

## Improvement of Lipid Profile and Antioxidant Status of Hyperlipidemic Albino Rats by Gamma-irradiated Safflower (*Carthamus tinctorius* L.)

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**H**YPER-LIPIDEMIA is a dominant risk factor that contributes to the development and progression of atherosclerosis. Safflower is rich in the essential omega-6 and omega-3 polyunsaturated fatty acids and phenolic compounds which are known to be effective for the treatment of hyperlipidemia. This study was performed to examine the efficacy of safflower to ameliorate the induced hyper-lipidemia in rats.

The results obtained revealed that rats fed on high fat diet (HFD) significantly induced an increase in lipid profile, glucose and some liver enzymes as well as elevation of malondialdehyde (MDA) associated with a significant decrease in high density lipoprotein (HDL-C), glutathione (GSH) content and some antioxidant enzymes activity. However, when rats received HFD containing either raw or irradiated safflower (1% w/w), a significant improvement in the above mentioned parameters was seen. In conclusion, safflower supplementation in diet of rats pointed out to a promising role of safflower, a natural product, on antioxidant enzymes, liver function and lipid profile of hyper-lipidemic rats, regardless if it is irradiated or not.

**Keywords:** Safflower, fat diet, lipid profiles, antioxidants,  $\gamma$ -rays.

Long-lasting high-fat diet causes the reduction of the activity of hepatic lipase and lipoprotein lipase and other lipid metabolic enzymes, leads to lipid metabolism disturbance and hyper-lipidemia (Lin *et al.*, 2005 and Li *et al.*, 2011). Hyper-lipidemia is a dominant risk factor that contributes to the development and progression of atherosclerosis which is the main reason for coronary heart disease, hypertension and cerebrovascular disease (Zhang *et al.*, 2011 and Herrera *et al.*, 2011).

The use of functional foods rich in polyunsaturated or monounsaturated fats in diets aimed to lowering plasma cholesterol levels, reducing the risk of coronary heart disease and preventing or treating many chronic diseases (Moon *et al.*, 2001). In addition, epidemiological studies have shown inverse relationships between consumption of diets rich in polyphenols and the cardiovascular risk. Much evidence indicates that the increased oxidative stress, such as oxidative modification of LDL, is deeply involved in the development of atherogenesis. So, it seems reasonable to attribute the cardioprotective effect of dietary polyphenols to their potent antioxidant activity (Halliwell *et al.*, 2005).

*Carthamus tinctorius L.* has been grown for centuries, primarily for its colourful petals to use as a food colouring and flavouring agent (Esendal, 2001). It has attracted significant interest as it is rich in the essential n-6 polyunsaturated fatty acid (PUFA) linoleic acid and n-3  $\alpha$ -linolenic acid. Numerous health organizations have recommendations for dietary linoleic acid intake, generally falling within the range of 3 -10 % of total energy consumption (Harris *et al.*, 2009 and Asp *et al.*, 2011). Several studies postulated that safflower flower is impressive for the treatment of inflammation, hyper-lipemia, arteriosclerosis, and osteoporosis, promoting blood coagulation, normalizing menstruation, and eliminating blood stasis (Zhao *et al.*, 2009).

Food irradiation has been recognized as a reliable and safe method for the preservation of food and for improving the hygienic quality and nutritional value of food (Al-Kaisey *et al.*, 2002). In addition to the control of microorganisms of numerous food commodities, ionizing irradiation can be used to reduce several carcinogenic agents (Fan and Mastovska, 2006).

Thus, the present study was designed to evaluate the possible beneficial effect of raw and irradiated safflower on serum lipid parameters and antioxidant enzymes in albino rats fed on fat-rich diet.

## **Material and Methods**

### ***Material***

Safflower flower and standard commercial rodent diet were purchased from local herbal market (Cairo, Egypt). The Safflower was crushed to coarse powder and sieved through No. 20 mesh size.

