

## Evaluation the Effect of Catechins Extract Intake for Iraqi Male Patients With Cardiovascular Diseases CVD.

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### Abstract

Cardiovascular diseases CVD are responsible for the majority of death in many countries, the term cardiovascular disease CVD includes several diseases such as: coronary artery diseases (angina, myocardial infarction MI, atherosclerosis) and stroke. These diseases cause elevation of TC, TG, LDL, VLDL, and MDA levels in plasma and decrease of HDL levels and PON activity in plasma, because of elevation in lipid peroxidation (LPO) activity.

Catechins is a water extract of green tea composed of: (-)epicatechin EC, ECG, EGC, EGCG, (+) catechin C and GC, these compounds play great roles against chronic diseases such as CVD. The effect of catechins extract on the preceding biochemical parameters was investigated in 40 volunteer male patients with CVD, catechins extract were given to patients three times daily for one month and all biochemical parameters were measured before and after administration with 250mg of catechins extract, then the results were compared with 30 volunteers healthy control.

The results indicated an elevation of HDL levels and PON activity, decrease of TC, TG, LDL, VLDL and MDA levels in plasma of male patients with CVD after administration of green tea catechins extract compared to control.

**Key words:** Catechins, Cardiovascular diseases CVD, Green tea.

### Introduction

Green tea (*Camellia Sinensis*; Theaceae) has attracted significant attention recently, for its health benefits for a variety of disorders. Historically, green tea has been consumed by the asian society, and is probably the most consumed beverage beside water. The beneficial effects of green tea are attributed to the polyphenolic compounds present in green tea, particularly the catechins, which make up 30% of the dry weight of green tea leaves and they exhibit biochemical and pharmacological activities including antioxidant activities[1].

These catechins belong to the flavonoids are present in higher quantities in green tea than in black tea, because of differences in the processing of tea leaves after harvest[2].

There are several polyphenolic catechins in green tea:

- (-) epicatechin (EC).
- (-) epicatechin -3-gallate (ECG).
- (-) epigallocatechin (EGC).
- (-) epigallocatechin-3-gallate (EGCG).
- (+) catechin (C).
- (+) galocatechin (CG).

(EGCG) the most abundant catechins in green tea, accounts for 65% of the total catechins content[1].( Fig.1)

The potential health benefits of green tea are mainly attributed to its antioxidant properties and the ability of its catechins to scavenge ROS. These properties are due to the presence of the hydroxyl groups on the B-ring in ungalloylated catechins (EC and EGC), (Fig.1) and in the B and C ring of galloylated catechins (ECG and EGCG)[3].

The presence of the 3,4,5-trihydroxy B-ring has been shown to be important for antioxidant and radical scavenging activity[2],[4].

The catechins have been shown to be more effective antioxidants than vitamins C and E,<sup>(5)</sup> and their order of effectiveness as radical scavengers is EGCG > (-) Epicatechin (EC) > (+) catechin (C) > vitamin E. The metal-chelating properties of green tea catechins are also important contributors to their antioxidative activity[4],[6].

Cardiovascular diseases CVD have no geographic and racial boundaries, they occur throughout the world, and are responsible for the majority of death in many countries. The term cardiovascular diseases CVD includes several diseases such as: coronary artery disease (angina, myocardial infarction MI, atherosclerosis), and stroke[7]. A deficient supply of oxygenated blood to the myocardium, causes central chest pain of varying intensity that may radiate to arms and jaws. The lumen of the blood vessels is usually narrowed by atheromatous plaques[8].

The major cause of cardiovascular diseases is atherosclerosis, the thickening and hardening of the artery walls caused by deposits of cholesterol-lipid-calcium plaque in the lining of the arteries.

It is well established that atherosclerosis is an inflammatory disorder[9]. LDL is believed to play a central role in initiating and promoting plaque formation it is deposited into sub endothelial space where it is taken up by various cells, including macrophages. This alters the gene and protein expression pattern of these cells and can promote an inflammatory response particularly when LDL becomes oxidized[10].

The following eight factors predispose individuals to atherosclerosis: age, sex, family history, dyslipidemia, smoking, hypertension, sedentary lifestyle, and diabetes mellitus[9].

