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EFFECT OF ADMINISTRATION OF α-TOCOPHEROL, NIGELLA SATIVA SEEDS TOGETHER WITH DEEP FRIED FAT ON GLUTATHIONE DEPENDENT ENZYMES AND LIPID PEROXIDES IN RATS LIVER

(With 3 Tables)

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دراسة تأثير تناول فيتامين هـ وبذور حبة البركة والزيت المغلى على نشاط جهاز الجلوتاثيون والدهون المؤكسدة فى كبد الفئران

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أجرى هذا البحث لدراسة تأثير إضافة فيتامين هـ وبذور حبة البركة (الحبة السوداء) على رفع كفاءة جهاز منع الأكسدة (الجلوتاثيون المختزل) فى الجسم لوقاية جدار الخلية من الأكسدة التى تحدث نتيجة تراكم المركبات السامة الناتجة من التناول المستمر للزيت المغلى. إستخدم لإجراء هذا البحث عدد ٧٠ فأرا ذكرا قسمت إلى سبعة مجموعات كل مجموعة تحتوى على (١٠) فئران . المجموعة الأولى مجموعة ضابطة (كنترول) تغذت على عليقة أساسية (ضابطة سالبة)، المجموعة الثانية تغذت على عليقة أساسية مضاف إليها ١٠٪ من زيت بذرة القطن الطازج، المجموعة الثالثة تغذت على عليقة أساسية مضاف إليها ١٠٪ من زيت بذرة القطن المغلى لعدة مرات ، المجموعة الرابعة تغذت على عليقة أساسية مضاف إليها ١٠٪ من زيت بذرة القطن المغلى مضاف إليها ٣٠٠ جرام فيتامين هـ لكل كجم عليقة ، المجموعة الخامسة تغذت على عليقة أساسية مضاف إليها ١٠٪ من زيت بذرة القطن المغلى مضاف إليها ٢٪ حبة البركة المجروشة، المجموعة السادسة تغذت على عليقة أساسية مضاف إليها فيتامين هـ (٣٠٠ جرام) لكل كجم عليقة، المجموعة السابعة تغذت على عليقة أساسية مضاف إليها ٢٪ حبة البركة المجروشة. وفى نهاية التجربة التى إستمرت ١٦ أسبوع تم تجميع عينات الكبد للمجاميع المختلفة وقياس نشاط جهاز الجلوتاثيون المختزل (جلوتاثيون بيروكسيديز وجلوتاثيون ريدكتيز وجلوتاثيون ترنسفيريز) كذلك قياس مستوى تأكسد الدهون ومستوى بعض المشتقات الحرة مثل الهيدروكسيل. وأوضحت النتائج: إن تناول الزيت المغلى له تأثير سام ينتج عنه زيادة فى المشتقات الحرة مثل الهيدروكسيل مما يترتب عليه زيادة تأكسد الدهون المكونة لجدار الخلية ونقص معنوى فى نشاط

أنزيمات منع الأكسدة التى تعتمد على الجلوتاثيون، إضافة المواد المانعة للأكسدة مثل فيتامين هـ أو حبة البركة السوداء مع الزيت المغلى تزيد من كفاءة جهاز منع الأكسدة وتقلل الأكسدة الخلوية التى ينتج عنها تهتك جدار الخلية ومكوناتها، وبذلك نوصى بعدم إستخدام الزيت المغلى الذى سبق غليه عدة مرات وذلك لفترات طويلة حيث أن له تأثير تراكمى على وظائف الأعضاء كما يوصى بتناول بعض الكميات من فيتامين هـ أو حبة البركة السوداء عند إستخدام الزيت المغلى للتقليل من التأثير السام له.

SUMMARY

In vivo studies were carried out to decide how vitamin E (dl. α-tocopherol acetate) and Nigella sativa seeds administrated orally combined with or without the deep fat frying (over heated oil) influence the glutathione dependent enzymes [(glutathione peroxidase (GP-ase), Glutathione reductase, (G-R-ase) and Glutathione transferase, (G-S-ase)], as well as reduced glutathione (GSH), Hydroxyl radical (OH) and lipid peroxidation (LP) of rat liver. It was found that, both vit. E and N.sativa seeds influence the action of deep fried fat but they are not able fully to prevent or eliminate its toxic effects.