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### ***High fat diet***

HFD (20g fat/ 100g diet): A wt of 20g fat (a mixture of 19g butter oil and 1g soybean oil) (Woods *et al.*, 2002) was added to 100g control diet.

### ***Irradiation process***

Powder of safflower was transferred into polyethylene bags and treated with 10 kGy of gamma rays, using a  $^{60}\text{Co}$  source at a dose rate of 4.75 kGy/ h at NCRRT, Cairo, Egypt.

### ***Fatty acids determination***

Total fatty acid (FA) concentrations in Safflower flower were determined according to Kinsella (1966).

### ***Animals***

The current experiments were conducted on male albino rats ( $150 \pm 20\text{g}$ ). The animals were housed under conditions of controlled temperature ( $30 \pm 2^\circ\text{C}$ ) with natural light. Food and water were provided *ad-libitum*.

### ***Experimental design***

The animals were randomly divided into four groups, each consisted of 7 rats. Group 1: rats were fed on normal diet for 10 weeks, served as a control, group 2: rats were fed on HFD for 10 weeks and group 3-4: rats were fed on HFD supplemented with 1% of either raw or  $\gamma$ -irradiated safflower powder. At the end of the experimental period, the rats in each group were fasted overnight, anaesthetized with diethyl ether and sacrificed. Blood samples were collected by heart puncture, allowed to coagulate and centrifuged to obtain serum for biochemical analysis.

### ***Biochemical Analysis***

Total lipids (TL), total cholesterol (TC), triglycerides (TG) and HDL-C were determined according to the procedure described by Frings *et al.* (1972), Allain *et al.* (1974), Fossati and Prencipel (1982) and Demacker *et al.* (1980), respectively. Low-density lipoprotein-cholesterol (LDL-C), very Low-density lipoprotein-cholesterol (vLDL-C) and atherogenic index were evaluated

according to Friedwald *et al.* (1972), Norbert (1995) and Harnafi *et al.* (2008) formulas, respectively by the following equations: LDL-C (mg/dl) = TC - (TG/5+HDL-C), vLDL-C (mg/dl) = TG/5 and the Ath. Index = (TC-HDL-C) / HDL-C. Serum glucose was evaluated by the method of Trinder (1969). The activity of serum aspartate transaminase (AST) and alanine transaminase (ALT) were assayed by the method of Reitman and Frankel (1957) while serum alkaline phosphatase activity (ALP) was assessed according to Kind and King (1954). MDA was determined by the method of Yoshioka *et al.* (1979). EDTA was used as anticoagulant for blood samples required to estimate GSH according to Gross *et al.* (1967). Catalase (CAT) activity was estimated by the method of Bergmeyer and Grabe (1987) and superoxide dismutase (SOD) was measured by using the method of Minami and Yoshikawa (1979).

### **Statistical analysis**

Statistical analyses were performed using computer program Statistical Packages for Social Science (SPSS, 1998), and values compared with each other using suitable tests.

## **Results**

The data showed that the main unsaturated fatty acids of raw safflower were linoleic (33.37%) and linolenic (4.78%) of total identified fatty acids.

The total saturated fatty acids (SFA) and total unsaturated fatty acids (USFA) were 0.72 and 40.56 % respectively, while in irradiated sample were 0.77 and 40.62% respectively. The total omega-6 fatty acids and total omega-3 fatty acids of raw safflower were (33.53 and 6.63%) while of  $\gamma$ - irradiated safflower were (33.85 and 6.40%).

The animals maintained on the HFD showed a significant high value of serum TL, TG, TC, LDL-C, vLDL-C and atherogenic index associated with a significant reduction in serum HDL-C compared to those of control animals. In comparison with rats fed HFD, the animals received the HFD fortified with either raw or  $\gamma$ -irradiated safflower revealed a significant decrease in the various measurements of lipids in serum, except the HDL-C which was significantly increased (Table 2).

