

## Some biochemical effects of *lupinus albus* L. seed oil on normal and alloxan induced diabetic mice

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### Abstract:

In this research *Lupin albus* L. seed oil had been extracted and then identification of it is contents from the fatty acids and lipids which was compared with standard fatty acids and lipids by using thin layer chromatograph.

Also the research has been dealt with the effects of increasing concentrations of (*Lupin albus* L.) seed oil on some biochemical parameters in serum and tissue of normal and alloxan-induced diabetic mice. When administrated orally (50,100,200) mg/Kg body weight of lupin seed oil the results indicated a significant decrease of serum glucose levels at 100 mg/kg body weight in normal and alloxan-induced diabetic mice the percentage of decrement was (-25.51% and -29.85%) respectively and then determined copper concentration in lupin oil by using atomic absorption spectrophotometer.

Also noticed that the level of cholesterol in serum of normal and alloxan induced diabetic mice was significantly lowerd, the percentage of decreasing was (-22.39 and -23.09%) respectively.

This research refer to significant decrease in liver glycogen content in normal mice of (-23.29%) after 180 min of administration of oil relative to control value.

On the other hand, lupin seed oil has non significant difference on the concentration of liver glutathione and heart malondialdehyde relative to control value.

**Key word:** lupinus albus, diabetes, cholesterol, glycogen, glutathione , malondialdehyde.

### Introduction:

Lupins have been cultivated for over 2000 years, originating around the Mediterranean and along the Nile valley where they were used for human consumption. Lupins have dijitate leaves [1], annual plant belonging to the class of leguminosae and of Rosales order, dicotyledons class and spermatophyta phylum, with flower terminating receme upto 1.5 m high [2]. Lupins whole seed contain protein , lipids fatty acids, ash, fibre, amino acids, carbohydrates, calcium, phosphorus, zinc, iron, copper and manganese [3]. Also the plant contains substantial amounts of antioxidants including vitamin E, vitamin C, thiamin, riboflavin and niacin [4,5,6].

Lupin hypoglycemic activity was described by (Trider, 1969)[7] which proposed it as a substitute for insulin in mild to medium diabetes mellitus. The hypoglycemic effect of lupin meal was described also in [8] which suggest the used of plums jam containing lupin meal for use as diabetic food for diabetics. Also lupins powdered seed in the form of cataplasma is used to treat ulcers and the seed decoction reduces blood pressure. Lupins is diuretic , seeds are used in veterinary medicine in treating skin disease [9]. When lupin seed was steeped in water for a day then biled, and added to the diet of rats , slightly superior growth [3]. Lupin seeds extracts by using phenolic compounds have antibacterial properties so that [10] suggest using lupin to inhibit bacteria growth. Its seeds used both for alimentary purposes (duo to their remarkable protein contents) and anti parasitic agents [2]. Lupins are thought to be a good substitute for soya beans due to its protein content, for incoporating into stock feed [11]. The main potential market for white lupin grain clearly lies in ruminat feed where its high oil and protein contents are of great value because of the absence of anti-nutritional factor [12].

The aim of the present study is to relates the used of lupin seed oil for the preparation of medicament , food supplements or foods for treatment of diabetes.

### Tools and Methods:

#### Oil extraction:

Lupin seed oil which prepared using 750 g of lupin powder added to 2 L of petroleum ether 60-80 °C in soxhlet apparatus for 12 h period at 40° ,the solvent was evaporated using rotatory evaporator to obtain the oil [13].

#### Oil identification by using thin layer chromatography (TLC):

The oil of lupin identificatied by using plate of TLC (supplied by Merck) into groups according to their polarity and examples from each group are included as representative standards [14].

#### Copper determination in oil:

Copper was determined in oil the lupin using atomic absorption spectrophotometer [15].

#### Animals used:

Fourty male albino mice (1.5-2) months old weighting (25-30) g each were used. They were housed under standard conditions of temperature 27 °C , humidity, and had free access to food and water. Animals were divided into eight groups (5 mice of each group). The mice were administered orally with oil at increasing concentrations (50,100,200) mg/Kg body weight of normal fated mice. The control group was administered physiological salin solution only. The blood samples were collected from orbital sinus puncture[16] of an ether anesthesia mice 3h of administration. Then the tissues (Livers and Hearts) of these mice were rapidly removed after scarification and washed with ice-cold saline and then kepted in the deep freeze until the glutathione concentration(GSH) and glycogen content were determined in livers and malondialdehyde (MDA) concentration was determined in hearts. The serum was separated by centrifugation at 4000g. Then , blood serum glucose level was determined using Randox kit for glucose oxidase methods [17]. Also the enzymatic method was applied to determine serum cholesterol level using PAP kit [18].

The glycogen content in liver was determined by colorimetric method [14]. MDA in tissues was determined depending on the reaction between MDA and thiobarbituric acid [19]. Hepatic glutathione were determined using modified Ellman method [20].

