Pakistan J. Zool., vol. 48(2), pp. 547-556, 2016.

Study of the Influence of Egyptian Apple Against Oxidative Stress in Gamma-Irradiated Rats

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ABSTRACT

Oxidative stress is known to be a key factor in several diseases and was reported as a result of radiation exposure in human and experimental animals. Apple could provide a cheap and readily available source of dietary antioxidants. Therefore, the present study was designed to determine the possible protective effects of dried apple powder (DAP) against gamma (γ-) radiation induced oxidative stress, biochemical and histopathological alterations in male rats fed on diet containing DAP (10%) for 6 weeks. Chromatographic analysis of DAP showed that it contains a variety of phenolic compounds. The present study revealed that γ-irradiation led to a significant increase in lipid peroxidation, significant decrease in glutathione content and antioxidant enzyme activities; increased liver function enzymes and serum lipid profile and histopathological disorders (rupture of the central vein with presence of haemorrhage and hepatic necrosis, accumulation of collagen fibres tissue in the central and portal vein, bile duct branches and between the hepatocytes). Rats fed on a diet containing 10% DAP showed significantly less severe damage and remarkable improvement in all of the measured parameters when compared to irradiated rats. It could be concluded that DAP attenuates the severity of radiation-induced liver biochemical and histopathological disorders probably due to the presence of phenolic compounds that possess antioxidant activity.

Article Information
Received 18 August 2015
Revised 17 October 2015
Accepted 24 October 2015
Available online 1 March 2016

Authors' Contributions
RGH performed all the experimental work including use of radiation facility. HMES worked on phenolic compounds of apple extract and statistically analyzed the data. Both the authors wrote the article.

Key words
Apple, irradiation, phenolic compounds, antioxidants enzymes.

INTRODUCTION

Radiation-related disorders are one of the challenging current health problems with far-reaching medical, social and economic consequences. Radiations are commonly used in a number of medical and industrial situations; however, their pro-oxidative effects limit their applications (Mihandoost *et al*., 2014). Moreover, the increased focus on treatment-related side-effects in cancer survivors and the need for medical countermeasures against radiologic or nuclear accidents or terrorism have resulted in a resurgence of interest in the mechanisms of and ways to modify radiation injury (Denham *et al*., 2001).

Radiation damage is largely caused by the over production of reactive oxygen species (ROS) resulting in oxidative stress that is known to be a key factor in several diseases. The liver performs hundreds of critical functions to maintain homeostasis and health. Increased production of ROS can compromise essential cellular functions, and probably contribute to liver fibrosis (Cheng *et al*., 2015).

Liver disorders are one of the serious health problems through the world. Liver fibrosis is a common sequel to diverse liver injuries. Without effective treatments, reversible liver fibrosis at an early stage leads to irreversible cirrhosis. Liver fibrosis is a reaction to chronic liver injury, and it is characterized by an excessive accumulation of extra-cellular matrix proteins including collagen. It is a common process during the majority of chronic liver diseases (Osada *et al*., 2004). Moreover, liver fibrosis represents the response of the liver to diverse chronic insults such as parasitic disease, chronic viral infection (hepatitis B and C), hereditary metal overload, toxic damage, and so forth. Because of the worldwide prevalence of these insults, liver fibrosis is common and is associated with significant morbidity and mortality (Chen *et al*., 2002).

Damage to liver cells (hepatocytes) causes impaired liver function; hence the investigation for new effective medicines without side effects is still ongoing. Natural remedies from traditional plants are seen as effective and safe alternative treatments for hepato-toxicity (Choi *et al*., 2011).

Fruits are considered rich sources of polyphenols, and widely known for their antioxidant properties. It is extensively reported that dietary intake of fruits, particularly those containing functional bioactive such as phenolic acids, tannins, flavonoids and nutrients is associated to reduce the risk of several chronic diseases (Abrosca *et al*., 2007). Among others, Apple (*Malus domestica* Borkh., Family Rosaceae) is one of the most important fruit crop of the temperate region and most frequently consumed in many regions of the world. Apple fruit has been reported as a potential source of

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0030-9923/2016/0002-0547 $ 8.00/0

polyphenolics, carbohydrates and antioxidants (Wolf and Liu, 2003) and represent a major source of dietary antioxidants. Several epidemiological studies reported that regular consumption of fresh apple as well as its processed products improves aging, diabetes, lower the risk of cardiovascular diseases, lung dysfunction, asthma, thrombotic stroke, liver, colon and lung cancer (Knekt *et al*., 2002; Francini and Sebastiani, 2013).

