Minia J. of Agric. Res. & Develop.
Vol. (٣٢) No. ٤ pp ٦١٣-٦٢٦,
٢٠١٢

FACULTY OF AGRICULTURE

PROTECTIVE INFLUENCE OF USING POLYPHENOL EXTRACT FROM BROCCOLI AS NATURAL ANTIOXIDANT AGAINST OXIDATION OF FRYING OIL IN RATS.

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Received ٢ Feb. ٢٠١٢ Accepted ٢٥ March ٢٠١٢

ABSTRACT

Chemical changes occur in frying oil (FO) includes oxidation. Broccoli rich with polyphenols have antioxidant properties which reduced concentrations of lipid peroxidation and anti-inflammatory of liver. The aim of the present work was to investigate the protective of using polyphenols extract from broccoli as natural antioxidant against oxidation of frying oil and increase the plasma antioxidant enzyme activities such as glutathione peroxides (GPx), catalase (CAT) and superoxide dismutase (SOD) in rats. Twenty four male rats were randomly divided in to four groups feeding diets containing frying oil used several times (١٠٪oil/١٠٠g diet).

The result revealed HPLC analysis of the polyphenols extract contained catechol, pyrogallol, catechin, caffiec and gallic acid as major compounds present 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) compared with the control. While rats feeding frying oil treat with polyphenols extract from broccoli occurred significant increase in the level of activity antioxidant enzymes (SOD & GPx)

and HDL-C compared to the control group. Histopathological studies showed a lot of changes in the liver of rats.

INTRODUCTION

In Egypt, with the bad economic situation and lack awareness of food in many homes often demand that oil previously fried is reused and this many constituted health risk to consumers. Frying oil used continuously at a high temperature is subjective to series of degradation reactions thermal oxidation. Oxidation of frying oil result primary products of lipid oxidation is hydro peroxides which referred to as peroxides (El-Noamany, ٢٠٠٠).

Oyewole & Olayinka, (٢٠٠٧) reported that oxidized oil has many risk properties related to toxic responses range from reduce gut absorption enlarged liver, kidney, fatty hepatic necrosis and even death. Antioxidants nutrients found in fruits, vegetables, and other foods are thought that help protect the body from free radical that may cause damage to the cells and weakening of the immune system *(Thompson et al., ٢٠٠٩ and James et al., ٢٠٠٧*).

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. Polyphenoles have both antioxidant and anti-inflammatory properties. *Lynnette, (٢٠٠١)* reported that polyphenols are a large and diverse class of compounds, many of which occur naturally in a range of food plants have antioxidant properties.

Young et al., (١٩٩٩) reported that broccoli rich with polyphenoles reduce the severity of liver injury in association with lower concentrations of lipid peroxidation. *Moreno et al., (٢٠٠٦)* said that broccoli 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). Xiuzhen et al., (٢٠٠٧)*

found that, bioactivities of dietary polyphenols increased several antioxidant enzymes activities such as CAT, GPx and SOD.

Therefore this study presents to the importance of studying the effectiveness of the use of extracts of natural food antioxidants, (polyphenol) of one selected consumed vegetable (broccoli), in order to validate the potentials of these vegetable 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:

Broccoli was purchased from a local supermarket, Cairo, Egypt in November ٢٠١١. The clean parts were dried at ٤٠° C in oven under 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 using magnetic estreria. The extract was filtered over whatman No.١ and then evaporated to dryness under vacuum.

Frying oil (sunflower٧٥٪ and soybean٢٥٪) was used in this experiment because it is less expensive and more available to consumer especially in rural areas in Egypt. Then ٠.٥ % of polyphenols extract from broccoli 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 (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 standard 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 (as shown below) which were fed with 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 balanced diet contain ١٠٪ frying oil (FO).

Group (٣): was fed on balanced diet contain ١٠٪ frying oil added to it TBHQ (FO+ TBHQ).

Group (٤): was fed on balanced diet contain ١٠٪ frying oil treatment with ٠.٥٪ polyphenols extract from broccoli (FO+ broccoli).

