

Effect of Alcoholic Catechin Extract on Hyperglycemia, Hyperlipidemia and Liver Functions in Alloxan Diabetic Mice

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Abstract:

The objective of this study is to estimate the effect of the hydro-ethanolic catechin extract toward blood glucose, lipid profile and liver functions in alloxan diabetic mice. 50 healthy mice (25-30 g) were divided into five groups of ten animals for each. Group A received normal saline as normal control group. To induce diabetes, alloxan (150 mg/kg), intraperitoneal (i.p.) single dose was injected to groups B, C, D and E. Group B represents diabetic control group. Groups C, D and E received ethanolic catechin extract (30 mg/kg and 40 mg/kg) for different periods of 1, 2 and 3 weeks as treated groups. Blood glucose, serum lipids [Total Cholesterol (TC), Triglycerides (TGs) and High Density Lipoproteins (HDL)], asparagine transaminase (AST), alanine transaminase (ALT) and alkaline phosphatase (ALP) were estimated after one, two and three weeks. Group B showed a significant increase in blood glucose, TC, TGs, AST, ALT and ALP as compared to group A. Groups C, D, and E showed a significant decrease in mentioned serum biochemical parameters in comparison to group B. In contrast, groups C, D and E showed significant increase in serum HDL as compared to B group. The results clearly revealed that ethanolic catechin extract possesses significant antihyperglycemic and antihyperlipidemic activities together with its ability to improve liver functions in alloxan diabetic mice.

Key words: Catechin extract, Alloxan diabetic mice, Hyperglycemia, Hyperlipidemia

Introduction:

Diabetes mellitus can be defined as a group of metabolic disease characterized by hyperglycemia result from insulin secretion, insulin action or both resulting in impaired function in carbohydrate, lipid and protein metabolism. It is a common disorder associated with markedly increased morbidity and mortality. [1]. Plant extracts have been used in traditional medicine for a number of ailments including metabolic disorders [2-4]. The effects of *Bryonia Laciniosa* Seed Extract and its saponin fraction on hyperglycemia and hyperlipidemia in Streptozotocin induced diabetes rats

were studied, the results were referred to the efficacy of this plant extract in the amelioration of diabetes and its associated complications. [5]. In another study, the effects of Aloe Vera leaf gel on hyperglycemia and hypercholesterolemia in type II diabetic patients were revealed that aloe gel may be a safe antihyperglycemic and antihypercholesterolemic agent for hyperlipidemia in type 2 diabetic patients.[6]. It was reported that the leaf extract of guava has traditionally been used for the treatment of diabetes, and that Guava leaf is considered useful as an

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alimentotherapy for chronic treatment in type II diabetes mellitus. [7].

The beneficial medicinal properties of green tea have only been elucidated in past two decades. [8]. Green tea extract is widely studied to determine its beneficial effects in prevention or treatment of human diseases.[9]. It was reported that catechin can reduce or prevent the onset of diseases such as cardiovascular and cancer diseases. [10-11]. Sabu *et al*, [12] were found that polyphenols of green tea extract were reduced of creatinine and urea nitrogen levels in alloxan- induced diabetic rats. In another study, Clark *et al*, [13] were referred that a significant reduction of the creatinine level in streptozotocin- induced diabetic rats was confirmed; in contrast, the blood urea level did not change. Furthermore, eligible studies showed a significant reduction in total cholesterol and low-density lipoproteins when individuals administrated a green tea beverage or extract. [14].

The administration of green tea polyphenols was showed markedly reduced the oxidation of low density lipoproteins. [15]. Many studies reported the medical importance of the plant extracts in treatment or prevent of different diseases. in recent study, it was found that artichoke leaf extract increase HDL and decrease total cholesterol and LDL-c in subjects with primary mild hypercholesterolemia.[16]. Clinical and experimental studies have established a positive correlation between catechines and cardiovascular health. catechines exert vascular protective effects through multiple mechanisms, including anti-oxidative, antihypertensive, antiinflammatory, antiproliferative, anti-thrombogenic, and lipid lowering effects. Catechins possess antioxidant activity, scavenge free radical, inhibit pro-oxidant enzymes and induce anti-oxidant

enzymes. Also, catechins are improving lipid profile by inhibiting the key enzymes involved in lipid biosynthesis and reducing intestinal lipid absorption. [17].