In view of these considerations, the objective of the present study was to evaluate the efficacy of dried apple powder (DAP) in the modulation of oxidative stress, biochemical and histopathological disorders in the liver of irradiated rats.

MATERIALS AND METHODS

Preparation of dried apple powder (DAP)

Fresh apples purchased from local market (Cairo, Egypt) were cut and dried in an air oven at 50ºC till complete dryness, weighted to calculate its moisture content. Then, dried apple was ground to fine powder (Abdel-Rahim and El-Beltagi, 2010).

Determination of phenolic compounds

Total phenolic content in the methanol extract of DAP was determined by high performance liquid chromatography (HPLC) according to the method of Goupy *et al*. (1999) at Central lab of Food Technology Research Institute, Agriculture Research Center, Egypt.

Methanolic extraction of polyphenols

20 mg of DAP was weighed into a test tube and extracted with 200 ml methanol (80%) containing 0.1% (v/v) of hydrochloric acid. The mixture was homogenized for 1 min and the homogenized mixture was kept at 45ºC in a water bath for 1 h. The homogenate was stored in tightly capped bottles for 24 h at 4ºC. The homogenate was centrifuged at room temperature for 20 min at 10000 rpm. The supernatant was removed from each tube and filtered by Whatman filter paper No. 2 and stored in separate amber screw-cap glass vials at -20ºC prior to analysis.

HPLC analysis of phenolic compounds

Phenolic compounds of apple analyzed with HPLC (Hewllet Packared series 1050) equipped with out-sampling injector, solvent degasser, ultraviolet (UV) detector set at 280nm and quarter HP pump (series 1050). The column temperature was maintained at 35°C.

Gradient separation was carried out with methanol and acetonitrile as a mobile phase at a flow rate of 1 ml/min. The phenolic standards from sigma Co. were dissolved in a mobile phase and injected into HPLC. Retention time and peak area were used to calculate the phenolic compounds concentration by the data analysis of Hewllet Packared software.

Radiation facility

Whole body gamma irradiation of rats at a dose level of 6Gy was performed using a Canadian Gamma Cell-40, (137Cs) (Atomic Energy of Canada Ltd, Ottawa, Ontario, Canada), located at the National Center for Radiation Research and Technology (NCRRT), in Nasr City, Cairo, Egypt. The dose rate of the irradiation process was 0.45 Gy/min at the time of the experiment calculated according to the dosimeter department in the NCRRT.

Experimental animals

Male albino rats Sprague Dawley (10±2 weeks old; 120±20 g) were purchased from the Egyptian Holding Company for Biological Products and Vaccines (Cairo, Egypt) and used for the different investigations carried out in the present study. Rats were acclimated to controlled laboratory conditions for two weeks. Rats were maintained on rodent diet and tap water *ad libitum*. The rodent control diet is composed of 15% casein, 10% corn oil, 5% cellulose, 4% salt mixture, 1% vitamins mixture and starch 65% (Philip *et al*., 1993).

All animal procedures were carried out in accordance with the Ethics Committee of the National Research Centre conformed to the Guide for the Care and Use of Laboratory Animals, published by the US National Institutes of Health (NIH publication No. 85–23, 1996).

Experimental design

Animals (28 rats) were randomly divided into 4 groups (seven rats in each group) as follows; Group 1: rats fed on a balanced diet for 6weeks, served as control group, Group 2: rats fed on a balanced diet with 10% dried apple for 6weeks (DAP group), Group 3: rats were exposed to whole-gamma-irradiation (6Gy) and fed on a balanced diet for 6weeks (Irradiated group; IRR) and Group 4: rats were exposed to whole-gamma-irradiation (6Gy) and fed on a balanced diet with 10% dried apple for 6weeks (IRR + DAP group).

At the end of the experiment, rats were fasted for 24 hours and anaesthetized with diethyl ether. Blood sample were collected though heart puncture and allowed to coagulate and centrifuged to obtain serum for biochemical analysis.

