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## THE EFFECT OF SOME NATURAL PRODUCTS ON BLOOD CHEMISTRY OF ALBINO RATS UNDER THE INFLUENCE OF IRON OVERLOAD

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### ABSTRACT

The present work was performed to evaluate the protective effect of green tea or licorice on blood chemistry under the influence of the undue effects of iron overload. Male albino rats were fed prepared basal diet containing 1500 mg of ferric iron / kg, iron overload diet was concomitantly administered with a daily oral dose of green tea aqueous extract equivalent to 3.5 mg /100g b wt / day or 100 mg / kg b wt / day of licorice aqueous extract for eight weeks. Iron overload exerted a well marked increase in serum iron level associated with a significant increase in lipid profile, serum aspartate amino transferase (AST) and alanine amino transferase (ALT) activities as well as a significant increase in serum urea and creatinine. The obtained results were statistically compared with iron adequate and iron deficient diet fed groups. The concomitant oral administration of green tea or licorice with iron overload diet exerted a significant decrease in serum iron levels; a more dramatic lowering effect was elicited by licorice, which must be taken into consideration. Apart from the negative effect of the licorice on HDL-cholesterol, the protective effect of the two test plants against the undue effects of iron overload was indicated by the well marked improvement in liver and kidney function as well as the reduction of increased lipid profile.

### INTRODUCTION

Iron is an essential element and its deficiency is a nutritional problem in both developed and developing countries. Consequently, many types of dietary iron supplementation are used. Some

researchers have suggested that the general population is consuming excess iron due to these supplements. They found that several types of diseases are provoked by short- or long-term exposure to iron in quantities above the capacity of the organism to protect itself against iron's reactivity and that iron's role in pathological processes is related to its ability to catalyze reactions that lead to the formation of oxygen free radicals (Fraga and oteiza, 2002). The redox activity of free iron is inhibited by the presence of iron chelators (Ferrali *et al.*, 2001).

Flavonoids represent one class of bioactive compounds that may have multiple beneficial effects on several chronic diseases. Flavonoids are polyphenols compounds that are synthesized by plants and that can be incorporated in animals through the diet. Interestingly, these compounds can play a double role in reducing the rate of oxidation, as they can participate in iron chelating (Jagetia *et al.*, 2004 and Pardo – Andreu 2008).

Tea is the second most consumed beverage in the world, water being the first. The chemical composition of green tea (*Camellia sinensis*), with respect to its main constituents, is similar to that of the plant's fresh leaves. Up to 30% of green tea's dry leaves are polyphenols. Most of these polyphenols are flavonols, which is usually called catechins. The main catechins in green tea are epigallocatechin- 3 gallate (EGCG), epicatechin-3 gallate, epigallocatechin, and epicatechin. Green green tea polyphenols can chelate metals ions like iron and copper to prevent their participation in Fenton and Harber – Weiss reactions (McKay and Blumberg 2002, and kim *et al.*, 2003).

The root of *Glycyrrhiza glabra* (licorice) is one of the most popular herbal medicines in the world due to its exceptional pharmacological properties recognized by the traditional medicine. Licorice contains glycyrrhizin, olean triterpenoids, glucose, and flavonoids. Glabridin is an isoflavan compound and one of the major active flavonoids in licorice (Chin *et al.*, 2007 ; Qing Yu *et al.*, 2008).

The present study was performed to evaluate the protective effect of the aqueous extracts of green tea or licorice as natural and traditional beverage on blood chemistry of albino rats under the influence of the undue effects of iron overload.

## MATERIALS AND METHODS

### Chemicals

Ferric citrate was purchased from Sigma-Aldrich Chemical co. All other chemicals and kits used were obtained at the highest purity available.

### Green tea

Green tea was obtained from the local market (Cairo, Egypt). Green tea aqueous extract was prepared according to Hassan and Ahmed (2004). Twenty gram of green tea leaves were added to 500 ml of boiled water and steeped for 5 minutes at room temperature. The extract was separated from the tea leaves by filtration and freeze – dried. Tea extract was dissolved in distilled water at a concentration of 0.7%. Each rat was orally administered 1ml tea solution per day. The dose was derived by exploration from mice according to Hassan and Ahmed, (2004) as described by Paget and Barnes, (1964).

### Licorice

Licorice was purchased from the local market (Cairo, Egypt). Licorice aqueous extract was prepared according to Lee *et al.*, (2007) by boiling 600 g of licorice in five liters of water for three hours. Then filtering the solution and freeze dried. Rats were administered either a vehicle (tap water) or licorice aqueous extract 100 mg / kg body weight / day (Lee *et al.*, 2007).

### Animals

Male albino rats weighing  $150 \pm 30$ g were kept under normal environmental conditions and acclimatized for two weeks prior to experimentation, during which they were allowed access to food (standard diet prepared according to NRC, 1995; the ferric premix was prepared from ferric citrate (16.5% Fe) as described by Cockell *et al.*, 2005) and water *ad libitum*.

### Experimental Design

The rats were randomly divided into equal seven groups (16 for each) according to iron status and medicinal plants treatment as follows:

Group I (Iron deficient group): fed a basal diet containing 10 mg ferric iron / kg diet (Trinder *et al.*, 2002); Group II (Iron adequate group): fed a basal diet containing 35 mg ferric iron / kg diet (Cockell *et al.*, 2005), Group III (Iron overload group): fed a basal diet

containing 1500 mg ferric iron / kg diet (Cockell *et al.*, 2005); Group IV: fed a basal diet containing 35 mg ferric iron / kg diet concomitant with a daily oral dose of green tea aqueous extract (3.5 mg / 100g b.wt.); Group V: fed a basal containing 1500 mg ferric iron / kg diet concomitant with a daily oral dose of green tea aqueous extract (3.5 mg / 100g b. wt.); Group VI: fed a basal diet containing 35 mg ferric iron / kg diet concomitant with a daily oral dose of licorice water extract (100 mg / kg b. wt.); Group VII: fed a basal diet containing 1500 mg ferric iron / kg diet concomitant with a daily oral dose of licorice water extract (100 mg / kg b.wt. ). The experimental period was lasted for eight weeks. Blood samples were individually collected at the end of 4 and 8 weeks. Serum was separated and kept at -20°C till be used.

