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EFFECT OF POLYPHENOLS EXTRACTED FROM RED BEET ON THE ACTIVITY OF ANTIOXIDANT ENZYMES IN RATS

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ABSTRACT

Recently, considerable attention has been focused on natural foods that inhibit, or retard diseases caused by oxidative processes because natural food ingredients are safer than synthetic ones. It was found that consumption of red beet regularly in daily diet may reduce the risk of degenerative diseases and improve public health because they contain a high percentage of polyphenols which have antioxidant and anti-inflammatory properties.

Therefore this the importance of studying the effectiveness using polyphenols extract from red beet as natural antioxidants for increase the antioxidant enzyme activities such as (SOD, CAT and GPx) in rats. Twenty four male rats were randomly divided in to four groups (control group, frying oil, frying oil was treat with tertbutylhydroquinone (TBHQ) and frying oil treat with ٠.٥٪ polyphenols extract from red beet). Rats fed on diets containing frying oil used several times (١٠٪oil/١٠٠g diet).

The result revealed that HPLC analysis of the polyphenols extract contained catechol, pyrogallol, catechin and caffiec as major compounds present a high proportion in addition to other compounds. Significant increase in the level of total lipid, cholesterol, triglycerides, LDL-C, HDL-C and a decrease in the

level of activity of antioxidant enzymes (SOD&CAT) in group fed frying oil (FO) in comparison to with the control. While rats feeding frying oil treat with polyphenols extract from red beet recorded significant increase in the level of activity of antioxidant enzymes (SOD, CAT&GPx) in comparison to the group which fed on the frying oil without the extract. Histopathological studies showed a lot of changes in the liver of rats.

INTRODUCTION

Antioxidants nutrients which found in fruits, vegetables, and other foods are thought that help in protecting the body from free radical that may cause damage in the cells and weakening of the immune system *(Thompson et al., ٢٠٠٩)*

Ferguson, (٢٠٠١) reported that polyphenols are a large and a diverse class of compounds have antioxidant and anti-inflammatory properties.

Moreno et al., (٢٠٠٦) found that red beet, when consumed regularly in our daily diet may reduce the risk of degenerative diseases and improve public health because they contain a high percentage of flavonoids.

Anti-oxidant enzymes such as glutathione peroxidase (GPx), Catalase (CAT) and superoxide desmutase (SOD) protected from harmful molecular cellular changes that occur due to free radicals which plays an important role in antioxidant defenses *(Minelli et al., ٢٠١٠).*

Han et al., (٢٠٠٧) found that, dietary polyphenols exhibit many biologically significant functions such as protection against oxidative stress and degenerative diseases. Dietary polyphenols may offer an indirect protection by activating endogenous defense systems and by modulating cellular signaling processes polyphenols increased several antioxidant enzymes activities such as CAT, GPx and SOD.

Therefore this study aimed to study the effectiveness use of polyphenols extracts as natural antioxidants from red beet, in order to validate the potentials of these vegetables as scavengers of free radicals where relations between the activities of antioxidants against

free radicals and improving public health by activating anti-oxidant enzymes.

MATERIALS AND METHODS

Materials:

Red beet was purchased from a local supermarket, Cairo, Egypt in November ٢٠١١. The clean parts were dried at ٤٠° C by oven vacuum drying in Agricultural Chemistry Dept., Faculty of Agriculture, Minia University according to *Yousif, (٢٠١٠).* Then ground to fine powder, the extraction was carried out by ethanol for ٣ h at ٤٠°C at ١:٥ ratio of powder to ethanol by used magnetic estreria. The extract was filter over whatman No.١ and then evaporator to dryness under vacuum.

Frying oil (sunflower٧٥٪ and soybean٢٥٪) was used in this experiment as low cost material and more available to consumer especially in rural areas in Egypt. Then ٠.٥ % of polyphenols extract from red beet was added to oil. The initial frying temperature was heated at ١٨٠° ± ٢ C. Continued to use frying oil to frying potatos for two hours, in the end of the frying experiment the oil was filtered, cool and kept in bottles. All oil samples stored in a freezer until used.

