds and sprouts may be attributed to the constituents found in the two forms alfalfa plant. From the phytochemical analysis, it was found that both alfalfa seeds and sprouts, in lower extent for most, contain flavonoids such as (quercetin, naringenin and rutin) and phenolic compounds(such as syringic, catechein, chlorogenic,p-coumaric, ferulic, gallic, ellagic, protocatechuic, , isoferulic, and caffeic). Compared to diabetic rats fed on diet supplemented with alfalfa seeds,and sprouts showed better improvements in serum insulin and glucose may be due to that alfalfa sprouts contain active compound such as syringic, catechein, chlorogenic and protocatechuic higher amounts than in alfalfa seeds. On the light of this, several studies concluded that quercetin(**Narenjkar *et al*., (2011) ,** ellagic acid and rutin**(Oyedemi *et al*., 2011**) and naringenin (**Tsai *et al* ., (2012)** caused significant decrease in the serum glucose.

Phenolic compounds have been found to be beneficial in controlling diabetes and many other diseases as evident from earlier studies (**Vasco *et al*., 2008**). The benefits towards many of these conditions come in part through the antioxidant characteristic of phenols **(Zinca and Vizireanu , 2013).** Both supplements showed presence of phenolic compounds. Several studies revealed the anti hyper glycemic effects of syringic acid (**Srinivasan *et al* ., 2014**)

mm,while rats fed on diet supplemented with 5% alfalfa sprouts exhibit reduction paw´s thickness by 24.18 % ,
whilerats fed on diet supplemented with 10% alfalfa sprouts exhibit reduction paw´s thickness by 39.45 % .
After 6 hours of inflammation induction , rats fed on diet supplemented with 5% alfalfa seeds exhibit reduction paw´s thickness by 12.18 %, while rats fed on diet supplemented with 10% alfalfa seeds exhibit reduction paw´s thickness by 15.09 % with mean value of 4.67±0.29 mm . Rats fed as diet supplemented with 5% alfalfa sprouts exhibit reduction paw´s thickness by 21.27 % with mean value of 4.33 ± 0.58 mm , while rats fed on diet supplemented with 10% alfalfa sprouts exhibit reduction paw´s thickness by 30.36 %.
Similarly, results of percent inflammation inhibition and paw´s thickness after one, four and six days of inflammation induction revealed that all treatments were able to reduce the incidence of inflammation especially10% alfalfa sprouts.

Concerning histological results, microscopically,liver of -ve control rats fed on normal diet revealed normal histological structure as shown in Fig.1 .Liver of rat from +ve control group showed obvious changes such as congestion of hyperplastic bile duct, and congested hepato-portal blood vessel .Liver of the diabetic group fed on diet supplemented with 5% alfalfa seeds revealed moderate changes in the liver tissue including dilated blood sinusoids with disorganized hepatic cords. Also, the diabetic group fed on diet supplemented with 10% alfalfa seeds revealed moderate changes in the liver tissue including vacuolated enlarged hepatocytes with narrowed blood sinusoids.
On the other hand, diabetic group fed on diet supplemented with 5% alfalfa sprouts indicated mild liver changes including vacuolated enlarged hepatocytes. Interestingly, microscopic examination of liver tissue of diabetic group fed on diet supplemented with 10% alfalfa sprouts showed apparently healthy hepatic parenchyma.

Discussion

Regarding bioactive compounds found in alfalfa seeds and sprouts, **Kundan *et al.*, (2011)** reported that extract phytochemical screening of *Medicago sativa* L. extract showed the presence of flavonoids. This study was undertaken to evaluate the anti-diabetic activity of alfalfa seeds and their sprouts and its usefulness in managing inflammation.

The present results were in agreement with **Gupta *et al.*, (2012),** and **Mbaka *et al.*, (2012**) who reported that, diabetic rats showed reduction in weight gain compared with non diabetic rats. **Liang *et al.*, (2013)** reported that when insulin is deficient and the cells cannot metabolize glucose for energy, the cells compensate by increasing their metabolism of proteins and fats. However,

improvement was observed in the group receiving 5% alfalfa seeds while the greatest improvement was observed in the group receiving10% alfalfa sprouts.

