HEPATOPROTECTIVE EFFECTS OF POMEGRANATE AND APPLE JUICES AGAINST N-NITROSODIETHYLAMINE-INDUCED HEPATOCELLULAR CARCINOMA IN MALE RATS

Dr. Amr A. Rezq

Nutrition and Food Sciences Department, Faculty of Home Economics, Helwan University, Cairo, Egypt

Dr. Aml F. Elgazar

Nutrition and Food Sciences Department, Faculty of Home Economics, Helwan University, Cairo, Egypt

المجلة العلمية المحكمة لدراسات وبحوث التربية النوعية

المجلد الثانى– العدد الأول– مسلسل العدد (٣)– يناير ٢٠١٦

رقم الإيداع بدار الكتب ٢٤٢٧٤ لسنة ٢٠١٦

ISSN-Print: 2356-8690 ISSN-Online: 2356-8690

موقع المجلة عبر بنك المعرفة المصري https://jsezu.journals.ekb.eg

البريد الإلكتروني للمجلة E-mail JSROSE@foe.zu.edu.eg

HEPATOPROTECTIVE EFFECTS OF POMEGRANATE AND APPLE JUICES AGAINST N-NITROSODIETHYLAMINE- INDUCED HEPATOCELLULAR CARCINOMA IN MALE RATS

Dr. Amr A. Rezq **Dr. Aml F. Elgazar**

Nutrition and Food Sciences Department, Faculty of Home Economics, Helwan University, Cairo, Egypt

Abstract :

Liver cancer represents a complex and fatal malignancy derived primarily due to oxidative stress and inflammation; it is the second most prevalent cause of death in underdeveloped and developing countries. The main objective of the present study was to investigate the hepatoprotective effect of pomegranate juice (PJ) and apple juice (AJ) against N-nitrosodiethylamine (NDEA)-induced hepatocellular carcinoma in male rats. Sixty adult male Sprague Dawley rats weighing 200 ± 5 g were used and randomized into 6 groups. Results showed that PJ and AJ antagonized NDEA-induced elevations of serum biochemical indices AST, ALT and ALP, and inhibited tumor biomarkers (alpha fetoprotein), TNF-α and NF-κ β and normalized the serum levels of total bilirubin, protein and albumin. The higher dose of PJ and AJ partly alleviated hepatic preneoplastic lesions induced by NDEA. The mechanistic studies revealed that PJ and AJ decreased the elevated in malondialdehyde, increased the content reduced glutathione and restored activities of SOD, GPx, and CAT antioxidant enzymes in hepatic tissue. In conclusion, PJ and AJ have a protective effect against NDEA-induced hepatocellular carcinoma.

Key words: Pomegranate; Apple; Hepatocellular carcinoma; Liver enzymes; Cancer biomarkers; Lipid peroxidation; Antioxidant enzymes.

Introduction :

Cancer remains the main cause of death worldwide, despite advances and the development in several medical scientific researches (Anand *et al.*, 2008). Liver cancer, predominantly hepatocellular carcinoma (HCC), represents a complex and fatal malignancy derived primarily as results of oxidative stress and inflammation. It is the second most common cause of

death in developing and underdeveloped countries (Jemal *et al.*, 2011). Environmental carcinogens are important risk factors leading to the formation of hepatocarcinoma. N-nitrosodiethylamine (NDEA) is found in many processed foods (cheese, steamed and fried fish, and meats) and in alcoholic beverages, tobacco products, cosmetics and agricultural chemicals. NDEA has been commonly used to induce hepatocellular carcinoma in experimental animals (Ansil *et al.*, 2014). The traditional therapy of hepatocarcinoma includes chemotherapy, radiation, surgical resection, ablation and liver implantation. However, this therapy gives a little hope for restoration of health because of poor diagnosis and serious side effects of chemical anticancer drugs (Jeong *et al.*, 2013). Therefore, the search for most effective and less toxic anticancer agents from natural products is necessary to prevent the process of hepatocarcinogenesis.

Regular consumption of fresh fruits and/or vegetable play an important role in the protection and/or prevention of reactive oxygen species (ROS)-associated toxicity and may reduce chronic diseases related to oxidative stress in human body (George *et al.*, 2013). Polyphenol-rich fruits such as pomegranate, apple, kiwi, banana and strawberry have antineoplastic effects in laboratory animal models (Thomas *et al.*, 2014) and can be used as potential weapons in the fight against many cancers. Pomegranate fruit is a rich source of many phytochemicals such as anthocyanins, flavonoids and ellagitannins which showed potent hepatoprotective and antioxidant (Bishayee *et al.*, 2011), anti-inflammatory (Bishayee *et al.*, 2013) and antitumor (Jaganathan *et al.*, 2014) properties. Pomegranate seeds have been reported to be promising for the treatment of certain diseases including cardiovascular disease, diabetes and some types of cancer (Viladomiu *et al.*, 2013). Apples are widely consumed fruits and a rich source of many phytochemicals such as carotenoids, flavonoids, triterpenoids and polyphenols. Previous studies have been linked the consumption of apple with reduced risk of some cancers, cardiovascular disease, asthma, and diabetes (Boyer and Liu, 2004). Apple skins are rich in luteolin flavonoid which was reported to possess antioxidant, anti-inflammatory and anticancer activity (Lin *et al.*, 2008). Phenolic compounds that abundant in apple have a variety of biological effects and may contribute to beneficial effects against cardiovascular diseases, diabetes, and cancer (Hyson, 2011 and Szaefer *et al.*, 2014).

The present study was carried out to investigate the hepatoprotective effects of pomegranate and apple juices against hepatocellular carcinoma induced by N-nitrosodiethylamine in rats.