### **Biochemical analysis**

The following parameters were determined: serum Iron, Total Iron Binding Capacity (TIBC) by Ceriotti and Ceriotti ,1980 and Tabacco *et al.*, 1981 methods, Transferrin saturation (TS %) was calculated as (Serum iron / TIBC × 100); Triacylglycerol (TAG) by Fossati and Prencipe 1982 method ; Total Cholesterol ( TC ) by Richmond 1973 method , High density lipoprotein– Cholesterol (HDL-C) by Warnick *et al.*,1983 method , ALT and AST by Reitman and Frankel, 1957 method , Urea, according to the method of Tabacco *et al.*, 1979 and Creatinine by Faulkner and king, 1976 method.

The results were evaluated by one-way ANOVA using SPSS software version 15.0.

## **RESULTS AND DISCUSSION**

### **Results**

Serum iron level was significantly decreased in iron deficient fed rats (Group I) in comparison with iron adequate group (Group II) at the same time interval. Such decrease was accompanied by a well marked increase in serum TIBC level and a significant decrease in transferrin saturation percent (TS %). On the other hand, iron overload fed rats (Group III) exhibited the highest serum iron level associated with a non significant decrease in TIBC and a significant increase in TS % (Table 1).

The concomitant administration of green tea or licorice with iron adequate diet (Groups IV and VI) restore the changes in serum iron levels, TIBC and TS % until the end of the fourth week. Meanwhile,

serum iron level tends to elucidate a significant decrease accompanied by a non significant increase in TIBC level as well as a significant decrease in TS % at the end of the experimental period. The administration of green tea or licorice with iron overload diet (Groups V and VII) exerted a well marked decrease in serum iron level associated with a significant increase in serum TIBC level and a significant decrease in TS % allover the experimental period (Table1).

Serum triacylglycerol (TAG) and total cholesterol levels were insignificantly changed in iron deficient fed rats when statistically compared with iron adequate fed rats (Group II). Similar findings were also observed when green tea or licorice concomitantly administered with iron adequate diet (Group IV and VI, Table 2). Meanwhile, feeding of iron overload diet induced a significant increase in serum TAG and total cholesterol levels in comparison with iron adequate fed rats at the same time intervals. This increment was significantly decreased with the concomitant administration of either green tea or licorice (Table 2).

Iron overloaded rats (Group III) showed a significant increase in HDL-C level at the end of the experimental period when statistically compared with iron adequate fed rats at the same time intervals. The concomitant administration of green tea with iron adequate or iron overload diet (Groups IV and V) exhibited a gradual increase in serum HDL-C level. On the contrary, the concomitant administrations of licorice with iron overload diet (Group VII) significantly decreased HDL-C level at the end of the experimental period (Table 2).

The administration of iron overload diet (Group III) caused a significant increase in both serum ALT and AST activities in comparison with iron adequate diet (Group II) at the same time intervals. The concomitant administration of green tea or licorice with iron overload diet ameliorates such effect. The obtained results revealed the most potent lowering effect of licorice (Table 3).

Serum urea and Creatinine were significantly elevated in iron overload fed rats when statistically compared with iron adequate fed rats (Group II) at the same time interval (Table 4). The concomitant administration of green tea or licorice displayed no effect on such increments. On the other hand, the concomitant administration of licorice with iron overload significantly decrease urea level (Table 4).

**Table (1), effect of Green tea or Licorice aqueous extract on serum iron ( $\mu\text{g}/\text{dl}$ ), total iron binding capacity ( $\mu\text{g}/\text{dl}$ ) and transferrin percent (TS %) under the effect of iron overload**

Time Groups	4 Weeks			8 Weeks		
	Serum iron	TIBC	% TS	Serum iron	TIBC	% TS
Group I	196.8 $\pm$ 4.8 <sup>A</sup>	883.2 $\pm$ 40.3 <sup>C</sup>	22.4 $\pm$ 0.6 <sup>A</sup>	165.1 $\pm$ 3.7 <sup>A</sup>	811.3 $\pm$ 29.0 <sup>C</sup>	20.4 $\pm$ 0.3 <sup>A</sup>
Group II	231 $\pm$ 5.4 <sup>B</sup>	657.7 $\pm$ 4.1 <sup>AB</sup>	35.1 $\pm$ 0.7 <sup>B</sup>	246.1 $\pm$ 6.8 <sup>C</sup>	670.8 $\pm$ 7.6 <sup>AB</sup>	36.7 $\pm$ 0.9 <sup>C</sup>
Group III	267.6 $\pm$ 9.1 <sup>C</sup>	611.7 $\pm$ 11.8 <sup>A</sup>	43.8 $\pm$ 1.2 <sup>E</sup>	312.4 $\pm$ 5.0 <sup>D</sup>	590.1 $\pm$ 6.6 <sup>A</sup>	52.9 $\pm$ 0.6 <sup>D</sup>
Group IV	216 $\pm$ 3.5 <sup>B</sup>	613.4 $\pm$ 10.1 <sup>A</sup>	35.3 $\pm$ 0.8 <sup>C</sup>	198.2 $\pm$ 4.5 <sup>B</sup>	707.8 $\pm$ 23.6 <sup>B</sup>	28.2 $\pm$ 1.5 <sup>B</sup>
Group V	228.3 $\pm$ 4.4 <sup>B</sup>	722.1 $\pm$ 30.8 <sup>B</sup>	32.0 $\pm$ 1.9 <sup>BC</sup>	205.1 $\pm$ 3.2 <sup>B</sup>	846.4 $\pm$ 51.6 <sup>C</sup>	24.8 $\pm$ 1.8 <sup>B</sup>
Group VI	225.2 $\pm$ 4.0 <sup>B</sup>	645.1 $\pm$ 18.5 <sup>AB</sup>	35.1 $\pm$ 1.4 <sup>C</sup>	195.9 $\pm$ 9.2 <sup>B</sup>	759.9 $\pm$ 44.5 <sup>BC</sup>	26.1 $\pm$ 1.5 <sup>B</sup>
Group VII	221.9 $\pm$ 5.7 <sup>B</sup>	907.2 $\pm$ 47.9 <sup>C</sup>	24.8 $\pm$ 1.4 <sup>AB</sup>	174.6 $\pm$ 6.4 <sup>AB</sup>	840.3 $\pm$ 16.4 <sup>C</sup>	20.8 $\pm$ 1.0 <sup>A</sup>