Biochemical analysis

The activity of serum aspartate transaminase (AST) and alanine transaminase (ALT) was estimated

according to Reitman and Frankel (1957) and serum alkaline phosphatase activity (ALP) was assessed according to Kind and King (1954). Total cholesterol (TC), triglycerides (TG) and high-density lipoprotein-cholesterol (HDL-C) were determined according to procedure described by Allain *et al*. (1974), Fossati and Prencipe (1982) and Demacker *et al*. (1980), respectively. Low-density lipoprotein-cholesterol, very low-density lipoprotein-cholesterol and risk ratio were evaluated according to Friedwald's formula (Friedwald *et al*., 1972) by the following equations: LDL-C (mg/dl) = TC-(TG/5+HDL-C), vLDL (mg/dl) = TG/5.

Moreover, the liver tissues was dissected and divided into two parts. One part for histopathological study and the other part was homogenate in saline solution. Liver homogenates were obtained using a tissue homogenizer. The homogenates (1:10 w/v) were prepared using a 100 mM KCl buffer (pH 7.0) containing EDTA 0.3 mM. All homogenates were centrifuged at 200×g for 20 minutes at 4°C, and the supernatants were used to estimate the level of thiobarbituric acid reactive substances (TBARS) (Yoshioka *et al*., 1979), glutathione (GSH) (Beutler *et al*., 1963), and for the assays of superoxide dismutase (SOD) (Minami and Yoshikawa, 1979) and catalase (CAT) activities (Johansson and Borg, 1988), respectively.

Histopathological examination

For histopathological study the tissue samples were taken rapidly from each rat, and fixed in 10% formalin. All the samples were dehydrated in ascending grades of ethanol, cleared in butanol and embedded in parablast. Sections of 5-6 μm thick sections were stained with haematoxylin and eosin (H&E) staining for general histological studies, and Masson's Trichrome stain for collagen fibres.

Statistical analysis

Results were presented as mean ± standard deviation (SD) (n=7). Experimental data were analyzed using one way analysis of variance (ANOVA). Duncan's multiple range test was used to determine significant differences between means using the computer program Statistical Packages for Social Science (SPSS, 1998). Differences between means were considered significant at $P < 0.05$.

RESULTS

The main phenolic compounds identified and quantified in DAP by HPLC analysis in mg/100g dry weight are: Gallic acid, 0; P- OH Benzoic, 0.93; Caffeine, 0.34; Catechin, 82; Vanilic, 0.73; Pyrogallol, 0; Caffeic, 0; Phenol, 53; Daidzin, 33; Rutin, 46; P-Coumaric, 61; Genistein, 179; Salicylic, 137; Ferulic, 93; Cinnamic, 241; Quercetin, 19; Euganol, 3; Chrysin, 6; Galangin, 2 and Pinostrobin, 11. The most abundant were cinnamic, genistein, salicylic, ferulic and catechin, respectively.

Exposure of rats to gamma-radiation (6 Gy) resulted in a significant elevation in the serum concentration of TC, TG, LDL-C and vLDL-C with a significant decrease in HDL-C concentration as compared to those of normal control group. Whereas, the addition of DAP to the diet of gamma-irradiated rats significantly reduced the TC, TG, LDL-C and vLDL-C levels and increased the level of HDL-C compared to irradiated group (Table I).

Hepatic enzyme

The results presented in Table I revealed a significant elevation in the activity of AST, ALT and ALP in irradiated group compared to control; whereas, the activity of liver enzymes were decreased in the group of irradiated rats receiving DAP compared to irradiated group.

Oxidative stress enzymes

The level of TBARS and GSH in the liver of control and experimental rats are presented in Table II. The results indicate that whole-gamma-irradiation (6Gy) provoked a significant rise in TBARS level and a significant decrease in GSH concentration in examined rat liver relative to the corresponding values of control rats. While, irradiated rats receiving DAP in the diet showed a significant decrease liver TBARS accompanied by significant rise in GSH concentration compared to irradiated group (Table II).

Also, the activity of the two antioxidant enzymes SOD and CAT exhibited a significant reduction in the liver of gamma irradiated-rats, compared to the control animals. On the other hand, a significant enhancement in the activity of SOD and CAT was recorded in the liver of irradiated-rats supplemented with DAP compared to irradiated group (Table II).