The aim of the present study was to investigate the effects of daily intraperitoneally injection of catechin extract at doses of 3 mg/kg and 4 mg/kg for 1-3 weeks on serum glucose, lipid profiles and liver function in alloxan – induced diabetic mice to confirm the curative properties of catechin extract toward diabetes and its complications in animals model.

Materials and Methods:

Materials:

Alloxan monohydrate was purchase from Sigma Chemical Company. Kits used in this work were purchase from Biomereix Company, France, and all other standard chemicals were obtained from common commercial suppliers.

Preparation of catechin extract:

Green tea was purchase from a local market and stored in dry atmosphere. Catechin extract was prepared by the modified method of Yang *et al*. [18]. Green tea leaves were ground in a miller, at temperature less than 50 C°. Green tea powder was extracted with 70% ethanol (1:10 w/v) for two days with constant stirring. Suspension was filtered through Whatman filter paper NO.1 to retain the clear solution, the residue was extracted again and the pooled tea extract was evaporated under vacuum below 50 C°. The product was left to dry at room temperature and the result extract powder was stored in a dry closed container.

Animals care:

Healthy adult albino males of Swiss albino strain were obtained from animal house of Biotechnology Research Center, Al-Nahrain University, 50 mice were used in this

study, the ages of the mice were in the range of 2.5 to 3 months old and the weight of each one is in the range of 25-30 grams. The animals were housed in small cages, which were cleaned weekly by washing with soap and tap water and sterilized with 70% ethyl alcohol throughout the period of the study. The room temperature was maintained at (24 ± 2) ° C, and the animals were exposed to 14 hours light program.

Induction of diabetes:

Diabetic mice were induced with diabetes by a single intraperitoneal injection (IP) of alloxan monohydrate in physiological saline at a dose of 150 mg/kg body weight in a volume of 0.1 ml. the diabetic state was confirmed through a period of 48 hours after alloxan injection by weight loss and hyperglycemia. The mice with a fasting blood glucose level higher than 200 mg/dl were included in the study. [1].

Experimental design:

Totally fifty mice (40 diabetic mice, 10 normal mice) were used for the experiment. Five groups consisting ten mice for each group were classified as follows:

Group A: normal mice injected with 0.1 ml of physiological saline (normal control, NC)

Group B: normal mice injected with alloxan (150mg/kg) to induce diabetes in mice (diabetic control, DC)

Group C: diabetic mice treated with 3mg/kg (five mice) and 4mg/kg (five mice) of catechin extract after one week from treated with alloxan.

Group D: diabetic mice treated with 3mg/kg (five mice) and 3mg/kg (five mice) of catechin extract after two weeks from treated with alloxan.

Group E: diabetic mice treated with 3mg/kg (five mice) and 4mg/kg (five mice) of catechin extract after three weeks from treated with alloxan.

General procedure:

Blood samples were collected periodically by heart puncture at the end of 1, 2 and 3 weeks after treated with catechin extract. The mice fasted over night and killed by cervical dislocation.

Biochemical measurements:

Serum glucose level was determined using the glucose liquicolor kit (GOD-PAP) method, depending on Reitman and Frankel method [19]. Activities of aspartate amino transferase (AST) and alanine amino transferase (ALT) were estimated according to Reitman S and Frankel S. A colorimetric method [20]. The activity of ALP was estimated according to the Kind and King method [21]. Total cholesterol, triglycerides and high density lipoprotein (HDL) levels were determined using standard kits of biomereix kit, France.

Statistical analysis:

The data of all parameters were expressed as mean \pm SD. statistical evaluation of data was performed using analysis of variance (ANOVA) [22] by SPSS program version 11. $P < 0.05$ was taken to indicate significance.

