

Protective Effect of Pomegranate and Strawberry Juices against Hepatocarcinoma Induced by Nitrosodiethylamine in Rats

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Abstract:

This study aimed to evaluate the protective effect of pomegranate juice (PJ) and strawberry juice (SJ) against hepatocarcinoma induced by nitrosodiethylamine (NDA) in rats. Forty two male rats were distributed into 6 equal groups. Group 1 was negative control and the other 5 groups were intoxicated by a single intraperitoneal dose (60 mg/kg B.wt.) of NDA in the 3rd week of experiment, followed by weekly 3 subcutaneous injections of CCl₄ till the end of experiment (8 weeks) to induce hepatocarcinoma. Group 2 was kept positive (intoxicated) control and groups 3, 4, 5 and 6 were orally given PJ and SJ at low (5%) and high (10%) concentrations for 8 weeks, respectively. Changes in serum biochemical parameters, tumor biomarkers and liver histology were used to evaluate the protective effect. Lipid peroxidation, antioxidant defense capacity and content of 8-hydroxy-2-deoxyguanosine (8-OHdG) in liver DNA (a marker of apoptosis) were measured to examine the mechanism of action. Oral administration of PJ and SJ inhibited NDA-induced elevations in serum biochemical aspartate aminotransferase (AST), alanine aminotransferase (ALT), alkaline phosphatase (ALP) and total bilirubin (T Bil) and in tumor biomarkers (alpha fetoprotein, tumor necrosis factor-alpha and nuclear factor-kappa beta) levels. The results showed that oral administration of PJ and SJ ameliorated hepatic preneoplastic lesions induced by NDA, counteracted hepatic lipid peroxidation, alleviated hepatic oxidative stress and antagonized NDA-induced elevation of 8-OHdG content in liver DNA. These findings denoted that PJ and SJ produce a protective effect against hepatocarcinoma induced by NDA. The protective mechanism of action might be due to inhibition of hepatic lipid peroxidation, lowering oxidative stress and induction of apoptosis.

Keywords: Pomegranate; Strawberry Hepatocarcinoma; Nitrosodiethylamine; Biochemical parameters; Histopathology; Lipid peroxidation; Antioxidant; Apoptosis.

INTRODUCTION

Liver cancer, particularly hepatocellular carcinoma (HCC), almost always develops as chronically diseased liver tissue, induced by long term exposure to an inflammatory stimulus, such as hepatitis virus C, excessive alcohol consumption, metabolic syndrome, food additives, water pollutants, environmental and industrial toxic chemicals, as well as several dietary carcinogens, such as aflatoxin and nitrosamine (Abnet, 2007). HCC is one of the most frequent malignant tumors and it is the second most common cause of death in developing and developed countries (Jemal *et al.*, 2011).

Nitrosodiethylamine (NDA) is found in many foodstuffs as cheese, steamed and fried fish, meat products, bacon, some beverages, tobacco products, cosmetics and agricultural chemicals (Levallois *et al.*, 2000). NDA is one of the most important environmental carcinogens as it induces carcinoma in experimental animals (Amin *et al.*, 2011 Dong *et al.*, 2014 and Velu *et al.*, 2016). NDA is known for its hepatotoxic, carcinogenic and mutagenic potential to cause tumors in the gastrointestinal tract, liver, skin and other organs. At a low dose (10 mg/kg body weight), NDA causes hepatic fibrosis (Kim *et al.*, 2005). The dose 60 mg/ kg B.wt in rats causes preneoplastic lesions of hepatocarcinoma (Velu *et al.*, 2016). The therapy of hepatocarcinoma including chemotherapy, radiation, surgical resection and ablation gives a little hope for restoration of health because of poor diagnosis and serious side effects of chemical anticancer drugs (Keshta, *et al.*, 2016). The search for more effective and less toxic anticancer agents form natural products is necessary to prevent hepatocarcinogenesis.

Pomegranate (*Punica granatum* L., Family *Punicaceae*) is an ancient unique fruit borne on a small, long living tree cultivated throughout the Mediterranean region. Several studies on the antioxidant (Basiri, 2015 and Bhol *et al.*, 2016); anticarcinogenic (Adhami *et al.*, 2009; Turrini *et al.*, 2015 and Vini and Sreeja, 2015), antidiabetic (Middha *et al.*, 2014) and anti-inflammatory (Li *et al.*, 2013 and Noori *et al.*, 2016) properties of pomegranate constituents have been published, focusing on its application for the treatment and prevention of cancer, cardiovascular disease, diabetes mellitus, and erectile dysfunction. Pomegranate, a fruit of promise, is considered as holy fruit for its therapeutic purposes since antiquity and it is used as an alternative medicine in Ayurveda worldwide (Onal *et al.*, 2016).