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 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 and Seidel, ١٩٨٣),* HDL-C *(Lopez-Virella et al., ١٩٧٧),* Cholesterol *(Richmond, ١٩٧٣),* Total Lipid *(Zollner and Kirsch., ١٩٦٢),* Triglycerides *(Fassati and Prencipe, ١٩٨٢),* GOT and GPT *(Reitman and Frankel., ١٩٥٧),* SOD *(Nishikimi et al., ١٩٧٢),* CAT *(Aebi,* ١٩٨٤) GPx *(Paglia and Valentine, ١٩٦٧).*

Statistical analysis:-

Statistical analysis was performed with SPSS computer program (SPSS, ١٩٩٠) soft ware. 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 broccoli. The results showed that the extract contained pyrogallol, protocatechouic, gallic, catechin and catechol at

amount ٢٧٩٦.٩, ١٨٦.٣١, ١٩.١٧, ٣٣٣.٣٩and ٢٦٥.٦٤ mg/١٠٠g respectively as major compounds present a high proportion in addition to other compounds. These result in agreement with *Young et al., (١٩٩٩)* who reported that broccoli is rich with polyphenols that reduce the severity of liver injury in association with lower concentrations of lipid peroxidation.

Table ١: Phenolic compound as determined by HPLC in broccoli extract:-

Phenolic compound	Concentration(mg/١٠٠g)
Gallic	١٩.١٧
Catechol	٢٦٥.٦٤
Pyrogallol	٢٧٩٦.٩
Protocatechouic	١٨٦.٣١
Caffeine	٧١.٨٣
Vanaillic	٧٤.٠٥
Caffiec	١٩.٥١
P-coumaric	١١.٣٠
Chrisin	١.٠١
Chlorogenic	٢١٦.٧٣
Syringic	٦٢.٥٢
Ferulic	٤٩.٠٢
Salicylic	٢١٧.١
Coumarin	١٧.٦٨
Catechin	٣٣٣.٣٩
Cinnamic	٢٣.٣٤
P-Benzoic	-----

Determined HPLC according to standard/Official methods (ISO) ١٩٩٩.

Serum constituents:-

Table (٢) showed a significant increase at P<٠.٠١ in the levels of TG of all group fed with diet containing frying oil compared control. These results were agreed with *Lu and Lo (١٩٩٥)* who stated that the frying oil diet resulted in a higher content of TG and cholesterol. Group fed with diet contain FO treated with broccoli

polyphenols extract tended to have the lowest level of TG compared to FO+ TBHQ. Accordingly, it can say that the level of TG present in groups has been decreased with additional polyphenols extracts of broccoli. *Bahadoran et al., (٢٠١٢)* reported, significant decrease of serum triglycerides in rats treated by broccoli powder.

Data in table (٢) showed a significant increase at P<٠.٠١ in the levels of CHL in all groups feeding with frying oil compared to control group. These results were agreed with Hamza and Mahmoud, (٢٠٠٩) who reported that the rats maintained on the HFD showed significant high value of serum CHL (١٧١.٦١ mg/dl) as compared to control (١١٤.٢٠ mg/dl). It was observed that the group feeding with FO treated with broccoli polyphenols extract tended to have the lowest level of CHL. Non-significantly increase in the levels of total lipids in all group, the highest levels of total lipid was observed in group fed with diet containing frying oil compared to control group. While group fed with diet contain FO treated with polyphenols extract from broccoli tended to have the lowest level of total lipids compared to FO+ TBHQ group, significant increase at (P<٠.٠١) in the levels of LDL-C in all groups fed with diet containing frying oil compared to control group. These results were agreed with the same authors who found increase significant value of LDL-C with HFD comparing to control. On other hand group fed with FO+ TBHQ recorded significant decrease. TBHQ has strong as antioxidant potentials (Hamza and Mahmoud, ٢٠٠٩). Treatment frying oil with polyphenols extracts decreased the level of LDL-C of rats group compared to another groups.

HDL-C showed in table (٢) significantly increase (P<٠.٠١) all groups fed with FO diet compared to control group. The highest level of HDL-C reached (٥٠.٥٤ mg/dl) in groups fed with FO without antioxidant. Polyphenols extract treatments led to significant increase in the level of HDL-C. The data suggest that increases HDL-C as a protecting mechanism against the peroxidative stress might be induced by the consumption of a diet containing the thermally oxidised oil *(Garrido et al., ٢٠٠٤).*

The influence protective of using polyphenol extract from broccoli as natural antioxidant

Table (٢) showed the level of GOT & GPT were significant increase at P<٠.٠١ for groups fed with frying oil compared to normal rats. Our results are in basic agreement with the results of *Darwish, (٢٠١٠)* who found increase in the level of GOT/GPT in rats fed with frying oil groups, while treatment with polyphenols extract led to significant decreased in the level of GOT and GPT. It is suggested that using polyphenols extract from broccoli for treating frying oil induce natural antioxidants phenol compound.

Table ٢: Effect of frying oil and frying oil treated with poly phenol extracts from broccoli on some biochemical parameters in rats.