Results and Discussion:

Table (1) show the results of the blood glucose level in normal control, diabetic control and the treated groups with catechin extract for one, two and three weeks at 3mg/kg (low dose) and 4mg/kg (high dose). The results show that the normal level of blood glucose is 98.23 mg/dl (state a). alloxan causes increasing of blood glucose level up to 193.21 mg/dl (state b). The treatments with catechin extract for both low and high doses decreasing the blood glucose level (state c) after one and two weeks of the treatment in comparison with diabetic control. The treatment of the diabetic mice for three weeks decreasing the level of blood glucose to the normal value in each of

low and high doses in comparison with normal control. Alloxan causes β -cell necrosis and induces “experimental diabetes” in many animal models. [23]. The destruction of β -cells during diabetes ultimately causes degradation or loss of structural proteins due to the unavailability of carbohydrates for energy metabolism. [24]. Insulin deficiency resulted from β -cells destruction ultimately results in increased production of glucose by the liver, and decreased utilization of glucose in peripheral tissues. [25]. The elevated blood glucose level observed in the diabetic mice was significantly decreased in the treated these groups

with catechin extract. These findings may be attributed to the role of catechin extract in stimulation of insulin from the remnant β -cells. The reduced glucose levels can be interpreted by many suggested mechanisms. [26].

Some of these mechanisms referred to that catechin extract might exert insulin-like effect on peripheral tissues by either promoting glucose uptake metabolism or by inhibiting hepatic gluconeogenesis [27,28], or by interfering with the absorption of glucose into the muscle and adipose tissues by stimulating regeneration of pancreatic β -cells [29].

Table (1): Serum glucose levels in normal control, diabetic control and the treated diabetic mice at low and high doses of catechins extract for different periods.

Groups	Low dose (30 mg/kg) Glucose mg/dl(Mean \pm SD)	High dose (40 mg/kg) Glucose mg/dl(Mean \pm SD)
Normal control	98.23 \pm 1.76 ^a	98.23 \pm 1.76 ^a
Diabetic control	193.21 \pm 4.53 ^b	193.21 \pm 4.53 ^b
Treatment for 1 week	143.83 \pm 11.63 ^c	138.43 \pm 14.3 ^c
Treatment for 2 weeks	123.20 \pm 13.04 ^c	118.71 \pm 6.43 ^d
Treatment for 3 weeks	105.52 \pm 4.87 ^a	104.22 \pm 4.83 ^a

Differences a, b, c are significant ($P < 0.05$) to comparison columns.

Tables (2) and (3) show the results of SGPT and SGOT and ALP of normal, diabetic and treated mice with catechin extract in both low (30 mg/kg) and high (40 mg/kg) doses. Values of these enzymes are increasing in diabetic mice (state b) in comparison to the normal control (state a), and begun to decrease during the treatment by catechin extract (state c). Under limited

treatment, enzymes activities were decreased to normal value (state a) or near that in comparison to diabetic control (state b). The treatment for three weeks of the diabetic mice is decreasing of the elevated values of these enzymes to the normal values in both low and high doses of catechin extract.

Table(2): Serum GOT (AST), GPT (ALT) and ALP levels in normal control, diabetic control and the treated diabetic mice at low dose (30 mg/kg) of catechin extract for different periods.

Groups	GPT U/l (Mean +SD)	GOT U/l (Mean +SD)	ALP U/l (Mean +SD)
Normal control	30.22 ±3.21 ^a	63.34±7.62 ^a	70.84± 8.02 ^a
Diabetic control	49.05 ±4.66 ^b	82.03±10.22 ^b	88.9 ±14.04 ^b
Treatment for 1 week	40.04±7.53 ^c	79.51±11.64 ^b	86.3 ±6.03 ^b
Treatment for 2 weeks	34.63 ±6.98 ^a	68.29±8.57 ^a	84.80 ±3.42 ^b
Treatment for 3 weeks	33.25 ±7.02 ^a	66.78±7.44 ^a	76.61 ±6.63 ^a

Differences a, b, c are significant (P <0.05) to compression columns.