Strawberry (*Fragaria ananassa*, Family *Rosaceae*) has been extensively used to treat a wide range of ailments in many cultures. Strawberry is a relevant source of bioactive compounds because of its high levels of vitamin C, foliate, and phenolic constituents (Flores *et al.*, 2016). The major class of phenolic compounds is represented by flavonoids (mainly anthocyanins, with flavonols), followed by hydrolyzable tannins (ellagitannins and gallotannins) and phenolic acids (hydroxybenzoic and hydroxycinnamic acids), with condensed tannins (proanthocyanidins) as minor constituents (Aaby *et al.*, 2005). Previous studies have shown that the strawberry that are rich in flavonoids, which are responsible for the antioxidant properties, as well as compounds isolated from strawberry were promising in cancer chemopreventive therapy (Somasagara *et al.*, 2012 and Casto *et al.*, 2013). The present study was designed to evaluate the protective effect and to examine the possible mechanisms of pomegranate and strawberry juices on hepatocellular carcinoma induced by N-Nitrosodiethylamine in rats.

MATERIALS and METHODS

Fruits:

Both Fully ripe pomegranate (*Punica granatum*, Family *Punicaceae*) and fresh Strawberry (*Fragaria ananassa*, Family *Rosaceae*) were purchased from a local fruit shop. Pomegranate seeds were hand separated from the outer peel and used for preparation of the juice by mixing in an electric blender. Strawberry fruits were washed, cut into small pieces and mixed in an electric blender to prepare the juice.

Chemicals and kits:

Diethylnitrosamine (NDA, Product number: N0258, molecular formula: $C_4H_{10}N_2O$) is a yellow liquid dispensed in 1 ml ampoules. It was purchased from Sigma-Aldrich Chemical Company (St. Louis, MO, USA). Carbon tetrachloride (CCl_4) was purchased from El-Gomhoryia Company, Egypt in the form of colorless solution. Enzyme linked immunosorbent assay (ELISA) kits were used for the determination of alpha fetoprotein (AFP), tumor necrosis factor alpha (TNF- α) and nuclear factor-kappa B (NF- κ B).

Rats:

Forty two adult male Sprague Dawley rats weighing 165-170 g body weight and 8-9 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.

Induction of hepatocarcinoma:

The preneoplastic lesions of hepatocarcinoma were induced by single intraperitoneal (IP) injection of nitrosodiethylamine (NDA) in a dose of 60 mg/kg b.wt dissolved in dimethyl sulfoxide (DMSO) in the 3rd week of experiment period (Velu *et al.*, 2016). This was followed by weekly subcutaneous injections (3 injections/week) of CCl₄ diluted with liquid paraffin (1:1, v: v) at 2ml/kg b.wt during the 4th week till the 8th week of the experiment period as described by (Sundraresan and Subramanian, 2008).

Experimental design:

Forty two adult Sprague Dawley rats randomly distributed into 6 groups, of 7 rats each, were used in this study. Group 1 was normal (negative) control and the other 5 groups were intoxicated by a single intraperitoneal dose (100 mg/kg) of NDA injected to rats in the 3rd week, followed by weekly subcutaneous injections (3 injections/week) of CCl₄ from the 4th week till the end of experiment (8 weeks) for induction of hepatocarcinoma. Group 2 was kept intoxicated (positive) control and groups 3, 4, 5 and 6 were orally given PJ and SJ at low (5%) and high (10%) concentrations, respectively, for 8 weeks. At the end of experimental period, the rats were euthanized by prolonged exposure to ether and blood samples were withdrawn for separating the serum by centrifugation at 4000 rpm for 15 min. Serum samples were kept frozen at -70 °C till biochemical analyses. Rats were sacrificed and half of liver was used for preparing tissue homogenates to be used for biochemical analyses. The other half of liver was preserved in 10% formalin solution till processed for the histopathological examination.