The level of $HbA_{1C}$was significantly increased in diabetic rats compared to normal rats representing 13.58 ± 1.48% and 5.53 ± 0.94 %, respectively (Table 5). The administration of alfalfa seeds at 5% and 10 % to the diet of diabetic rats resulted in significant ($p \leq 0.05$) decrease in HbA_{1C} levels compared to the diabetic untreated positive control group representing 11.75± 0.91% and 10.65± 1.04 %vs. 13.58 ± 1.48%, respectively. On the contrary, the administration of alfalfa sprouts at the same levels to the diet of diabetic rats resulted in significant decrease in HbA_{1C}level compared to the diabetic untreated positive control group representing 9.90± 1.64% and 7.98± 0.83%vs. 13.58 ± 1.48%, respectively. It can be concluded that 10% of alfalfa sprout is the most effective level in the diet which give the best results in glycogen and glycated haemoglobin.

Induction of diabetes caused significant elevation in both alanine amino transferase(ALT) and aspratate amino transferase (AST) values in serum of positive control group compared to the negative control group representing51.87 ± 2.26u/l vs. 26.58 ± 1.29u/l , respectively for ALT concentration and 95.50 ± 5.41 u/l vs. 48.43± 1.39 u/l for AST levels, respectively(Table 6). The concentration of ALT significantly decreased in the diabetic groups supplemented with alfalfa seeds or its sprouts at 5% and 10% levels of intake compared with the positive control group. The highest reduction in ALT concentration was observed when rats were fed diet supplemented with 10%alfalfa sprouts followed by 5% alfalfa sprouts.

The addition of the tested two levels of alfalfa seeds and its sprouts caused significant reduction in AST concentrations compared to the positive control group(Table 6). AST values were 82.70± 2.09 u/l and 67.92± 3.71 u/l for 5% and 10% alfalfa seeds groups , respectively ,while they were 64.58 ±3.49 u/l and 52.12 ±2.21u/l for 5% and 10% alfalfa sprouts groups, respectively. The most pronounce improvement in AST activities was observed in the group of diabetic rats supplemented with 10% alfalfa sprouts in the diet .No significant difference was observed between this group and AST value of negative control group representing 52.12 ± 2.21u/lvs. 48.43 ± 1.39u/l , respectively.

Diet supplemented with the two levels of alfalfa seeds as well as sprouts significantly decreased the paw´s thickness of experimental rats after induction of pedal inflammation by formalin compared by the diabetic group. After 3 hours of inflammation induction , rats fed diet supplemented with 5% alfalfa seeds exhibit reduction paw´s thickness by 12.54% with mean value of 4.81±0.29mm, while rats fed on diet supplemented with 10% alfalfa seeds exhibit reduction paw´s thickness by 11.81% with mean value of 4.85±0.32

Feed efficiency ratio (FER) of negative control group was significantly higher than that of positive control group (P≤ 0.05) with a mean value of 0.095 ± 0.008and 0.030 ± 0.005, respectively. Diabetic rats fed on diets supplemented with alfalfa seeds and its sprouts at the two levels of intake showed significant increase in FER with mean values of 0.043± 0.004, 0.050 ± 0.006 ,0.058±0.004 and 0.065±0.003 ,respectively compared to the positive control group.
The lowest improvement in FER was observed in the diabetic group received 5% alfalfa seeds, while the greatest improvement was observed in the diabetic group received 10% alfalfa sprouts compared with the positive control group.