Parameters	CG	FO	FO+ TBHQ	FO + broccoli extract
Total lipids(mg/dl)	٥٢١.٠٨^{a} ± ١٣٧.٩	٧٣٧.٥٣^{c} ± ٨٧.٠٩	٧٠٦.٢٥^{b} ± ١٦٢.٤٣	٥٦٢.٤٣^{ab} ±١٤٦.٨
Total cholesterol (mg/dl)	٦٨.٦٩^{a} ± ٤٩.٢٦	١٦٢.٨٧^{c} ±١٣.٣٩	١٣٤.٩٢^{bc} ± ١٠.٣	١١١.٦٩^{b} ± ١.٤٦
Triglycerides (mg/dl)	١٦٩.٠٤^{a} ± ١١.٤٦	٢٤٧.٣٨^{c} ± ٢٦.٧٦	٢٢٧.٠٦^{b} ± ٣٨.١٤	١٧٧.٠٥^{ab} ± ٢٥.٣٣
HDL-C (mg/dl)	٢٥.٣٥^{a} ± ٣.١	٥٠.٥٤^{c} ± ٤.٨	٢٦.١٦^{ab} ± ٣.٥	٣٥.١٢^{b} ± ٩.٠
LDL-C (mg/dl)	٩٧.٢٦^{a} ± ١٤.٢	١٦٢.٢٤^{c} ± ١١.٤	١١٨.٢٧^{bc} ± ١٠.٦	١١١.١٣^{b} ± ٧.٥
GOT (U/ml)	٢١.٦٧^{ab} ± ٢.٣	٢٩.١٧^{c} ± ٢.٤	٢٣.١٧^{b}±٥.٤	١٩^{a} ± ٢.٥
GPT (U/ml)	١٢.٣٣^{b} ± ١.٠٣	١٣.٣٣^{c} ± ٢.٠٦	١١.١٧^{ab} ± ١.٣	١٠.٥^{a} ± ١.٠٤

a, b, c = Means with the same letter are not significantly different (P> ٠.٠٥)
Values are expressed as mean ± S.D (n=٦)

The present result in table (٣) shows decrease in the level of SOD & CAT activity in FO group compared to normal rats. 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 of frying oil and frying oil treatment with poly phenol extracts from broccoli on SOD, GPx and CAT activity in rats.

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 ± ٠.٤٠٠	٢٠٧.٩٤ ± ٧١.٠٤
(FO+TBHQ)	٢٠.٨١ ± ١٦.٨٣	٢.٨٧ ± ٠.٤٤	٢٢٤.٣٥ ± ٤٣.٤٣
(FO+ broccoli)	١٩.٠١ ± ٦.٨١	٣.٥٢ ± ٠.٧٣	٢٤٠.٣٨ ± ٢٦.٩٦

a, b, c = Means with the same letter are not significantly different (P> ٠.٠٥)
Values are expressed as mean ± S.D (n=٦)

- SOD: superoxide desmutase.
- GPX: glutathione peroxidase.
- CAT: Catalase.

Rats fed with FO treated with polyphenols extract showed improvement in the SOD & GPx activity compared to control group, *Xiuzhen et al., (٢٠٠٧)* reported that bioactivities of dietary polyphenols increased several antioxidant enzymes activities. The highest level of SOD activity showed in group fed with FO+TBHQ may be cause overload lipid peroxidation (*El shall, et al., ٢٠٠٩).* Significant increase (P<٠.٠٥) in the level of GPx activity after rats fed with frying oil compared to control group. However feeding with FO treated with polyphenols extract showed significant increase (P<٠.٠٥) in the level of GPx activity compared to control group, that fed with FO without treatment.

In the end of experiment it could be concluding that using polyphenols extracts (included natural antioxidants phenol compound

as catechin, gallic acid and caffeic) from broccoli for treating frying oil might play protective role against through enhancing reducing lipid peroxidation and increasing the activities of antioxidant enzymes such CAT, GPx and SOD.

Histopathological studies:

Histopathological examination for 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.