Biochemical analyses:

Serum liver enzymes aspartate aminotransferase and alanine aminotransferase (Bergmeyer *et al.*, 1978) and alkaline phosphatase (Roy, 1970) were chemically determined using specific kits. Serum total protein (TP), albumin (Alb) and globulins (Glb) were determined according to the method of (Fernandez *et al.*, 1966). Serum total bilirubin (TBil) was estimated as described by (Stiehl, 1982) and alpha fetoprotein (as a traditional tumor marker) levels were determined as described by (Gibbs *et al.*, 1987). Serum levels of tumor necrosis factor-alpha (as a proinflammatory cytokine) 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 kit.

Lipid peroxidation and activities of antioxidant enzymes:

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). Reduced glutathione (GSH) content in liver homogenate was determined colorimetrically by the method modified by Bulaj *et al.*, (1998). Activities of antioxidant enzymes glutathione peroxidase (Paglia and Valentine, 1979); superoxide dismutase (Spitz and Oberley, 1989) and catalase (Sinha, 1972) were colorimetrically determined using commercial assay kits.

Quantification of 8-hydroxy-2-deoxyguanosine (8-OHdG):

Genomic DNA in liver was extracted from frozen tissues using a commercial DNeasy tissue kit. The contents of 8-OHdG in liver DNA were determined using an ELISA kit. Briefly, the 8-OHdG antibody and the sample were added to ELISA reading plate which had been precoated with 8-OHdG antigen. The content of 8-OHdG in the sample competes with the 8-OHdG antibody binding sites in the plate. The average concentration of 8-OHdG per nanogram (ng) of liver DNA for each group was calculated. The sample DNA assays were performed in duplicate (Zhang *et al.*, 2013).

Histological procedure:

The other part of livers of the sacrificed rats was preserved in 10 % neutral formalin solution. The fixed specimens were trimmed, washed and dehydrated in ascending grades of alcohol. Tissue specimens were then cleared in xylene, embedded in paraffin, sectioned at 4-6 microns thickness, stained with Hematoxylin and Eosin (H&E stain) and examined under the microscope (Carleton , 1976).

Statistical analysis:

Data were presented as means \pm SD. The statistical analysis was carried out using one way analysis of variance (ANOVA) test followed by Tukey test for multiple comparisons according to Snedecor and(Cochran ,1986). Significances between groups were tested at $P < 0.05$.

RESULTS

Single intraperitoneal injection of nitrosodiethylamine (NDA) at 60 mg/kg b.wt and subcutaneous injections of CCL4 (2ml/kg b.wt) in rats significantly increased serum levels of liver enzymes aspartate aminotransferase (AST), alanine amino-transferase (ATL) and alkaline phosphatase (ALP) when compared with the negative control rats, indicating incidence of liver damage and exit of enzymes into circulation. Oral administration of PJ and SJ at both 5 and 10 % concentrations daily for 8 weeks significantly lowered the elevated serum AST, ALT and ALP levels when compared to the positive control group as recorded in table (1).

Rats intoxicated with NDA and CCL4 had significant decreases in serum total protein (TP), albumin (Alb) and globulin (Glb) but an increase in total bilirubin (TBil) levels when compared with the negative control group. Oral administration of PJ and SJ at both 5 and 10 % concentrations daily for 8 weeks significantly normalized serum levels of TP, Alb, Glb and TBil when compared to the positive control group as depicted in table (2).

Table (1): Effects of pomegranate juice (PJ) and strawberry (SJ) on serum levels of aspartate aminotransferase (AST), alanine aminotransferase (ATL) and alkaline phosphatase (ALP) in rats intoxicated with nitrosodiethylamine.

Parameters Groups	AST (U/L)	ALT (U/L)	ALP (U/L)
Negative Control	45.0 ± 2.16d	37.0 ± 2.25d	99.5 ± 3.25d
Positive Control	186.0 ± 7.33a†	165.0 ± 3.54a†	149.0 ± 6.17a†
PJ (5%)	179.0 ± 3.13b§	153.0 ± 2.43b§	140.0 ± 4.56b§
PJ (10%)	175.0 ± 3.62b	142.0 ± 3.61b	135.0 ± 4.11b
SJ (5%)	153.0 ± 3.42c	140.0 ± 2.44c	130.0 ± 4.56c
SJ (10%)	144.0 ± 2.15c	135.0 ± 3.72c	119.0 ± 3.35c

Means ± SE with different superscript letters (a, b, c, d) in the same column are significant at $P < 0.05$ or $P < 0.01$ using one way ANOVA test. (n = 7 rats)

† Significant when compared with the negative control group.

