Protective Effect of Broccoli and Red Cabbage Against Hepatocellular Carcinoma Induced by N- Nitrosodiethylamine in Rats

Aml F. M. Morsy*[a], Hodaa S. Ibrahim[a] and M. A. Shalaby[b]

a Nutrition and Food Science Department, Faculty of Home Economics, Helwan University, Egypt.
b Pharmacology Department Faculty of Veterinary Medicine, Cairo University, Egypt.
* dr_aml_fawzy@yahoo.com

Abstract: The hepatoprotective effect of broccoli and red cabbage extracts against hepatocellur carcinoma induced by N- Nitrosodiethyamine (NDEA) in male rats were studied. Four groups of rats were used; group (1) was used as a negative control (normal), while rats of the other groups were given NDEA as a single interperoitenial dose with subcutaneous injection of carbon tetrachloride (CCl_4) once weekly for six weeks to induce hepatocellular carcinoma. Group (2) was left as a positive control, while groups (3) and (4) were pretreated with broccoli and red cabbage 10%extract, for 12 weeks, respectively. At the end of the experiment, blood samples were taken for biochemical analysis and liver tissues were histopathologically examined. The obtained results revealed that rats with hepatocellur carcinoma (HCC) had significant increase in serum levels of AST, ALT, ALP, total protein, albumin, total and direct bilirubin and malondialdehyide (MDA), as well as significant decrease in reduced glutathione (GSH), glutathione peroxidase (GPX), superoxide dismutase (SOD) and catalase (CAT) enzymes, compared to the normal control group. Liver sections of rats with HCC showed fatty infiltration of hepatocytes, cytomegaly with karyomegaly as well as vesicular active nuclei and presence of more than one nucleolus in some hepatocytes. Oral administration of broccoli and red cabbage extracts caused significant reduction in serum levels of AST, ALT, ALP, total protein, albumin, total and direct bilirubin as well as MDA and produced significant increase in GSH, GPX, SOD and CAT, compared to the positive group. Liver of these rats revealed only slight hydropic degeneration of hepatocytes, while other sections showed apparent normal hepatocytes. This study concluded that broccoli and red cabbage have a protective effect against hepatocellular carcinoma in rats, therefore this study recommends increased dietary intake of broccoli and red cabbage may be beneficial for patients with liver cancer as a preventative measures.

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1. Introduction:

Hepatocellular carcinoma (HCC) is the most common form of liver cancer in adults, which accounts for about 75% of primary liver cancers. It is the 5th most common cancer in worldwide and represents 83% of all cases (Ferlay et al., 2001). In Egypt, HCC is frequent accounting for 13% of all cancer types (Inas, 2005). Liver cancers have different growth patterns; the first type begins as a single tumor that grows larger in hepatic tissue. The second type is spread through the liver almost from the beginning and is not confined to a single tumor. This is seen most often in people with liver cirrhosis. In the third type, the cancer develops as nodules in several parts of the liver (Strauss, 1995).

Risk factors for HCC include hepatitis B virus (HBV), hepatitis C virus (HCV) and aflatoxins are assumed to play an important role in the high incidence of HCC in Egypt. HBV vaccination of children and high-risk population must be the priority in reducing the incidence of HCC. Measures to reduce food spoilage by fungi and the associated dietary exposure to aflatoxins are a desirable public health goal (Wild and Hall, 2000).

Numerous compounds in the human diet have chemoprotective properties against chemical carcinogens (Wattenberg, 1990). Intake of a diet rich in fruits and vegetables is associated with a lowering risk of certain types of cancer (Steinmetz and Potter, 1991). Green leafy vegetables of all varieties and cruciferous plants such as cabbage, Brussels sprouts, cauliflower and broccoli are rich in anti-carcinogens (Block et al., 1992; Wargovich, 1999). Cruciferous vegetables contain a number of bioactive components such as folate, vitamin C, tocopherols, carotenoids, flavonoids and polyphenols (Price et al., 1998; Kurilich et al., 1999).

Broccoli is a plant of family *Brassicaceae* (formerly *Cruciferae)* which has large green flower heads (Murray and Lara, 2005). Recently, Elizabeth and Marcela, (2009) suggested that broccoli can decrease the risk for incidence of cancer. It contains many bioactive, including vitamins C and E, quercetin and kaempferol glycosides.