Liver histopathological studies

Liver of control rats consists of well defined hexagonal or pentagonal classic hepatic lobules each traversed by a central vein; polygonal medium sized hepatocytes are arranged in plates radiating, branching and anatomising from the central vein. Each hepatocyte contains a single central rounded vesicular nucleus; some of the hepatocytes are binucleated. The cytoplasm contains basophilic granules. The sinusoids are composed of endothelial cells and contain phagocytic cells known as Von kupffer cells. Liver sections from control group

Table I.- Effect of DAP supplementation on lipid profile and hepatic enzymes of different animal groups.

Parameters*	Control	DAP	Irradiated	IRR+ DAP
Lipid profile				
TC (mg/dl)	149±12.03[a]	141±13.73[a]	217±17.41[c]	168±10.99[b]
TG (mg/dl)	108±9.62[a]	103±9.30[a]	180±7.21[c]	134±9.17[b]
HDL-C (mg/dl)	46±4.82[a]	47±4.35[a]	38±4.64[c]	43±4.85[b]
LDL-C (mg/dl)	81±5.88[a]	73±5.59[a]	143±6.78[c]	98±6.99[b]
vLDL-C (mg/dl)	21±3.74[a]	20±3.58[a]	36±4.11[c]	26±3.74[b]
Hepatic enzyme				
AST (U/ml)	36.13±3.79[a]	34.98±4.45[a]	59.82±6.04[c]	41.53±4.88[b]
ALT (U/ml)	25.19±2.23[a]	25.54±2.09[a]	38.30±2.01[c]	30.45±2.31[b]
ALP(U/100ml)	8.42±1.27[a]	8.39±1.69[a]	15.11±1.35[c]	11.83±1.40[b]

*Abbreviations used: DAP, dried apple powder; HDL-C, high-density lipoprotein-cholesterol; IRR, irradiated; LDL-C low density lipoprotein cholesterol; TC, total cholesterol; TG, triglycerides; VLDL-C, very low density lipoprotein cholesterol.
Values are expressed as means ± S.D. (n=7).
Values in the same row with different superscript are differing significantly at P<0.05.

Table II.- Effect of DAP supplementation on the liver TBARS, GSH levels and SOD and CAT activities of different animal groups.

Groups	TBARS (n mol/g tissue)	GSH (mg/g tissue)	SOD (U/mg protein)	CAT (U/mg protein)
Control	163± 8.27[a]	53.3± 5.91[a]	48.66 ± 9.22[a]	16.32 ± 0.97[a]
DAP	157± 12.24[a]	55.4± 7.05[a]	50.85 ± 6.65[a]	16.86 ± 0.29[a]
IRR	252± 13.86[c]	24.5 ± 5.80[c]	28.32 ± 4.79[b]	10.26 ± 0.34[c]
IRR+ DAP	180± 15.16[b]	47.7± 7.18[b]	47.17± 9.33[a]	14.18 ± 0.90[b]

Values are expressed as means ± S.D. (n=7).
Values in the same row with different superscript are differing significantly at P<0.05.

showed normal structure which shows normal lobular architecture with central vein and radiating hepatic cords (Fig. 1A). The same observation was observed when the experimental animals were treated by DAP (Fig. 1B). Liver tissue in rats exposed to 6Gy γ-radiation (IRR group) showed gradual vacuolization, cytoplasmic disintegration and pyknotic nuclei in liver hepatocytes. Hepatic Necrosis and great rupture of the central vein with presence of Haemorrhage were also observed in liver tissue (Fig. 1C). However, treatment of the irradiated rats with DAP (IRR+DAP group) predicts great amelioration and recovery of the central vein (CV) and hepatic cells (Fig. 1D).

Collagen fibres deposition in liver tissue

Light microscopic examination of liver sections of control rats stained with *Masson's trichrome stain* represent the normal distribution of collagen fibres (which stained blue) in the portal vein (PV) areas. The thin collagen fibres make the supporting walls of the blood vessel, bile ducts and hepatocytes (Fig. 2A).

Treatment of control rat with DAP shows normal distribution of collagen fibres around the central vein branch (Fig. 2B).

Great accumulation of collagen fibres tissue in the central vein, portal vein, bile ducts branches and between the hepatocytes was observed in liver tissue after the exposure of experimental animals to 6Gy γ-radiation (Fig.2C). Treatment of the previous group with DAP records the normal distribution of collagen fibres around the hepatic vessels (Fig. 2D).