Table(3): Serum GPT (ALT), GOT (AST) and ALP levels in normal control, diabetic control and the treated diabetic mice at high dose (40 mg/kg) of catechin extract for different periods.

Groups	GPT U/l (Mean +SD)	GOT U/l (Mean +SD)	ALP U/l (Mean +SD)
Normal control	30.22 ±3.21 ^a	63.34 ±7.62 ^a	70.84± 8.02 ^a
Diabetic control	49.05 ±4.66 ^b	82.03 ±10.22 ^b	88.9 ±14.04 ^b
Treatment for 1 week	38.81 ±2.89 ^c	75.42 ±7.81 ^c	80.39 ±4.62 ^b
Treatment for 2 weeks	32.46 ±2.15 ^a	65.08 ±6.47 ^a	77.71 ±6.92 ^{ab}
Treatment for 3 weeks	32.33 ±3.08 ^a	63.29 ±5.76 ^a	71.63 ±5.08 ^a

Differences a, b, c are significant (P <0.05) to compression columns.

The normal value for each of the studied enzymes in the treated diabetic mice has been attained at the third week of the treatment. In diabetes, the elevated activity of ALT represents the hepatocellular damage which is usually accompanied by an increase in AST and ALP activities. Furthermore, the activities of ALT, AST and ALP have been used as an indicator of liver function. Thus, the decreased of activity of these enzymes in treated diabetic mice by catechin extract is evidence of the prevention of cellular and tissue damage under diabetic conditions. These results may support

the optimized lipid metabolism in the liver of diabetic mice [30, 31].

Tables 4 and 5 show levels of serum TGs, total cholesterol and HDL-c in normal, diabetic and the treated diabetic mice in both low and high doses of catechin extract for 1-3 weeks. The results show that the normal value of TGs of the treated diabetic mice has been attained at the second week of the treatment in both low and high doses of catechin extract, while the normal value of cholesterol was obtained at the third week of the treatment in both low and high doses of catechin extract.

Table(4): Serum TGs, cholesterol and HDL-c levels in normal control, diabetic control and the treated diabetic mice at low dose (30 mg/kg) of catechins extract for different periods.

Groups	TGs mg/dl(Mean±SD)	Chol. mg/dl(Mean ±SD)	HDL mg/dl(Mean ±SD)
Normal control	168.4 ±4.31 ^a	200.31 ±6.02 ^a	93.20 ±3.40 ^a
Diabetic control	210.4 ±3.38 ^b	263.40 ±3.08 ^b	68.30 ±4.61 ^b
Treatment for 1 week	192.2 ±2.36 ^c	253.11 ±4.41 ^c	70.60±6.43 ^b
Treatment for 2 weeks	173.6 ±6.70 ^a	242.08 ±6.81 ^c	81.00 ±4.56 ^c
Treatment for 3 weeks	174.8 ±3.09 ^a	208.20 ±3.36 ^a	83.20 ±6.02 ^a

Differences a, b, c are significant (P <0.05) to compression columns.

Table(5): Serum TGs, cholesterol and HDL-c levels in normal control, diabetic control and the treated diabetic mice at high dose (40 mg/kg) of catechins extract for different periods.

Groups	TGs mg/dl(Mean±SD)	Chol. mg/dl(Mean ±SD)	HDL mg/dl(Mean ±SD)
Normal control	168.4 ±4.31 ^a	200.31 ±6.02 ^a	93.20 ±3.40 ^a
Diabetic control	210.4 ±3.38 ^b	263.40 ±3.08 ^b	68.30 ±4.61 ^b
Treatment for 1 week	184.4 ±2.36 ^c	243.11 ±3.61 ^c	74.30 ±6.69 ^c
Treatment for 2 weeks	177.6 ±3.80 ^a	230.02 ±6.81 ^c	77.40 ±6.02 ^c
Treatment for 3 weeks	175.6 ±6.65 ^a	209.30 ±3.65 ^a	86.63 ±4.49 ^a

Differences a, b, c are significant (P <0.05) to compression columns.