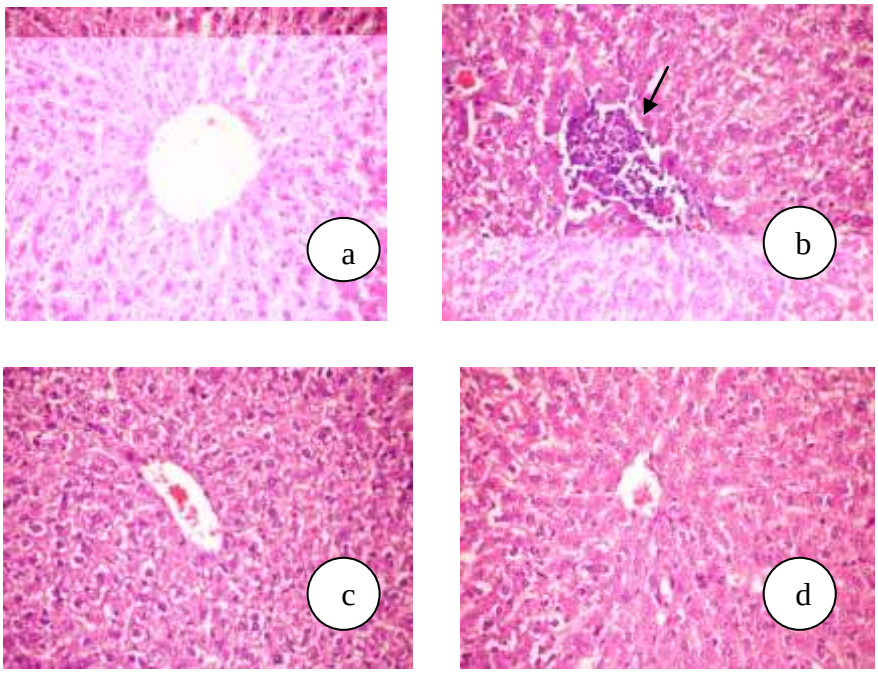

photo ١:- A photomicrograph of the liver sections of the control rat(a), frying oil diet (b), Frying oil + TBHQ(c), Frying oil+ polyphenole extract from broccoli (d) (H&E×٤٠٠).

These results are in agreement with that of *Darwish, (٢٠١٠)* who found feeding rats with frying oil showed, kupffer cell and congested central vein.

Totani and Ojir, (٢٠٠٧) reported that many dark-red patches, necrosis, and bleeding were found in the livers of ٧٥٪ of the experimental rats which had extremely which high (GOT) (GPT) values. This result in agreement with that explained previously in the current study reaviling 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.

Liver of rats fed with diet containing frying oil treated with polyphenols extract from broccoli shown in photo (١d) reavel apparent normal hepatocytes. Our results were in agreement with *Young et al., (١٩٩٩)* who reported that broccoli rich with polyphenols reduce the severity of liver injury.

It can be concluded that polyphenols compound reduced markers of liver damage in rats fed with frying oil, with concomitant elevation in antioxidant enzymes level indicating a possible protective effect of polyphenols compound activities enzymes may be an important and novel strategy for hepatic protection against oxidative.

Acknowledgement:-

The authors wish to thank the staff of Agricultural chemistry Dep. Faculty of Agriculture. Minia Univ.

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The influence protective of using polyphenol extract from broccoli as natural antioxidant

التأثير الوقائى لاستخدام مستخلص البوليفينول من البروكلى كمضاد أكسدة طبيعى ضد أكسدة زيت القلى فى الفئران.

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تحدث تغيرات كيميائية في زيت القلي ويشمل الأكسدة. البروكلي غني بالبوليفينول له خصائص مضادة للأكسدة والتى تخفض من بيروكسيد الدهون والتهاب الكبد.

وكان الهدف من هذا العمل استخدام مادة البوليفينول المستخلص من البروكلى كمضادات أكسدة طبيعية ضد أكسدة زيت القلي، لزيادة نشاط الانزيمات المضادة للأكسدة مثل سوبر اكسيد ديسموتيزوالجلوتاثيون بيروكسيد والكتاليز فى فئران التجارب.

أجرى تحليل كيميائى بجهاز HPLC للتعرف علي مكونات المركبات الفينولية التى يشار اليها بالتأثير ووجد أنها تحتوى على مركبات حمض البيروجلليك و حامض الجاليك والكاتيكول وحمض الكافيك كمركبات تتواجد بنسبة عالية بالإضافة الى مركبات أخرى.

ولقد لوحظ انة عند تغذية الفئران على عليقة تحتوى على زيت القلى المستخدم عدة مرات (١٠% /١٠٠جرام عليقة) حدوث زيادة معنوية فى مستوى الدهون الكلية، الكولسترول، الجليسريدات الثلاثية، LDL-C، HDL-C و حدوث انخفاض فى مستوى نشاط الانزيمات المضادة للاكسدة (SOD, CAT) مقارنة بالمجموعة الضابطة. بينما عند تغذية الفئران بزيت القلى المعالج بالبوليفينول المستخلص من البروكلى حدثت زيادة معنوية فى مستوى نشاط الأنزيمات المضادة للأكسدة (SOD,GPx) و HDL-C مقارنة بالمجموعة الضابطة. وأوضحت الدراسات الهستوباثولوجية كثيرا من التغيرات فى كبد الفئران.