DISCUSSION

Growing evidence suggests dietary antioxidants to play an important role in mitigating the damaging effects of oxidative stress on cells, preserving health and even reversing the progression of chronic diseases. Together, these effects combine to protect the body from many of the reversible consequences of aging (Jung *et al*., 2009).

Apples are a rich source of phenolic constituents, which are distributed in the peel, core and pulp. Content

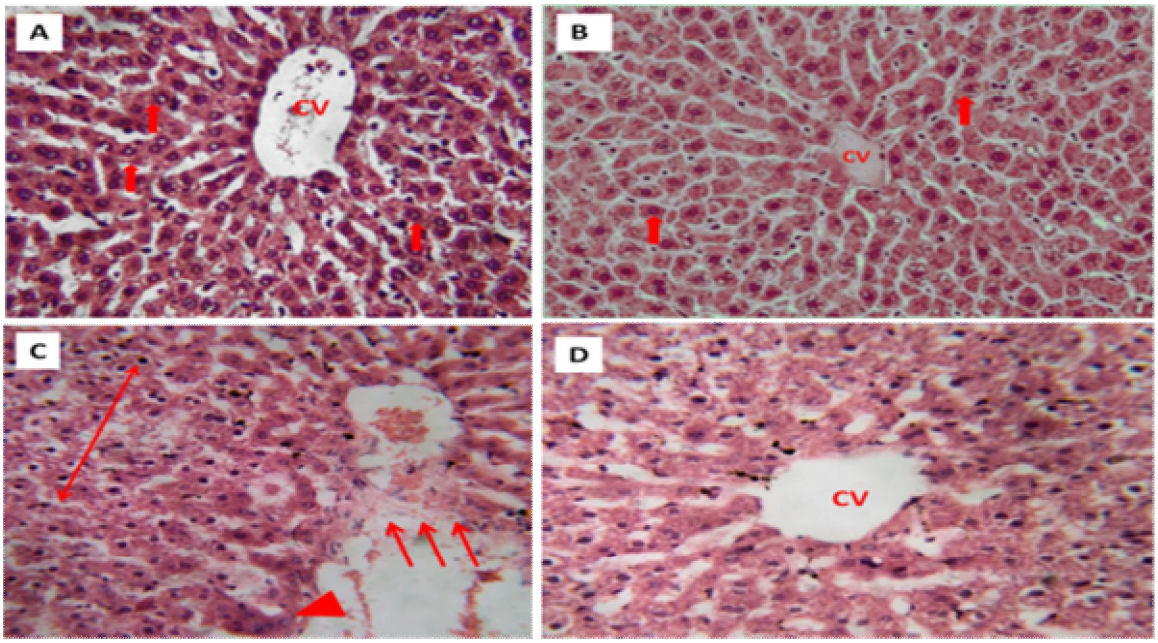

Fig. 1. Histological structure of a rat liver. **A,** control rat showing the normal lobular pattern of the liver with a centrilobular vein (CV) and radiating irregular anatomising plates of hepatocytes with their nuclei (↑) and intervening blood sinusoids; **B,** rat treated with DAP showing the normal appearance of liver tissue; **C,** rat exposed to 6 Gy γ-radiation showing gradual vacuolization, cytoplasmic disintegration and pyknotic nuclei (↕). Hepatic necrosis (◄) and great rupture of the central vein with the presence of haemorrhage are also observed (↑↑↑); **D,** rat exposed to 6Gy γ-radiation and treated with DAP showing great amelioration and recovery of the central vein branch (CV) and hepatic cells. Lipid and fatty features are still recorded. Stain: H and E; magnification: X 400.

and composition of phenolic compounds vary strongly in dependence of the apple variety, area of cultivation, and time and year of harvest (Lata and Tomala, 2007). The present study revealed that Egyptian Anna apple have considerable number of healthy compounds namely polyphenols and flavonoid. Many different phenolic compounds have been identified in apples. The two main subtypes of polyphenols are flavonoids and phenolic acids. Some of the most important flavonoids in apples are: quercetins, present in glycosylated forms (flavonols); rutin and genistein. Some of the most common phenolic acids in apples are: caffeic acid, present in esterified form with quinic acid (chlorogenic acid); and p-coumaric acid, present in esterified form with quinic acid (pcoumarylquinic acid), salicylic, and ferulic (Thielen *et al*., 2005).