§ Significant when compared with the positive e control group

Table (2)

Effects of pomegranate juice (PJ) and strawberry (SJ) on serum levels of total proteins (TP), albumin (Alb), globulin (Glb) and total bilirubin (TBil) in rats intoxicated with nitrosodiethylamine.

Parameters Groups	TP (g/dL)	Alb (g/dL)	Glb (g/dL)	TBil (mg/dL)
Negative Control	8.40±0.03 ^a	4.30±0.14 ^a	3.50±0.10 ^a	0.36±0.01 ^d
Positive Control	4.53±0.02 ^{d†}	2.15±0.12 ^{d†}	1.95±0.02 ^{d†}	2.50±0.03 ^{a†}
PJ (5%)	5.55±0.07 ^{e§}	2.20±0.13 ^{e§}	2.60±0.04 ^{b§}	2.38±0.01 ^{e§}
PJ (10%)	6.44±0.03 ^c	2.77±0.16 ^c	2.65±0.01 ^b	1.79±0.02 ^b
SJ (5%)	7.85±0.05 ^b	3.60±0.11 ^b	3.10±0.02 ^c	0.95±0.01 ^c
SJ (10%)	8.35±0.04 ^b	4.10±0.21 ^b	3.20±0.01 ^c	0.85±0.02 ^c

Means ± SE with different superscript letters (a, b, c, d) in the same column are significant at $P < 0.05$ using one way ANOVA test. (n = 7 rats)

† Significant when compared with the -ve control group.

§ Significant when compared with the +ve control group.

Intraperitoneal injection of NDA and subcutaneous injection of CCL4 in rats significantly elevated serum levels of alpha fetoprotein (AFP), tumor necrosis factor-alpha (TNF- α) and nuclear factor-kappa beta (NF- κ β) when compared with the negative control group. Oral administration of PJ and SJ at both 5 and 10 % concentrations daily for 8 weeks significantly lowered the elevated serum AFP, TNF- α and NF- κ β levels when compared to the positive group as recorded in table (3).

Table (3):

Effects of pomegranate juice (PJ) and strawberry (SJ) on serum levels of alpha fetoprotein (AFP), tumor necrosis factor- alpha (TNF- α) and nuclear factor-kappa beta (NF- κ β) in rats intoxicated with nitrosodiethylamine.

Parameters Groups	AFP (ng/ml)	TNF- α (ng/ml)	NF- κ β (ng/ml)
Negative Control	3.24 ± 0.01d	1.95 ± 0.01d	110.0 ± 3.11d
Positive Control	8.44 ± 0.08a†	4.82 ± 0.07a†	177.5 ± 8.57a†
PJ (5%)	6.22 ± 0.04b§	3.55 ± 0.01b§	159.4 ± 6.38b§
PJ (10%)	5.99 ± 0.05b	3.50 ± 0.02b	150.6 ± 5.42b
SJ (5%)	4.32 ± 0.02c	2.86 ± 0.02c	125.0 ± 7.01c
SJ (10%)	3.35 ± 0.01c	2.52 ± 0.01c	115.5 ± 5.21c

Means ± SE with different superscript letters (a, b, c, d) in the same column are significant at $P < 0.05$ using one way ANOVA test. (n = 7 rats)

† Significant when compared with the -ve control group.

§ Significant when compared with the +ve control group.

Rats intoxicated by NDA and CCL4 had significant high content of hepatic malondialdehyde (MDA) and low content of reduced glutathione (GSH) when compared with the negative control group. Oral administration of PJ and SJ at both 5 and 10 % concentrations significantly normalized hepatic contents of MDA and GSH when compared to the positive control group, as depicted in table (4).

Activities of hepatic glutathione peroxidase (GPx), superoxide dismutase (SOD), and catalase (CAT) antioxidant enzymes in rats intoxicated with NDA and CCL4 were significantly suppressed as compared with the negative control group. Oral administration of PJ and SJ at both 5 and 10 % concentrations significantly enhanced the activity of inhibited hepatic SOD, GPx and CAT enzymes when compared with the positive control group as recorded in table (5).