Materials and Methods:

Materials:

Fruits :

Fully ripe fresh pomegranate (*Punica granatum* L., Family *Punicaceae*) and apple (*Malus domestica*, L. Family, *Rosaceae*) were purchased from a local fruit market, Cairo, Egypt. One kilogram (1000 g) of pomegranate seeds and whole apple fruit including skin were homogenized with 1000 ml distilled water for 10 min, filtered through a double layer of gauze to obtain fruit juices and kept in a refrigerator till use.

Chemicals and kits:

N-nitrosodiethylamine (Synonym: Diethylnitrosamine (DNA), Product number: N0258, molecular formula: $C_4H_{10}N_2O$, NDEA) is a yellow liquid dispensed in 1ml ampoules. It was purchased from Sigma-Aldrich Chemical Company (St. Louis, MO, USA). Carbon tetrachloride (CCl4) was purchased from El-Gomhoryia Company, Egypt in the form of colorless solution. Enzyme linked immunosorbent assay (ELISA) kits for the determination of alpha fetoprotein (AFP), tumor necrosis factor alpha (TNF-α) and nuclear factor-kappa B (NF-κ B) were procured from Glory Science Co., Taiwan. The other biochemical kits were obtained from Biodiagnostics Company, Dokki, Egypt.

Rats:

Sixty adult male Sprague Dawley rats weighing 200 ± 5 g b.wt and 12-13 weeks old were used in this study. Animals were obtained from the Laboratory Animal House, Agricultural Research Center, Egypt. Rats were housed in a well ventilated laboratory room under standard conditions of 24 °C temperature, 50-52% relative humidity and 12 hr light/12 hr dark cycles. Basal diet and water were provided *ad libitum* to rats.

Methods :

Preparation of basal diet:

The dietary supply of protein, fat, carbohydrates, vitamins and minerals was in accordance with the recommended dietary allowances for rats according to Reeves *et al.* (1993). It consists of casein 20%, soybean oil 5%, Choline chloride 0.20%, vitamin mixture 1.0%, mineral mixture 4.0%, fibers 5%, L-Cystine 0.18%, sucrose 10% and the reminder was corn starch.

Induction of hepatocellular carcinoma:

The preneoplastic lesions of hepatocellular carcinoma were induced by single intraperitoneal (IP) injection of N-nitrosodiethylamine (NDEA) in a dose of 100 mg/kg of body weight dissolved in dimethyl sulfoxide (DMSO) in the 3rd week of experiment period. This was followed by weekly subcutaneous injections (3 injections/week) of CCl_4 diluted with liquid paraffin (1:1, V: V) with 2ml/kg of body weight during the 4th week till the 6th week of the experiment period as described by Sundraresan and Subramanian (2003).

Design of the experiment:

Sixty adult Sprague Dawley rats were used in this study and randomly distributed into 6 groups, of 10 rats each. Group 1 was normal (negative) control, while the other 5 groups were treated with single intraperitoneal dose (100 mg/kg) of NDEA injected to rats in the 3rd week, followed by weekly subcutaneous injections (3 injections/week) of CCl4 from the 4th week till the 6th week of experiment period for induction of hepatocellular carcinoma. Group 2 was kept as positive control group and groups 3 and 4 were orally pretreated with pomegranate juice at 2 doses 100 and 200 ml/kg b.wt, respectively, and groups 5 and 6 were orally pretreated with apple juice at doses 100 and 200 ml/kg b.wt, respectively, for 8 weeks.

At the end of experiment period, the rats were euthanized by prolonged exposure to ether and blood samples were collected, left to clot a room temperature and centrifuged at 4000 rpm for 15 min. Serum samples were collected and kept frozen at -70 Ċ till biochemical analyses. Rats were sacrificed and a portion of livers was used for preparing tissue homogenates to be used for biochemical analyses. The other part of livers was preserved in 10% formalin solution till processed for the histopathological examination.

Biochemical analyses:

Serum aspartate aminotransferase (AST) and alanine aminotransferase (ALT) were carried out by the methods described by Bergmeyer *et al.* (1978) and alkaline phosphatase (ALP) by Roy (1970). Serum total protein (TP) was chemically estimated according to the methods of Okokon *et al.* (2013). Serum albumin (Alb) and globulins (Glb) were determined according to the method of Fernandez *et al.* (1966). Serum total bilirubin (TBr) levels were estimated as described by Stiehl (1982). Levels of serum alpha fetoprotein (as a traditional tumor marker) were determined as described by Gibbs *et al.* (1987). Serum tumor necrosis factor- alpha (as a proinflammatory cytokine) levels were quantified as described by Pennica *et al.* (1985) and nuclear factor-kappa beta (as a transcription factor) levels were quantified as described by Adams (2009) using ELISA kits (Glory Science Company, Taiwan.) according to instructions of the producer.

Lipid peroxidation and antioxidant enzymes of liver tissues assay:

One gram of frozen liver tissue was washed with ice-cooled 0.9% NaCl solution and homogenized in 100 ml of ice-cooled 1.5% potassium chloride solution and 50 mmol potassium phosphate buffer solutions (pH 7.4) to yield 1% homogenate (W/V). Liver homogenates were centrifuged at 4000 rpm for 10 min at 4°C. The supernatants were used for estimation of lipid peroxidation (LPO) as described by Ohkawa *et al.* (1979). The technique is based on the reaction of thiobarbituric acid with malondialdehyde (MDA) in acidic media at 95°C for 45 min to form thiobarbituric acid reactive substance (TBARS) and expressed as MDA content. Reduced glutathione (GSH) content in liver homogenate was determined colorimetrically by the method described by Sedlak and Lindsay, (1968). The tissue levels of MDA, GSH, GPx, SOD and CAT were chemically assayed using commercial assay kits. Activities of antioxidant enzymes glutathione peroxidase were determined according to the method of Paglia and Valentaine (1976), superoxide dismutase and catalase were colorimetrically determined by applying the method of Spitz and Oberley, (1989) and Sinha, (1972), respectively.