The present results revealed that the levels of TC, TG, LDL and vLDL-C in serum were significantly higher in irradiated rats, than those of the control group. On the other hand, radiation exposure resulted in a significant decrease in HDL-C level. Significant increase in the levels of serum lipid profile and LDL are demonstrated post radiation exposure of rats, possibly as a result of liver injury (Sierens *et al*., 2002).This indicates that ionizing-radiation-induced oxidative stress, which might alter hepatic lipid metabolism and serum lipoproteins. It seems that there is an association between radiation-induced oxidative stress and elevated levels of lipid fractions and LDL (Baynes, 1991). This association is similarly observed in other conditions, characterized by increased oxidative stress (Makhlouf and Makhlouf 2012). Therefore, it is suggested that oxidative stress might be an important determinant of altered lipid metabolism, due to radiation exposure (Sierens *et al*., 2002).

Administration of DAP to irradiated rats resulted in significant declines in serum lipid profile, LDL-C and vLDL-C, associated with remarkable elevation in HDL-C, as compared to γ-irradiated group.

The physiological effects of dried apple powder as antioxidant take place via its content like flavonoid, phenolics and the dietary fiber therefore suggesting their role in prevention of coronary heart disease (Wan *et al*., 2001), including atherosclerosis. Wan *et al*. (2001) reported that flavonoids may decrease the risk of cardiovascular disease by lowering LDL: HDL ratio and reducing oxidized LDL in human, and make LDL less susceptible to oxidative stress. Flavonoids may work by