Results were expressed as mean values  $\pm$  SE, (n= 6).

The presence of different capital letters in the same column indicating a significant difference between groups using one way ANOVA and Duncan's multiple comparison test at  $p < 0.05$ .

**Table (2), effect of Green tea or Licorice aqueous extract on serum triacylglycerol (TAG), total Cholesterol (TC) and HDL- Cholesterol (HDL-C) under the effect of iron overload**

Time Groups	TAG (mg / dl)		Cholesterol (mg / dl)		HDL-C (mg / dl)	
	4 Weeks	8 Weeks	4 Weeks	8 Weeks	4 Weeks	8 Weeks
Group I	55.8 $\pm$ 2.6 <sup>A</sup>	57.9 $\pm$ 1.6 <sup>A</sup>	62.2 $\pm$ 1.0 <sup>B</sup>	62.6 $\pm$ 1.3 <sup>A</sup>	43.9 $\pm$ 3.4 <sup>A</sup>	39.4 $\pm$ 3.0 <sup>A</sup>
Group II	55.1 $\pm$ 1.9 <sup>A</sup>	56.4 $\pm$ 2.2 <sup>A</sup>	66.6 $\pm$ 1.9 <sup>BC</sup>	62.15 $\pm$ 1.0 <sup>A</sup>	42.5 $\pm$ 3.7 <sup>A</sup>	49.5 $\pm$ 3.6 <sup>AB</sup>
Group III	114.7 $\pm$ 6.8 <sup>C</sup>	112.0 $\pm$ 6.5 <sup>C</sup>	99.7 $\pm$ 3.0 <sup>E</sup>	96.5 $\pm$ 2.5 <sup>D</sup>	44.2 $\pm$ 3.8 <sup>A</sup>	65.2 $\pm$ 4.1 <sup>C</sup>
Group IV	53.1 $\pm$ 3.1 <sup>A</sup>	54.6 $\pm$ 4.4 <sup>A</sup>	67.3 $\pm$ 2.1 <sup>BC</sup>	60.5 $\pm$ 2.2 <sup>A</sup>	54.9 $\pm$ 2.4 <sup>B</sup>	61.4 $\pm$ 4.6 <sup>C</sup>
Group V	82.1 $\pm$ 3.5 <sup>B</sup>	78.3 $\pm$ 4.1 <sup>B</sup>	76.5 $\pm$ 2.2 <sup>D</sup>	83.6 $\pm$ 3.7 <sup>C</sup>	48.1 $\pm$ 2.1 <sup>AB</sup>	58.3 $\pm$ 2.4 <sup>BC</sup>
Group VI	53.3 $\pm$ 2.2 <sup>A</sup>	55.5 $\pm$ 2.0 <sup>A</sup>	47.8 $\pm$ 3.2 <sup>A</sup>	56.95 $\pm$ 3.6 <sup>A</sup>	40.7 $\pm$ 2.5 <sup>A</sup>	54.2 $\pm$ 5.2 <sup>BC</sup>
Group VII	92.7 $\pm$ 4.9 <sup>B</sup>	86.7 $\pm$ 6.3 <sup>B</sup>	71.3 $\pm$ 1.9 <sup>CD</sup>	72.3 $\pm$ 4.3 <sup>B</sup>	45.3 $\pm$ 2.8 <sup>A</sup>	42.7 $\pm$ 2.6 <sup>A</sup>

Results were expressed as mean values  $\pm$  SE, (n= 6).

The presence of different capital letters in the same column indicating a significant difference between groups by using one way ANOVA and Duncan's multiple comparison test at  $p < 0.05$ .

**Table (3), effect of Green tea or Licorice aqueous extract on serum ALT and AST enzymatic activity under the effect of iron overload**

Time Groups	ALT(U/L)		AST(U/L)	
	4 Weeks	8 Weeks	4 Weeks	8 Weeks
Group I	26.5 ± 0.84 <sup>A</sup>	23.9 ± 1.7 <sup>A</sup>	75.1 ± 4.5 <sup>A</sup>	79.7 ± 4.1 <sup>AB</sup>
Group II	35.75 ± 1.7 <sup>BC</sup>	34.6 ± 1.6 <sup>B</sup>	89.4 ± 1.2 <sup>BC</sup>	88.3 ± 1.9 <sup>B</sup>
Group III	43.4 ± 2.2 <sup>D</sup>	53.17 ± 2.1 <sup>C</sup>	91.5 ± 0.5 <sup>BC</sup>	109.3 ± 1.3 <sup>C</sup>
Group IV	37.7 ± 1.9 <sup>C</sup>	38.7 ± 2.4 <sup>B</sup>	87.3 ± 3.6 <sup>BC</sup>	85.3 ± 3.2 <sup>A<sup>B</sup></sup>
Group V	48.0 ± 1.2 <sup>E</sup>	39.1 ± 1.4 <sup>B</sup>	94.7 ± 2.5 <sup>C</sup>	84.0 ± 3.0 <sup>A<sup>B</sup></sup>
Group VI	31.8 ± 1.2 <sup>B</sup>	35.9 ± 1.7 <sup>B</sup>	86.0 ± 1.9 <sup>B</sup>	85.3 ± 2.4 <sup>A<sup>B</sup></sup>
Group VII	37.5 ± 0.72 <sup>C</sup>	27.5 ± 0.96 <sup>A</sup>	87.5 ± 2.2 <sup>BC</sup>	79.2 ± 2.2 <sup>A</sup>

Results were expressed as mean values ± SE, (n= 6).