***Key words**: Nigella sativa seeds -œ-Tocopherol-Enzymes-Rat liver*

INTRODUCTION

Cellular damage due to lipid peroxidation has a role in various disease states such as coronary atherosclerosis (Kok, 1991), diabetes mellitus (Sugawara, 1992) as well as normal aging (Ji et al., 1991).

Sheehy et al. (1994) reported that consumption of heated sunflower oil by chicks reduces α-tocopherol status and increase susceptibility of muscles to lipid peroxidation. In vivo studies showed that, α-tocopherol is an effective antioxidant that has the capacity to scavenge O_2, H_2O_2, OH, O and lipid-free radicals (Fukuzawa et al., 1985), and it is necessary for stabilization of various easily oxidizable components of the cells (Draper et al., 1964). Moreover, it is considered as one of the principal biological defense against lipid peroxidation as it reacts with hydroxyl radicals (Maiorino et al., 1989). It was shown that chronic ingestion of oxidized lipid may compromise free radical scavenging activity in vivo, by depleting α-tocopherol in the gastrointestinal tract or possibly in plasma and other tissues (Sheehy et al., 1994).

When lipid peroxidation is induced, glutathione dependent-enzymes inhibit it only if Vit. E is present (Ursini et al., 1982). This led Maiorino et al. (1989) to suggest that there is a cooperation between peroxidase and free radical scavenger.

Nigella sativa is a herbaceous plant belonging to family Ranunculacea, commonly used for many medical purposes. Its seeds have anthelmintic activity against tape worms (Agarwal et al., 1979), antibacterial and antifungal properties (Rathee et al., 1982).

Recently, Mandour and Rady (1997) found that the adminstration of Nigella sativa revealed a significant decrease in the concentrations of all amino acids except histidine which showed a significant increase in ducklings.

On the other hand, Houghton (1995) reported that oil of Nigella sativa seeds inhibited the non-enzymatic peroxidation in ox brain phospholipid liposomes, that suggests antioxidant activity of this oil. Nearly similar results were observed by Rady et al. (1997) for chicken erythrocyte glutathione redox system.

For these, reasons, therefore, the present work deals with how Vit. E and Nigella sativa, in vivo, influence the redox system changes brought about by the deep fat frying oil (over heated oil).

MATERIAL AND METHODS

Seventy male albino rats were obtained from the Egyptian organization for biological products and vaccines, Helwan; their weight was about 50 g and age from 4 to 6 weeks.

The animals were acclimatized to our laboratory conditions before being used. All rats were maintained on homogenous basal diet (Table 1), free access to fresh water was allowed. The rats were caged together under the same environmental conditions throughout the experimental period. (16 weeks).

The rats were classified into 7 groups each comprised of 10 rats as follows:

Group (1): Act as control kept on basal diet.

Group (2): Negative control group kept on basal diet mixed 10% unheated cotton seed oil.

Group (3): kept on basal diet mixed 10% over heated cotton seed oil (deep-fat frying).

Group (4): kept on basal diet and over-heated cotton seed oil 10% and vit.E (300 mg of dL-α-tocopherol acetate /kg diet).

Group (5): kept on basal diet mixed with 300 mg of dL-α-tocopherol acetate /kg diet.

Group (6): kept on basal diet mixed with 10% over heated cotton seed oil and 2% crushed Nigella sativa seeds.

Group (7): kept on basal diet mixed with 2% crushed Nigella sativa seeds.

Table 1.

Ingredient	% of basal diet
Yellow corn	50
Wheat bran	19-20
Soybean oil	28
Common salt	0.5
Mineral mix	1.1
Vit. mix	0.4

Vit mix./kg diet:

Vit. A (4000,000 IU), vit.D_3 (1000,000 IU), vit. E (300,000 IU), vit. K (0.5 mg/kg), niacin 20 mg, folic acid 1 mg, Cal.Pantothenate 20 mg, riboflavin 3 mg, thiamin 4 mg, vit B_6 6 mg/kg, vit B_{12} 0.05 mg, choline chloride 1000 mg. according to NRC (1984) and these vitamins were manufactured by Alex. Pharm. and Chem. Company.