Histopathological Examination:

Livers of all rats were taken and fixed in 10% neutral formalin solution. The fixed specimens were then trimmed, washed and dehydrated in ascending grades of alcohol. Specimens were then embedded in paraffin, sectioned at 4-6 microns thickness, stained with Hematoxylen and Eosin and then examined microscopically as described by Bancroft and Gamble (2002).

Statistical analysis:

Data were presented as means ± standard errors (SE). The statistical analysis was performed using computerized statistical package of social sciences (SPSS) program (SPSS. 16.00 software for Windows) with one-way analysis of variance (ANOVA) followed by Duncan's multiple range tests (Senedecor and Cochran, 1981).

Results:

As shown in Table 1, single intraperitoneal injection of NDEA (100mg/kg b.wt) followed by CCL_4 injection (subcutaneous) in rats significantly elevated serum levels of liver enzymes (AST, ATL and ALP) compared with that of normal control rats. Oral administration of pomegranate juice (PJ) and apple juice (AJ), each at 100 and 200 ml/kg, to NDEA and CCL_4-intoxicated rats significantly lowered the high serum levels of AST, ALT and ALP enzymes compared to that of the positive control group (intoxicated rats).

Table (1): Serum levels of AST, ATL and ALP in rats injected with NDEA and CCl_4

Groups	Parameters as Mean ± SE		
	AST (U/L)	ALT (U/L)	ALP (U/L)
Group (1): Negative control	43.50 ± 2.11^{d}	38.00 ± 1.10^{d}	97.50 ± 1.24^{d}
Group (2): Positive control	133.00 ± 4.12^{a}	128.50 ± 4.30^{a}	118.50 ± 3.45^{a}
Group (3): PJ (100 ml/kg b.wt)	95.00 ± 2.16^{c}	90.20 ± 2.23^{c}	100.00 ± 3.16^{c}
Group (4): PJ (200 ml/kg b.wt)	92.60 ± 2.32^{c}	87.90 ± 2.31^{c}	101.00 ± 3.11^{c}
Group (5):			

AJ (100 ml/kg b.wt)	119.00 ± 4.00[b]	117.00 ±3.20[b]	105.50 ± 2.16[b]
Group (6): AJ (200 ml/kg b.wt)	118.20 ± 3.55[b]	114.50 ±3.71[b]	103.00 ± 2.25[b]

Means± SE with different superscript letters in the same column are significant at $P < 0.05$.

Results in Table 2 illustrated that intoxicated rats with NDEA and CCL_4 had significant decreases in serum TP, Alb and Glb and an increase in TBr levels when compared with the normal control group. PJ and AJ when given orally to the intoxicated rats significantly increased serum levels of TP, Alb, Glb and decreased serum levels of TBr compared to that of the positive control group (intoxicated rats).

As shown in Table 3, Injected rats with NDEA and CCL_4 alone had significant increased of serum tumor biomarkers AFP, TNF-α and NF-κ β levels, compared with that of the normal control group. Oral administration of PJ and AJ to Injected rats with NDEA and CCL_4 significantly decreased the elevated serum AFP, TNF-α and NF-κ β levels compared to that of the positive control group.

Table 4 illustrates that treated rats with NDEA and CCL_4 alone had significant high MDA and low GSH concentrations in liver tissues compared with that of the normal control group. Oral administration of PJ and AJ to intoxicated rats significantly lowered hepatic MDA concentration and increased GSH concentration as compared to the positive control group.

Activities of GPx, SOD, and CAT antioxidant enzymes in NDEA-treated rats were significantly suppressed as compared with the normal control group. Oral administration of PJ and AJ to intoxicated rats significantly increased the activity of inhibited hepatic SOD, GPx and CAT enzymes as compared to the positive control group (Table 5).

Table (2): Serum levels of TP, Alb,Glb and TBil in rats injected with NDEA and CCl_4

Groups	Parameters as Mean ± SE			
	TP (g/dL)	**Alb (g/dL)**	**Glb (g/dL)**	**TBr (mg/dL)**
Group (1):	7.90±0.02[a]	4.10±0.11[a]	3.40±0.20[a]	0.38±0.01[d]

Negative control				
Group (2): Positive control	3.33±0.02^{d}	2.25±0.12^{d}	1.60±0.02^{d}	3.15±0.03^{a}
Group (3): PJ (100 ml/kg b.wt)	5.55±0.03^{b}	3.80±0.13^{b}	2.40±0.04^{c}	2.70±0.03^{b}
Group (4): PJ (200 ml/kg b.wt)	6.43±0.04^{b}	3.95±0.14^{b}	2.65±0.01^{c}	2.42±0.01^{b}
Group (5); AJ (100 ml/kg b.wt)	4.80±0.04^{c}	3.10±0.11^{c}	3.00±0.02^{b}	1.29±0.02^{c}
Group (6): AJ (200 ml/kg b.wt)	5.30±0.02^{c}	3.25±0.13^{c}	3.10±0.01^{b}	1.56±0.01^{c}

Means ±SE with different superscript letters in the same column are significant at P <0.05.

