

## Study the Effects of Polyphenolic Cocoa beans Extracts (CE) in Streptozotocin-induced diabetic mice.

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Date of acceptance 1/3 / 2010

### Abstract

Diabetes Mellitus is a group of metabolic diseases characterized by increasing of glucose level in plasma compared with normal value (hyperglycemia). This disease also causes elevation of lipid profile levels except HDL (High density lipoproteins) which increased relatively.

The effects of the polyphenolic mixture (catechins, epicatechins, procyanidin B<sub>1</sub>, procyanidin B<sub>2</sub> and procyanidin C<sub>1</sub>) on total cholesterol (TC), triacylglycerol (TG), high density lipoprotein (HDL) and low density lipoprotein (LDL) were studied in (30) streptozotocin-induced diabetic mice with (20-25)gm weight.

Mice were given (30 mg/mL) of Polyphenolic Cocoa beans Extracts (CE) once daily for (7) days before Streptozotocin STZ injection and for (21 day) there after. At (day7) (TC), (TG) and (LDL) levels were elevated ( $P < 0.001$ ) and (HDL) level was decreased ( $P < 0.001$ ) compared with control group because of the effect of streptozotocin.

A decreasing of (TC), (TG) and (LDL) ( $P < 0.05$ ) and increasing of HDL levels ( $P < 0.05$ ) at (day 21) were proved because of the protective effect of polyphenols against oxidative stress.

**Key words:** Study, Cocoa beans, diabetic mice, Polyphenolic .

### Introduction

Diabetes Mellitus, a state of chronic hyperglycemia is a common disease causes an elevation in glucose, total cholesterol (TC), triacylglycerol (TG), low density lipoproteins (LDL), very low density lipoproteins (VLDL) and malondialdehyde (MDA) levels and decreasing of high density lipoproteins (HDL) level because the elevation of oxidative stress[1]

The increased lipid peroxidation may contribute to the pathogenesis of the diabetic complications such as atherosclerosis and heart disease[1,2]

Cocoa beans have been reported to be a rich source of

polyphenols, especially catechin and its derivatives[2].

These compounds inhibit the diabetogenic action of streptozotocin (STZ) in animal studies because these compounds are reactive antioxidants [2,3]

Earliest studies established that the major polyphenols in cocoa beans are catechin, epicatechin, the dimmers epicatechin-catechin (procyanidin B<sub>1</sub>) and epicatechin-epicatechin (procyanidin B<sub>2</sub>) and the trimer epicatechin-epicatechin (procyanidin C<sub>1</sub>)[2].

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Catechins have protective effects against markers associated with the atherogenic process and to have antioxidant capacity higher than of other known antioxidants such as vitamin C and E[3]

Several studies in human and mice showed that a diet containing cocoa polyphenols extract (CE) reduced the glucose and lipid profile levels in STZ-induced diabetic mices[2]

The present study has been conducted on the protective effect of (CE) against (STZ) diabetogenic action[1,3].

## Materials and Methods

### 1. Preparation of ethanolic extract.[1,2]

The Malaysian cocoa (*Theobroma cocoa*) beans were purchased from a specific herbal shop in Al Mansur/Baghdad were roasted in an air oven for 25 min at (135-150)°C in the center of chemical researches in Ministry of sciences and Technology. After cooling to room temperature, the roasted beans were deshelled and cotyledone were ground and defatted with petroleum ether, the cotyledons were air dried to remove the solvent residue. The extract was prepared by treating the powder with ethanol (70%) for (3h), the residue was removed and the resulting extract was lyophilized. This extract was considered to be cocoa polyphenols-rich that used for total phenolic determination and the animal study. Polyphenols content was determined by folin ciocaltue assay, ethanol and sodium carbonate solution. The absorbance was recorded at (725 nm).

### 2. Animal groups and analysis:-

- Thirty mice (20-25) gm weight were kept at room temperature (about 25°) and allowed free access to respective diets and water, in a specific laboratory cooperative with post-graduates students in college of education (Ibn-al-Haitham).
- The animals were given (30 mg/ml) of polyphenolic compounds mixture:- (catechin, epicatechin, procyanidin B<sub>1</sub>, procyanidin B<sub>2</sub> and procyanidin C<sub>1</sub>) once daily for (7 days) before STZ (150 mg/kg) injection and for 21 days there after, at (days 7) mice were given the above polyphenolic extract (1 h) before STZ injection.
- At days (0, 7, 21) plasma lipid profile were measured using enzymic methods.