Red Cabbage *(Brassica oleracea* var. *capitata* f. *rubra)* is a type of cabbage, also known as Red Kraut or Blue Kraut after preparation (Michaelis et al., 2008). The breakdown products of glucosinolates, which present in it such as isothiocyanates are considered responsible for the chemopreventive properties of red cabbage (Lynn et al., 2006).

The aim of the present study was to investigate the hepatoprotective effect of broccoli and red cabbage extracts against hepatocellur carcinoma induced by N - Nitrosodiethylamine in male rats.

2. Materials and methods:

2.1 Material:

2.1.1 Rats and Diet:

Male albino rats of Sprague Dawley strain weighing 175±5g were used. Their age between 14-16 weeks old and were purchased from the Laboratory Animal Colony, Ministry of Health and Population, Helwan, Egypt. Basal diet constituents were obtained from El-Gomhorya Company, Cairo, Egypt.

2.1. 2 Chemicals:

N-Nitrosodiethyamine (NDEA) was purchased from Sigma Chemical Company, USA. Carbon tetrachloride (CCl_4) was obtained from El-Gomhorya Company, Cairo, Egypt. Biochemical kits for serum analysis were purchased from the Gamma Trade Company for Pharmaceutical and Chemicals, Dokki, Egypt.

2. 1.3 Plants:

Broccoli and red cabbage were purchased from the local market, Cairo, Egypt. The plants were authenticated in the Botany Department, Faculty of Agriculture, Cairo University.

2. 2 Methods:

2.2.1 Preparation of plant extracts:-

Broccoli and red cabbage vegetables were cleaned, air dried and grinded into a fine powder. The powdered plants were extracted with 90% ethyl alcohol using Soxhlet apparatus and concentrated at low temperature(50Ċ) using a Rotary evaporator apparatus (manufactured in Basil, Switzerland) according to the method described by Mohamed, (2002). Each dried ethanol extract was dissolved separately in a mixture of carboxy methylcellulose and few drops of Tween 80 as a suspending agent to obtain 10% concentration liquid extract.

2. 2.2 Preparation of basal diet:

The basal diet (AIN-93M) was prepared according to Reeves et al., (1993). Diet was formulated to meet the recommended nutrients levels for rats.

2. 2.3 Experimental Design:

Forty male albino rats were fed on the basal diet and water was provided *ad libitum.* Animals were maintained under standard conditions of humidity (50-60%), temperature (20-25°C) and light (12-h light: 12-h dark cycle) for one week before starting the experimental for acclimatization. Rats were divided into four groups of ten animals each as follows:

Group (1): Served as a control negative (normal rats) and fed on basal diet for 12 weeks.

Group (2): Kept as a control positive (with HCC) and fed on basal diet for 12 weeks.

Group (3): Fed on the basal diet and given orally 10% broccoli extract using stomach tube for 12 weeks.

Group (4): Fed on the basal diet and given red cabbage 10% extract orally by stomach tube for 12 weeks.

In first six weeks of experimental period, animals of groups (2), (3) and (4) were given a single intraperitoneal dose of NDEA (200 mg/kg b.wt.) followed by carbon tetrachloride (CCl4) tetrachloride (CCl4) given subcutaneously once weekly in a dose of 200 mg/kg b.wt. during the other 6 weeks for induction of HCC as described by Sundaresan and Subramanian (2003). At the end of the experimental period, blood samples were collected from the portal vein into dry clean centrifuge tubes for serum separation. Serum samples were frozen at -10°C until chemical analysis. Liver of sacrificed rats were kept in 10% formalin solution till processed for histopathological examination.

2.2.4 Biochemical analysis:

2. 2.4.1 Determination of liver functions:

Serum asprtate aminotransferase (AST) and alanine aminotransferase (ALT) activities were determined according to the method described by Kaplan, (1984). Serum alkaline phosphatase (ALP) was calorimetrically determined according to the method described by Roy (1970). Serum total protein concentration was calorimetrically determined according to the method described by Koller, (1984). Serum concentrations of albumin, total and direct bilirubin were determined as described by Kaplan, (1984).

2.2.4.2 Determination of malondialdehyde and reduced glutathione:

Serum malondialdehyde (MDA) was determined by the method of Draper and Hadly, (1990). Reduced glutathione concentration (GSH) in was measured by the method described by Beutler and Kelly, (1963).