Table (3): Serum levels of AFP, TNF-α and NF-κ β in rats injected with NDEA and CCl_4

Groups	**Parameters as Mean ± SE**		
	AFP (ng/ml)	**TNF-α (ng/ ml)**	**NF-κ β (ng/ml)**
Group (1): Negative control	3.66 ± 0.02^{d}	1.66 ± 0.01^{d}	106.50 ± 3.11^{d}
Group (2): Positive control	9.92 ± 0.03^{a}	4.65 ± 0.03^{a}	125.00 ± 2.66^{a}
Group (3): PJ (100 ml/kg)	5.95 ± 0.02^{c}	2.65 ± 0.01^{c}	109.40± 2.38^{c}
Group (4): PJ (200 ml/kg)	4.16 ± 0.05^{c}	2.15 ± 0.02^{c}	108.60 ± 3.42^{c}
Group (5): AJ (100 ml/kg)	6.22 ± 0.02^{b}	3.16 ± 0.02^{b}	115.40 ± 2.01^{b}
Group (6): AJ (200 ml/kg)	6.15 ± 0.01^{b}	3.32 ± 0.01^{b}	114.50 ± 3.21^{b}

Means ±SE with different superscript letters in the same column are significant at P < 0.05.

Table (4): Liver tissues of MDA and GSH contents in rats injected with NDEA and CCl_4

Groups	Parameters as Mean ± SE	
	MDA (µmol/gm protein)	GSH (µmol/gm protein)
Group (1): Negative control	48.50 ± 0.40^{d}	11.60 ± 0.31^{a}
Group (2): Positive control	78. 35 ± 0.50^{a}	5.22 ± 0.15^{d}
Group (3): PJ (100 ml/kg b.wt)	56.62 ± 0.30^{c}	9.22 ± 0.51^{b}
Group (4): PJ (200 ml/kg b.wt)	55.37 ± 0.30^{c}	9.89 ± 0.42^{b}
Group (5): AJ (100 ml/kg b.wt)	66.50 ± 0.40^{b}	7.12 ± 0.25^{c}
Group (6): AJ (200 ml/kg b.wt)	65.20 ± 0.50^{b}	7.75 ± 0.22^{c}

Means ± SE with different superscript letters in the same column are significant at $P < 0.05$

Table (5): Hepatic GPx, SOD and CAT activities in rats injected with NDEA and CCl_4

Groups	Parameters as Mean ± SE		
	GPx (nmol/min/mg protein)	SOD (U/mg protein)	CAT (nmol/min/mg protein)
Group (1) Negative control	66.00 ± 3.11^{a}	0.99 ± 0.03^{a}	0.180 ± 0.03^{a}
Group (2): Positive control	48.00 ± 2.15^{d}	0.32 ± 0.01^{d}	0.142 ± 0.01^{d}
Group (3): PJ (100 ml/kg b.wt)	60.13 ± 3.23^{b}	0.72 ± 0.02^{b}	0.170 ± 0.02^{b}
Group (4) PJ (200 ml/kg b.wt)	61.16 ± 3.15^{b}	0.84 ± 0.01^{b}	0.177 ± 0.03^{b}
Group (5):			

AJ (100 ml/kg b.wt)	52.23 ± 3.13^{c}	0.70 ± 0.03^{c}	0.161 ± 0.02^{c}
Group (6): AJ (200 ml/kg b.wt)	53.12 ± 2.12^{c}	0.73 ± 0.02^{c}	0.165 ± 0.03^{c}

Means ± SE with different superscript letters in the same column are significant at $P < 0.05$

Unit of GPx = nmol of GSH utilized/min/mg protein.

Unit of CAT = nmol of H2O2 utilized/min/mg protein.

The histopathological examination of liver sections of normal control rats showed normal histological structure of hepatic lobule with normal hepatocytes, portal vein and sinusoids (Fig.1). Liver sections of injected rats with NEDA and CCL_4 showed compact hepatocellular carcinoma and fat droplets in hepatocytes as demonstrated in Fig. 2, while some of other hepatic sections exhibited large polymorphic nuclei of hepatocytes with sporadic necrosis of hepatocytes as shown in Fig. 3, as well as large fat droplets and marked necrosis of the cells were seen (Fig.4). Liver sections of rats administered orally the large dose of pomegranate juice showed alleviation of preneoplastic lesions induced by NEDA and presence of mild necrosis of hepatocytes around the central vein (Fig.5). Liver sections of rats given orally the large dose apple juice revealed amelioration of preneoplastic lesions induced by NEDA and presence of marked necrosis and fibrosis of hepatocytes (Fig. 6).

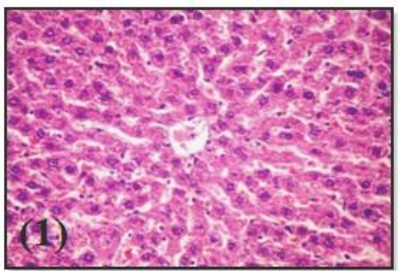

Fig. 1. Photomicrograph of liver section of a normal control rat showing normal histological structure of hepatic lobule with normal central vein, hepatocytes and sinusoids. (H & E X 200).

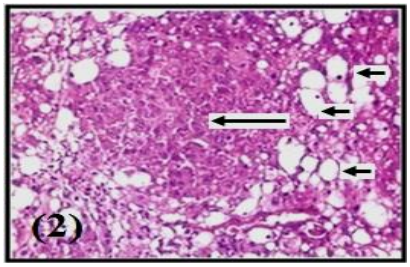

Fig. 2. Photomicrograph of liver section of a rat injected with NDEA and CCL4 showing compact hepatocarcinoma (long arrow) with fat droplets in hepatocytes (short arrows). (H & E X 400).