The presence of different capital letters in the same column indicating a significant difference between groups by using one way ANOVA and Duncan's multiple comparison test at  $p < 0.05$ .

**Table (4), effect of Green tea or Licorice aqueous extract on serum urea and creatinine under the effect of iron overload**

Time Group	Urea (mg/dl)		Creatinine (mg/dl)	
	4 Weeks	8 Weeks	4 Weeks	8 Weeks
Group I	43.1 ± 2.3 <sup>A</sup>	50.3 ± 3.8 <sup>A</sup>	0.45 ± 0.03 <sup>A</sup>	0.46 ± 0.05 <sup>A</sup>
Group II	48.6 ± 1.8 <sup>BC</sup>	54.0 ± 1.5 <sup>A</sup>	0.49 ± 0.04 <sup>AB</sup>	0.60 ± 0.03 <sup>BC</sup>
Group III	61.9 ± 3.4 <sup>D</sup>	75.5 ± 2.8 <sup>B</sup>	0.58 ± 0.03 <sup>BC</sup>	0.82 ± 0.03 <sup>E</sup>
Group IV	46.7 ± 3.2 <sup>A<sup>B</sup></sup>	51.4 ± 3.6 <sup>A</sup>	0.54 ± 0.04 <sup>ABC</sup>	0.52 ± 0.05 <sup>AB</sup>
Group V	52.2 ± 3.5 <sup>AB<sup>C</sup></sup>	70.0 ± 2.2 <sup>CB</sup>	0.70 ± 0.02 <sup>D</sup>	0.70 ± 0.04 <sup>CDE</sup>
Group VI	55.7 ± 3.5 <sup>BCD</sup>	55.2 ± 1.6 <sup>A</sup>	0.63 ± 0.06 <sup>CD</sup>	0.63 ± 0.04 <sup>BCD</sup>
Group VII	58.3 ± 4.5 <sup>CD</sup>	57.8 ± 2.3 <sup>A</sup>	0.73 ± 0.02 <sup>D</sup>	0.76 ± 0.06 <sup>DE</sup>

Results were expressed as mean values ± SE, (n= 6).

The presence of different capital letters in the same column indicating a significant difference between groups by using one way ANOVA and Duncan's multiple comparison test at  $p < 0.05$ .

## Discussion

The obtained results are going to be discussed under the following two categories, assessment of the undue effects of iron overload on blood chemistry and evaluation of the protective effect green tea or licorice aqueous extract.

### **A-Assessment of the undue effects of iron overload on blood chemistry**

It has been reported that, when dietary or genetic disorders induce iron overload, the iron binding of plasma is completely saturated which is measured indirectly by TS % (Yamanishi *et al.*, 2003). This study shows a significant increase in serum iron level associated with a significant increase in TS % when iron is supplemented to diet in excessive amount (1500 mg of ferric iron / kg diet). Such that increase in TS % suggesting, transferrin become nearly full saturated and the supplementation with excess ferric citrate could be considered to some extent appropriate to study the event link to change in blood chemistry.

The administration of iron overload diet induced a significant increase in serum triacylglycerol (TAG) and total cholesterol levels. The gained results are in agreement with those obtained by Dabbagh *et al.*, (1994), Whittaker *et al.*, (1996), Kirk *et al.*, (2001) and Lafay *et al.*, (2005). It has been previously reported that, in iron overloaded rats the increase in plasma total cholesterol level was associated with a dramatic decrease in plasma ascorbic acid (Odumosu and Wilson, 1979 and Dabbagh *et al.*, 1994). The increase in total cholesterol level in iron overloaded rats could be possibly explained by the decrease in cholesterol 7  $\alpha$  - hydroxylase activity, an enzyme involved in the catabolism of cholesterol and the synthesis of bile acids and that enzyme requires ascorbic acid as a cofactor (Turley *et al.*, 1976). Our finding disagree with the results obtained by Turbino – Ribeiro *et al.*, (2003) who reported that, overdose of iron dextran treatments induced a reduction in serum cholesterol and no effect on serum triacylglycerol (TAG). It may be pointed out that, this disagreement may be due to the difference in iron compounds and to the level of iron load used which may be a determinant factor in inducing increase in lipid profile.

Apart from the effect of licorice co administration with iron overload diet, the current study shows a significant increase in HDL –



C at the end of the experimental period. Difference in lipid and plasma lipoprotein metabolism among various species makes it difficult to compare the various animal models. Rabbits for instance do not have hepatic lipase or a protein analogous to human apolipoprotein AII. Rats and mice, unlike human and other species do not have plasma cholesterly ester transfer proteins. Thus, most serum cholesterol is present as part of HDL – C (Rubies – Prat *et al.*, 1982 and Tall, 1993). When, only experiments with rats are considered, there is consensus that excess iron increases HDL – C as was observed by Dabbagh *et al.*, (1994) and Turbino – Ribeiro *et al.*, (2003) with rats that consumed a cholesterol containing diet and by Brunet *et al.*, (1999) with rats that were fed a diet without cholesterol.

Feeding animal with excess iron significantly increase the activity of ALT and AST. This finding is in agreement with that obtained by Asare *et al.*, (2006), El Bahar *et al.*, (2007) and Pardo-Andreu *et al.*, (2008). These authors reported that the liver is one of the major sites of iron deposition in iron overload conditions and massive deposition in hepatocellular leading to hepatic fibrosis and cirrhosis.