In contrast, the normal value of total cholesterol and HDL-c was attained at the third week of the treatment for both low and high doses of catechin extract. The results show that HDL value in diabetic state was decrease (state b) in comparison of normal control (state a). The treatment by catechin extract after 3 weeks increases the value of HDL to near of normal value in comparison of diabetic control.

In alloxan-induced diabetes, the increasing in plasma cholesterol, triglycerides and decreases in HDL levels is usually associated with an increase in glucose level. In this case, insulin deficient state turns into the activation of hormone-sensitive lipase (HSL) which is resulted to enhance the release of free fatty acids from adipose tissue. Consequently, excess fatty acids in the blood produced by the alloxan-induced diabetes converts into phospholipids and cholesterol in the liver. These two substances, along with excess triglycerides formed in the liver, may be discharged into the blood in the form of lipoproteins [32]. Thus, hyperlipidemia may be regarded as a consequence of the activated actions of lipolytic hormones on fat depots. In spite the presence of hyperlipidemia case, which results by alloxan action, the treatment with the catechin extract was normalized the serum lipid status together with its action in normalized serum glucose level. Our findings are in agree with literature. [33-37].

In conclusion, the *catechin* extract possesses antihyperglycemic and antihyper-lipidemic activities. In addition, *catechin* extract could also ameliorate the impaired liver functions by inhibit its disorders associated with alloxan diabetes. These findings suggesting that catechin extract might be useful for the treatment of alloxan – diabetic mice model, which may be generally applicable on diabetes by further studies.

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تأثير مستخلص الكاتشين الكحولي على فرط السكر وفرط الدهون ووظائف الكبد في الفئران المستحدثة لداء السكري بالالوكزان

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الخلاصة:

الهدف من هذه الدراسة هو تقييم تأثير مستخلص الكاتشين الكحولي تجاه مستوى كلوكوز الدم ونمط الدهون ووظائف الكبد في الفئران المستحدثة لداء السكري بالالوكزان. تم تقسيم 50 فارة صحية (25- 30 غم) الى خمسة مجاميع ، 10 فئران لكل مجموعة. استلمت المجموعة A المحلول الملحي كمجموعة ضابطة طبيعية. تم زرق المجاميع الاربعة B, C, D, E بمادة الالوكزان (150 ملغم / كغم) من وزن الجسم عن طريق الصفاق لاستحداث السكري. تمثل المجموعة B المجموعة الضابطة للفئران المستحدثة لداء السكري بالالوكزان. و تم زرق المجاميع E, D, C المستحدثة لداء السكري بمستخلص الكاتشين وبجرعتين (30 ملغم/كغم و 40 ملغم /كغم من وزن الجسم) لفترات مختلفة (1 و 2 و 3 اسابيع) كمجاميع معالجة. تم تقدير مستويات سكر الدم ودهون المصل (الكوليستيرول ، الكليسيريدات الثلاثية والبروتينات الدهنية العالية الكثافة) والاسباراجين ترانس امينيز والالنين ترانس امينيز و الفوسفاتيز القاعدي بعد 1 اسبوع و 2 اسبوع و 3 اسبوع من فترة الاصابة بالسكري المستحدث بالالوكزان. اظهرت المجموعة B زيادة معنوية في مستويات كلوكوز الدم و الكوليستيرول و الكليسيريدات الثلاثية و الاسباراجين ترانس امينيز و الالنين ترانس امينيز و الفوسفاتيز القاعدي مقارنة بالمجموعة A. و اظهرت المجاميع E, D, C نقصا معنويا في متغيرات المصل البايوكيميائية المقاسة المذكورة مقارنة مع المجموعة B. على العكس من ذلك ، اظهرت المجاميع E, D, C زيادة معنوية في مستوى البروتينات الدهنية عالية الكثافة بالمقارنة مع المجموعة B. بينت النتائج بوضوح ان مستخلص الكاتشين الكحولي يمتلك فعالية لتقليل فرط السكر وفرط الدهون وله القابلية على تحسين وظائف الكبد في حالة السكري المستحدث بالالوكزان.