Animals and experimental design:

Twenty four albino male rats with body weight of ١١٥±٥g were obtained from Agricultural Faculty, Minia Univ. Egypt and were acclaimed for one week prior to experiment during which they were fed on slandered laboratory chow and water. They were housed in groups of six each in universal poly propylene cages at room temperature (٢٥± ٢C°). The rats were randomly divided to four groups they were fed diets containing frying oil used several times (١٠٪oil/١٠٠g diet) according to *Saka et al.,(* ٢٠٠٢) for ٣٠ days:-

Group (١): control group was fed on balanced diet (CG).
Group (٢): was fed on balance diet contain ١٠٪ frying oil (FO).
Group (٣): was fed on balance diet contain ١٠٪ frying oil treated with TBHQ (FO+ TBHQ).
Group (٤): was fed on balance diet contain ١٠٪ frying oil treated with ٠.٥٪ polyphenols extract from red beet (FO+ red beet).

Effect of polyphenols on the activity of antioxidant enzymes in rats

The animals were sacrificed at the end of the biological experiment; the blood was collected from the orbital plexus under other anesthesia. Blood was allowed to clot and then centrifuged at ٣٠٠٠ rpm for ١٥ min and serum kept at ٢٠°C until required.

Biochemical analysis:-

The following parameters were determined in serum of rats at the end of the experimental period: LDL-C *(Wieland & Seidel, ١٩٨٣)*, HDL-C *(Lopez et al., ١٩٧٧)*, Cholesterol *(Richmond, ١٩٧٣)*, Total Lipid *(Zollner and Kirsch., ١٩٦٢)*, Triglycerides *(Fassati & Prencipe, ١٩٨٢)*, GOT and GPT *(Reitman & Frankel., ١٩٥٧)*, SOD *(Nishikimi et al., ١٩٧٢)*, CAT *(Aebi, ١٩٨٤)* GPx *(Paglia & Valentine, ١٩٦٧).*

Statistical analysis:-

Statistical analysis was performed with SPSS computer program *(SPSS, ١٩٩٠)*. Data were analyzed using one way analysis of variance (ANOVA). Results are reported as mean values ± SD and difference were considered significant at $p<٠.٠٥$.

RESULTS AND DISCUSSION

Chemical composition of phenolic compound:

Table (١) presents the phenolic compound composition of ethanolic extracts of red beet. The results showed that the extract contained pyrogallol, protocatechouic, catechin and catechol at amount ٣٢٨٢.٩, ٥٢.٠١, ٣٥٠.٦٧and ٩٣.٤٧ mg/١٠٠g respectively as major compounds present a high proportion, in addition to other compounds. These result in agreement with *Kujala et al., (٢٠٠٠)* who reported that the red beet root part containing the largest amount of total phenolic.

Serum constituents:

Data in Table (٢) showed a significant increase ($P<٠.٠١$) in the levels of CHL in all group feeding frying oil in comparison control group. These results were in agreement with *Hamza and Mahmoud, (٢٠٠٩)* whom reported that the rats maintained on the HFD showed significant high value of serum CHL (١٧١.٦١ mg/dl) in comparison to control (١١٤.٢٠ mg/dl). While it was observed that the group feeding FO treated with red beet polyphenols extract tended to have the lowest

level of CHL. Non-significant increase in the levels of total lipids in all group.

Table ١: Phenolic compound was determined by High-performance liquid chromatography (HPLC) in red beet extract:

Phenolic compound	Concentration(mg/١٠٠g)
Gallic	------
Catechol	٩٣.٤٧
Pyrogallol	٣٢٨٢.٩
Protocatechouic	٥٢.٠١
Caffeine	٤٢.٦٤
Vanaillic	٢٩.٧٧
Caffiec	٧.٢٣
P-coumaric	١٠.٣٩
Chrisin	٠.٣٨
Chlorogenic	٤٦.٧٧
Syringic	١٣.٢٤
Ferulic	٣٣.٨٠
Salicylic	٤٠.٣٧
Coumarin	-----
Catechin	٣٥٠.٦٧
Cinnamic	١٤.٩٠
P-Benzoic	-----

Determined by HPLC according to used standard⁄Official methods (ISO) ١٩٩٩.