The increase in serum urea and creatinine as a consequence of iron overload confirmed the previous reports of Chopra *et al.*, (2004) and El Bahar *et al.*, (2007). These authors reported that, the intrapritoneal injection of excess iron to rats or mice resulted in an elevation of plasma blood urea nitrogen and creatinine, suggesting the impairment of kidney function. Dimitriou *et al.*, (2000) concluded that, iron overload leads to intralysosomal storage of iron in the kidney, which brings about distinct changes in the behavior of the organelles in subcellular fractionation studies. Lysosomes become more fragile and show increased density, suggesting that, iron overload modify the constituents of the lysosomal membrane.

### **B- Evaluation of the protective effect green tea or licorice aqueous extract**

The gradual decrease in serum iron level that was accompanied by a gradual increase in TIBC and a marked decrease in TS % after the concomitant administration of green tea aqueous extract with iron overload diet could be attributed to the green tea polyphenols. Catechins possess well established metal – chelating agent properties, structurally important features defining their chelating potential are

the 3', 4' dihydroxyl group in the B ring as well as the gallate(Hider *et al.*, 2001) , which may neutralize ferric ion to form redox inactive iron. Similar findings were also concluded by Srichairatanakool *et al.*, 2006 and Thephinlap *et al.*, 2007, with the investigation of either crude green tea aqueous extract or with seperated epigallocatechin – 3 – gallate (EGCG) and epicatechin – 3 – gallate (ECG) from green tea.

The obtained results revealed that the concomitant oral administration of licorice aqueous extract with iron overload diet exerted a more potent decreasing effect on serum iron level as observed by the gradual decrease in TS % that could be reflect a drastic decrease in iron storage. The lowering effect of licorice aqueous extract may be attributed to the chelating properties of the glabridin and hispaglabridins A and B isoflavanes on excess iron in the plasma (Takeda *et al.*, 1996), or may be ascribed to the presence of 18  $\beta$  glycyrrhetic acid (the major hydrolyzed metabolite of glycyrrhizin in the intestine) that may affect iron absorption. This explanation need to further investigation to avoid the gradual decrease in iron storage and to determine the exact mechanisms that by which licorice can induce alterations in the iron overload status.

The regulation of plasma cholesterol levels involves factors that influence both extracellular and intracellular cholesterol metabolism as 3 – hydroxy – 3 methyl glutaryl – coenzyme A (HMG – Co A) reductase and acyl coenzyme A: cholesterol O – acyl transferase (ACAT), which is the enzyme that catalyzes the intracellular esterification of cholesterol and hepatic VLDLs- cholesterol secretion (Shin *et al.*, 1999). HMG – Co A reductase and ACAT inhibitors (such as green tea catechins and licorice isoprenyl flavonoids) are able to block this regulatory enzyme of cholesterol synthesis and are very effective in lowering serum cholesterol in most animal species including humans (Kim *et al.*, 2005 and Choi *et al.*, 2007).

Aqueous extract of green tea significantly reduced the levels of triacylglycerol (TAG) and serum total cholesterol, when simultaneously administered with iron overload diet. This lowering effect may be mainly attributed to the green tea polyphenols, particularly catechins. This finding is in agreement with that reported by Chan *et al.*, (1999).

Raederstoff *et al.*, 2003 reported that EGCG reduced total cholesterol and LDL plasma levels with non significant change in serum triacylglycerol and HDL – cholesterol. These authors suggested

that, one of the underlying mechanisms by which EGCG affects lipid metabolism is the interfering with the micellar solubilization of cholesterol in the digestive tract, which then in turn decreases cholesterol absorption. Yokozawa *et al.*, 2002, reported that the administration of green tea polyphenols effectively inhibited LDL – cholesterol oxidation and elevated serum antioxidant activity. Furthermore, green tea polyphenols increased the levels of HDL – cholesterol. In that concern, the obtained data in the current study revealed a progressive non significant increase in HDL – cholesterol when green tea aqueous extract simultaneously administrated with iron overload fed diet. This effect seemed to be in agreement with Araya *et al.*, 2001, who reported that flavonoids increased plasma HDL – cholesterol. According to these authors, the increase indicated in plasma HDL – cholesterol may be either due to the stabilizing effect of polyphenols flavonoids on plasma lipoprotein or the systemic effect of flavonoids to modulate various enzyme activities that can affect lipoprotein metabolism leading to an augmentation of HDL – cholesterol.

Licorice water extract induced the same reducing effect on serum TAG and cholesterol levels when concomitantly administrated with iron overload diet. The hypolipidemic effect of licorice extract may be attributed to glabridin isoflavan (Rosenblat *et al.*, 1999). It may be pointed out that, licorice aqueous extract displayed a non – significant fluctuating decreasing effect on HDL – cholesterol level when administrated with excess iron.

There is a body of evidence that, there are circumstances in which HDL may not be protective and may be in fact paradoxically promote vascular inflammation and oxidation of low – density lipoprotein (Ansell *et al.*, 2006 and 2007). It has been reported that, the isoprenyl flavonoids, that was isolated from the ethanolic extract of licorice root, may interfere with the production of lipoprotein and accumulation of cholesteryl ester (Choi *et al.*, 2007). Navab *et al.*, (2007) reported that oxidative alteration of the main protein of HDL apolipoprotein A1 impaired its capacity to promote cholesterol efflux from monocyte macrophages, which in turn reverse cholesterol transport and HDL antioxidant and anti – inflammatory function (Gorden and Fifkind *et al.*, 1989; Duell *et al.*, 1991).

