Effects of Soybean Seed on Glucose Levels, Lipid Profiles and Histological Structures of the Liver in Alloxan-Induced Diabetic Albino Rats

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Abstract:
The effects of soybean seeds on glucose levels, lipid profiles and histological structure of the Liver were investigated**.** For this purpose, forty male albino rats were divided into four groups each of 10 rats, as following (G1): Normal control rats, (G2): Diabetic rats which injected with alloxan (150 mg/ kg), (G3): Normal rats were treated with soybean seeds at 20% of daily food, (G4): diabetic rats were treated with soybean seeds at 20% of daily food. All treatment was once daily and lasted for four weeks. The animals were taken then collect blood by heart puncture, and scarified directly; then liver was taken. Serum glucose concentration increased significantly ($P \leq 0.05$) in diabetic rats. Soybean treatment decreased the elevated glucose concentration significantly ($P \leq 0.05$) in treated diabetic rats; however, their glucose concentrations were still significantly higher ($P \leq 0.05$) than those of the control group. 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. Administration of soybean seeds resulted in a significant increase in body weight with a reduction in levels of triglyceride and low density lipoprotein. Histopathological changes on liver of G2 showed fatty change in hepatocyte,While there is no histopathogenic effect except moderat prolifiration of kuppfer cell in the section of G3, also Section of liver of G4 Showed, no pathogenic effect, and removing of fatty change.

Key Words: Soybean, Alloxan, Lipid profiles, Structures of liver, Rats

Introduction
More than 400 medicinal plants are present world wide for the treatment of diabetes mellitus, while only few of them have been subjected to scientific authentication as anti-diabetic agents [1]. 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 [2]. Soybean protein administration reduced cholesterol; triglyceride, and Low density lipoprotein levels in healthy persons [3] as well as in diabetic patients [4], similar effect was also noticed in rats [5]. Soybean is an extremely rich source of protein and fat, and a good source of energy, vitamins and minerals [6] and it has been shown to be hypocholesterolemic in animals [7]. Soybean is not only protein rich, but also a good source of minerals like phosphorous, calcium, iron and soluble fiber. Soybean proteins complement cereal proteins to provide source of dietary protein of vegetable origin for human beings [8]. Isoflavones present in Soybean include genistein and daidzein. Soybean proteins have been shown to reduce the risk of cardiovascular diseases by lowering blood pressure, blood cholesterol and triglycerides [9, 10]. Isoflavones are flavonoids found in mainly in legumes, and in highest amounts in soybeans. Soybeans contain from 0.1 to 5 mg total isoflavones per gram of whole bean [11]. In whole soybeans and many soy foods, isoflavones exist as hydrophilic glucoside conjugates: 6”-O-malonylglucosides, 6”-O-acetylglucosides, and β- glucosides [11]. During digestion, these are hydrolyzed to the more bioavailable aglycone form [12].

Materials and Experimental design:

Animals and Experimental design:
Forty male albino rats, 3-4 weeks old and 150-190 gm body weight were housed in cages and maintained under laboratory controlled of temperature (25 ±2) and light (12 hour light and 12 hour dark), pelleted food and tap water were given. The animals were divided into four groups each of 10 rats. **Group1**: normal control rats.**Group2**: Diabetic rats.**Group3**: normal rats were treated with soybean seeds at a dose of 20% from daily food. **Group 4**: diabetic rats were treated with soybean seeds at a dose of 20% from daily food. All treatment was once daily and lasted for four weeks.

Preparation of pellet:
Soybean seeds were purchased from the local market in Baghdad; they were dried on the filter paper and powdered in mixer. The pellet contains 50% bran, and 25% white flour, 20% powder of Soybean, and 5% of vitamin [13]

Induction of Diabetes:
Rats were fasted for 48 hour before inducing diabetes with alloxan. Diabetes was induced by a single subcutaneous injection of alloxan in a dose of 150 mg/kg of body weight [14].

Blood Sample Collection:
Blood was obtained by heart puncture with the help of sterilized needle and sterilized syringe and transferred into the appropriate sterilized micro centrifuge tube allowed to clot, serum was separated after centrifugation at 1500 rpm for 15 minuets, serum lipid profiles were measured using colorimetric assay kits (Bicon, Diagnostic Gmbh, Burbach, Germany).