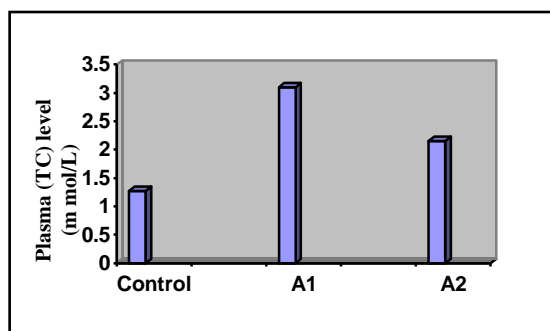
## Results

**Figure (1):-** The effect of polyphenols (30 mg/ml) on total cholesterol (TC) level in streptozotocin (STZ)-induced diabetic mice where:-

**Control group:-** healthy mice after (0) days of treatment.

**A1 group:-** diabetic mice given polyphenols for (7 days) after (STZ) injection.

**A2 group:-** diabetic mice given polyphenols for (21 days).



**Table(1):- The effect of polyphenols (30 mg/ml) on (TC) of studied groups.**

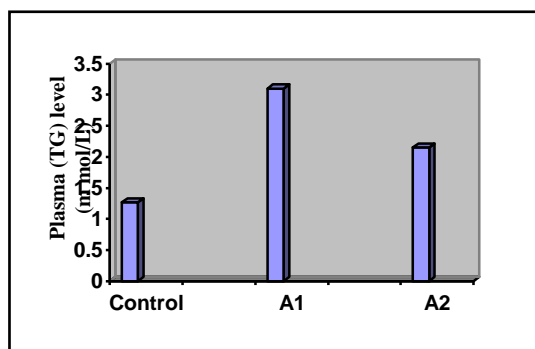
Subject	Number	Mean (TC) m mol/L	± S.D	T.Test
Control	30	1.27	0.60	
A <sub>1</sub>	30	3.10	0.91	P < 0.001
A <sub>2</sub>	30	2.15	0.73	P < 0.05

**Figure (2):-** The effect of polyphenols (30 mg/ml) on triacylglycerol (TG) level in streptozotocin (STZ)-induced diabetic mice where:-

**Control group:-** healthy mice after (0)days of treatment.

**A<sub>1</sub> group:-** diabetic mice given polyphenols for (7 days) after (STZ) injection.

**A<sub>2</sub> group:-** diabetic mice given polyphenols for (21 days).

**Table(2):- The effect of polyphenols (30 mg/ml) on (TG) of studied groups.**

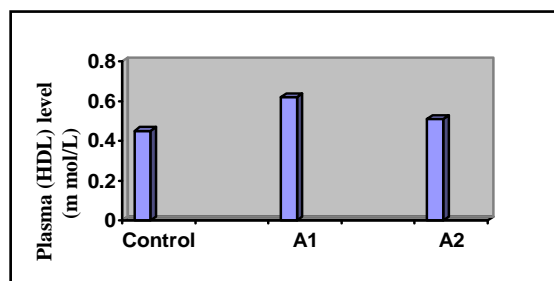
Subject	Number	Mean (TG) m mol/L	± S.D	T.Test
Control	30	0.55	0.023	
A <sub>1</sub>	30	0.71	0.098	P < 0.001
A <sub>2</sub>	30	0.62	0.045	P < 0.05

**Figure (3):-** The effect of polyphenols (30 mg/ml) on high density lipoproteins (HDL) level in streptozotocin (STZ)-induced diabetic mice where:-

**Control group:-** healthy mice after (0)days of treatment.

**A<sub>1</sub> group:-** diabetic mice given polyphenols for (7 days) after (STZ) injection.

**A<sub>2</sub> group:-** diabetic mice given polyphenols for (21 days).

**Table(3):- The effect of polyphenols (30 mg/ml) on (HDL) of studied groups.**

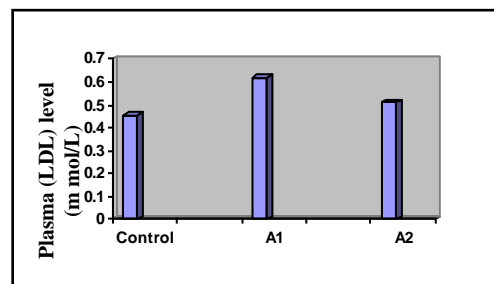
Subject	Number	Mean (HDL) m mol/L	± S.D	T.Test
Control	30	0.23	0.04	
A <sub>1</sub>	30	0.99	0.12	P < 0.001
A <sub>2</sub>	30	0.72	0.08	P < 0.05

**Figure (4):-** The effect of polyphenols (30 mg/ml) on low density lipoprotein (LDL) level in streptozotocin (STZ)-induced diabetic mice where:-

**Control group:-** healthy mice after (0)days of treatment.

**A<sub>1</sub> group:-** diabetic mice given polyphenols for (7 days) after (STZ) injection.

**A<sub>2</sub> group:-** diabetic mice given polyphenols for (21 days).



**Table(4):- The effect of polyphenols (30 mg/ml) on (LDL) of studied groups.**

Subject	Number	Mean (LDL) m mol/L	± S.D	T.Test
Control	30	0.45	0.033	
A <sub>1</sub>	30	0.62	0.052	P < 0.001
A <sub>2</sub>	30	0.51	0.48	P < 0.05

**Discussion:****1- Determination of total cholesterol (TC) level.**

Figure (1) and table (1) represents the reactive antioxidant effect of cocoa beans extract (CE) on (TC) level in STZ-induced diabetic mice. Plasma (TC) level in the group A<sub>1</sub> was significantly elevated at day (7) after (STZ) injection compared with day (0). This increasing due to STZ is a specific  $\beta$ -cell toxin and used to induce hyperglycemia in rats and mice. It is taken up by pancreatic  $\beta$ -cells via a glucose transporter (GLVT<sub>2</sub>) and causes alkylation of deoxyribonucleic acid (DNA). This process is more important for the diabetogenicity of (STZ). At this point, glucose would be oxidized to reactive oxygen species such as hydrogen peroxide (H<sub>2</sub>O<sub>2</sub>) and superoxide radical O<sub>2</sub><sup>-</sup> that cause lipid peroxidation (LPO)[1,2].