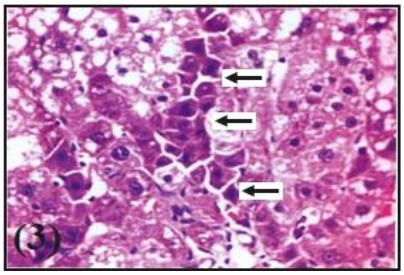

Fig. 3. Photomicrograph of liver section of a rat injected with NDEA and CCL4 showing large polymorphic nuclei of hepatocytes (arrows) with marked necrosis of hepatocytes (H & E X 400).

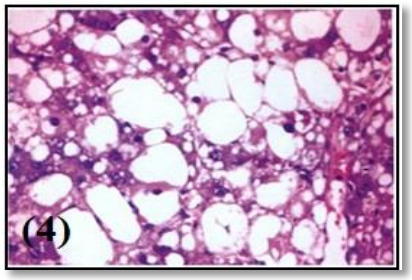

Fig. 4. Photomicrograph of liver section of a rat injected with NDEA and CCL4 showing marked necrosis of hepatocytes and presence of large fat droplets (H & E X 400).

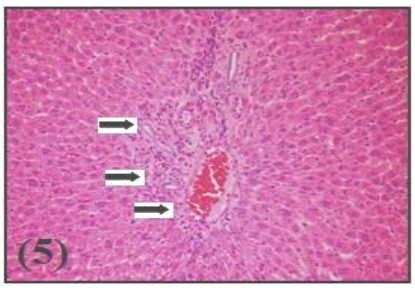

Fig. 5: Photomicrograph of liver section of a rat administered with the large dose (200 mg/kg) of pomegranate juice showing alleviation of preneoplastic lesions induced by NEDA and presence of mild necrosis of hepatocytes around the central vein (arrows) (H &E X 200).

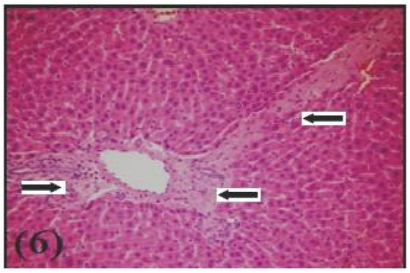

Fig. 6: Photomicrograph of liver section of a rat administered with the large dose (200 mg/kg) of apple juice showing amelioration of preneoplastic lesions induced by NEDA and presence of marked necrosis and fibrosis of hepatocytes (H & E X200).

Discussion :

Cancer and other degenerative diseases are inversely associated with the regular consumption of fruits and vegetables. Fruits and vegetables with antineoplastic activity have recently gained much attention. The biological value of these plant materials depends on the presence of bioactive constituents, especially those with hepatoprotective, antioxidant and anti-inflammatory properties (**Thomas *et al.*, 2014**). However, the

mechanisms underlying the anticancer activity of plant materials are still in need for further investigations.

N-nitrosodiethylamine is a potent carcinogen widely existing in the environment (Subramanian *et al.*, 2007). Carbon tetrachloride (CCL_4) is a selective hepatotoxic chemical agent commonly used for induction of liver damage in experimental animals. CCL_4 produces reactive free radicals (trichloromethyl radical, CCl3) which initiate cell damage via either covalent binding to cell membrane proteins or by induction of lipid peroxidation (Li *et al.*, 2013). Lipid peroxidation is associated with hepatic cell damage leading to liver cirrhosis and fibrosis (Parola *et al.*, 1992).

The results of this study revealed that intoxication of rats by NDEA and CCL_4 induced preneoplastic lesions of hepatocarcinoma, elevated serum AST, ALT and ALP, total bilirubin, tumor markers AFP, TNF-α and NF-κ β levels and lowered serum total protein, albumin and globulin levels. There were also increased in hepatic lipid peroxidation and decreased activities of tissue antioxidant enzymes. These serum biochemical alterations were parallel to preneoplastic lesions seen upon examination of liver sections. These results were similar to the previous reports by Jeong *et al.*, (2013) and Zhao *et al.*, (2014). In addition, previous authors reported that intoxication of rats with NDEA and CCL_4 induces hepatocellular carcinoma associated with serum and tissue biochemical alterations nearly similar to those founded in the presented study. In contrast, Pomegranate juice (PJ) administered orally to NDEA- and CCL_4-intoxicated rats induced good anticancer activity against hepatocarcinoma. This activity of PJ was evident by normalization of liver enzymes, total protein and bilirubin and serum biomarkers of hepatocarcinoma (AFP, TNF-α, and NF-κ β) to nearly levels of normal control group. The anticancer activity of PJ agreed with that reports by Cayir *et al.*, (2011) and Bishayee *et al.*, (2013) who concluded that pomegranate can be used for the prevention of hepatocarcinogenesis as it inhibited the inflammatory cascade in NDEA-injected rats via modulation of nuclear factor-kappa β (NF-κ β)-regulated inflammatory pathway. Also they attributed the anticancer activity of PJ to presence of bioactive antioxidant phytochemicals in the juice (Celik *et al.*, 2009 and Sadeghi ***et al.***, 2009) and its ability to inhibit lipid peroxidation (Cayir *et al.*, 2011).

The alterations in serum biomarkers of hepatocarcinoma induced by PJ in this study were parallel to amelioration of histopathological preneoplastic lesions seen in liver of NDEA-intoxicated rats, denoting an anticancer activity. Moreover, ellagic acid, a component of pomegranate seeds juice was found to suppress androgen-dependent prostate cancer via induction of apoptosis of cancer cells (Naiki-Ito *et al.*, 2014). However, pomegranate peel extract was reported to be effective against azoxymethane-induced colon cancer in rats (Waly *et al.*, 2012).