Mineral mix./kg diet:

Each kg of diet contain: $MgSO_4$ 255 g, NaCl 100 g, $FeSO_4$ 50 g, $ZnSO_4$ 11 g $MnSO_4$ 5.07 g, $CuSO_4$ 1.6 g, cobalt chloride 0.8 g, KI 0.5 g, chromic chloride 0.2 g.

Collection and examination of the samples:

After 16 weeks from the beginning of the experiment and overnight fasting, the rats were killed by decapitation, livers were removed and homogenized in saline solution, using a potter Elvehjem homogenizer for about 3 min.

The homogenates were used for determination of the following parameters:

- Reduced glutathione (GSH) according to the method described by Sedlak and lindsay (1968) using Ellman's reagent.
- Hydroxyl radical (OH) was measured using deoxyribose method as described by Olah et al. (1990).

- Lipid peroxidation (LP): Malondialdhyde (MDA) was as an indicator for lipid peroxidation. It was determined by the method described by Placer et al.(1966). Calibration curve was prepared using Malondialdehyde diethyl acetate (Merck, Germany).
- Glutathione peroxidase (GP-ase, EC 1.11.1.9) was determined chemically using cumen hydroperoxide as a co-substrate. Enzyme quantity regarded as enzyme unit (IU) which transformed 1 micromole substrate in the minute (Chiu et al., 1976, Sedlak and Lindsay, 1968 and Matkovics et al., 1988).
- Glutathione reductase (GR-ase; EC 1.6.4.2), The activity was measured according to the method described by Bergmeyer et al. (1988) using $NADPH_2$. Enzyme unit is the amount of the enzyme using 1 micromole of $NADPH_2$ in one minute and expressed as nanomoles of $NADPH_2$ oxidized/minute/mg protein.
- Glutathione transferase (G-S-ase; EC 2.51.18): The enzyme activity is expressed as micromole of 5-chloro-1,3-dinitrobenzene (CDNB) conjugated/minute/mg protein according to the method of Vessey et al. (1984) in the presence of reduced glutathione.
- Protein content, was measured by the method described by Lowery et al. (1951) using Folin-phenol reagent. Calibration curve was prepared with bovine serum albumin.

Statistical analysis:

The data obtained were statistically analyzed according to Snedecor and Cochran, (1982).

RESULTS

Table 2 shows that the hepatic tissues of rats supplemented with deep fat frying combined with or without Vit. E and/or Nigella sativa seeds posesses the lowest values (0.026, 0.034 and 0.036 µg/g wt) respectively of reduced glutathione (GSH) compared to the control ones (0.044 µg/g wt). Furthermore, Table 2 demonstrated that the lipid peroxidation was significantly ($P \leq 0.05$) decreased in rats treated by Nigella sativa seed and Nigella sativa seeds plus deep fat fyring from 98.8 to 60.4 nm MDA/g wt and from 98.8 to 66.4 nm MDA/g wt respectively. On the other hand, the highest level of hydroxyl radical (78 nm/g wt) was noticed in the liver of rats given the deep fat fyring. Concerning, the glutathione dependent enzymes, Table 3 revealed a significant ($P \leq 0.05$) decrease in the GP-ase activity in all treated

groups reached to the lowest level (0.035 U/mg prot.) in the liver of rat given the deep fat fyring. While, the activities of both GR-ase and G-S-tr-ase were significantly ($P \leq 0.05$) reduced from 8.4 to 4.8 U/mg prot. and from 0.43 to 0.34 U/mg prot. respectively in hepatic tissues of rats supplemented with deep fat frying.

DISCUSSION

The effect of the deep fat frying (over heated oil) with or without addition of α-tocopherol or Nigella sativa seeds on the glutathione dependent enzymes and other parameters was studied. It was found that, the liver redox system is changed by feeding the deep fat frying. Our results demonstrated that the following sequence of effectiveness can be established for Vit. E and Nigella sativa which were used to counteract the toxic effects of over heated oil. The combined adminstration of Vit. E and Nigella sativa seeds with over heated oil increased significantly the GSH values if compared with there in over heated oil treated group. On the other hand, all groups supplemented with Nigella sativa seeds showed a significant decrement in lipid peroxidation, while their values are increased in all other treated groups, Table 2.

The coadminstrated Nigella sativa seeds and over heated oil increased the activities of glutathione reductase and glutathione transferase to the normal levels, Table 3. However it showed a slight enhancing action on the glutathione peroxidase, as it still lower than there in control group.