So stored lipids would be hydrolysed from tissues to act as energy source instead of glucose, therefore (TC) level was increased compared with day (0).[4,5]

At (day 21) total cholesterol was normalized because catechins or cocoa beans extract (CE) act as antioxidants by activate enzymes that inhibit (LPO) such as kinases and inhibit enzymes that activate oxidative stress such as xanthine oxidase[6].

**1- Determination of Triacylglycerol (TG) level.**

The plasma triacylglycerol level in group A<sub>1</sub> was significantly higher at day (7) after STZ injection compared

with day (0) (figure 2) and (table 2) because the increasing of hydrolysed lipids which act as energy source instead of glucose[4].

At (day 21)(TG) level in group A<sub>2</sub> was decreased relatively because the reactive roles of catechins as antioxidants[7,8].

**2- Determination of high density lipoprotein (HDL) level.**

The plasma (HDL) level in group A<sub>1</sub> was significantly decreased at day (7) after STZ injection compared with day (0) (figure 3) and (table 3) because the increasing of cholesterol ester transfer protein (CETP) activity. The last transfer cholesterol ester from HDL to VLDL, the result is free HDL filtrated from kidney.[9]

At (day 21) HDL elevated relatively because of catechins action as antioxidants.[7]

**3- Determination of low density lipoprotein (LDL) level.**

The plasma (LD) level in group A<sub>1</sub> was relatively increased at (day 7) after STZ injection compared with day (0) (figure 4) and (table 4) because the impairment of LDL receptors in cellular membranes (oxidative modification of LDL) and precipitation in arterial walls.<sup>(10)</sup>

At (day 21) LDL would be decreased relatively because the antioxidant effects of catechins.<sup>(8)</sup>

**Conclusion**

Cocoa beans extract (CE) containing polyphenolic compounds mixture (catechins, epicatechins, procyanidin B<sub>1</sub>, procyanidin B<sub>2</sub> and procyanidin C<sub>1</sub>) have a protective effect against hyperglycemia in STZ-induced diabetic mice.

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## دراسة تأثيرات مستخلص حبيبات الكاكاو البولي فينولية في الفئران المصابة بالسكري المحث بالستربتوزوتوسين

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### الخلاصة :-

يعد مرض السكري مجموعة من الامراض الايضية المتزامنة والتي تتصف بارتفاع مستوى الكلوكرز في بلازما الدم مقارنة بالقيم الطبيعية لدى الاصحاء. ان هذا المرض يتسبب ايضاً في الارتفاع مستويات الدهون ما عدا البروتينات الشحمية عالية الكثافة والتي يرتفع مستواها نسبياً.

ان تأثيرات مزيج البولي فينولات ( الكاتيشين , الايبيكاتيشين , البروسياندين B<sub>1</sub> , البروسياندين B<sub>2</sub> , والبروسياندين C<sub>1</sub> ) على الكوليسترول الكلي , الكليسيريدات الثلاثية , البروتينات الشحمية عالية الكثافة والبروتينات الشحمية واطئة الكثافة تم دراستها في الفئران المصابة بالسكري المحث بالستربتوزوتوسين ذات أوزان ( 20 - 25 ) غم.

ان هذه الفئران عوملت بمستخلص الكاكاو البولي فينولي ( 30 ملغم/مل ) ولمرة واحدة يومياً لمدة سبعة أيام قبل ان يتم الحقن بالستربتوزوتوسين ( في اليوم السابع ) ثم استمرت المعاملة بالمركبات البولي فينولية خلال الواحد والعشرين يوماً القادمة. في اليوم السابع يلاحظ ارتفاعاً في مستويات الكوليسترول الكلي , الكليسيريدات الثلاثية والبروتينات الدهنية واطئة الكثافة (  $P < 0.001$  ) وانخفاضاً في مستوى البروتينات الدهنية عالية الكثافة (  $P < 0.001$  ) مقارنة مع مجموعة السيطرة بسبب تأثير الستربتوزوتوسين.

ولقد تم اثبات انخفاض مستويات الكوليسترول الكلي , الكليسيريدات الثلاثية والبروتينات الشحمية واطئة الكثافة (  $P < 0.05$  ) وارتفاعاً في مستوى البروتينات الشحمية عالية الكثافة (  $P < 0.05$  ) في اليوم الحادي والعشرين بسبب التأثيرات الدفاعية للبولى فينولات المضادة لشدة الاكسدة.