Histopathological examination:
Specimens of liver were fixed in 10% neutral formalin solution, and then processed for routine technique for embedding in paraffin. Blocks were

sectioned with haematoxyline and eosin for histopathological examination [15].

Statistical analysis

Data are expressed as the mean ± SE. The statistical significance was carried out using one- way analysis of variance test followed by Duncan's Multiple Range Test (SPSS statistical software package) [١٦]. A possibility of *P* value ($p< 0.05$) was considered as significant differences between means.

Result and Discussion:

Administration of soybean seeds induced in a significant increase in body weight , also areduction in the levels of triglyceride , low density lipoprotein (Table1) . In this study, significant reduced was noticed in the body weight in diabetes induced experimentally by alloxan compared with the control .These results are agrees with results of [١٧,18]. The reduction of body weight in diabetic rats is due to dehydration and catabolism of fats and proteins [19], increased catabolic reaction leading to muscle wasting can be the cause of the reduced body weight gain in diabetic rats [20].While, significant effect of Soy beam on body weight showed an increase of body weight, similar observation has been reported in diabetic rats [17], but disagree with results of [21] in diabetic rats. Soybean contains high percentage of protein, isoflavone, or fiber that increases metabolic processes in body [22]. Diabetes induced by alloxan lead after four weeks to a significant increase in levels of cholesterol, triglyceride, low density lipoprotein (LDL-), and total lipids, with decreased significantly both body weight and (HDL-) high density lipoprotein (Table1). The extracts exhibited significant reduction of serum cholesterol level in alloxan-treated rats. The abnormal high concentration of serum lipids in the diabetic rat is mainly due to increase in the mobilization of free fatty acids from the peripheral fat depots [23]. Maintenance of serum cholesterol profile indicates that phytoestrogens and saponins in soybean may exert their role in maintenance [24]. Soy foods contain a good amino acid profile, and comparison with ideal patterns published by the food and Nutrition Board [25] shows adequate quantities of the essential amino acids like histidine, isoleucine , leucine, lysine , phenylalanine tyrosine , threoninem trytophan, and valine. Lysine concentration is especially high , and is important for world nutrition, because soy protein is an inexpensive protein source [26] Serum glucose concentration increased significantly ($P \leq 0.05$) in diabetic rats. Soybean treatment decreased the elevated glucose concentration significantly ($P \leq 0.05$) in treated diabetic rats; however, their glucose concentrations were still significantly higher ($P \leq 0.05$) than those of the control group. A reduction in the serum glucose levels of the groups fed with soybean-supplemented diets was observed in this work. This may imply that soybean could reduce the incidence of hyperglycemia. Soluble fibers from soybean resist digestion and absorption and are thus used to regulate glucose absorption and elevation in diabetes [27].Consumption of foods containing soybean and soybean constituents has been associated with reduced heart disease risk factors, reduced diabetes. Isoflavone compounds found in soybean, especially genistein may help to stay lean by causing us to produce fewer and smaller fat cells [28]. Potassium to sodium (ratio 3/1 – 11/1) makes soybean an ideal food for diabetes mellitus patients [29]. Soybean has also been shown to promote serum insulin production [30]. It has been demonstrated that soya protein helps persons with diabetes prevent kidney diseases and improve the cholesterol profile [31].

Table1. the mean and SE of body weight(b.w) and serum: glucose, triglycerides, VLDL-, and HDL-cholesterol concentrations (mg/dl) of rats before and after treated.

Treated / Test	G1	G2	G3	G4
	Mean ± SE	Mean ± SE	Mean ± SE	Mean ± SE
b.w. (Before treated) g	155.4±2.1679a	155.4±5.549a	157.6±4.560a	155.8 ±3.11 a
b.w. (After treated) g	190.6± 1.581 a	146.8±1.303b	223.2±2.387b	175.75±3.962b
Glucose (mg/dl)	110.2±3.633a	228.4±2.449b	112±3.937c	157.6±7.162b
S.triglycerides (mg/dl)	43.4±2.701 a	69.8±4.549b	37.2±7.854b	37.2±7.854 b
Cholesterol (mg/dl)	80.6±4.878a	91.4±2.701 b	74.8±3.563 b	72.6±7.162 b
HDL- (mg/dl)	37.6±2.828 a	65.4±2.880 b	50.6±4.159 b	39.6±6.268 b
LDL- (mg/dl)	28.6±4.332a	38±5.147 b	31.6±3.258b	34.3 ± 4.124c
VLDL- (mg/dl)	11±1.581 a	15.6±1.816 b	8.72±0.878 b	9±1.303 b