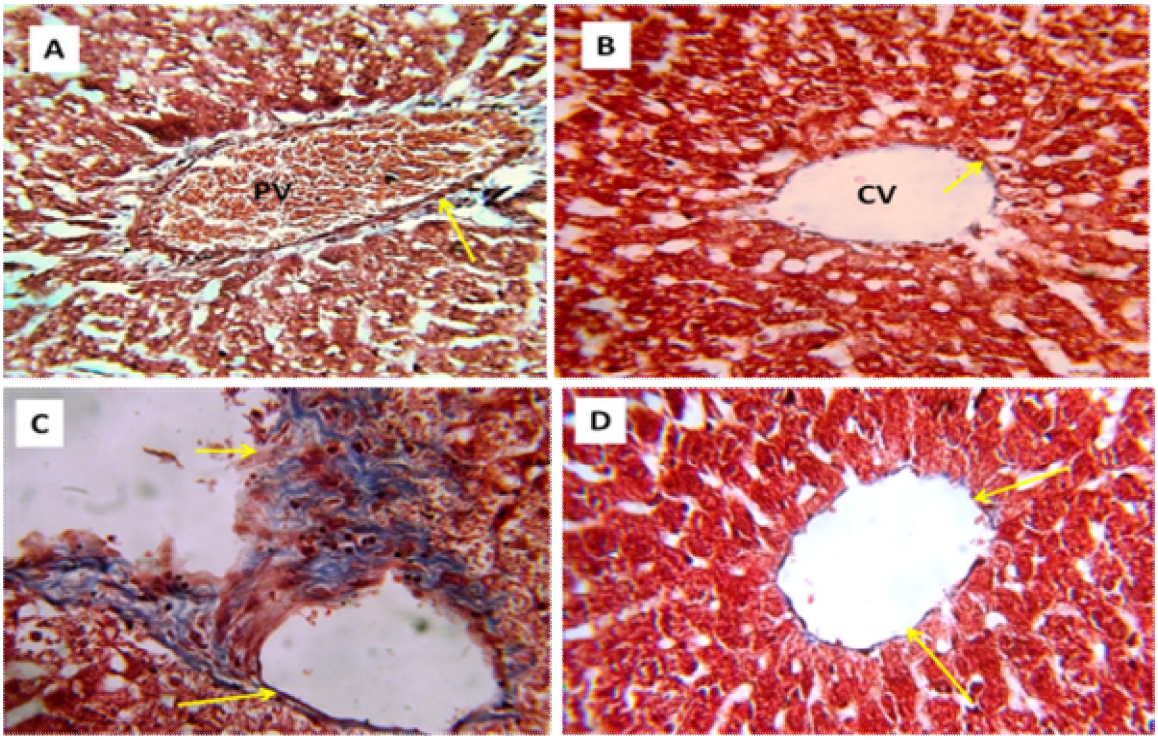

Fig. 2. Histological structure of rat liver showing the distribution of collagen fibres. **A,** Sections of the control group showing normal distribution of collagen fibres (which stained blue) in the portal vein (PV) areas. Note: thin collagen fibres supporting the walls of the blood vessel, bile ducts and hepatocytes; **B,** rat treated with DAP showing normal distribution of collagen fibres around the central vein (CV); **C,** 6Gy γ- irradiation group showing great deposition of collagen fibres in branches of central vein, portal vein, bile ducts and between the hepatocytes (→); **D,** 6Gy γ- irradiation group treated with DAP showing the normal distribution of collagen fibre around the branches of hepatic vessels (↑). Stain: Masson's trichrome stain; Magnification: X400.

making liver cells more efficient, to remove LDL-C from blood by increasing the LDL receptor densities in liver, and by binding to apolipoprotein B (Baum *et al*., 1998). Also, the increase in HDL-C concentration could protect the LDL against oxidation *in vivo* because lipids in HDL are preferentially oxidized, before those in LDL (Bowry *et al*., 1992). Moreover, phenolic and the dietary fiber in apples significantly lowered lipid oxidation both in humans and rats and lowered cholesterol in human. Apple phytochemicals have been shown to have roles in the sterol or polyunsaturated fatty acids modulation of cholesterol synthesis and absorption (Yi-Fang and Liu, 2005).

Several enzymes in blood are considered as indicators of hepatic dysfunction and damage and the leakage of hepatic enzymes such as AST, ALT and GGT into blood are routinely used as a reliable biochemical index for hepatocellular damage. In the current study, it could be noticed that γ-irradiation caused a significant increase in the activities of hepatic enzymes in serum. The increase hepatic enzymes activity by radiation may be due to the damage of cellular membranes of hepatocytes, which in turn, leads to an increase in the permeability of cell membranes, and facilitates the passage of cytoplasmic enzymes outside the cells, leading to the increase in the aminotransferase in liver and blood serum (Ali *et al*., 2012). Also, it is proposed that oxidative stress is linked to the histopathological disorders and organ damage, following exposure to ionizing radiation (Halliwell and Whiteman, 2004).

However, the activity of liver enzymes was decreased, as a result of dried apple powder administration to γ-irradiated rats. DAP exhibit protective and curative effects against liver damage and fibrosis, via decreased lipid peroxidation and inhibited pro-inflammatory gene expression (Akazome, 2004).

The present study shows that γ- irradiation produced significant oxidative damage following radiation exposure. This damage is indicated by the significant enhancement of TBARS level (a marker of lipid peroxidation) in hepatic tissues accompanied by depletion in the enzymatic antioxidants CAT and SOD

activities and the non-enzymatic antioxidant GSH level. In the current study, the elevated level of TBARS in γ-irradiated rats might be due to the interaction of free radicals with polyunsaturated fatty acids in the phospholipids portion of cellular membranes (Adaramoye *et al*., 2012). The antioxidant enzymes both preserve cellular stability and play an important role in keeping free radicals away (Kavasoğlu *et al*., 2015). Therefore, the decrease in the activities of CAT and SOD could be as a result of their utilization by the enhanced production of ROS, which interacts with the enzyme molecules causing their denaturation and partial inactivation (Park *et al*., 2009). The significant reduction in GSH level following radiation exposure in the present study is in accordance with Mathur and Sharma (2012), who concluded that the reduction in GSH content was attributed to the enhanced utilization of the antioxidant system as an attempt to detoxify the free radicals generated by radiation.

In the present study, irradiated rats treated with DAP showed a significant decrease in the level of TBARS content, with concomitant significant increase in the activity of SOD and CAT, and in the content of GSH. Thus, DAP has potential as an anti-peroxidative agent, and as an antioxidant. Yi-Fang and Liu (2005) reported that DAP contained vitamin C and low levels of vitamin E, in addition to quercetin and cinnamic flavonoids; all of which are powerful natural antioxidants that increase antioxidant enzymes, and decrease TBARS concentration and improve lipid profiles in rats. In addition, DAP contains many trace elements including Cu, Mn, Zn and Fe, which are necessary components of SOD (Heghedűş-Mîndru *et al*., 2014). Among these, Cu, Mn and Fe are prosthetic groups of SOD and play a decisive role in its enzyme activity. Zn stabilizes the structure of SOD. Se is an important element of GSH-Px, which regulates lipid metabolism, prevents fatty liver formation, and improves antioxidant ability in rats (Nagasako-Akazome *et al*., 2007).