Paraoxonase (PON), E.C (3.1.8.1) acts as an important antioxidant enzyme against oxidative stress, the enzyme has been implicated in the pathogenesis of a number of disorders including cardiovascular diseases, atherosclerosis and cancer[11].

The aim of the present study was to evaluate the effect of catechins extracted and purified from green tea leaves, on the plasma lipid profile (TC, TG, HDL, LDL, VLDL), MDA and PON activity in males patients with cardiovascular diseases CVD before and after administration of catechins extract.

## Material and Methods

### Chemicals:

All laboratory chemicals and reagents used were of analar grade.

### Patients Groups:

Forty male patients with cardiovascular diseases enrolled in this study with ages ranged from (35-50) years who are suffering from cardiovascular disease CVD for two years and higher, these samples were collected from volunteers in different laboratories. Patients groups are classified into two groups:-

\* **Group B** : include patients before administration of green tea catechins extract.

\* **Group A** : include patients after administration of 250mg green tea catechins extract, (three times daily for one month).



Thirty healthy male volunteers are included in this study as control group C, their ages range (35-50) years. Non of the controls has atherosclerotic vascular disease, diabetic (DM), renal disease, history of allergy, is heavy smoker or alcoholic consumption.

#### **Specimen Collection:**

Ten ml. of venous blood have been taken from control group and patients, before and after taking catechins extract, by using plastic disposable syringes after 12 hours of fasting. These tubes contain EDTA, the blood samples were allowed to stand for 10min. in an incubator at 37°C then centrifuged at 3000 RPM for 15min. to isolate plasma from blood cells, each sample was stored at deep freezer -20°C until time of analysis.

#### **Extraction and Identification of Catechins:**

Green tea leaves were finely ground into a powder with a homogenizer or mortar and pestle. The powder 100g were mixed with 2 liters of water previously brought to boiling point average temperature of water was approximately 80°C, the sample was then stirred with a magnetic stirrer for 20min. The cold sample was filtered through a 0.45 Millipore nylon filter[12].

The purified aqueous extract is spray-dried to give a very fine yellowish-brown color, water soluble powder identified by HPLC-UV. to give catechins[13].

#### **Catechins extract samples:**

Catechins was a form of capsules containing (250mg) that was extracted from green tea leaves, these capsules are given to patients with, cardiovascular diseases CVD, one capsule three time daily during a period of one month, and parameters are measured before and after treatment with catechins extract.

##### **A- Determination of plasma total cholesterol TC levels[14]:**

The concentration of total cholesterol TC was measured by enzymatic method[14], with commercially available kit, (Bio Merieux France). TC is determined spectrophotometrically at  $\lambda_{max}$ . 510nm.

##### **B- Determination of plasma Triacylglycerol TG levels[15].**

Plasma triacylglycerol TG concentration was measured by enzymatic method[15], by using available kit, (Bio Merieux France). The concentration of TG was determined at  $\lambda_{max}$ . 500nm.

##### **C- Determination of plasma HDL levels[16].**

HDL was measured with the phosphotungstic acid in the presence of magnesium ion  $Mg^{2+}$  precipitation method[16], and using commercially available kit, (Bio Merieux France), absorbance was recorded at  $\lambda_{max}$ . 510nm.

##### **D- Determination of plasma VLDL levels[17] and LDL levels[18].**

VLDL was determined according to the equation[17].

$$VLDL \text{ mg/dl} = TG \text{ mg/dl} / 5.$$

LDL was determined according to the equation[18].

$$LDL \text{ mg/dl} = TC \text{ mg/dl} - [HDL \text{ mg/dl} + VLDL \text{ mg/dl}].$$

##### **E- Determination of plasma MDA levels[19].**

Malondialdehyde is an end secondary product of lipid peroxidation of unsaturated fatty acid. It's determination is based on the formation of colored complex upon reaction with (TBA) thiobarbutyric acid.

##### **F- Estimation of plasma paraoxonase PON activity[20].**

PON activity in plasma was determined by using phenyl acetate as substrate, substrate hydrolysis rate was measured at 270nm.