Different letters indicate significant differences (P <0.05)
Similar letters indicate statistically no significant differences.
SE: Standard Error

Histopathological changes on liver of G2 showed fatty change in hepatocyte,While there is no pathogenic effect just moderat prolifiration of kuppfer cell in the section of G3, also Section of liver of G4 Showed, no pathogenic effect, and removing of fatty,change Studies in animal models [32] have shown that soy protein consumption may directly influence the hepatic metabolism of cholesterol by

increasing the activity of 3- hydroxy-3-methylglutaryl coenzyme A (HMG CoA) reductase, thereby inhibiting hepatic cholesterol synthesis. Lovati *et al* [32] reported a seven fold increase in LDL receptor activity in humans, resulting in increased clearance of cholesterol from the blood in patients with raised serum cholesterol concentrations who consumed a soy protein diet compared to a standard low-lipid diet with animal protein These results were confirmed by other researchers, as reported by Potter[33]. However, the hypothesis of an activation of LDL receptors in liver cells is still controversial and more extensive studies are needed to ascertain the cholesterol-lowering mechanism of soy beans. Section of liver in diabitic rat(G4) treated with soea bean Shows, no pathogenic effect, and removing of fatty change because Soy contains many active components that may be responsible for an array of health benefits [34]. This suggests that soybean incorporation in the diet had no adverse effect on the organs. Earlier study on the histology of the liver and kidney of rats fed with soybean supplemented diet showed normal and well preserved sections of the organs [35]. These components include the active peptides [32], saponins, sterols, phospholipids, fiber, fat and isoflavones [36]. Soy peptides (i.e. 7S gobulin, specifically the α' subunit, and active peptides of ~15 amino acid composition) and isoflavones (i.e. daidzein and genistein, the 2 main isoflavones in soy) have been the most widely studied, especially for their role in modulating lipid [37] Soy isoflavones can, however, provide antioxidant activity that may protect against cardiovascular disease. LDL oxidation is known to promote aggregation of fatty streaks in arteries. Inhibition of lipid peroxidation by soy isoflavones may reduce risk of atherosclerosis [38].

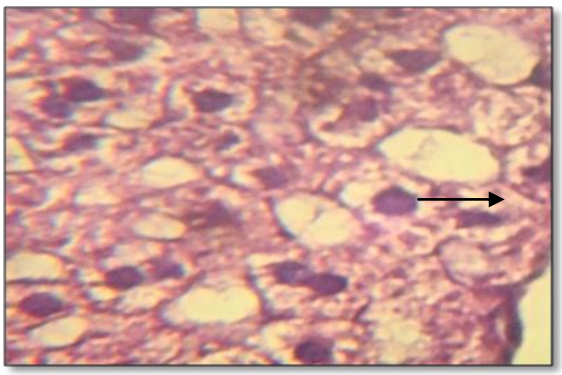

Figure.1: Section of liver of control G1 showed normal central vein, normal hepatocytes and normal arrangement of liver plate (400X).

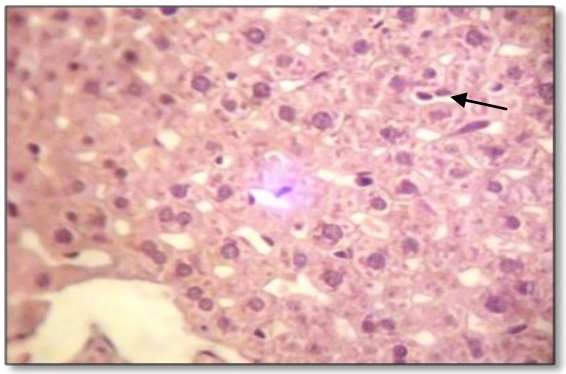

Figure. 2: Section of liver of (G2) shows (→) fatty change in hepatocyte (400X).