The highest levels of total lipid was recorded in group fed diet containing frying oil in comparison control group. While, group fed diet contain FO treated with polyphenols extract from red beet tended to have the lowest level of total lipids in comparison to FO+ TBHQ group. Significant increase (P<٠.٠١) in the levels of TG in all groups fed diet containing frying oil in comparison to control. These results were in agreement with *Lu & Lo, (١٩٩٥)* whom stated that the frying oil diet resulted in a higher content of TG and cholesterol. Group fed diet contain FO treated with red beet polyphenols extract tended to have the lowest level of TG in comparison to FO+ TBHQ. Accordingly, it can say that the level of TG present in the

experimental groups has been decreased with additional polyphenols extracts of red beet.

Results of Table (٢) showed a significant increase (P<٠.٠١) in the levels of LDL-C in all groups fed diet containing frying oil in comparison control group. These results coincided with results of *Hamza & Mahmoud, (٢٠٠٩)* whom found that the rats maintained on the HFD showed increase significant value LDL-C with HFD comparing to control. On the other hand group fed FO+ TBHQ recorded significant decrease. The same authors mentioned above reported that tertbutylhydroquinone (TBHQ) have strong as antioxidant potentials. Treatment frying oil with polyphenols extracts observed decreased in the level of LDL-C comparing to another groups.

Table ٢: Effect on serum lipid contents, GOT and GPT in rats fed frying oil and frying oil treatment with poly phenol extracts of (red beet).

Parameters	CG	FO	FO+ TBHQ	FO + red beet extract
Total lipids(mg/dl)	٥٢١.٠٨ ± ١٣٧.٩	٧٣٧.٥٣± ٨٧.٠٩	٧٠٦.٢٥ ± ١٦٢.٤٣	٦٢٥.٢٣ ± ١٥٧.٤
Total cholesterol (mg/dl)	٦٨.٦٩ ± ٤٩.٢٦	١٦٢.٨٧±١٣.٣٩	١٣٤.٩٢ ± ١٠.٣	١٢٤.٣٧±١٠.١
Triglycerides (mg/dl)	١٦٩.٠٤ ± ١١.٤٦	٢٤٧.٣٨± ٢٦.٧٦	٢٢٧.٠٦ ± ٣٨.١٤	٢٢١.٢٧±١٧.٤٤٠
HDL-C (mg/dl)	٢٥.٣٥ ± ٣.١	٥٠.٥٤ ± ٤.٨	٢٦.١٦ ± ٣.٥	٢٩.٢٥±١١.١
LDL-C (mg/dl)	٩٧.٢٦ ± ١٤.٢	١٦٢.٢٤ ± ١١.٤	١١٨.٢٧ ± ١٠.٦	١٢٣.٥٩ ± ١٠.٦
GOT (U/ml)	٢١.٦٧ ± ٢.٣	٢٩.١٧ ± ٢.٤	٢٣.١٧ ±٥.٤	٢٠.٣٣ ± ٥.٠٣
GPT (U/ml)	١٢.٣٣ ± ١.٠٣	١٣.٣٣ ± ٢.٠٦	١١.١٧ ± ١.٣	١٠.٥ ± ١.٠٤

- HDL-c: High density lipoprotein cholesterol.
- LDL-c: Low density lipoprotein cholesterol.
- Each value is the mean of ٦ replicates ± SD.

Significant increase showed (P<٠.٠١) in levels of HDL-C in all groups fed FO diet comparing to control group Table (٢). The highest level of HDL-C reached ٥٠.٥٤ mg/dl in groups fed FO without

antioxidant. Polyphenols extract treatments led to significant increase in the level of HDL-C. It is clear that increase HDL-C as a protecting mechanism against the peroxidative stress induced by the consumption of a diet containing the thermally oxidised oil (Garrido et al., ٢٠٠٤).

GOT & GPT were significantly increased (P<٠.٠١) in groups fed frying oil comparing to control group of rats. Our results are in basic agreement with the results of *Darwish, (٢٠١٠)* who found increase in GOT/GPT for frying oil groups.While treatment with polyphenols extract led to significant decrease in the level of GOT and GPT. It is clear that polyphenols extract from red beet that treated frying oil included natural antioxidants phenol compound.