**TABLE 1. Fatty acid composition of raw and  $\gamma$ -irradiated safflower.**

Fatty acids	% raw	% irradiated
Myristic (14:0)	0.04	0.05
Palmitic (16:0)	0.05	0.05
Palmitolic (16:1)	0.08	0.07
Stearic (18:0)	0.32	0.36
Oleic (18:1)	0.32	0.30
Linoleic (18:2)	33.37	33.68
Linolenic (18:3)	4.78	4.63
Arachidic (20:0)	0.12	0.09
Arachidonic (20:4)	0.16	0.17
Eicosapentanoic (20:5)	0.20	0.19
Behenic (22:0)	0.15	0.17
Docosahexaenoic (22:6)	1.65	1.58
Lignoceric (24:0)	0.04	0.05
Total SFA	0.72	0.77
Total UFA	40.56	40.62
Total MUFA	0.40	0.37
Total PUFA	40.16	40.25
n-6	33.53	33.85
n-3	6.63	6.40
n-6/n-3	5.06	5.29
Others	58.72	58.61

Total MUFA: Total mono unsaturated fatty acids.

Total n-6: Total omega-6= (C18:2+ C20:4).

Total n-3: Total omega-3= (C18:3+ C20:5+ C22:6).

**TABLE 2. Effect of feeding rats on HFD enriched with either raw or  $\gamma$ -irradiated safflower on serum lipid profiles.**

Animal groups	TL mg/dl	TC mg/dl	TG mg/dl	HDL-C mg/dl	LDL-C mg/dl	v-LDL mg/dl	Ath. index
Control	494.35 $\pm$ 15.11 <sup>a</sup>	119.22 $\pm$ 6.62 <sup>a</sup>	112.21 $\pm$ 7.68 <sup>a</sup>	51.24 $\pm$ 5.46 <sup>a</sup>	45.52 $\pm$ 6.14 <sup>a</sup>	22.44 $\pm$ 0.56 <sup>a</sup>	1.33 $\pm$ 0.11 <sup>a</sup>
HFD	864.11 $\pm$ 6.45 <sup>c</sup>	177.61 $\pm$ 9.72 <sup>c</sup>	220.11 $\pm$ 9.26 <sup>c</sup>	32.91 $\pm$ 4.56 <sup>c</sup>	100.68 $\pm$ 6.32 <sup>c</sup>	44.02 $\pm$ 0.83 <sup>c</sup>	4.39 $\pm$ 0.23 <sup>c</sup>
HFD+ Saff.	673.31 $\pm$ 5.93 <sup>b</sup>	137.68 $\pm$ 6.19 <sup>b</sup>	177.52 $\pm$ 6.94 <sup>b</sup>	45.88 $\pm$ 5.55 <sup>b</sup>	56.30 $\pm$ 4.36 <sup>b</sup>	35.50 $\pm$ 0.48 <sup>b</sup>	2.00 $\pm$ 0.13 <sup>b</sup>
HFD+ irr.Saff.	661.37 $\pm$ 7.81 <sup>b</sup>	134.48 $\pm$ 5.66 <sup>b</sup>	171.23 $\pm$ 6.47 <sup>b</sup>	45.13 $\pm$ 4.9 <sup>b</sup>	55.11 $\pm$ 4.47 <sup>b</sup>	34.25 $\pm$ 0.41 <sup>b</sup>	1.98 $\pm$ 0.17 <sup>b</sup>

Values are expressed as means  $\pm$  S.E. (n=7).

Values in the same column with different superscripts are differing significantly at  $P < 0.05$ .

HFD= high fat diet, Saff. = Safflower, irr. Saff. = irradiated Safflower.

The results recorded in Table 3 exhibited a significant elevation in AST, ALT, and ALP level as well as glucose concentration in HFD group compared with those of control and all treated groups. While a significant depression in liver function enzymes and glucose levels was observed in treated rats as compared to HFD group.