The positive protective effect of the two tested extracts in recovering the reduction that induced in hepatic function is indicated

by the well marked decrease in ALT and AST enzymatic activities. These findings confirmed the results obtained by Jagetia *et al.*, (2004), Lafay *et al.*, (2005) and Pardo – Andreu *et al.*, (2008). The previous authors proved the effect of various flavonoids compounds in improving the impaired liver function resulted from excess iron. The reduction indicated in ALT and AST activities could be referred to the flavonoids components that found in the two tested extracts. Sakata *et al.*, 2004 and Roomi *et al.*, 2008 demonstrated the potential inhibitory effect of EGCG on the proliferation of hepatic stellate cells as well as the hepatoprotective effect of crude green tea polyphenols against acetoaminophen induced liver and kidney functions.

The hepatoprotective effect of licorice extract ascribed to the presence of 18  $\beta$  glycyrrhetic acid, the aglycone found in glycyrrhizin and liquiritigenin (Gumprich *et al.*, 2005 and Kim *et al.*, 2009).

It has been reported that, the effect of iron on kidney tissue involves chemical modification of the constituent of the lysosomal membrane such as lipid peroxidation (Dimitriou *et al.*, 2000); this finding could explain the disturbance that induced in serum urea and creatinine in the current study. The concomitant administration of the two tested extracts induced a significant decrease in serum urea levels. It may be pointed out that, the co - administration of the two tested extracts with excess iron displayed a non significant decrease in serum creatinine levels. The results in the current study are in agreement with that obtained by Yokozawa *et al.*, 1999 who proved the effectiveness of green tea extract in improving the decline that induced in the kidney function. Yokozawa *et al.*, 2003 and Mohamadin *et al.*, 2005 reported that, the administration of green tea extract significantly improved kidney function in a dose – dependent manner.

## REFERENCES

- Ansell BJ, Fonarow GC and Fogelman AM (2006): High-density lipoprotein: is it always atheroprotective. *Curr Atheroscler Rep*, 8: 405- 411.
- Ansell BJ, Fonarow GC and Fogelman A M (2007): The paradox of dysfunctional high-density lipoprotein. *Curr Opin Lipidol*, 18: 427-434.

- Araya J, Rodrigo R, Orellana M and Rivera G. (2001): Red wine raises plasma HDL and preserves long-chain polyunsaturated fatty acids in rat kidney and erythrocytes. *Br. J. Nutr.*, 86: 189-195.
- Asare GA, Mossanda SK, Kew MC, Paterson AC, Kahler-Venter CP and Siziba K (2006): Hepatocellular carcinoma caused by iron overload: A possible mechanism of direct hepatocarcinogenicity. *Toxicol*, 219: 41-52.
- Brunet S, Thibault L, Delvin E, Yotov W, Bendayan M and Levy E (1999): Dietary iron overload and induced lipid peroxidation are associated with impaired plasma lipid transport and hepatic sterol metabolism in rats. *Hepato*, 29: 1809-1817.
- Ceriotti F and Ceriotti G (1980): Improved direct determination of serum iron and total iron binding capacity. *Clin Chem.*, 26: 327-331.
- Chan PT, Fong WP, Cheung YL, Huong Y, HO WK and Chen ZY (1999): Jasmine green tea epicatechins are hypolipidemic in hamsters (*Mesocricetus auratus*) fed a high fat diet. *J. Nutr.*, 129: 1094-1101.
- Chin YW, Jung HA, Liu Y, Su BN, Castoro JA, Keller WJ, Pereira MA and Kinghorn AD (2007): Anti – oxidant constituents of the roots and stolons of licorice (*Glycyrrhiza glabra*). *J. Agr. Food Chem.*, 55: 4691– 4697.
- Choi J, Rho MC, Lee SW, Kwon OE, Park HR, Kang JY, Lee SH, Lee HS, Bae KH, and Kim YK (2007): Glabrol, an acyl-coenzymeA: Cholesterol acyl transferase inhibitors from licorice roots. *J. Ethnopharmacol.*, 110: 563-6.
- Chopra K, Singh D and Chander V (2004): Nephrotoxicity and its prevention by catechin in ferric nitrilotriacetate promoted oxidative stress in rats. *Hum. Exp. Toxicol.*, 23: 137-43.
- Cockell KA, Wotherspoon ATL, Belonje B, Fritz ME, Madere R, Hidioglou N, plouffe LJ, Ratnayake WMN and Kubow S (2005): Limited effects of combined dietary copper deficiency / iron overload on oxidative stress parameters in rats liver and plasma. *J. Nutr Biochem.*, 16: 750 – 756.
- Dabbagh AJ, Mannion T, Lynch SM and Frei B (1994): The effect of iron overload on rat plasma and liver oxidant status in vivo. *Biochem. J.*, 300: 799-803.