The induction of diabetes with alloxan was performed by subcutaneous injection of alloxan monohydrate, which was immediately dissolved in 0.02 ml sterile physiological saline solution at a dose of 150 mg/Kg body weight. The diabetic state was monitored by tested the glucosurea using tes-tape, Eli Lilly and company, USA [16]. Then the resulting diabetic animals were grouped into groups (5,6,7,8) after fasting for a period of 16 h, then (6,7,8) groups were administered orally with an oil at 50,100 and 200 mg/Kg body weight. After 3h of administration blood collection and tissues were taken

and performed as indicated previously with fasted normal mice. All assays were done in duplicate.

#### Statistical :

Significance of differences between mean values was analyzed by Duncan (uses harmonic mean sample size=3.00 and "F" test) [21].

The statistical methods used to analyze the data include mean, standard deviation and maximum, while (F) test was used to compare between groups at ( $p \leq 0.05$ ).

#### Results and Discussion

##### TLC technique:

The fresh dried plant "Lupins Albus" was used for phytochemical analysis by using plate of TLC to inditificated oil of lupin from mixture of three different solvents (petroleum ether B.p: 60-80 °C): diethyl ether : and glacial acetic acid (80:20:1) ratio as a mobile phase[14].

**Table (1): (Rf) values of lupin oils comparing with standard sample of lipids and fatty acids**

	Carbon chain length	Compound	Rf value standard sample	Rf value of Lupin oil
Staurated fatty acid	C12	Lauric	0.69	0.0
	C14	Myristic	0.32	0.0
	C16	Palmitic	0.51	0.51
	C18	Stearic	0.65	0.0
	C20	Arachidic	0.87	0.87
Unsaturated fatty acid	C18:1	Oleic	0.47	0.47
	C18:2	Linoleic	0.53	0.55
Phospholipid		Lecithin phosphotidylecholin	0.73	0.72
Vit A		Vit A	0.89	0.89
Waxes Honey waxes		Myricyl palmitede	0.31	0.32
Sterols		Cholesterol	0.64	0.64

Detection of oils on TLC plates was normally carried out by spraying the plate with 2,7 dichlorofluorescein.

In order to find the type of fatty acids and lipids. Indentification was based on comparing the relative retention (Rf) of lupin oils with relative retention (Rf) of standard sample analysed under the same experimental conditions as shown in table (1). These results were with agreement with other researchers [22] which conclude that the fatty acid composition of white lupin was useful for human consumption , the results indicated that oleic and arachidic acids in seed oil were very high in lupin [23].

On the other hand , Hansen and Czochanska and were found that the composition of lupin seed oil contains triglycerides, phospholipids, free sterols, glycolipids, sterol and wax esters while they were found that the main fatty acids in the total oil extract were linoleic, oleic and palmitic [24].

#### Effect of lupin seed oil on the some biochemical parameters:

The mean values of serum (Glucose and cholesterol) and liver [GSH concentration and glycogen content] and malondialdehyde (MDA) concentration in heart for control and lupin seed oil –treated normal and alloxan-induced diabetic mice were shown in Table (2).

The result indicated that the dose of oil which was response the experiment is 100 mg/Kg body weight and

there is a significant decrease of serum glucose levels in untreated and treated with alloxan diabetic mice the percentage of decrement was (-25.51%) and (-29.85%) respectively compared with control value as show in table (2) .This decrease may be due to an increase peripheral glucose utilization which increases the release of insulin and /or inhibition of the proximal tubular reabsorption meachanism for glucose in the kidney [25]. The result of the present study show that the lupin seed oil may be possess active constituents capable of lowering blood glucose in both diabetic and non diabetic animals, this effect of lupin oil may be due to it's ability to increase glycolysis or decrease glyconeogenesis or may then cause inhibition of glycogenolysis in the liver. These results were in agreement with other studies [26,7] which they suggest the use of lupin as therapeutical agent , in particular as hypoglycemic agent.Also lupin seed oil content of Cu at concentration (0.4 µg/g) which was similar to [3]. The presence of Cu at this concentration plays an important role in insulin synthesis [27] which lead to decrease in serum glucose level.

Also the results in Table (2) indicated that the lupin seed oil at a dose of 100 mg/Kg body weight decreases the level of cholesterol. The percentage of decrement was (-22.39%) relative to control and (-23.09%) relative to diabetic control. This result was similar to [28], this decrease may be due to inactivation of the hydroxy

methyl glutaryl CoA reductase (HMG –CoA reductase) , a thiol group enzyme that is necessary for cholesterol synthesis [16,29].

In the present study, the mice treated with oil of 100mg /Kg body weight showed a significant decrease in glycogen content in normal mice of (-23.29%) after 180 min of administration of oil relative to control group, as

shown in table (2), these results are similar to that results obtained by other [28] which observed that lupin diets increased plasma glucagons hormone levels, this hormone has an opposite effect for that of insulin.

On the other hand, lupin seed oil has non significant difference on the concentration of liver glutathione and heart malondialdehyde relative control value.