## **Results and Discussion**

### **A- Catechins extract action on plasma total cholesterol TC levels.**

The effect of catechins extract 250mg on plasma TC levels Table-1, Fig.2 showed a significant decrease for group A (patients after treatment with catechins extract),  $205 \pm 15.2$  mg/dl, compared with group B (patients before treatment with catechins extract),  $238 \pm 16.4$  mg/dl but never achieved the normal value (control).

The results are explained based on that catechins extract activate enzyme that regulate lipid metabolism and inhibit lipid peroxidation LPO, and decreasing enzymes activities that activate oxidative stress[20].

The antioxidant activity of green tea catechins is due to their ability to affect the enzyme of the antioxidant system, catechins scavenge the ROO<sup>•</sup> radical by blocking the radical chain reaction and thus prevent the peroxidation process[1].

A recent study have shown that EGCG administration is associated with a simultaneous decrease in TC levels, there is no doubt that high levels of cholesterol are associated with atherosclerosis and coronary heart disease CHD. Hyper Cholesterolemia is usually associated more with angina pectoris than with myocardial infarction MI[21].

#### **B- Catechins extract action on plasma triacylglycerol TG levels.**

Data in table-1 and figure-3 represent the value of the plasma TG levels in control and patients groups *in vivo* study, the effect of catechins extract 250mg on plasma TG levels showed a significant decrease for group A (patients after treatment with catechins extract),  $134 \pm 12.5$  mg/dl compared with group B (patients before treatment with catechins extract)  $174 \pm 17$  mg/dl, but never achieved the normal value (control).

In various tissues such as skeletal muscles, heart and adipose tissue, lipoprotein lipase LPL hydrolyzes TG on chylomicrons, the free fatty acids and glycerol generated by the hydrolysis of TG, can then be taken up cells and used as a source of energy[22].

The interpretation for our results could be explained as catechins extracts 250mg leads to increase of LPL activity in patients group A, this enzyme catalyzes the hydrolysis of TG. TG are being considered as an independent risk factor of cardiovascular diseases CVD and lipid metabolic syndrome by dysregulation of TG/HDL axis[23].

#### **C- Catechins extract action on plasma HDL levels.**

The effect of catechins extract 250mg on plasma HDL levels, table-1, figure-4 showed a significant increase for group A (patients after treatment with catechins extract)  $43 \pm 3.6$  mg/dl compared with group B (patients before treatment with catechins extract)  $36.9 \pm 7.2$  mg/dl, but never achieved the value of control.

One important cardioprotective function of HDL is to remove cholesterol from lipid-laden macrophages in the artery walls. HDL also exerts anti-inflammatory effects that might inhibit atherogenesis. However, HDL has been proposed to be dysfunctional in humans with established cardiovascular diseases CVD[24]. Low level of HDL reported in this study is in agreement with patients of CVD, HDL facilitates enzymatic conversion to cholesterol esters which moves to the core of the HDL particle and returns to the liver[25].

Our observations indicates that green tea catechin extract, activates LCAT and PAF-AH platlet activated factor acyl transferase. These enzymes inhibited lipid peroxidation, catechins extract also activate paraoxonase PON activity, this enzyme catalyzes the hydrolysis of lipid peroxides, cholesteryl linoleate hydroperoxides in oxidized HD[26]. There is an inverse relationship between CVD risk and HDL concentration. HDL level is needed to be increased through exercise, weight loss and carbohydrate free diet. Cigarette smoking (Ten cigarettes per day) increases the CVD risk three-fold due to reduce level of HDL[27].

#### **D- Catechins extract action on plasma VLDL levels.**

The effect of catechins extract 250mg on plasma VLDL levels. (Table-1, figure-5) showed a decrease for group A ((patients after treatment with catechins extract),  $26.8 \pm 2.4$  mg/dl compared with group B (patients before treatment with catechins extract),  $34 \pm 3.4$  mg/dl.

VLDL is produced by the liver and contains apo B-100, apo E and apo Cs; like chylomicrone, they are also rich in TG. They are the major carriers of endogenous TG and

transfer TG from the liver to peripheral tissue<sup>(22)</sup>. Because the largest amount of TG are positioned on VLDL, therefore our results can be explained just like our interpretation about the effect of catechins extract action on plasma TG levels.