- Dimitriou E, Kairis M, Sarafidou J and Michelakakis H(2000): Iron overload and kidney lysosomes. *Biochimica et Biophysica Acta.*, 1501: 138-148.
- Duell PB, Oram JF, and Bierman EL (1991): Non enzymatic glycosylation of HDL and impaired HDL-receptor-mediated cholesterol efflux, *Diabetes*, 40: 377-384.
- El – Bahar SM, Korshom MA, Mandour AA, Elbessomy AA and Lebdah MA (2007): The protective effect of turmeric on iron overload in albino rats. *The Egyptian Journal of Biochemistry and Molecular Biology (EJ B)* 25: 94 – 113.
- Faulkner WR and king JW (1976): In *fundamentals of clinical chemistry*. 2<sup>nd</sup>. Ed. (N.W. Ttletz, Ed.), Sounders, Phlladelphia, PP: 994 –998.
- Ferrali M, Donati D, Bambagioni S, Fontani M, Giorgi G, and Pietrangelo A (2001): 3-Hydroxy- (4H)-benzopyran-4-ones as potential iron chelating agent in vivo. *Bioorg. Med. Chem*, 9: 3041-3047.
- Fossati P and Prencipe L (1982): The determination of triglyceride using enzymatic method. *Clin. Chem.*, 28: 2077
- Fraga CG and Oteiza PI (2002): Iron toxicity and antioxidant nutrients. *Toxicol*, 180:23-32.
- Gorden DJ and Fifkind, BM (1989): High density lipoprotein-the clinical implications of recent studies. *N. Eng. J. Med.*, 321: 1311-1316.
- Gumpricht E, Dahl R, Deveraux MW and Sokol RJ (2005): Licorice compounds glycyrrhizin and 18  $\beta$ -glycyrrhetic acids are potent modulators of bile acid-induced cytotoxicity in rat hepatocyte. *J. Biol. Chem.*, 280-10556-10563.
- Hassan AM and Ahmed KA (2004): Chemo preventive effect of green and black tea (cytogenetically and histopathologically) on benzo – A – pyrene treated mice. *Vet. Med. J. Giza.*, 52 (4): 457 – 473.
- Hider RC, Liu ZD and Khodr HH (2001): Metal chelation of polyphenols. *Method Enzymol*, 335: 109-203.
- Jagetia, GC, Reddy TK, Venkatesha VA and Kedlaya R (2004): Influence of naringin on ferric iron induced oxidative damage in vitro. *Clinca Chemic. Acta*, 347: 189-197.
- Kim JH, Kang BH and Jeong JM (2003): Antioxidant antimutagenic and chemopreventive activities of a phyto – extract mixture

- derived from various vegetables, fruits and oriental herbs. *Food. Sci. Biotechnol.*, 12: 631 – 638.
- Kim SJ, Bok SH, Lee MK, Park Y B, Kim HJ and Choi MS(2005): Lipid lowering efficacy of 3, 4-di (OH)-phenylpropionic-L leucine in high cholesterol fed rats. *J. Biochem. Mol. Toxicol.*, 19: 25-31.
- Kim. YW, Hang H E, Lee MG, Hwang SJ, Kim SC, Lee CH and Kim SG (2009): Liquiritigenin, a flavonoid aglycone from licorice, has a choleric effect and the ability to induce hepatic transporters and phase-11-enzymes. *Am. J. Physiol. Gastrointest. Liver Physiol.*, 296, G3: 72-81.
- Kirk, EA, Heinecke JW and Le Boeuf RC (2001): Iron overload diminishes atherosclerosis in apo E-deficient mice. *J. Clin. Invest*, 107: 1545-1553.
- Lafay S, Gueux E, Rayssiguier Y, Mazur A, Remesy C and Scalbert A (2005): Caffeic acid inhibits oxidative stress and Reduces hypercholesterolemia induced by iron overload in rats. *Int. J. Vit. Nutr Res.*, 75: 119-125.
- Lee JR, Park SJ, Kwon YK, Jee SY, Seo J, Lee HS, Kwon TK and Kim SC. (2007): Hepatoprotective activity of licorice water extract against cadmium – induced toxicity in rats. *e CAM*, Advance Access publication 9 August, : 195-201.
- Mckay DL and Blumberg JB (2002): The role of tea in human health: An update. *J. Am. Coll. Nutr.*, 21: 1 – 13.
- Mohamadin AM, El-Beshbishy HA and El-Mahdy MA (2005): Green tea attenuates cyclosporine A induced oxidative stress in rats. *Pharmacol. Res*, 51: 51- 57.
- Navab M, Yu R, Gharavi N, Huang W, Erza N, Lotfizadeh A, Anantharamaiah G M, Alipour N, Van Lenten BJ, Reddy ST and Marelli D (2007): High-density lipoprotein: antioxidant and anti-inflammatory properties. *Curr Atheroscler. Rep.*, 9: 244-248.
- NRC.(1995) : Nutrition Requirements of Laboratory Animals, Fourth Revised Edition, Institute for Laboratory Animal Research, National Research Council, National Institute of Health, Academic Press, Washington DC, USA.
- Odumosu A and Wilson CW (1979): Tissue ascorbic acid fenfluramine, and changes in fat metabolism. *Int. J. Obes.*, 3: 123-131.

- Paget GE and Barnes JM (1964): In "Toxicity tests" volume (1) chapter (6) pp 135. Editor: Laurance DR and Bacharach AL. Academic press, London and New York.
- Pardo-Andreu GL, Barrios MF, Curti C, Hernández I, Merino N, Lemus Y, Martínez I, Riaño A and Delgado R (2008): Protective effects of *Mangifera indica* L extract (Vimang), and its major component mangiferin, on iron-induced oxidative damage to rat serum and liver. *Pharmacol Res.*, 57: 79-86.
- Qing Yu X, Xue CC, Zhou ZW, Li C G, Du YM, Liang J and Zhou SF (2008): In Vitro and vivo neuroprotective effect and mechanisms of glabridin, a major active isoflavan from *Glycyrrhiza glabra* (licorice). *Life Sci.*, 82: 68 – 78.
- Raederstoff DG, Schlachter MF, Elste V and Weber P (2003): Effect of EGCG on lipid absorption and plasma lipid levels in rats. *J. Nut. Biochem.*, 14:326-332.
- Reitman S and Frankel S (1957): A colorimetric method for the determination of serum glutamic oxaloacetic and glutamic pyruvic transaminases. *Am. J. Clin. Path.*, 28: 56-63.
- Richmond W (1973): Preparation and properties of a cholesterol oxidase from *Nocardia* Sp., and its application to the enzyme assay of total cholesterol in serum. *Clin. Chem*, 19: 1350 - 1356.
- Roomi MW, Kalinovskiy K, Ivanov V, Rath M and Niedzwiecki A (2008): A nutrient mixture prevents acetoaminophen hepatic and renal toxicity in ICR mice. *Hum. Exp. Toxicol.*, 27: 223-230.
- Rosenblat M, Belinky P, Vaya J, Levy R, Hayek T, Coleman R, Merchav S and Aviram M (1999): Macrophage enrichment with the isoflavan glabridin inhibits NADPH oxidase - induced cell-mediated oxidation of low density lipoprotein: A possible role for protein kinase C. *J. Biol. Chem.*, 274: 13790-13799.
- Rubies-Prat J, Masdeu S, Nubiola AR, Chacon P, Holguera C and Masana L(1982): High-density lipoprotein cholesterol and phospholipids, and apoprotein A in serum of patients with liver disease. *Clin. Chem.*, 28: 525-527.
- Sakata R, Ueno T, Nakamura T, akamoto M, Torimura T and Sata M (2004): Green tea polyphenols epigallocatechin-3-gallate inhibits platelet-derived growth factor-induced proliferation of human hepatic stellate cell line L190. *J. Hepatol.*, 40: 52-59.
- Shin YW, Bok SH, Jeong TS, Bae KH, Jeung NH, Choi MS, Lee SH and Park YB (1999): Hypocholesterolemic effect of naringen