2.2.4.3Determination of antioxidant enzymes:

The serum levels of glutathione peroxidase (GPX), superoxide dismutase (SOD) and catalase (CAT) were determined by autoanalyzer (Roche-Hitachi, Japan) according to the methods described by Hissin and Hilf, (1976); Kakkar et al., (1984); Sinha, (1972), respectively.

2. 2.5 Histopathological examination:

Liver of the scarified rats were taken and immersed in 10% formalin solution. The fixed specimens were then trimmed, washed and dehydrated in ascending grades of alcohol. Specimens were then cleared in xylol, embedded in paraffin, sectioned at 4-6 microns thickness, and stained with Heamtoxylin and Eosin stain for examination of the liver as described by Carleton, (1979).

2.2.6 Statistical analysis

The obtained results were expressed as Mean ± SE. Data were evaluated statistically with computerized SPSS package program (SPSS 9.00 software for Windows) using one-way analysis of variance (ANOVA). Significant differences among means were estimated at $p<0.05$ according to Snedecor and Cochran, (1986).

3. Results:

Data in Table (1) show that rats with HCC (positive control group) had significant ($p<0.05$) increase in serum level of AST (195.00±9.72 U/L), compared to normal rats (104.00±5.91 U/L). Oral administration of broccoli and red cabbage extract caused significant reduction ($p<0.05$) in serum level of AST (143.00±6.55 and 137.00±5.64 U/L, respectively) as compared to the positive control group (195.00±9.72 U/L). There were no significant changes in AST serum level between rats given broccoli or red cabbage extracts.

Table (1): Effect of oral administration of broccoli and red cabbage extracts on serum concentrations of AST, ALT and ALP of hepatocellular carcinoma rats.

(n=10 rats)

Groups	Liver enzymes (Mean ±SE)		
	AST (U/L)	ALT (U/L)	ALP (U/L)
Negative control	104.00±5.91 e	74.70±1.37 d	188.97±1.48 d
Positive control (HCC)	195.00±9.72 a	150.00±3.33 a	286.09±4.80 a
Broccoli extract (10%)	143.00±6. 55 cd	84.40±3.35 cd	193.34±2.62 d
Red cabbage extract (10%)	137.00±5.64 d	95.00±3.87 bc	211.79±1.31 c

Means with different superscripts letters are significant at $p<0.05$.

Results of hepatocellular carcinoma rats revealed that rats with HCC (positive group) had significant increase ($p<0.05$) in serum level of ALT (150.00± 3.33 U/L), compared to the negative control group (74.70±1.37 U/L). Oral administration of broccoli and red cabbage extracts to rats inflicted with HCC induced significant ($p<0.05$) reduction of levels in serum ALT (84.40±3.35 and 95.00±3.87, respectively) as compared to the control positive group. There were non-significant changes in serum levels of ALT between rats given orally either broccoli or red cabbage extract.

Tabulated results showed that serum level of ALP significantly ($p<0.05$) increased in positive control rats (286.04± 4.80 U/L) as compared to the negative control rats (188.97±1.48 U/L) as expected. Rats given orally broccoli and red cabbage extracts showed significant reduction ($p<0.05$) in serum level of ALP (193.34±2.62 and 211.79±1.31U/L, respectively), compared to the positive control group.

Results in Table (2) showed that rats with HCC had significant ($p<0.05$) increase in serum level of total protein and albumin (8.04±0.26 and 7.88±0.43 g/dL, respectively), compared to the normal rats (6.80 ± 0.26 and 4.88±0.10 g/dL, respectively). Oral administration of broccoli and red cabbage extracts caused significant ($p<0.05$) decrease in serum level of total protein and albumin as compared to the positive control group.

Table (2): Effect of oral administration of broccoli and red cabbage extracts on serum concentrations of total protein and albumin of hepatocellular carcinoma rats.

(n=1 0 rats)

Groups	Parameter (Mean ±SE)	
	Total protein (g/dL)	Total albumin (g/dL)
Negative control	6.80±0.26 b	4.88±0.10 d
Positive control (HCC)	8.04±0.26 a	7.88±0.43 a
Broccoli extract (10%)	6.49±0.33 b	5.25±0.11 cd
Red cabbage extract (10%)	6.63±0.11 b	5.64±0.19 bc

Means with different superscripts letters are significant at $p<0.05$.