**TABLE 3. Effect of feeding rats on HFD enriched with either raw or  $\gamma$ -irradiated safflower on glucose level and liver enzymes activity.**

Animal groups	Glucose mg/dl	ALP U/L	ALT U/ml	AST U/ml
<b>Control</b>	88.17 $\pm$ 5.53 <sup>a</sup>	81.64 $\pm$ 4.28 <sup>a</sup>	13.52 $\pm$ 0.57 <sup>a</sup>	23.93 $\pm$ 1.12 <sup>a</sup>
<b>HFD</b>	186.58 $\pm$ 6.07 <sup>c</sup>	144.05 $\pm$ 5.14 <sup>c</sup>	29.08 $\pm$ 1.07 <sup>c</sup>	50.84 $\pm$ 2.17 <sup>c</sup>
<b>HFD+ Saff.</b>	100.45 $\pm$ 2.56 <sup>b</sup>	101.51 $\pm$ 6.25 <sup>b</sup>	18.89 $\pm$ 1.46 <sup>b</sup>	30.82 $\pm$ 1.28 <sup>b</sup>
<b>HFD+ irr. Saff.</b>	102.57 $\pm$ 2.73 <sup>b</sup>	98.80 $\pm$ 4.93 <sup>b</sup>	18.35 $\pm$ 0.65 <sup>b</sup>	30.71 $\pm$ 1.42 <sup>b</sup>

Legends as in Table 2.

Due to keeping rats on HFD, a significant increase in MDA as well as a remarkable reduced level of GSH and activity of SOD and CAT was noticed as compared with those of rats received control diet (Table 4). In contrast, giving rats HFD plus raw or  $\gamma$ -irradiated safflower caused a significant decreased level of MDA and significant elevated concentration of GSH, SOD and CAT activity.

**TABLE 4. Effect of feeding rats on HFD enriched with either raw or  $\gamma$ -irradiated safflower on MDA, GSH, CAT and SOD activity.**

Animal groups	MDA n mol/ml	GSH mg/dl	SOD u/ml	CAT u/ml
<b>Control</b>	68.40 $\pm$ 6.56 <sup>a</sup>	62.61 $\pm$ 4.5 <sup>a</sup>	5.78 $\pm$ 0.65 <sup>a</sup>	37.66 $\pm$ 2.45 <sup>a</sup>
<b>HFD</b>	129.51 $\pm$ 9.12 <sup>c</sup>	44.51 $\pm$ 4.28 <sup>c</sup>	4.17 $\pm$ 0.41 <sup>c</sup>	22.58 $\pm$ 3.18 <sup>c</sup>
<b>HFD+ Saff.</b>	80.03 $\pm$ 7.15 <sup>b</sup>	57.15 $\pm$ 5.7 <sup>b</sup>	5.51 $\pm$ 0.38 <sup>b</sup>	33.62 $\pm$ 3.67 <sup>b</sup>
<b>HFD+ irr. Saff.</b>	79.23 $\pm$ 5.68 <sup>b</sup>	58.17 $\pm$ 4.35 <sup>b</sup>	5.58 $\pm$ 0.43 <sup>b</sup>	33.74 $\pm$ 2.8 <sup>b</sup>

Legends as in Table 2.

## Discussion

High-fat intake has been shown to play a relevant part in the obesity epidemic and it is considered as one of the most important risk factors involved in different diseases (Herrera *et al.*, 2011) such as cardiovascular disease and insulin resistance and diabetes. (Buettner *et al.*, 2007). Safflower and its major and unique phenolic constituents, serotonin hydroxycinnamic acid amides (serotonin derivatives), essential n-6 linoleic acid and n-3  $\alpha$ -linolenic acid, were documented to be protective against LDL oxidation and atherogenesis (Koyama *et al.*, 2006).