In this work, studying the protective mechanisms of PJ revealed that its anticancer activity might be due to inhibition of hepatic lipid peroxidation and enhancement of antioxidant capacity. These mechanisms were evident by the increase in hepatic GSH and the decrease in MDA concentrations (both biomarkers of lipid peroxidation) and enhancement of activities of antioxidant enzymes in liver tissues. In this concern, Celik *et al.*, (2009) and Sadeghi *et al.*, (2009) attributed the protective activity of PJ against hepatocarcinoma in rats to its antioxidant phytochemicals. Cayir *et al.*, (2011) suggested that the protective effect of pomegranate oil against hepatotoxicity and nephrotoxicity in rats might due to its inhibition of lipid peroxidation. Moreover, Bishayee *et al.*, (2013) attributed the anticancer effect of pomegranate to the anti-inflammatory activity of its bioactive constituents via modulation of NF-κ β-regulated inflammatory pathway.

Concerning apple juice (AJ), the current results denoted that AJ had an anticancer activity against NDEA and CCL_4- induced hepatocarcinoma in rats. However, the anticancer effect of AJ was less potent than that of PJ as evident from biochemical results and histopathological examination. The anticancer effect of AJ agreed with that reported by Liu *et al.*, (2001) who mentioned that Fuji apple extracts inhibited Hep G2 cell proliferation by 39% and red delicious extracts inhibited cell proliferation by 57% *in vitro*. Boyer and Liu (2004) reported that consumption of apple reduced risk of some cancers and Lin *et al.*, (2008) reported that apple skin is rich in luteolin flavonoid which was reported to possess antioxidant and anti-inflammatory effects and is linked to the anticancer activity against lung, liver and breast cancers. In addition, Hyson, (2011) demonstrated that diets high in fruits especially, apples are associated with reduced lung, liver, stomach, and colon cancers. Recently, Szaefer

et al., (2014) mentioned that phenolic compounds are abundant in apple and have a variety of biological effects including anticancer activity. Studying the mechanisms of anticancer activity of AJ in this work denoted that its antihepatocellular carcinoma in rats might be to its reduction in lipid peroxidation, (as indicated by decreased concentration of MDA in liver tissues); antioxidant (as indicated by increased activity of hepatic antioxidant enzymes) and anti-inflammatory (via modulation of NF-κ β-regulated inflammatory pathway) effects. Inhibition of lipid peroxidation by apple was previously recorded by Ogino *et al.*, (2007) and antioxidant activity of apple was reported by Avci *et al.*, (2007) and Lin *et al.*, (2008). The anti-inflammatory effect of apple juice was confirmed by previous findings of Szaefer *et al.*, (2014) in rats.

Conclusion :

In conclusion, oral administration of pomegranate and apple juices to NDEA- and CCL_4- intoxicated rats produces a potent protective effect against hepatocarcinoma. The mechanisms underlying this effect may be due to the inhibition of lipid peroxidation, enhancement of hepatic antioxidant defense capacity as well as its anti-inflammatory activity via modulation of NF-κ β-regulated inflammatory pathway. Therefore, regular intake of pomegranate and apple in form of fresh fruit and/ or fresh juices may be beneficial for patients suffering from hepatocarcinoma. Moreover, isolation of phytochemicals of pomegranate and apple is necessary to search for safe natural agents to be manufactured for the prevention of hepatocarcinoma.

References :

Adams, P.D. (2009). Healing and hurting: molecular mechanisms, function, pathologies of cellular senescence. Mol. Cell., 36(1): 2-14.

Anand, P., Kunnumakkara, A.B., Sundaram, C., Kuzhuvelil, B. H., Sheeja, T. T., Oiki, S. L., Bokyung S. and Bharat, B. (2016). Aggarwal. Cancer is a preventable disease that requires major lifestyle changes. Pharm Res., 25(9):2097-2116.

Ansil, P.N., Nitha, A., Prabha, S.P. and Latha, M.S. (2014). Curative effect of Amorphophallus campanulatus (Roxb.) tuber on N-Nitrosodiethylamine-induced hepatocellular carcinoma in rats. J. Environ. Pathol. Toxicol. Oncol., 33:205-219, 2014.

Avci, A., Atli, T., Eruder, I., Varli, M., Devrim, E. Turgay, S. and Durak I. (2007). Effects of apple consumption on plasma and erythrocyte antioxidant parameters in elderly subjects. Exp Aging Res., 33(4):429-437.

Bancroft, J. D. and Gamble, M. (2002). Theory and practice of histological techniques, 5th edn. Churchill Livingstone, London, New York and Philadelphia.

Bergmeyer, H.U., Schreiber, P. and Wahlefeld, A.W. (1978). Optimization of methods for aspartate and alanine aminotransferase. Clin. Chem., 24(1): 58-61.

Bishayee, A., Bhatia, D., Thoppil, R.J., Darvesh, A.S., Nevo. E. and Lansky, E.P. (2011). Pomegranate-mediated chemoprevention of experimental hepatocarcino-genesis involves Nrf2-regulated antioxidant mechanisms. Carcinogenesis. 32(6):888-896.

Bishayee, A., Thoppil, R.J., Darvesh, A.S., Ohanyan, V., Meszaros, J.G. Bhatia, D. (2013). Pomegranate phytoconstituents blunts the inflammatory cascade in a chemically induced rodent model of hepatocellular carcinogenesis. J. Nutri. Biochem., 24(1): 178-187.

Boyer, J. and Liu, R.H. (2004). Apple phytochemicals and their health benefits. Nutri. J., 3: 5-10.

Cayir, K., Karadeniz, A., Simsek, N., Yildrim, S., Karakus, E. Kara, A and Sengul, E. (2011). Pomegranate seed extract attenuates

chemotherapy-induced acute nephrotoxicity and hepatotoxicity in rats. J. Med. Food.,14(10): 1254-1262.