The present result in Table (٣) showed decrease in the level of SOD & CAT activity in FO group comparing to control set. That decrease of antioxidant enzyme may be due to rapid consumption and exhaustion of storage of this enzyme in fighting free radicals in rats *(Noeman, et al., ٢٠١١).* This coincides with data previously observed by *Quiles et al., (٢٠٠٢)* who found that rat intake of fried oil led to higher levels of lipid peroxidation and a lower concentration of plasma antioxidants.

Table ٣: Effect on SOD, GPx and CAT activity in rats fed frying oil and frying oil treatment with polyphenols extract of (red beet):

Group	SOD U/ml	GPX mU/ml	CAT U/l
(CG)	١٤.٥٤ ± ٦.١٦	٢.٥٣ ± ٠.٥٦	٣٣٩.٣٩ ± ٢٩.٦٣
(FO)	٩.٨ ± ٥.١٢	٢.٧١ ± ٠.٤٠ ٠	٢٠٧.٩٤ ± ٧١.٠٤
(FO+TBHQ)	٢٠.٨١ ± ١٦.٨٣	٢.٨٧ ± ٠.٤٤	٢٢٤.٣٥ ± ٤٣.٤٣
(FO+ red beet)	١٣.٩٩ ± ٨.١١	٣.٢٧ ± ٠.٥٣	٢٣٠.٢٥ ± ٤٦.٠٣

- SOD: superoxide desmutase.
- GPX: glutathione peroxidase.
- CAT: Catalase.
- Each value is the mean of ٦ replicates ± SD.

Rats fed FO treated with polyphenols extract showed improvement in the SOD & CAT activity comparing to control group, *Han et al., (٢٠٠١)* who reported that bioactivities of dietary polyphenols

increased several antioxidant enzymes activities. The highest level of SOD activity showed in group fed FO+TBHQ which may be cause overload lipid peroxidation *(El shall, et al., ٢٠٠٩).* Significant increase (P<٠.٠٥) in the level of GPx activity was found after rats fed frying oil comparing to control group. However feeding FO treatment with polyphenols extract showed significant increase (P<٠.٠٥) in the level of GPx activity after rats fed frying oil comparing to control group.

In the end of experiment it could be concluded that addition polyphenols extracted of red beet to frying oil work as natural antioxidants phenolic compound play a protective role through reducing lipid peroxidation and increasing the activities of antioxidant enzymes such CAT, GPx and SOD.

Histopathological studies:

Histopathological examination of liver for the different groups was compared with each others. Photo (١a) showed the normal liver section properties in control group. Photo (١b) showed the hydropic degeneration of hepatocytes and multiple focal areas of hepatic necrosis associated with leucocytic cells infiltration lobule, kupffer cells activation and portal infiltration with leucocytic in the group fed on frying oil. This result are in agreement with results of *Darwish, (٢٠١٠)* who found that feeding rats with frying oil showed kupffer cell and congested central vein. *Totani & Ojiri, (٢٠٠٧)* also reported that many dark-red patches, necrosis, and bleeding were found in the livers of ٧٥٪ of the experimental rats; these rats had extremely high GOT and GPT values. That result are in agreement with previous explanation in the current study that FO group has high level of GOT&GPT. Slight hydropic degeneration of hepatocytes shows in photo (١c) for rats fed frying oil with (TBHQ).

Treating the frying oil with polyphenols extract from red beet shown in photo (١d) the hydropic degeneration of hepatocytes in the liver. It can be concluded that polyphenols compound reduced markers of liver damage in rats fed frying oil (FO), It is clear also that antioxidants may play a protective role in enzymes activities that are important for hepatic protection against oxidative.