Rats with HCC showed significant ($p<0.05$) increases in serum levels of total and direct bilirubin (0.71±0.01 and 0.94±0.02 mg/dL, respectively) as compared to negative control group (0.45±0.0 1 and 0.59±0.01mg/dL, respectively). Broccoli and red cabbage extracts significantly ($p<0.05$) decreased the serum levels of total and direct bilirubin, compared to the positive control group as shown in Table (3).

Results in Table (4) showed that rats with HCC had significant ($p<0.05$) increase in MDA (2.81±0.02 µmol/dL), however, GSH levels was decrease (25.42±0.25 µmol/dL), compared to the normal rats. Oral administration of broccoli and red cabbage extracts produced a significant ($p<0.05$) reductions in MDA (1.65±0.01 and 1.81±0.02 µmol/dL, respectively) and an increase in GSH levels (36.80±0.21 and 34.60±0.15 µmol/dL, respectively), compared to the positive control group.

Table (3): Effect of oral administration of broccoli and red cabbage extracts on serum concentrations of total and direct bilirubin of hepatocellular carcinoma rats.

(n=1 0 rats)

Groups	Parameter (Mean ±SE)	
	Total bilirubin (mg/dL)	Direct bilirubin (mg/dL)
Negative control	0.45 ± 0.01^{d}	0.59 ± 0.01^{c}
Positive control (HCC)	0.71 ± 0.01^{a}	0.94 ± 0.02^{a}
Broccoli extract (10%)	0.47 ± 0.01^{cd}	0.61 ± 0.02^{c}
Red cabbage extract (10%)	0.46 ± 0.01^{d}	0.61 ± 0.01^{c}

Means with different superscripts letters are significant at $p<0.05$.

Table (4): Effect of oral administration of broccoli and red cabbage extracts on serum concentrations of malondialdehyide and reduced glutathione of hepatocellular carcinoma rats.

(n=10 rats)

Groups	Parameter (Mean ±SE)	
	MDA (µmol/dL)	GSH (µmol/dL)
Negative control	1.23 ± 0.03^{f}	40.21 ± 0.12^{a}
Positive control (HCC)	2.81 ± 0.02^{a}	25.42 ± 0.25^{e}
Broccoli extract (10%)	1.65 ± 0.01^{d}	36.80 ± 0.21^{c}
Red cabbage extract (10%)	1.81 ± 0.02^{c}	34.60 ± 0.15^{d}

Means with different superscripts letters are significant at $p<0.05$

Antioxidant enzymes levels in serum of rats was significantly reduced in HCC rats compared to negative untreated group as shown in Table (5). Rats given orally broccoli and red cabbage extract showed significant ($p<0.05$) increases in serum levels of antioxidant enzymes as compared to the positive control group.

Histopathological examination of the liver of normal rats revealed normal histological structure of hepatic lobule as shown in Figure (1). Livers of rats with HCC showed fatty infiltration of hepatocytes, cytomegaly with karyomegaly as well as vesicular active nuclei and presence of more than one nucleolus as shown in Figure (2). Oral administration of broccoli extract at 10% to HCC rats revealed only slight hydropic degeneration of hepatocytes as shown in Figure (3). Other liver sections of the same group showed apparent normal heptocytes. Liver sections of rats with HCC given orally red cabbage extract at 10% revealed slight hydropic degeneration of some hepatocytes as shown in Figure (6), while other liver sections from the same group showed apparent normal heptocytes.

Table (5): Effect of oral administration of broccoli and red cabbage extracts on serum concentrations of antioxidant enzymes of hepatocellular carcinoma rats.

(n=10 rats)

Groups	Paramet		
	GPX (mmol/dL)	SOD (U/dL)	CAT (mmol/dL)
Negative control	18.70 ± 0.13^{a}	95.75 ± 0.22^{a}	67.00 ± 0.56^{a}
Positive control (HCC)	8.25 ± 0.14^{f}	55.45 ± 0.19^{f}	42.15 ± 0.60^{f}
Broccoli extract (10%)	13.95 ± 0.02^{c}	86.66 ± 0.16^{b}	63.60 ± 0.56^{c}
Red cabbage extract 10%)	9.25 ± 0.02^{e}	68.61 ± 0.10^{e}	51.65 ± 0.24^{e}

Means with different superscripts letters are significant at $p<0.05$.