Celik, I., Temur, A. and Isik, I. (2009). Hepatoprotective role and antioxidant capacity of pomegranate (Punica granatum) flowers infusion against trichloroacetic acid- exposed in rats. Food Chem. Toxicol., 47(1): 145-149.

Fernandez, A., Sobel, C. and Goldenberg, H. (1966). An improved method for determination of serum albumin and globulin. Clin. Chem., 12(4): 194-205.

George, T.W., Waroonphan, S., Niwat, C., Gordon, M.H. and Lovegrove, J.A. (2013). Effects of acute consumption of a fruit and vegetable pure-based drink on vasodilatation and oxidative status. Br. J. Nutr., 109(8):1442-1452.

Gibbs, P.E., Zielinski, R., Boyd, C. and Dugaiczyk, A. (1987). Structure, polymorphism, and novel repeated DNA elements revealed by a complete sequence of human alpha fetoprotein gene. Biochem., 26(5): 1332-1343.

Hyson, D.A. (2011). A comprehensive review of apples and apple components and their relationship to human health. Adv. Nutri., 2(5): 408-420.

Jaganathan SK, Vellayappan MV, Narasimhan G, Supriyanto E. (2014). Role of pomegranate and citrus fruit juices in colon cancer prevention. World J. Gastroentrol., 20(16):4618-4625.

Jemal, A., Bray, F., Center, M.M., Ferlay, J., Ward, E. and Forman, D. (2011). Global cancer statistics. CA Cancer Clin. J., 61(2): 69-90.

Jeong, J.H., Jeong, H.R., Jo, Y.N., Kim, H.J., Shin, J.H. and Heo, H.J. (2013). Ameliorating effects of aged garlic extracts against Aβ-induced neurotoxicity and cognitive impairment. BMC Compel. Altern. Med., 13: 268-274.

Li, R., Xu, L., Liang, T., Li, Y., Zhang. S. and Duan, X. (2013). Puerarin mediates hepatoprotection against CCl4-induced hepatic fibrosis rats through attenuation of inflammation response and amelioration of metabolic function. Food Chem. Toxicol., 52: 69-75.

Lin, Y., Shi, R., Wang, X. and Shen, H. (2008). Luteolin, a flavonoid with potentials for cancer prevention and therapy. Curr. Cancer Drug Targets. 8(7): 634-646.

Liu, R.H., Eberhardt, M. and Lee, C. (2001). Antioxidant and antiproliferative activities of selected New York apple cultivars. New York Fruit Quarterly. 9(2):15-17.

Naiki-Ito, A., Chewonarin, T., Tang, M., Pitchakarn, P., Kuno, T., Ogawa, K., Asamoto, M., Shirai, T. and Takahashi, S. (2014). Ellagic acid, a component of pomegranate fruit juice, suppresses androgen-dependent prostate carcinogenesis via induction of apoptosis. Prostate. 57 (2): 151-160.

Ogino, Y., Osada, K., Nakamura, S., Ohta, Y., Kanda, T. and Sugano, M. (2007). Absorption of dietary cholesterol oxidation products and their downstream metabolic effects are reduced by dietary apple phenols. Lipids. 42 (2): 151-161.

Ohkawa, H., Ohishi, N. and Yagi, K. (1979). Assay for lipid peroxides in animal tissues by thiobarbituric acid reaction. Analyt. Biochem., 95 (2): 351–358.

Okokon, J.E., Nwafor, P.A., Charles, U. and Dar, A. (2013). Choudhary MI. Antioxidative and hepatoprotective effects of ethanol root extract of *Hippocratea Africana* against paracetamol-induced liver injury. Pharm. Biol., 51 (7): 872-880.

Paglia, D.F. and Valentaine, W.N. (1976). Studies on glutathione and glutathione characterization of erythrocytes glutathione peroxidase. J. Lab. Clin. Med., 70(1):158-169.

Parola, P., Leonarduzzi, G., Biasi, F., Albano, E. and Biocca, M.E. (1992). Vitamin E dietary supplementation protects against CCl4-induced chronic damage and cirrhosis. *Hepatol.*, 16(4):1014-1021.

Pennica, D., Nedwin, G.E., Hayflick, J.S., Seeburg, P.H., Agarwal, B.B. and Goeddel, D.V. (1985). Human tumor necrosis factor: precursor, structure, and homology to lymphotoxin. Nature., 312 (5996): 724-729.

Reeves, P.G., Nielson, F.H. and Fahmy, G.C. (1993). AIN-93 purified diets for laboratory rodents: final report of the American Institute of

Nutrition adhoc writing committee on the reformulation of the AIN-76A rodent diet. J. Nutri., 123(11): 1939-1951.

Roy, S.E. (1970). Colorimetric determination of serum alkaline phosphatase. Clin. Chem., 16(5): 431-432.

Sadeghi, N., Jannat, B., Oveisi, M.R., Hajimahmoodi, M. Photovat, M. (2009). Antioxidant activity of Iranian pomegranate *(Punica granatum* L.) seed extract*s.* J. Agric. Sci. Technol., 11(12):633-638.

Sedlak, J. and Lindsay, R.H. (1968). Estimation of total, protein-bound, non protein– bound sulfhydryl groups in tissues with Ellman's reagent. *Anal.* Biochem., 25(1): 192-205.

Senedecor, G.W. and Cochran, W.G. (1981). Statistical methods. 7th. Ed. Iowa, USA: Iowa Uni. Press. Ames., p. 175-91.

Sinha, K.A. (1972). Colorimetric assay of catalase enzyme. Anal. Biochem., 47: 28- 330.

Spitz, D.R. and Oberley, L.W. (1989). An assay for superoxide dismutase activity in mammalian tissue homogenates. Anal. Biochem., 179(1): 8-18.