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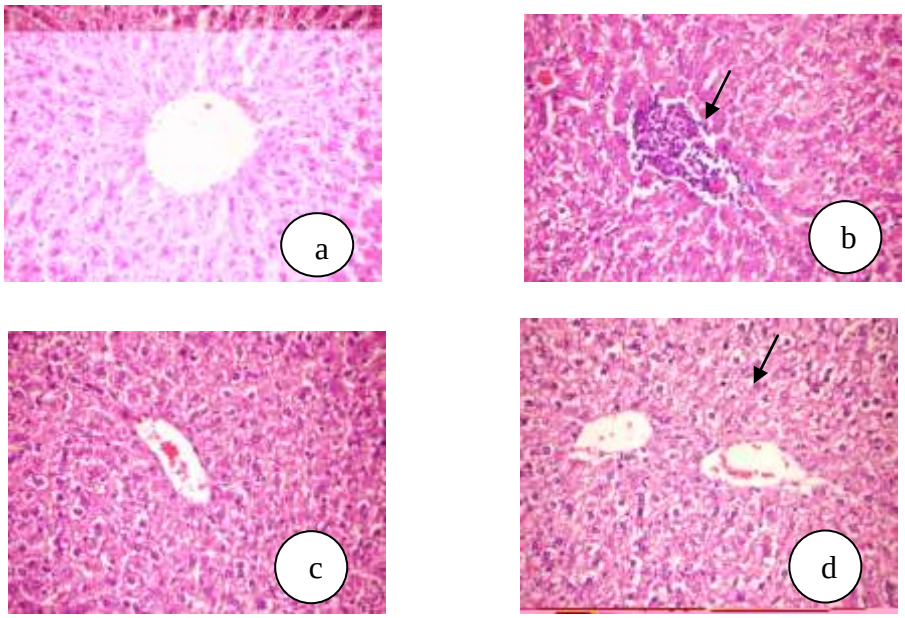

Photo (١):- A photomicrograph of the liver sections of the control rat(a), frying oil diet (b), Frying oil+ TBHQ(c), Frying oil+ polyphenole extract from red beet (d) (H&E×٤٠٠).

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Effect of polyphenols on the activity of antioxidant enzymes in rats

تأثير البوليفينول المستخلص من البنجر الأحمر على نشاط الإنزيمات المضادة للأكسدة في الفئران.

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انصب اهتمام كبير في الآونة الأخيرة على الأغذية الطبيعية التي تمنع أو تؤخر الأمراض الناجمة عن عمليات الأكسدة لأن المكونات الغذائية الطبيعية هي أكثر أمانا من تلك الاصطناعية .عندما يستهلك البنجر الأحمر بانتظام في غذائنا اليومي فأنة يقلل من خطر أمراض الشيخوخة ويحسن الصحة العامة لأنه يحتوي على نسبة عالية من مادة البوليفينول التي لها خصائص مضادة للأكسدة والالتهابات.

ولذلك كانت أهمية دراسة فاعلية استخدام مستخلص البوليفينول من البنجر الأحمر كمضادات أكسدة طبيعية لزيادة نشاط الإنزيمات المضادة للأكسدة مثل سوبر اكسيد ديسموتيزوالجلوتاثيون بيروكسيد والكتاليز فى فئران التجارب .

أجرى تحليل كيميائي بجهاز HPLC للتعرف علي مكونات المركبات الفينولية المذكورة أنفا ووجد أنها تحتوى على مركبات مثل حمض البيروجلليك والكاتيكول وحمض الكافيك كمركبات تتواجد بنسبة عالية بالإضافة إلى مركبات أخرى.

وقد لوحظ انه عند تغذية الفئران على عليقه تحتوى على زيت القلي المستخدم عدة مرات (١٠% /١٠٠جرام عليقه) حدوث زيادة معنوية في مستوى الدهون الكلية، الكولسترول، الجليسريدات الثلاثية، LDL-C، HDL-C و حدوث انخفاض في مستوى نشاط الإنزيمات المضادة للاكسدة (SOD&CAT) مقارنة بالمجموعة الضابطة. بينما عند تغذية الفئران بزيت القلى المعالج بالبوليفينول المستخلص من البنجر الأحمر حدثت زيادة معنوية فى مستوى نشاط الإنزيمات المضادة للأكسدة (SOD, GPx &CAT) مقارنة بالمجموعة التي تغذت على عليقه تحتوى على زيت القلي المستخدم عدة مرات بدون مستخلص . وأوضحت الدراسات الهستوباثولوجية كثيرا من التغيرات في كبد الفئران.