- associated with hepatic cholesterol regulation enzyme. *Int. J. Vitam. Nutr. Res.* 69: 341– 347.
- Srichairatanakool S, Ounjaijean S, Thephinlap C, Khunsuwan U, Phisalpong C and Fucharoen S (2006): Iron-chelating and free radical scavenging activities of microwave-processed green tea in iron overload. *Hemoglobin*, 3030: 311-327.
- Tabacco A, Meiattini F, Moda E and Tarlip P (1979). Simplified enzymatic colorimetric serum urea nitrogen determination. *Clin. Chem.*, 25:336.
- Tabacco A, Moda E, Tarli P and Neri P (1981): An improved highly sensitive method for the determination of serum iron using chromazurol B. *Clin Chem Acta*, 114:287-290
- Takeda S, Ishihara K, Wakui Y, Amagaya S, Maruno M, Akao T and Kobashi K. (1996): Bioavailability study of glycyrrhetic acid after oral administration of glycyrrhizin in rats; relevance to the intestinal bacterial hydrolysis. *J Pharm Pharmacol*, 48: 902–5
- Tall AR (1993): Plasma cholesteryl ester transfer protein. *J. Lipid Res.*, 34: 1255-1274.
- Thephinlap C, Ounjaijean S, Khansuwan U, Fucharoen S, Porter JB and Srichairatanakool S (2007): Epigallocatechin –3 – gallate and epicatechin– 3–gallate from green tea decrease plasma non – transferrin bound iron and erythrocyte oxidative stress. *Med. Chem.*, 3: 289 – 96.
- Trinder D, Olynyk JK, Sly WS and Morgan EH (2002): Iron uptake from plasma transferrin by the duodenum is impaired in the Hfe Knockout mouse. *Proc Natl Acad Sci., USA* 99: 5622.
- Turbino-Ribeiro SML, Silva ME, Chianca DA, Paula JHD, Cardoso, LM, Colombari E and Pedrosa ML (2003): Iron overload in hypercholesterolemic rats affects iron homeostasis and serum lipids but not blood pressure. *J. Nutr.*, 133: 15-20.
- Turley SD, West CE and Horton BJ (1976): The role of ascorbic acid in the regulation of cholesterol metabolism and in the pathogenesis of atherosclerosis. *Atherosclerosis*, 24: 1-18.
- Warnick GR, Benderson V and Albers N. (1983): Selected Methods *Clin Chem.*, 10: 91 – 99.
- Whittaker P, Wamer WG, Chanderbhan RF, and Dunkel VC (1996): Effects of alpha-tocopherol and beta-carotene on hepatic lipid peroxidation and blood lipids in rats with dietary iron overload. *Nutr Cancer*, 25: 119-128.

- Yamanishi H, Iyama S, Yamaguchi Y, Kanakura Y and Iwatani Y (2003): Total iron-binding capacity calculated from serum transferrin concentration or serum iron concentration and unsaturated iron-binding capacity. Clin. Chem., 49: 175-8.
- Yokozawa E, Nakagawa T, Lee KI, Cho EJ, Terasawa K and Takeuchi S (1999): Effects of green tea tannin on cisplatin-induced nephropathy in LLC-PK1 cells and rats. J. Pharm. Pharmacol., 51: 1325-1331.
- Yokozawa T, Nakagawa T and Kitani K (2002): Antioxidative activity of green tea polyphenol in cholesterol-fed rats. J. Agr. Food Chem., 50: 3549-3552
- Yokozawa T, Cho EJ and Nakagawa T (2003): Influence of green tea polyphenol in rats with arginine induced renal failure. J. Agr. Food Chem., 51: 2421-5.

### تأثير بعض المنتجات الطبيعية على كيمياء الدم في حالة تراكم الحديد في الجرذان البيضاء

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استهدفت هذه الدراسة تأثير الجرعات الزائدة من الحديد الضار وكذلك تقييم التأثير الوقائي لكل من الشاي الاخضر والعرقسوس على بعض المكونات البيوكيميائية في الدم حيث ادت الجرعة الزائدة من الحديد (1500 ميلليجرام من الحديد لكل كيلو من الغذاء الاساسي) الى احداث ارتفاع معنوي في مستوى الحديد بمصل الدم ونسبة تشبع الترانسفيرين مع نقص غير معنوي في قدرة اتحاد الحديد الكلية وارتفاع مستوى الدهون الثلاثية والكوليستيرول الكلي وخلل في وظائف كل من الكبد والكلية. وكان لاعطاء مستخلصي الشاي الاخضر (3.5 ميلليجرام لكل مائة جرام من وزن الجسم) والعرقسوس (100 ميلليجرام لكل كيلو جرام من وزن الجسم) تأثير مخفض لنسبة الحديد في الدم وتقليل نسبة الدهون الثلاثية والكوليستيرول الكلي و احداث تحسن في وظائف كل من الكبد والكلية .