The present study showed that gamma radiation at 6Gy induced liver fibrosis with many histopathological alterations. Liver fibrosis is a reaction to chronic liver injury, and it is characterized by an excessive accumulation of extra-cellular matrix proteins including collagen. It is a common process during the majority of chronic liver diseases (Osada *et al*., 2004). Fibrosis can lead to impairment of liver function, development of hepato cellular carcinoma, and portal hypertension with all its associated complications.

Tetrahydrobiopterin (BH4) is one of the critical cellular non-enzymatic redox sensitive antioxidants and oxidation of BH4 to dihydrobiopterin (BH2) and other oxidized bio-pterin species causes endothelial nitric oxide synthases (eNOS) to produce higher superoxide levels instead of nitric oxide (NO), a phenomenon popularly termed as "eNOS uncoupling" resulting in increased oxidative stress (Crabtree *et al*., 2009). The oxidation of BH4 to BH2 also further uncouples eNOS due to the ability of BH2 to compete and displace BH4 from eNOS. BH4 insufficiency-dependent eNOS uncoupling has been suggested as an etiologic factor in the progression and the subsequent development of various neurological, cardiovascular and fibrotic diseases (Kietadisorn *et al*., 2012). BH4 treatment suppresses these oxidative stress-dependent adverse pathophysiological conditions (Moens and Kass, 2007). Therefore, maintenance of cellular BH4 homeostasis is critical for normal physiological functioning. This critical balance of BH4 can be altered by differential intrinsic as well as extrinsic stimuli, including radiation.

The molecular mechanism of liver injury as a consequence of radiation exposure, especially in the context of concomitant chemotherapy, is not well understood. Gamma radiation-induced BH4 deficiency promotes formation of peroxynitrite, which is known to play a major role in tissue fibrosis (Anscher *et al*., 1990); consequently it could directly increase fibrotic response of this tissue following radiation. Furthermore, it has been demonstrated that liver fibrosis from late radiation effects correlated with the increase in intracellular transforming growth factor-β1 (TGFβ1) levels (Flechsig *et al*., 2012). TGFβ1 activation and over expression following chronic inflammation has been shown to increase significantly in the radiation exposed livers (Anscher *et al*., 1990). Because peroxynitrite also augments the release of matrix metalloproteinase, a critical factor for fibrotic development it is logical to hypothesize that decreased levels of BH4 following radiation exposure might indirectly contribute to liver fibrosis following radiation as well (Tatsuya *et al*., 2001).

Microscopic studies revealed that the livers of rats receiving DAP showed alleviation in fibrosis. The liver collagen and reticulum contents were lower in rats treated with DAP. Additionally, Heo *et al*. (2008) evaluated the effects of DAP on experimental liver fibrosis induced by γ-radiation in rats. They demonstrated that the histopathological score of fibrosis, liver function were significantly improved in rats treated with radiation plus DAP, compared with those irradiated only.

Moreover, Auclair *et al*. (2010) reported that DAP treatment can alleviate inflammation and inhibit liver fibrosis through multiple mechanisms; it is effective in the treatment of chronic liver diseases. Moreover, while the mechanism underlying the protective benefits of DAP has remained unclear, the attempts at explanation of its protective activity proposed its antioxidant properties

(Auclair *et al*., 2010). Furthermore, DAP reduced expressions of transforming growth factor-β1 (TGF-β1) and collagen I mRNA. In addition, DAP is able to ameliorate liver injury and prevent rats from radiation-induced liver fibrosis by suppressing oxidative stress (Heo *et al*., 2008).

Finally, it can be concluded that DAP has a potential activity against γ-radiation induced oxidative stress and liver fibrosis. Therefore, it may be useful against liver fibrosis, cirrhosis, and dysfunction induced by radiation exposure and different pathogenic factors.

ACKNOWLEDGEMENT

Authors are grateful to Neamat H. Ahmed, Professor of Cell Biology and Histology, Atomic Energy Authority, for her help and support to carry out this work.

Conflict of interest statement

Authors wish to confirm that there are no known conflicts of interest associated with this publication and there has been no significant financial support for this work that could have influenced its outcome.

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