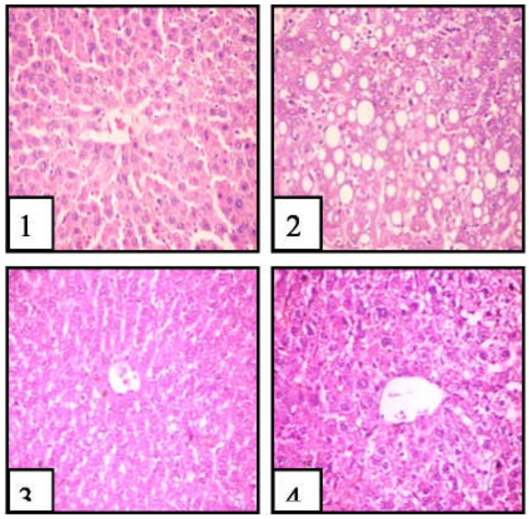

Figure (1): liver of control rat, showing the normal histological structure of hepatic lobular. (H and E x 200).
Figure (2): liver of positive group, showing fatty infiltration of hepatocytes, cytomegaly with karyomegale, viscular active nuclei and more than one nucleolus. (H and E x 200).
Figure (3): liver of rats with HCC treated with broccoli extract showing slight hydropic degeneration of heptocytes. (H and E x 200).
Figure (4): liver of rats with HCC rats treated with red cabbage extract showing slight hydropic degeneration of heptocytes. (H and E x 200).

4. Discussion:

The present study aimed to investigate the hepatoprotective effect of broccoli and red cabbage extracts at 10% against hepatocellular carcinoma induced by NDEA in rats. The biomarkers used in this study provide the measures of carcinogen exposure in rats as an area of high risk for development of hepatocellular carcinoma. Results of this study showed that rats with HCC had significant increase in serum levels of AST, ALT, ALP, total protein, total and direct bilirubin as well as MDA. However, there were significant reductions in GSH, GPX, SOD and CAT, compared to the normal rats. The increase in these parameters in rats with HCC might be attributed to the injured structural integrity of the liver as they are released into the circulation after cellular damage induced by CCl4 and NDEA. These results agreed with those obtained by Pevicharova et al., (1997) who found that activities of AST, ALT and ALP were increased significantly following N-nitroso compounds treatment in rats. Moreover, Vozarova et al., (2002) mentioned that the elevated activities of AST, ALT and ALP enzymes were signs of impaired liver function in response to NDEA administration. Bansal et al., (2005) attributed the elevation of liver enzymes to their release from the cytoplasm into the blood circulation after rupture of the plasma membrane and cellular damage. Mittal et al., (2006) reported that NDEA administration caused a substantial liver damage as evidenced by the increases in the activities of AST and ALT enzymes in the treated rats.

Data in this study showed an increased in serum MDA concentration which may be related to the increased in free radicals. This finding was consistent with the observation that the free radicals reduced the activity of the endogenous antioxidant enzyme SOD (Conner and Grisham, 1996). It is known that free radical scavenging enzyme such as SOD protects the biological systems from oxidative stress. The current study showed a significant decrease in the activity of SOD in groups of rats administrated with NDEA (positive control). This reduction could be attributed to an enhanced production of free radicals during NDEA metabolism. Zwart et al., (1999) reported that lipid peroxidation produced several toxic byproducts such as MDA which can attack cellular targets including DNA, inducing mutagenecity and carcinogenicity. Administration of NDEA depleted the level of glutathione (GSH) in this study. Such depletion agreed with that reported by Kweon et al., (2003); Bansal et al., (2005); Sivaramakrishnan et al., (2007) and Pradeep et al., (2007) in their study. Since glutathione is required to maintain the normal reduced state of cells and to counteract all the deleterious effects of oxidative stress. Thus GSH is involved in many cellular processes including the detoxification of endogenous and exogenous compounds. NDEA is electrophilic carcinogens that interact with the large nucleophilic pool of GSH thereby reducing the macromolecule and carcinogen interaction (Ketterer and Meyer, 1989). The depletion of liver GSH in NDEA-treated rats may be responsible for the increased in lipid peroxidation. A significant decrease in the activities of GSH dependent enzymes, GPX and CAT in NDEA-treated rats may be due to decreased expression of these antioxidants during hepatocellular damage. Furthermore, the decreased levels of cellular GSH caused a reduction in their activities as GSH is a vital co-factor for these enzymes. The obtained results were in accordance with that reports by Kweon et al., (2003) who demonstrated that NDEA induced hepatocellular injury by a substantial fall in hepatic GSH, GPX and CAT activity, which then improved by administration of antioxidants. Boitier et al., (1995) reported that in hepatocellular carcinoma there is a disturbance between oxidant and antioxidant balance, which is tilted towards oxidant side.