Serum insulin concentration of normal rats fed on basal diet (negative control group) was 13.94±1.80µ/ml, while it was 7.24±0.69 µ/ml for diabetic rats fed on basal diet (positive control group) as shown in Table(5) . Serum insulin concentration was decreased significantly by inducing diabetes. When diabetic rats were fed on diet supplemented with 5 and 10% alfalfa seeds, serum insulin concentrations were significantly improved with mean values of 11.23±1.57 µ/ml and 11.66± 1.78 µ/ml, respectively compared to the positive control group. The same trend was observed in serum insulin concentrations of rats had diets supplemented with 5% and 10% alfalfa sprout. Diet supplementation with 10% alfalfa sprout had the best serum insulin concentration among all tested groups compared with the negative control group.
Positive control group showed significant increase in serum glucose level representing 235.83±22.72 mg/dl compared with negative control (92.17±9.13 mg/dl) (Table 5). Fasting blood glucose concentration in diabetic rats had fallen down following the administration of alfalfa seeds and its sprouts in the diet at the two levels of intake compared with the positive control group representing 151.00±12.43mg/dl, 149.33±15.58mg/dl,140.00±9.55mg/dl and 121.50± 12.72 mg/dl for 5%, 10% seeds and 5% and 10% alfalfa sprouts ,respectively. Interestingly, the results revealed that the most pronounced reduction in fasting blood glucose level was observed when rats were fed on diet containing 10% alfalfa sprouts.

Positive control diabetic group showed significant lower serum glycogen concentration than that of the negative normal control group 175.62± 2.60 ng/L vs.65.78± 4.91ng/L, respectively (Table 5). When rats were fed on alfalfa seeds or sprouts at any level of intake, their serum glycogen concentrations were increased significantly compared with positive control group. Results showed that serum glycogen levels in the diabetes treated groups fed on diets supplemented with 5% or 10% alfalfa seeds and 5% or 10% alfalfa sprouts were 110.00± 5.50ng/L, 117.85±9.88ng/L,120.02± 6.34ng/L and154.51±2.85ng/L , respectively . The lowest significant glycogen

Results

The chemical analysis of alfalfa seeds and its sprouts(Table1) revealed that protein contents were 40.274% vs. 41.622% , concentrations reached fat were 19.158% vs. 13.215% , while carbohydrate were 29.823% vs. 31.352% , crude fiber 15.450 % vs. 15.180% , moisture 07.245 % vs. 10.273% and ash 03.498% vs. 03.536%, respectively.

Alfalfa sprouts had higherflavonoids compounds than alfalfa seeds (Table2) . Flavonoid compounds found in alfalfa seeds and their sprouts include for example romarinic, quercetin ,narenginin, kampferol , luteolin and rutin. The highest concentration of flavonoid compounds was found in alfalfa seeds as rutin with mean value of 78.43 ppm , which was decreased in the sprouts (20.70 ppm) . However, luteolin content was found in alfalfa seeds with concentration of 33.07 ppm, which was increased in alfalfa sprouts with value of 57.86 ppm.

Alfalfa sprouts are higher than alfalfa seeds in most of the active phenolic compounds (Table3). Phenolic compounds found in alfalfa seeds and its sprouts include for example syringic (1582.19 vs.45351.08 ppm) ,gallic (10.80 vs . 9.40 ppm) , protocatechuic (33.42 vs. 233.06 ppm) , catechein (107.32 vs.839.47 ppm) , epicatechen (59.88 vs. 10.72 ppm) , caffeic (16.82 vs. 1.24 ppm) , p-coumaric (5.24 vs. 7.14 ppm), ferulic (39.97 vs. 7.05 ppm) , iso-ferulic (13.26 vs. 4.32 ppm) and ellagic (10.05 vs. 59.06 ppm) , respectively.

Syringic compounds had the highest concentration in both alfalfa seeds and their sprouts. Followed by catechein compound .The third concentration was chlorogenic in both seeds and sprouts .