Table (4):

Effects of pomegranate juice (PJ) and strawberry (SJ) on hepatic malondialdehyde (MDA) and reduced glutathione (GSH) contents in rats intoxicated with nitrosodiethylamine.

Parameters Groups	MDA ($\mu\text{mol/gm protein}$)	GSH ($\mu\text{mol/gm protein}$)
Negative Control	48.32 ± 0.3^d	11.50 ± 0.61^a
Positive Control	$85.54 \pm 0.6^{a\dagger}$	$6.62 \pm 0.25^{d\dagger}$
PJ (5%)	$72.32 \pm 0.7^{b\dagger}$	$8.22 \pm 0.51^{c\dagger}$
PJ (10%)	69.92 ± 0.6^b	8.89 ± 0.42^c
SJ (5%)	59.32 ± 0.4^b	10.62 ± 0.65^c
SJ (10%)	52.21 ± 0.5^b	10.72 ± 0.62^c

Means \pm SE in the same column with different superscript letters (a, b, c, d) are significant at $P < 0.05$ using one way ANOVA test. (n = 7 rats)

\dagger Significant when compared with the -ve control group.

\S Significant when compared with the +ve control group.

Table (5):

Effect of pomegranate juice (PJ) and strawberry (SJ) on activities of hepatic glutathione peroxidase (GPx), superoxide dismutase (SOD) and catalase

Parameters Groups	GPx (nmol/min/mg protein)	SOD (U/mg protein)	CAT (nmol/min/mg protein)
Negative Control	$66.10 \pm 4.21a$	$0.85 \pm 0.04a$	$0.195 \pm 0.03a$
Positive Control	$47.22 \pm 2.15d^{\dagger}$	$0.47 \pm 0.01d^{\dagger}$	$0.155 \pm 0.01d^{\dagger}$
PJ (5%)	$55.13 \pm 3.23b^{\S}$	$0.56 \pm 0.02b^{\S}$	$0.175 \pm 0.02b^{\S}$
PJ (10%)	$59.16 \pm 3.35b$	$0.59 \pm 0.01b$	$0.180 \pm 0.03b$
SJ (5%)	$60.23 \pm 4.73b$	$0.77 \pm 0.03b$	$0.185 \pm 0.02b$
SJ (10%)	$63.34 \pm 4.52b$	$0.81 \pm 0.02b$	$0.190 \pm 0.03b$

(CAT) in rats intoxicated with nitrosodiethylamine.

Means \pm SE with different superscript letters (a, b, c, d) in the same column are significant at $P < 0.05$ using one way ANOVA test. (n = 7 rats)

\dagger Significant when compared with the -ve control group.

\S Significant when compared with the +ve control group.

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

Unit of CAT = nmol of H_2O_2 utilized/min/mg protein.

In rats intoxicated with NDA AND CCL4, the content of apoptosis marker 8-hydroxy-2-deoxyguanosine (8-OHdG) in liver DNA was elevated to 2.4 fold as compared to the negative control group. Oral administration of PJ and SJ at both 5 and 10 % concentrations reduced the high content of 8-OHdG to 68, 61, 47 and 41 ng/mg DNA, respectively versus to 84 ng/mg DNA in the positive control group as demonstrated in figure (1).