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Results occurred in Table 1. point to the fatty acid composition of both raw and irradiated safflower which in agreement with those deduced by Zahran *et al.* (2007). No real effect of gamma-irradiation at dose 10 KGy on the chemical constituents (Abdel-Khalek, 2008). The content of fatty acid did not change in the range of dose under study. These observations concord with those of (Oraei *et al.*, 2011) who studied the effect of gamma-irradiation on the major fatty acids (oleic, linoleic, Linolenic, palmitic, stearic) and found no effect of  $\gamma$ -irradiation on major fatty acid composition.

In the present study, rats fed HFD have higher concentration of TL, TC, TG, LDL-C, vLDL-C and Ath. index accompanied by lower level of HDL-C than those consumed control diet (Table 2). Younies (2008) reported that HFD induced a significant rise in TL, TC, TG, LDL-C and Ath. index while a significant decline was recorded in serum HDL-C. Hyper-lipidemia is a result of an oxidative abuse due to free radicals formed by the interaction of HFD (Shyamala *et al.*, 2005). The effect of HFD in this study, on the lipid profile, was in coincidence with the previous study of Hamza and Mahmoud (2009). Ji and Gong (2008) declared significant increment in plasma TG, phospholipids and TC of rats fed on HFD. However, HDL-C showed a distinct diminution in plasma of HFD animals, versus the control group.

As shown in this experiment, rats fed HFD supplemented with raw or  $\gamma$ -irradiated safflower have lower concentration of TL, TC, TG, LDL-C, vLDL-C and atherogenic index and higher level of HDL-C than the rats fed on HFD only. Several hypotheses have been advanced for the cholesterol-lowering effect of safflower rich in PUFAs, including the stimulation of cholesterol excretion into the intestine and oxidation of cholesterol to bile acids (Gotto *et al.*, 1990). Moreover, this cholesterol-lowering effect may be as a consequence of a shift in distribution of cholesterol from the plasma into the tissues because of increased catabolic rate of LDL-C due to up-regulation of LDL receptor by PUFAs and down-regulation by saturated fatty acids (Spady *et al.*, 1993).

Omega-3 PUFA ( $\alpha$ - linolenic acid) content of safflower can reduce TG concentration through the inhibition of hepatic vLDL-TG synthesis and secretion that is secondary to a decrease in TG synthesis. This decrease in vLDL-TG secretion may be due to the decrease in the expression of hepatic

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gene transcription factor sterol regulatory element binding proteins (SREBP-1c) which is the key switch in controlling lipogenesis, (Asp *et al.*, 2011).

The enzymatic activity seen in Table 3. indicated that AST, ALT and ALP values were elevated in the serum of the HFD fed rats. This could be owing to leakage of the enzymes into the serum as a result of damage to the integrity of the heart and liver. Elevated serum activity of these enzymes has been reported to be indicators of calculated risk of cardiovascular disease, Otunola *et al.* (2010). Addition of raw or  $\gamma$ -irradiated safflower to HFD caused a significant decline in the activity of liver enzymes. A possible mechanism of the reduced activity of the tested enzymes and the hepatoprotective effect of safflower may be related to its antioxidant effect because of the phenolic and flavonoids compounds (Kim *et al.*, 2007). Previous study allowed that polyphenols can inhibit nitrosation and that flavonoids have a hepatoprotective activity (Orhan *et al.*, 2007).

It could be noticed that glucose level increased in serum of HFD fed rats (Table 3). HFD would induce free radical production which induced oxidative stress. A relationship between glucose concentration and oxidative stress has been shown in red blood cells (Deladino *et al.*, 2008). Raw or  $\gamma$ -irradiated safflower significantly lowered serum glucose concentration suggesting its glucose lowering property and the hypoglycemic effect of its phenolic content and isoflavones (Kim *et al.*, 2007).

In the present study it has been found that MDA was significantly increased in rats fed HFD, compared to those fed normal diet. However, GSH content and SOD and CAT activity reflected a significant reduction (Table 4). These results are consistent with those reported by Shyamala *et al.* (2005), so, elevated level of MDA of HFD rat due to the excessive formation of free radicals and activation of lipid peroxidation system. Most studies provided evidence of increased TBARS level in the myocardium of hyper-lipidemic rabbits (Lapenna *et al.*, 1992), while decreased GSH content in the tested organs of mice fed with high-fat was pronounced (Ming *et al.*, 2009).