The mean value of feed intake (FI) of the negative control rats was 15.12±1.05 g/day but when rats were become diabetic (positive control), their FI was increased significantly with a mean value of 20.17±0.93 g / day(Table 4). Diabetic rats fed diet supplemented with5% and 10% alfalfa seeds showed significant decrease in FI compared to the positive control rats. The most pronounced improvement in FI was observed in the group of diabetic rats supplemented with 5% and 10% alfalfa sprouts as no significant difference was observed between them and that of negative control group.

Regarding body weight gain (BWG) in Table 4, positive control group showed significant loss in body weight compared to the negative control group. BWG values of diabetic groups supplemented with 5% and10% of alfalfa seeds and its sprouts were significantly increased compared favorably to that of positive control group in the following magnitude of increasing order 21.33±1.21 g >23.67±2.16 g > 26.42 ± 1.36 g >28.00 ± 1.67g vs. 17.00 ± 2.61 g ,respectively . Alfalfa sprout supplementation at 5 and 10 % induced better BWG compared to 5 and 10 % alfalfa seeds supplementation.

Induction of Inflammation:

At the last week of the feeding trial, three rats were selected from each of the five diabeticsubgroup then 0.1 ml of formalin (4%) was injected in the left hind paw of ether anaesthetized rats in order to induce inflammation **(Northover and Subramanian, 1962)**. The paw´s thickness was measured just before and 3 h, 6 h, 1 day, 2 days, 4 days, and 6 days after formalin injection using skin caliber. The anti-inflammatory effect of alfalfa seeds powder and its sprouts was assessed by calculating percent change in the thickness of rats´ paws compared with the positive control group at the same time.

Blood Samples and Tissue Collection:

At the end of the feeding trial, rats were fasted over-night, lightly anaesthetized by diethyl ether.

Blood samples were collected from hepatic portal vein into dry clean centrifuge tubes. Blood samples were left to clot at room temperature, and then centrifuged for 15 minutes at 3000r.p.m**.** Sera were transferred into dry clean eppendorf tubes and kept frozen at -20^{o} C till biochemical analysis.

Livers were removed immediately after sacrificing , excised, rinsed, blotted dry with tissue paper, and kept in formalin solution (10%) for histological examination.

Biological Evaluation and Biochemical Analysis:

Feed intake (FI) was recorded every day throughout the experimental period. Body weight gain (BWG) was determined according to **Chapman *et al.*, (1959**).

Fasting serum insulin and glucose were determined according to **Temple *et al.*, (1992)** and **Trinder(1969) ,**respectively**.** Serum glycogen was determined according to **Mendel *et al.*, (1954)** whileglycatedhaemoglobin **(**HbA_{1c}**)** was determined according to **Trivelli *et al.*, (1971).** Serum alanine amino transferase**(**ALT) andserum aspartate amino transferase (AST) levels were analyzed according to **Reitman and Frankel, (1975**).

Histopathological Examination:

Specimens from liver were washed, dehydrated in ascending grades of ethyl alcohol,cleared in xylene and embedded in paraffin wax. Sections of 5-6µm in thickness were cut out, deparaffinized and stained with Haematoxylin and Eosin (H&E) for examination under the light microscope **(Bancroft *et al.*, 1994).**

Statistical Analysis:

Data were statistically analyzed using SPSS, PC statistical software (version 16). Results were expressed as mean ± SD. Differences among groups were analyzed by analysis of variance (ANOVA) using Duncan's test. A p value of ≤ 0.05 was considered statistically significant according to **Armitage and Berry, (1987)**.

2. Methods:

Preparation of alfalfa dried seeds and their sprouts:

Alfalfa seeds were cleaned, washed, air dried and well grinded to get the fine powder**.** In order to obtain the alfalfa sprouts, alfalfa seeds were cleaned, washed, soaked overnight then water was poured out. The seeds were let to sit for five days and were rinsed twice a day with cold water and were exposed to the sun for 15 minutes after the fifth day. Finely grounded into the powder. Fresh alfalfa sprouts were freeze-dried at biology department, animal research institute, Giza, Egypt using Freeze-Dryer Lyophilizer (Heidolph)at -50ºC. Fresh alfalfa sprouts(100 gm)yielded 20 g freeze dried powder.