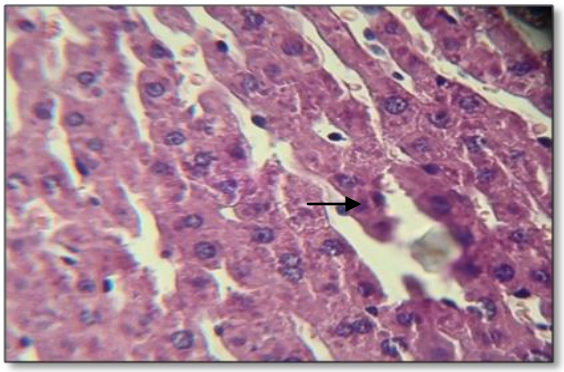

Figure.3: Section of liver of rat (G3) treated with soea bean Shows, there is no pathogenic effect just moderat prolifiration of kuppfer cell (400X).

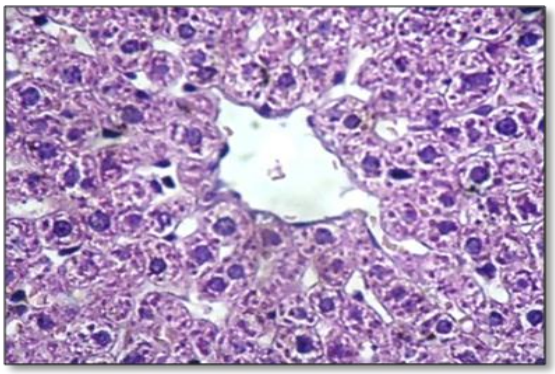

Figure 4: Section of liver of diabitic rat (G4) treated with soea bean shows no pathological effect .(400X).

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تأثير بذورِ فول الصويا في مستوى السُكّرِ و مستويات الدهون و التركيب النسجي للكبدِ في ذكور الجرذانِ البيض المصابة بداء السكر المستحدث بالالوكزان

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الملخص

تم التحري عن تأثيرالمعاملة ببذورفول الصويا في مستوى السكر ومستويات الدهون. قسمت ٤٠ من ذكور الجرذان البيض البالغة الى أربع مجاميع كُلّ مِنْها ١٠ جرذانِ كالاتي: (G1) جرذان السيطرةِ الطبيعيةِ ، (G2) جرذان مصابة بالسكر أَيّ حَقنتْ ب١٥٠ ملغرام/ كيلوغرام من الالوكزان ، (G3) جرذان طبيعية عولجتْ ببذورِ فول الصويا بنسبة ٢٠% من الطعام، (G) جرذان مصابة بالسكر عولجتْ ببذورِ فول الصويا ٢٠% من الطعام اليومي . كانتْ المعالجة يوميةً ولمدة أربعة أسابيعِ. جمع الدم من الحيوانات بطريقة طعنة القلب، وتم تشريح الحيوانات ودراسة التغيرات النسجية للكبد.لوحظ وجود زيادة معنوية في وزن الحيوانات المعاملة ببذور فول الصويا لوحده، في حين قلت اوزان الحيوانات المصابة بالسكر بشكل معنوي، بينما لوحظ تحسن لاوزان الحيوانات المصابة بالسكر بعد معالجتها ببذور فول الصويا، وارتفاع تركيزُ السكر في مصلِ الدم بشكل ملحوظ في الجرذان المصابة بالسكر. بينما انخفض تركيزَ السكرالمرتفعِ بشكل ملحوظ بعد معالجةُ الجرذانِ المصابة بالسكر بفول الصويا . كما لوحظ وجود زيادة معنوية في مستويات الكولوستيرولِ، والكليسيريدات الثلاثية، ومستوى الشحوم ذات الكثافة الواطئة ومستوى الشحوم ذات الكثافة العالية في الحيوانات المصابة بالسكر. بينما اظهرت النتائج انخفاض معنوي في مستويات الكولوستيرولِ، والكليسيريدات الثلاثية، ومستوى الشحوم ذات الكثافة الواطئة ومستوى الشحوم ذات الكثافة العالية في الحيوانات المصابة بالسكريعد معاملتها ببذور فول الصويا .أظهرت نتائج الفحص النسجي للكبد في الحيوانات المصابة بالسكر تجمع الخلايا الدهنية ، بينما لم يظهرهناك تأثير واضح في الحيوانات المعاملة بفول الصويا وكذلك تحسن واضح في المجموعة المعالجة بفول الصويا وازالة التجمعات الدهنية في نسيج الكبد.

كلمات مفتاحية : فول الصويا، السكري، الالوكسان، مستويات الدهون، الجرذان.