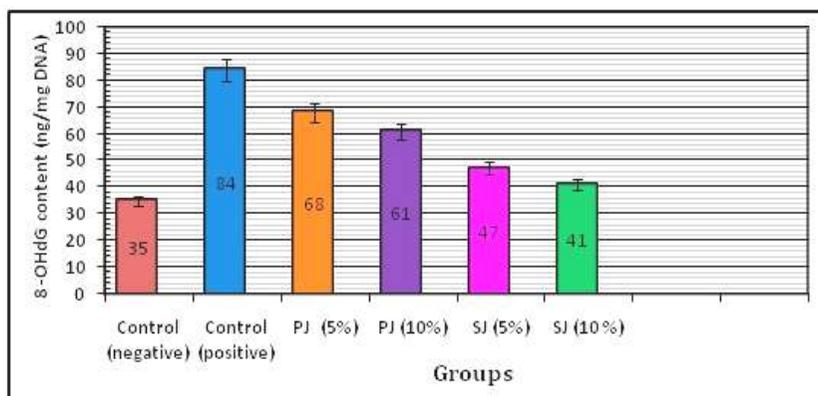
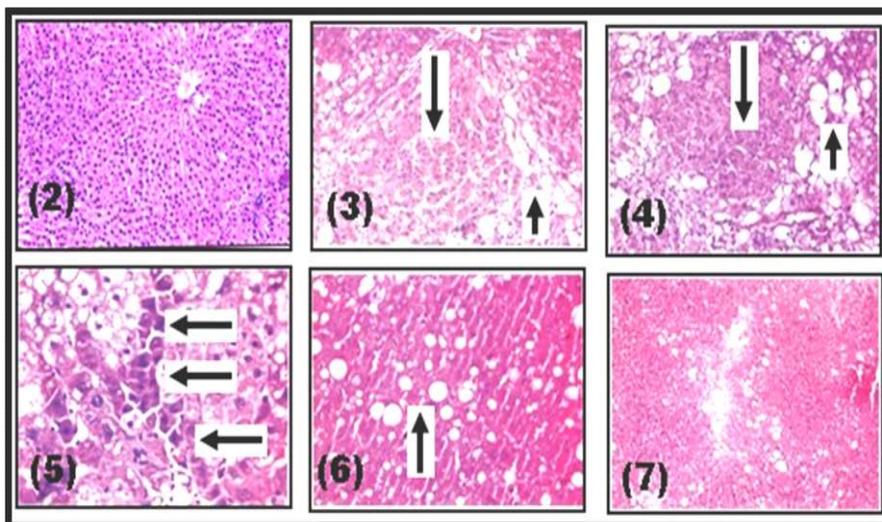


Fig (1): Effects of pomegranate and strawberry juices both at low (5%) and high (10%) concentrations on content of 8-hydroxy-2deoxyguanosine (8-OHdG) in liver DNA.

Histopathological examination of liver sections of rats in negative control group showed normal histological structure of hepatic lobule with normal hepatocytes, portal vein and sinusoids (Fig.2). Liver sections of rats intoxicated with nitrosodiethylamine (NDA) and CCL4 showed trabecular hepatocellular carcinoma (long arrow) with fat droplets (short arrow) in tumor cells (Fig. 3). Some liver sections revealed compact hepatocarcinoma (long arrow) with fat globules in tumor cells (short arrow), enlarged nuclei and increased cell mitosis (Fig.4). Polymorphism of nuclei of hepatocytes (arrows) was seen as shown in Fig. (5). Oral administration of large (10%) concentration of pomegranate juice caused regression of preneoplastic lesions induced by NDA and CCL4 and presence only of mild fat droplets was seen (Fig.6). Examination of liver sections of rats given the large (10%) concentration of strawberry juice showed amelioration of preneoplastic lesions as shown in Fig.(7)



Legend of Figures:

Fig. (2): C.S. of liver of a negative control rat showing normal histological structure of hepatic lobule with normal central vein, hepatocytes and sinusoids. (H&E X 200).

Fig. (3): C.S. of liver of a rat injected with NDA and CCL4 showing trabecular hepatocarcinoma (long arrow) with fat droplets (short arrow) in tumor cells. (H&E X 200).

Fig. (4): C.S. of liver of a rat injected with NDA and CCL4 showing compact hepatocarcinoma (long arrow) with fat droplets in tumor cells, and enlarged nuclei with increased cell mitosis. (H&E X 200).

Fig. (5): C.S. of liver of a rat injected with NDA and CCL4 showing polymorphism of nuclei of hepatocytes (arrows). (H&E X 400)

Fig. (6): C.S. of liver of a rat given large (10%) concentration of pomegranate juice showing regression of preneoplastic lesions induced by NDA and CCL4 and presence only of mild fat droplets. (H&E X 200).

Fig. (7): C.S. of liver of a rat given large (10%) concentration of strawberry juice showing amelioration of preneoplastic lesions induced by NDA and CCL4 (H&E X 200).

DISCUSSION

There is a great need to search for much safe natural materials to be developed for the prevention and therapy of hepatocarcinoma. Fruits and vegetables with anticancer activity have gained much attention (Brown, 2012). The biological value of the plant materials depends on presence of bioactive constituents, especially those of antioxidant properties. The mechanisms underlying the anticancer activity of plant materials are still need for further investigations. Therefore, the aim of the present study was to evaluate the protective effect and to examine the possible mechanisms of action of pomegranate and strawberry juices against hepatocarcinoma induced by N-nitrosodiethylamine (NDA) in rats.