#### E- Catechins extract action on plasma LDL levels.

The effect of 250mg of catechins extract on plasma LDL levels (Table-1, figure-6) showed a significant decrease for group A (patients after treatment with catechins extract), 125.3±10.4 mg/dl compared with group B (patients before treatment with catechins extract), 167.1±9.6 mg/dl, but never achieved the value of control.

LDL particles are the major lipoprotein responsible for the delivery of exogenous cholesterol to peripheral cells due to efficient uptake of LDL by the LDL receptors. The accumulation of LDL in the plasma leads to the development of atherosclerotic CVD[28].

Increase of LDL levels for group B (patients before treatment with catechins extract) due to elevation of lipid peroxidation, but after oral administration by 250mg of catechins extract the levels of LDL in patients were decreased about normal value. The role of catechins extract could be explained basing on its contents (EGCG and epicatechine EC are very strong active antioxidants that depress oxidative stress maximally by activated Monoprotein kinase[29].

#### F- Catechins extract action on plasma MDA levels.

Data in table-1 and figure-7 represent the value of the plasma MDA levels in control and patients groups, the effect of 250mg of catechins extract on plasma MDA levels showed a significant high decrease for group A (patients after treatment with catechins extract), 0.65±0.07nmol/dl compared with group B (patients before treatment with catechins extract), 1.89±0.19nmol/dl, but never achieve the value of control.

Reactive oxygen species ROS degrade polyunsaturated lipid, forming Malondialdehyde MDA, this compound is a reactive and is one of the many reactive electrophile species that cause toxic stress in cell and form advanced lipoxidation end-product, MDA one of the most frequently used indicators of lipid peroxidation in patients with CVD[30]. The interpretation of our results could be explained on the contents of catechins extract (EGCG, (-) epicatechin, ECG and EGC), these compounds involved in activation of several enzymes activities which regulate lipid metabolism and also inhibition of inzymes activities catalyzed lipid peroxidation[31].

#### G- Catechins extract action on plasma paraoxonase PON activity.

The effect of catechins extract 250mg on plasma paraoxonase PON activity, table-1 and figure-8 showed a significant high increase for group A (patients after treatment with catechin extract), 115±14.8 U/ml. compared with group B (patients before treatment with catechins extract), 61±7.3 U/ml. , but never achieved the value of group control.

PON activity are implicated in lowering the risk of developing CVD and atherosclerosis. PON activity prevent the formation of atherogenic oxidized-LDL, the form of LDL present in foam cells of an atheromatous plaque[32]. The explanation of our results was based on the role of green tea catechins extract and its contents (EGCG, (-) epicatechins, ECG and EGC) that could act through their antioxidant character that leads to elevate the activity of paraoxonase. PON activity protect against the formation of atherosclerotic plaques by decreasing lipid peroxidation[33].

## Conclusion

The interpretation of the results of our study indicated that the green tea catechins extract have a major role to play in improving lipid profile parameters and have a significant cardio protective effect in male patients with CVD.

Hopefully, the coming years will see the regular use of green tea catechins as an invaluable component of the management strategy to control disorder of lipid and cholesterol metabolism.

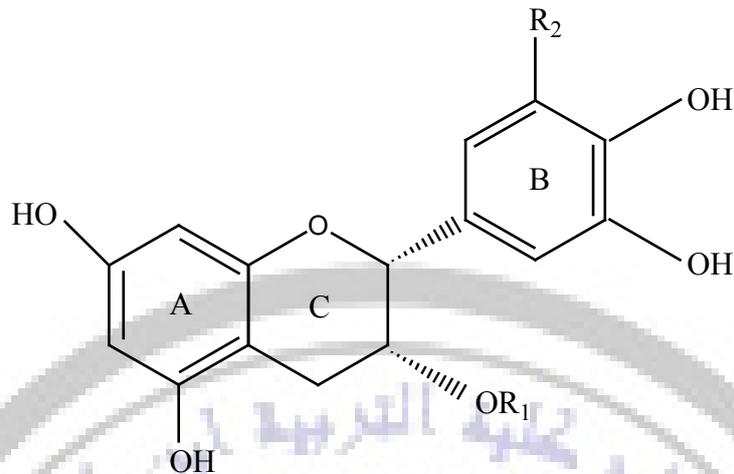
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**Table(1): leveles of lipids profile, MDA and PON activity in plasma of patients groups (Before and after treatment with catechins extract) and control.**