Stiehl, A. (1982). Hyperbilirubinemia in liver disease. Fortschritte der Medizin., 100 (18): 842–845.

Subramanian, P., Mirunalini, S., Dakshayani, K.P., Pandi-Perumal, S.R., Trakht, I. and Cardinali, D.P. (2007). Prevention by melatonin of hepatocarcinogenesis in rats injected with N-nitrosodiethylamine. J. Pineal. Res., 43(3): 305-312.

Sundraresan, S. and Subramanian, P. (2003). S-allylcysteine inhibits circulatory lipid peroxidation in N-nitrosodiethylamine-induced hepatocarcinogenesis. Pol. J. Pharmacol., 55(1): 37-42.

Szaefer, H., Krajka-Kuzniak, V. Ignatowicz, E., Adamska, T. and Markowski, J. (2014). The effect of cloudy apple juice on hepatic and mammary gland phase I and II enzymes induced by DMBA in female Sprague Dawley rats. Drug Chem. Toxicol., 37(4): 472-479.

Thomas, R., Williams, M., Sharma, H., Chaudry, A. and Bellamy, P. A. (2014). Double blind, placebo-controlled randomized trial evaluating the effect of a polyphenol-rich whole food supplement on PSA

progression in men with prostate cancer. Prost. Cancer Prost. Dis., 17(2):180-186.

Viladomiu, M., Hontecillas, R., Lu, P., Bassaganya-Riera, J. (2013). Preventive and prophylactic mechanisms of action of pomegranate bioactive constituents. Evid. Based Conpelement. Alternat. Med. http://dx.doi. org/ 10.1155/ 2013/789764.

Waly, M.I., Ali, A., Guizani, N., Al-Rawahi, A.S., Farooq, S.A. and Raman, M.S. (2012). Pomegranate (Punica granatum) peel extract efficacy as a dietary antioxidant against Azoxymethane-induced colon cancer in rat. Asian Pac. J. Cancer Prev., 13(8):4051- 4055.

Zhao, J.A., Peng, L., Geng, C.Z., Liu, Y.P., Wang, X. Yang, H.C. and Wang, S.J. (2014). Preventive effect of hydrazinocurcumin on carcinogenesis of diethylnitrosamine-induced hepatocarcinoma in male SD rats. Asian Pac. J. cancer Prev., 15(5):2115-2121.

التأثير الوقائى لعصير الرمان والتفاح ضد سرطان الكبد المُحدث بالنيتروز داي ايثيل امين في ذكور الفئران

د. عمرو عبد المرضي محمد – د. أمل فوزي محمود الجزار

قسم التغذية وعلوم الاطعمة – كلية الاقتصاد المنزلي – جامعة حلوان

الملخص:

يمثل سرطان الكبد والأورام الخبيثة المعقدة المستمدة أساسا من عمليات الاكسدة والالتهاب. ذلك هو السبب الرئيسي الثاني الأكثر انتشارا للوفاة في الدول المتخلفة والنامية. والهدف الرئيسي من هذه الدراسة التأثير الواقي لعصير الرمان والتفاح ضد سرطان الكبد المحُدث بمادة النيتروز داي اثيل امين في فئران التجارب. وقد تم استخدام عدد ٦٠ فأر ذكر يزن كل منها ٢٠٠ ± ٥ جم وتقسيمها الي ست (٦) مجموعات واستخدمت المجموعة الأولي كمجموعة ضابطة سالبة بينما تم حقن المجموعات الخمسة الآخري تحت الغشاء البريتوني في الأسبوع الثالث بمادة النيتروز داي ايثيل امين بجرعة ١٠٠ ملجم/كجم من وزن الجسم، ثم تبعها الحقن تحت الجلد بمادة رابع كلوريد الكربون بداية من الاسبوع الرابع حتي السادس وذلك لإحداث سرطان الكبد. واستخدمت المجموعة الثانية كمجموعة ضابطة موجبة بينما المجموعات ٣، ٤، ٥ و٦ تم إعطائها من قبل عن طريق الفم عصير الرمان و التفاح بجرعات ١٠٠ و ٢٠٠مل/كجم من وزن الجسم واستمرت التجربة ٨ اسابيع. وقد اظهرت النتائج ان عصير الرمان والتفاح لهما تأثير مضاد للنيتروز داي اثيل امين المسبب في ارتفاع مستويات سيرم الدم من اسبرتات امينوترانسفيريز، الانين امينوترامينيز و الاكلين فوسفاتييز. كما أظهرت النتائج ان عصير الرمان والتفاح لهما تأثير مثبط للالفافيتوبروتين (كمؤشر سرطان الكبد) ويُحسن من مسويات البليروبين والبروتينات الكلية والالبيومن في سيرم الدم. والجرعات المرتفعة من عصير التفاح والرمان لها تأثير اقوي جزائياً في منع ظهور الاورام في الكبد نتيجة الحقن بالنيتروزداي ايثيل امبن. كم أظهرت النتأئج أن عصير الرمان والتفاح أدي الي خفض مستوي المالونداي الدهيد وزيادة مستوي الجلوتاثون المختزل ونشاط انزيمات السوبر اوكسيد ديسميوتيز و الجلوتاثون بيروكسيديز والكتاليز في انسجة الكبد. وفي الختام اكدت الدراسة ان عصير التفاح والرمان لهما تأثير ايجابي في الوقاية من سرطان الكبد المُحدث بالنيتروزداي ايثيل امين.

الكلمات الاسترشادية: الرمان، التفاح، سرطان الكبد، انزيمات الكبد، المؤشرات الحيوية للسرطان، الليبيد بيروكسيد، مضادات الأكسدة، الفحص الهستوباثولوجي.