**Table (2): Mean blood serum (glucose and cholesterol) level in mg/100 ml  $\pm$  SE and liver (glycogen mg/g and GSH nmole/g) and heart MDA nmole/g after oral administration of different doses of lupin seed oils in normal and alloxan-induced mice.**

Group No.	Treatment	Serum glucose level mg/100ml $\pm$ SE	Change %	Serum cholesterol level mg/100ml $\pm$ SE	Change %	liver Glycogen mg/g $\pm$ SE	Change %	liver GSH nmole/g $\pm$ SE	Change %	MDA heart nmole/g $\pm$ SE	Change %
1	Normal saline control	96.73 $\pm$ 1.47 de	---	162.1 $\pm$ 1.9 d	---	2.82 $\pm$ 1.9 f	---	4276.2 $\pm$ 6.1 e	---	281.35 $\pm$ 6.3 c	---
2	Seed oil 50 mg	83 $\pm$ 2.13 c	-10.78	144.4 $\pm$ 2.7 c	-10.92	2.69 $\pm$ 0.01 b	-12.18	4302.5 $\pm$ 12.7 e	4.82	265.47 $\pm$ 3.5 ba	-4.1
3	Seed oil 100 mg	65 $\pm$ 3 a	-25.51	125.8 $\pm$ 3.4 a	-22.39	1.93 $\pm$ 0.04 a	-23.29	4319.8 $\pm$ 5 ef	6.41	241.92 $\pm$ 5.5 a	-9.3
4	Seed oil 200 mg	90 $\pm$ 2.8 f	-4.6	134.73 $\pm$ 2.36 b	-16.88	2.71 $\pm$ 0.02 e	-10.28	4278.86 $\pm$ 3.2 f	2.01	253.42 $\pm$ 3.4 b	-7.21
5	Diabetic control	185.13 $\pm$ 2.15 g	---	183.53 $\pm$ 1.79 e	---	3.11 $\pm$ 0.03 d	---	2890.8 $\pm$ 24.4 a	---	456.77 $\pm$ 2.59 g	---
6	Diabetic plus 50 mg	154.06 $\pm$ 1.47 d	-13.78	147.76 $\pm$ 2.89 c	-14.35	2.91 $\pm$ 0.05 df	-8.84	2873.3 $\pm$ 4 a	-11.31	439.89 $\pm$ 4.5 f	-8.3
7	Diabetic plus 100 mg	129.86 $\pm$ 3.29 b	-29.85	132.7 $\pm$ 1.7 ab	-23.09	2.88 $\pm$ 0.04 e	-10.7	2863.2 $\pm$ 4 ab	-15.4	446.34 $\pm$ 2.6 d	-5.11
8	Diabetic plus 200 mg	158.6 $\pm$ 0.66 de	-11.63	143.86 $\pm$ 3.7 c	-16.62	2.85 $\pm$ 0.04 fd	-13.09	2884 $\pm$ 7.6 b	-8.2	452.2 $\pm$ 3.7 db	-2.0

- vertically different letter meaning that there is a significant difference at ( $p \leq 0.05$ ).
- The values are mean  $\pm$ SE of 4 mice each group.

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## بعض التأثيرات الكيموحيوية لزيت ثمار الترمس على الفئران السليمة والمصابة بداء السكر

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### الملخص:

تم في هذا البحث استخلاص زيت ثمار الترمس ومن ثم شخّصت محتوياته من الأحماض الدهنية والدهون مقارنة مع أحماض دهنية ودهون قياسية باستخدام تقنية كروماتوغرافيا الطبقة الرقيقة .

كما تناول البحث تأثير تراكيز متصاعدة لزيت ثمار الترمس على بعض المتغيرات البايوكيميائية في مصل وأنسجة الفئران السليمة والمصابة بداء السكري التجريبي المستحدث بالالوكسان، إذ أدى التجريع عبر الفم بتراكيز (٥٠، ٢٠٠، ١٠٠٠) ملغم/كغم من وزن الجسم من زيت ثمار الترمس الى انخفاض معنوي في مستوى كلوكوز مصل الفئران السليمة والمصابة بداء السكري المستحدث بالالوكسان بواقع (- ٢٥,٥١%) و (- ٢٩,٨٥%) على التوالي ومن ثم تم استخدام تقنية الامتصاص الذري اللهبى لايجاد تركيز النحاس في زيت ثمار الترمس. كما لوحظ ان هنالك انخفاض معنوي في مستوى الكوليسترول في مصل الفئران السليمة والمصابة بداء السكر التجريبي المستحدث بالالوكسان بواقع (- ٢٢,٣٩%) و (- ٢٣,٠٩%) على التوالي ، وكما يشير البحث الى انخفاض معنوي في كلايكون الكبد في الفئران السليمة بواقع (- ٢٣,٢٩%) بعد مضي ١٨٠ دقيقة من التجريع مقارنة بالقيم الضابطة.

من ناحية اخرى فان ليس لزيت ثمار الترمس تأثير على تراكيز كلوتاتايون الكبد ومالوتالديهايد القلب مقارنة بالقيم الضابطة..

**الكلمات الدالة:** الترمس ، السكري ، الكوليسترول ، الكلوتاتايون.