Nitrosodiethylamine (NDA) has been commonly used as an experimental carcinogen as it induces hepatic preneoplastic lesions in rats (Cortinovis *et al.*, 1991). Carbon tetrachloride (CCl₄) is a selective hepatotoxic chemical agent that commonly used for induction of hepatitis in rats (Li *et al.*, 2013). CCl₄ 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. Lipid peroxidation is associated with hepatic cell damage and leads to liver cirrhosis and fibrosis (Barrera, 2012). In this study, intoxication of rats by NDA and CCL₄, induced hepatocellular carcinoma manifested by high serum levels of tumor markers alpha fetoprotein (AFP), tumor necrosis factor-alpha (TNF- α) and nuclear factor-kappa beta (NF- κ β). There were also increased lipid peroxidation, high content of 8-hydroxy-2-deoxyguanosine (8-OHdG) in liver DNA and oxidative stress evident by decreased activities of hepatic antioxidant enzymes in liver tissue. The serum and tissue biochemical alterations were parallel to preneoplastic lesions seen upon histopathological examination of liver sections. These results were similar to the previous reports by (Sundraresan and Subramanian, 2008 and Zhang *et al.*, 2013). The previous authors concluded that intoxication of rats with NDA and CCL₄ induces hepatocellular carcinoma characterized by nearly similar serum and tissue biochemical alterations as well as histopathological hepatic precancerous lesions that reported in this study.

The present results revealed that oral administration of pomegranate juice to rats intoxicated by NDA and CCL₄ exhibited an anticancer activity against hepatocarcinoma. This activity was manifested by restoration of serum liver enzymes, total protein and bilirubin to nearly normal levels; inhibition of lipid peroxidation and oxidative stress as well as regression of biomarkers of hepatocarcinoma. These findings agreed with the previous reports by Adhami *et al.*, (2009); Bishayee *et al.*, (2011); Turrini *et al.*, (2015) and (Vini and Sreeja, 2016). The previous authors concluded that pomegranate juice and extract produce an anticancer activity against hepatocarcinoma. Moreover, Turrini *et al.*, (2015) attributed the anticancer activity to presence of antioxidant polyphenols in pomegranate juice. In addition, Zahin *et al.*,

(2014) reported antioxidant and antigenotoxic properties of punicalagin and ellagic acid that found in pomegranate (*Punica granatum*) as its major constituents. Investigating the possible protective mechanisms of pomegranate revealed that these mechanisms might be via the inhibition of hepatic lipid peroxidation, enhancement of antioxidant capacity and induction of apoptosis. These mechanisms were evident from the increase in hepatic reduced glutathione (GSH) and the decrease in malondialdehyde (MDA) contents (biomarkers of lipid peroxidation), the decrease of 8-OHdG content (a marker of apoptosis) in liver DNA and the enhancement of activities of antioxidant enzymes (biomarkers of oxidative stress) in liver tissues. These results were

similar to those previously mentioned by Zahin *et al.*, (2014) and Turrini *et al.*, (2015). The alterations in serum and tissue biochemical parameters induced by in pomegranate juice were parallel to regression and amelioration of histopathological preneoplastic lesions seen in liver of NDA-intoxicated rats, denoting an anticancer activity. Moreover, the amelioration of precancerous lesions by pomegranate juice was in accordance with that previously reported by Bishayee *et al.*, (2011); Turrini *et al.*, (2015) and Vini and Sreeja (2016).

Similar results of pomegranate juice were reported for strawberry juice in this study. The anticancer activity of strawberry juice against hepatocellular carcinoma was nearly similar to that reported by Somasagara *et al.*, (2012) who reported that extracts of strawberry fruits induce intrinsic pathway of apoptosis in breast cancer cells and inhibits tumor progression in mice. Moreover, Casto *et al.*, (2013) concluded that strawberry induces chemoprevention of oral cancer by lyophilized strawberries. Flores *et al.*, (2016) mentioned that strawberry is a relevant source of bioactive compounds because of its high levels of vitamin C, foliate, and many phenolic constituents. Previous studies have shown that the strawberry that are rich in flavonoids, which are responsible for the antioxidant properties, as well as compounds isolated from strawberry were promising in cancer chemopreventive therapy (Somasagara *et al.*, 2012 and Casto *et al.*, 2013).