With regard to the action of deep fat frying and antioxidant substances (dL-α-tocopherol acetate and Nigella sativa seeds), it was found that, chronic ingestion of heated and oxygenated oil containing a large amount of oxidized lipid led to accumulate a small fat in liver cells and caused focal hepato cellular coaulative necrosis (Shibayama, 1992), facilitates electron-exchange (electron-acceptor nature) (Sheehy et al., 1993) and compromise free radical scavenging activity "in vivo" by depleting α-tocopherol in the gastrointestinal tract or possibly in plasma and other tissues and causes in intensive LP (Sheehy et al., 1994). In addition the action of Vit. E is believed to inhibit LP via donation of hydrogen atoms to a lipid peroxyl radical forming lipid hydroperoxide and reversible oxidized Vit. E (Fukuzawa et al., 1985) while, over load of Vit. E can turn-over in the cell in oxidized form and cause alteration in membrane proteins and become prooxidant (Rady et al., 1992 and Khilo et al., 1995).

It is obvious that Nigella sativa too acts as proton-electron donor, has a protective effect against chemical carcinogenesis and/or retard the

carcinogenic process. Regarding to its effect on the lipid profile it can inhibit the cyclooxygenase and S-lipooxygenase path ways arachidonate metabolism and this led to inhibit eicosanoid generation and lipid peroxidation (Haughton et al., 1995); El-Gazaar 1997).

In the light of these observations, it seemed important to reevaluated factors that may protect against oxidative-damage of cell membrane. The presence of glutathione redox system, that has an inhibitory effect against peroxidation of membrane lipids through the reduction of endogenously formed hydroperoxides of unsaturated fatty acids (Fujii et al.,1984).

We are continuing our experiment to obtain a deep understanding of the molecular mechanism of toxic action of deep fat frying and also to attempt to eliminate the toxic symptoms.

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Table 2.

Influence of deep fat frying combined with or without dl α-tochopherol acetate and Nigella sativa seeds on the level of reduced glutathione and oxidized lipids

	BD	BD + Fresh oil	BD + DFF	BD+DFF + Vit. E	BD + Vit E	BD+DFF + N.S.S.	BD + N.S.S.
GSH µg/g wt	0.044 ± 0.003^{A}	0.038 ± 0.006^{B}	0.026 ± 0.005^{C}	0.034 ± 0.007^{B}	0.038 ± 0.007^{AB}	0.036 ± 0.007 B	0.039 ± 0.008^{AB}
LP nmol MDA/g wt	79.78 ± 7.95^{AB}	94.24 ± 6.10^{A}	98.79 ± 10.19^{A}	84.6 ± 5.43^{A}	81.75 ± 10.64^{A}	66.38 ± 2.24 B	60.39 ± 3.63^{BC}
OH nm/g wt	68.2 ± 2.89^{B}	70.16 ± 1.68^{AB}	78.14 ± 2.91^{A}	70.6 ± 2.06^{AB}	67.29 ± 3.5^{AB}	62.35 ± 3.7 AB	63.9 ± 2.1^{AB}

Table 3.

Influence of deep fat frying combined with or without dl α-tochopherol acetate and Nigella sativa seeds on the glutathione dependent enzymes

	BD	BD + Fresh oil	BD + DFF	BD+DFF + Vit. E	BD + Vit E	BD+DFF + N.S.S.	BD + N.S.S.
Gp-ase U/mg prot	0.069 ± 0.008^{A}	0.055 ± 0.009^{B}	0.035 ± 0.008^{D}	0.052 ± 0.009^{B}	0.057 ± 0.007^{B}	0.048 ± 0.013^{C}	0.056 ± 0.009^{B}
GR-ase U/mg prot	8.36 ± 1.34^{A}	5.79 ± 1.04^{B}	4.85 ± 0.53^{B}	8.77 ± 1.63^{A}	8.79 ± 1.64^{A}	8.12 ± 0.59^{A}	8.1 ± 1.07^{A}
G-S-tr-ase µg/mg prot	0.43 ± 0.09^{A}	0.52 ± 0.08^{A}	0.34 ± 0.03^{B}	0.42 ± 0.08^{A}	0.45 ± 0.08^{A}	0.41 ± 0.05^{A}	0.42 ± 0.08^{A}