Raw or  $\gamma$ -irradiated safflower supplementation along with HFD reduced lipid peroxidation in the body as shown by the reduction in MDA level and enhances the antioxidant status as denoted by increasing GSH content and SOD

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and CAT activity. The effects of safflower are regarded to safflower polyphenols which composed of flavones, lignin, and serotonin derivatives. These phenolic compounds have radical scavenging activity and inhibit lipid peroxidation (Barnes, 2010 and Kim *et al.*, 2007). Furthermore, Lee *et al.* (2003) reported that 80 mg isoflavones/day decreased plasma malondialdehyde and increased total antioxidant status in hyper-cholesterolemic postmenopausal women. Linoleic, as a major fatty acid of safflower, efficiently scavenged reactive oxygen species (Zahran *et al.*, 2007), reduced MDA (Santos-Zago *et al.* 2007) and regulated lipid metabolism in various tissues by modulating lipid oxidation, lipolysis and *de novo* lipogenesis (Evans *et al.*, 2002).

In conclusion, it could be observed that safflower herb itself has a hypolipidemic effect regardless of being  $\gamma$ -irradiated or not because the irradiation did not show significant variation compared to the non-irradiated herb. Therefore, supplementation of either raw or gamma irradiated safflower to hyper-lipidemic rats is effective in decreasing the oxidative stress by increasing the activity of antioxidant enzymes (CAT and SOD), non-enzymes; GSH and limiting lipid peroxidation process by reducing MDA level as well as ameliorating the hyper-lipidemia-induced change in the lipid profile and liver function.

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## تحسين صورة الدهون ومضادات الأكسدة في الجردان المصابة بزيادة دهون الدم باستخدام العصفر المعالج بأشعة جاما

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٢٩ مدينة نصر ، القاهرة ، مصر .

تعتبر زيادة دهون الدم من أحد الأسباب الضارة التي تؤدي إلى حدوث أمراض تصلب الشرايين يتميز عشب العصفر بأنه غنى في الأحماض الدهنية الغير مشبعة من النوع اوميغا-٦ واوميغا-٣ ومركبات الفينولات التي تعتبر مواد فعالة لعلاج أمراض تصلب الشرايين و زيادة دهون الدم. وقد تم إجراء هذا البحث لدراسة فاعلية عشب العصفر في تحسين الأضرار الناتجة من زيادة الدهون المستحدثة في الجردان. أظهرت النتائج أن التغذية على عليقة عالية الدهن أدت إلى زيادة معنوية في قيم كلا من الليبيدات الكلية ، الدهون الثلاثية ، الكوليسترول الكلى ، الليبوبروتينات منخفضة الكثافة، مستوى الجلوكوز في الدم و بعض إنزيمات الكبد مثل اسبرتيت ترانس امينيز (AST) ، الالانين ترانس امينيز (ALT) ، الفوسفاتيز القلوى (ALP) بالإضافة إلى زيادة في مستوى المالونالدهيد وارتبط ذلك بانخفاض ملحوظ فى الليبوبروتينات عالية الكثافة والمحتوى من الجلوتاثيون ونقص في بعض إنزيمات الدم المضادة للأكسدة. أدت التغذية على عليقة عالية الدهن تحتوي على ١% عصفر مشعع او غير مشعع إلى تحسن ملحوظ في جميع القياسات البيوكيميائية السابقة. نتيجة لذلك، يمكن القول أن استخدام العصفر كمنتج طبيعي قد يكون له دور في تحسين إنزيمات مضادات الأكسدة وقياسات الدهون في الجردان المحدث بها زيادة في دهون الدم بغض النظر عن كونه مشععا أو غير مشعع.