Biochemical Parameters	Studied Groups	B	A	Controls
		Patients group before treatment with catechins extract. No. = 40	Patients group after treatment with catechins extract. No. = 40	No. = 30
TC (mg/dl.)		238±16.4	205±15.2	190±11.2
TG(mg/dl.)		174±17	134±12.5	120±12
HDL(mg/dl.)		36.9±7.2	43.9±3.6	45.7±7.6
VLDL(mg/dl.)		34±3.4	26.8±2.4	24±2.8
LDL(mg/dl.)		167.1±9.6	125.3±10.4	120±7.7
MDA(nmol/dl.)		1.89±0.19	0.65±0.07	0.41±0.08
PON(U/ml.)		61±7.3	115±14.8	140±13.1



Epicatechin:  $R_1 = R_2 = H$

Epigallocatechin;  $R_1 = H, R_2 = OH$

Epicatechin-3-gallate;  $R_1 = Galloy, R_2 = H$

epigallocatechin-3-gallate:  $R_1 = Galloy, R_2 = OH$

Fig. (1): Structure of polyphenolic catechins present in green tea[1].

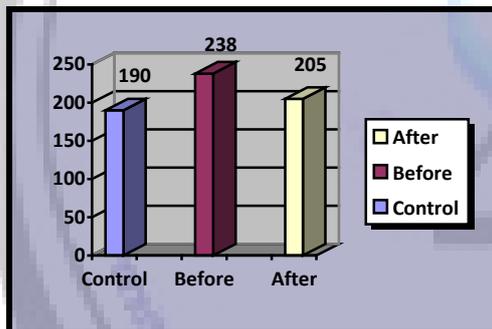


Fig. (2): Levels of TC in plasma of patients groups (Before and after treatment with catechins extract) and control.

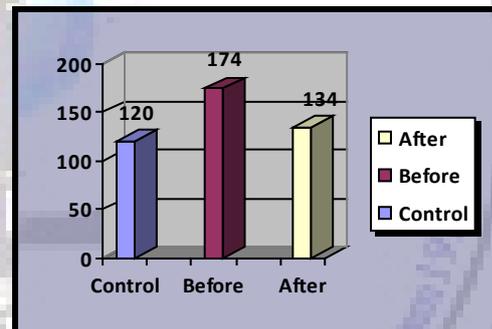


Fig. (3): Levels of TG in plasma of patients groups (Before and after treatment with catechins extract) and control.

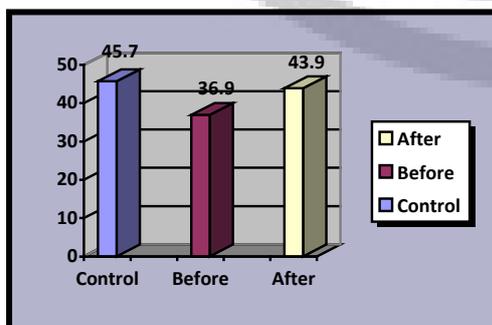


Fig. (4): Levels of HDL in plasma of patients groups (Before and after treatment with catechins extract) and control.

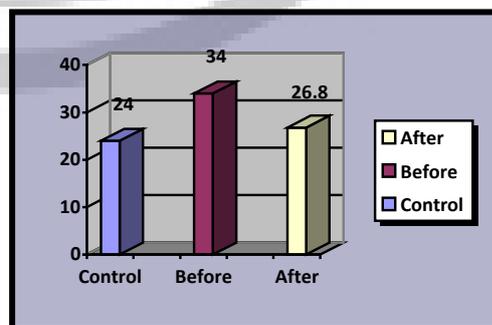


Fig. (5): Levels of VLDL in plasma of patients groups (Before and after treatment with catechins extract) and control.