Experimental Animals and diet:

Fifty six adult male albino rats of Sprague-Dawley strain weighting (160-170g) were used. Rats were accessed to the experimental diets and water *ad-libitum*. All rats were fed on the basal diet one week prior to the starting of the experiment as acclimatization period.

The basal diet (AIN-93G) was formulated according to **Reeves *et al*., (1993**). Salt mixture was prepared according to **Hegested *et al*., (1941)** and vitamin mixture was prepared according to **Campbell (1963)** .

Induction of Diabetes Mellitus:

Diabetes was induced by inter-peritoneal injection with freshly prepared alloxan monohydrate (120 mg / body weight of rat) dissolved in 0.1 M sodium citrate buffer (pH 3.0). After a period of 72 h, rats with fasting blood glucose >150 mg/ dl were considered to be diabetic **(Venkatesh*et al* .,2008 , Neeli *et al* .,2007**).

Experimental Design:

After acclimatization period, rats were divided into two main groups.The first main group (n=6) was fed on the basal diet during the experimental period (four weeks) and kept as a negative control (-ve). The rest of the animals (n = 50) were injected with alloxan for diabetes induction and were divided into five subgroups (10 rats each) fed on the following diet schema for four weeks:

Subgroup (1): Diabetic rats were fed on basal diet (positive control).

Subgroup (2): Diabetic rats were fed on basal diet supplemented with 5% alfalfa seeds powder.

Subgroup (3): Diabetic rats were fed on basal diet supplemented with 10% alfalfa seeds powder.

Subgroup (4): Diabetic rats were fed on basal diet supplemented with 5 % freeze dried alfalfa sprouts.

Subgroup (5): Diabetic rats were fed on basal diet supplemented with 10 % freeze dried alfalfa sprouts.

The Hypoglycemic and Anti-inflammatory Effects of Alfalfa (*Medicago sativa L.)* Plant in Rats

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Introduction

Diabetes mellitus, is one of the most common endocrine metabolic disorders has caused significant morbidity and mortality **(Patel *et al*.,2012).**The number of diabetic patients in 2014 was 347 million in worldwide, and the expected number in 2030 is 552 million, which represents an increase of more than 50**% (ADA, 2014)** .More than 80% of people with diabetes live in low- and middle-income countries **(WHO , 2014)**. Untreated, diabetes can lead to cardiovascular disease, blindness and kidney failure **(WHO,2012)** . The causes are complex, but are in large part due to rapid increases in overweight, obesity and physical inactivity (**WHO ,2013)**.

Alfalfa (*Medicago sativa* L.) is a genus plant distributed widely throughout the world. The common name of alfalfa is Lucerne and it is called the "father of all plants" **(Caunii *et al*.,2012)** .Although alfalfa is very important and well known as a livestock forage , in some parts of the world these leaves are eaten raw or cooked as a vegetable in human diet. Seeds and sprouts are a favorite salad ingredient and has been indicated to be helpful in lowering cholesterol levels in animal and human studies **(Colodny *et al*., 2001)** and reduce inflammation **(Hong*et al* ., 2009_a).**
As a result of alfalfa medicinal functions, it has become very important lately. The plant has been reported to have antioxidant effects **(Kundan and Anupam, 2011)**. So, this study was conducted to explore the functional role of alfalfa seeds and its sprouts in controlling blood glucose levels and treating inflammation in diabetic rats.

Materials and Methods

1. Materials:

Alloxan monohydrate, casein, cellulose, vitamin mixture, mineral mixture and formalin were obtained from El-Gomhoria Company, Cairo, Egypt. Alfalfa seeds (*Medicago sativa* L.) were obtained from agriculture research center , Giza , Egypt. Rats were obtained from Helwan Station, Cairo ,Egypt. Kits for biochemical analysis were obtained from Alkane company ,Cairo ,Egypt.