In conclusion, the results denote that oral administration of pomegranate and strawberry juice to NDA- intoxicated rats exhibits a protective effect against hepatocarcinoma. The mechanisms underlying this activity might be due to inhibition of lipid peroxidation, lowering oxidative stress and induction of apoptosis. These results confirm the traditional use of pomegranate and strawberry in folk medicine for the treatment of many types of cancers such as liver, lung, stomach and breast cancers. The study recommends that intake of pomegranate and strawberry juice as a drink may be useful for patients who suffer from hepatocarcinoma due to oxidative stress.

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التأثير الواقي لعصائر الرمان والفراولة ضد سرطان الكبد الناجم عن

نيتروز داى إيثيل أمين في الفئران

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الملخص العربي

استهدف هذا البحث دراسة التأثير الواقي وفحص آليات العمل لعصائر الرمان والفراولة ضد سرطان الكبد الناجم عن نيتروز داى إيثيل أمين فى الفئران. فتم توزيع عدد ٤٢ من ذكور الفئران البالغة بطريقة عشوائية إلى ٦ مجموعات كل منها ٧ فئران. المجموعة الأولى كانت ضابطة سالبة (فئران غير مصابة) والمجموعة الثانية ضابطة موجبة (فئران مصابة)، والمجموعات الثالثة والرابعة والخامسة والسادسة كانت فئران مصابة بالسرطان وسبق إعطائها عن طريق الفم عصير الرمان والفراولة وكل منهما بتركيز صغير (٥ %) وكبير (١٠ %) على التوالي لمدة ٨ اسابيع. وتم إحداث الإصابة بالسرطان بحقن الفئران فى التجويف البريتونى بجرعة مفردة (٦٠ مجم/كجم) من نيتروز داى إيثيل أمين فى الأسبوع الثالث من فترة التجربة وتلى ذلك حقن الفئران تحت الجلد برابع كلوريد الكربون (مخفف مع زيت البرافين بنسبة ١:١) بكمية ٢سم/كجم ثلاث حقنات فى الأسبوع أثناء الأسابيع الرابع والخامس والسادس والسابع والثامن. وفى نهاية فترة التجربة تم تجميع عينات من الدم لعمل التحليلات البيوكيميائية فى المصل ، وتم أخذ نصف الأكياد لقياس دلائل أكسدة الدهون ونشاط الإنزيمات المضادة للأكسدة (سوبر أوكسيد دثميوتيز - جلوتاثيون بيروكسيداز - كاتاليز) وتركيز مركب ٨-هيدروكسى- دى اوكسى جوانوزين (دليل موت الخلايا المبرمج) فى نسيج الكبد. واستخدم النصف الآخر لإجراء الفحص الهستوباثولوجى للكبد. وأظهرت النتائج أن إعطاء عصير الرمان والفراولة عن طريق الفم لمدة ٨ اسابيع أدى إلى نقص معنوى فى مستوى إنزيمات الكبد المرتفعة (اسبرتات أمينو ترانسفيريز، الانين أمينو ترانسفيريز والكالين فوسفاتيز) والبليروبين الكلى فى المصل ، بينما أدى إلى زيادة بروتينات الدم. وأدى أيضا إلى نقص معنوي فى دلائل سرطان الكبد بالمصل بينما أدى إلى زيادة نشاط الإنزيمات المضادة للأكسدة فى نسيج الكبد ونقص تركيز مركب ٨-هيدروكسى - دى اوكسى جوانوزين. وأظهر الفحص الهستوباثولوجى وجود تحسن أو إختفاء التغيرات المرضية المحدثة بمركب نيتروز داى إيثيل أمين فى نسيج كبد الفئران. ودلت النتائج على أن عصير الرمان والفراولة لهما تأثير واقى ضد سرطان الكبد. وأشارت النتائج إلى أن آليات عمل عصير الرمان والفراولة كمضاد للسرطان قد تكون عن طريق منع أكسدة الدهون بنسيج الكبد وزيادة نشاط الإنزيمات المضادة للأكسدة وإحداث موت مبرمج فى خلايا الكبد. وتوصى هذه الدراسة أن شرب عصير الرمان والفراولة قد يكون مفيدا للمرضى الذين يعانون من سرطان الكبد.