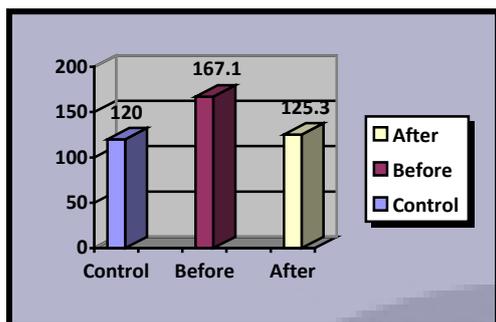


Fig.(6): Levels of LDL in plasma of patients groups (Before and after treatment with catechins extract) and control.

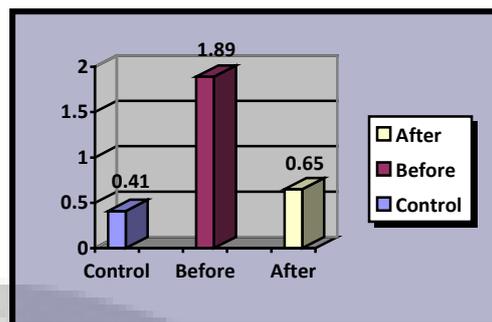


Fig. (7): Levels of MDA in plasma of patients groups (Before and after treatment with catechins extract) and control.

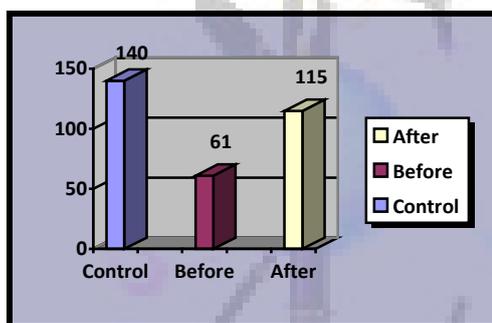


Fig. (8) : Levels of PON activity in plasma of patients groups (Before and after treatment with catechins extract) and control.



## تقييم تأثير مستخلص الكاتيشين المعطى للمرضى العراقيين من الذكور والمصابين بأمراض الأوعية القلبية CVD

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قسم الكيمياء ، كلية التربية - ابن الهيثم ، جامعة بغداد

استلم البحث في: 31 تموز 2011 قبل البحث في: 16 تشرين الثاني 2011

### الخلاصة

تعد الأمراض القلبية الوعائية السبب الأساسي وراء الوفيات في عدد كبير من البلدان المختلفة. وتشمل الأمراض القلبية الوعائية أمراض عديدة مثل الذبحة الصدرية ، احتشاء عضلة القلب ، تصلب الشرايين والجلطة. هذه الأمراض هي السبب وراء ارتفاع مستويات الـ TC, TG, LDL, VLDL, MDA وانخفاض مستويات HDL ونشاط انزيم PON في بلازما الدم ، وهذا نتيجة لارتفاع فعاليات الاكسدة الفوقية للدهون.

مستخلص الكاتيشين المائي من اوراق الشاي الاخضر يتكون من :- ( - ) الايبي كاتيشين ( EC ، ECG ، EGC ، EGCG ) ، (+) كاتيشين C والـ GC . لهذه المركبات دور فعال وكبير للوقاية من الامراض المزمنة مثل الـ CVD .

ان تأثير مستخلص الكاتيشين على الدوال الكيموحياتية سابقة الذكر ، قد تم دراستها على 40 مريضاً متطوعاً من الذكور المصابين بالـ CVD، إذ تم اعطائهم مستخلص الكاتيشين ثلاث مرات يومياً ولمدة شهر واحد كما قيست جميع الدوال الكيموحياتية قبل وبعد العلاج بـ 250mg من مستخلص الكاتيشين. تم كذلك مقارنة النتائج مع 30 من المتطوعين الاصحاء الذكور.

اشارت نتائج الدراسة الى ارتفاع في مستويات الـ HDL والنشاط الانزيمي للـ PON ، وبالمقابل لوحظ انخفاض في مستويات الـ TC, TG, LDL, VLDL, MDA في بلازما دم المرضى المتطوعين من الذكور والمصابين بالـ CVD بعد إعطائهم مستخلص كاتيشين أوراق الشاي الأخضر.

الكلمات المفتاحية : الكاتيشين ، الامراض القلبية الوعائية ، الشاي الاخضر