Previous researches indicated that NEDA induced hepatocarcinogenesis in Wistar rats. It significantly elevated thiobarbituric acid reactive substances in the circulation of rats. Carcinoma indicated the higher levels of lipid peroxidation, which was accompanied by significantly decreased levels of antioxidants (reduced glutathione, glutathione peroxidase, superoxide dismutase and catalase) enzymes, as compared to the controls. Lipid peroxidation has been implicated as a major cause in cancer development (Sundaresan and Subramanian, 2003). A study by Dakshayani et al., (2005) demonstrated that the oxidative stress may be the reason for the elevated lipid peroxidation level in the liver of NDEA treated animals. These results were confirmed by Bansal et al., (2005) who reported that liver is the main site of NDEA metabolism, the production of ROS in liver may be responsible for its carcinogenic effects. In addition, Mittal et al., (2006) concluded that nitrosamines caused the generation of reactive ROS resulting in oxidative stress which alter the antioxidant defense system in the tissues.

With regard to the effect of NDEA on liver structure in rats, our results were agreed with Lijinsky, (1992) who showed that NDEA caused a wide range of tumors in all animal species and induce cancer in a variety of rodent organs, especially the liver. Devi et al., (2000) revealed that disarrangement of normal hepatic cells with centrilobular necrosis vacuolization of cytoplasm and fatty degeneration were observed in carbon tetrachloride intoxicated mice. This suggested that prolonged cell damage by chronic inflammation is critical in cancer development. Over production of nitric oxide has been implicated in the tissue damage caused by inflammation, contributing the tumor promotion (Nishikawa et al., 1998). Moreover, Singh et al., (2004) reported that oxidative stress caused by reactive oxygen species generated after administration of NDEA has been reported in membrane lipid peroxidation, and has been associated with various stages of tumor formation process.

This study revealed oral administration of broccoli and red cabbage extracts caused significant decrease in serum levels of AST, ALT, ALP, total protein, total albumin, total and direct bilirubin and MDA. However, there were significant increases in the levels of GPX, SOD and CAT as well as improved liver structure, compared to the positive group. Consequently, administration of broccoli or red cabbage extracts could prevent or decreased the incidences of hepatocarcinogenesis in rats induced by NDEA. These results agreed with Wargovich, (1999) who reported that cruciferous vegetables namely cabbage, Brussels sprouts, cauliflower and broccoli are rich in anti-carcinogens. A possible mechanism of reduced activities of the tested enzymes and hepatoprotective effect of broccoli and red cabbage extract may be related to their antioxidant effect of the phenolic and flavonoids compounds. Previous study reported that polyphenols can inhibit nitrosation and flavonoides have hepatoprotective activities (Orhan et al., 2007). Since flavonoids are a group of potentially chemoprotective compounds and have similar structures that consist of 2 phenolic benzene rings linked to a heterocyclic pyre or pyrone (Aherne and O'Brien, 2002). It has many biological effects that play a role in cancer prevention, including free radical scavenging, antimutagenic and antiproliferative properties, regulation of cell signaling and cell cycle, and inhibition of angiogenesis (Moon et al., 2006). In vitro and vivo experimental studies suggested that flavonoids influence signal transduction pathways (Frigo et al., 2002), and inhibit proliferation in human cancer cell lines (Manthey and Guthrie, 2002).

Cruciferous vegetables contain several chemical compounds that may modulate the carcinogenic process. These compounds act as antioxidants or as inhibitors and/or inducers of phase I and phase II enzymes (Fong et al., 1990; Bradlow et al., 1991). Phytonutrients in Crucifers vegetables work at a much deeper level and actually signal genes to increase production of enzymes involved in the detoxification. Lampe and Peterson, (2002) revealed that anti-carcinogenic actions of cruciferous vegetables are attributed to their content of glucosinolates (GLS). Fowke et al., (2003) reported that cruciferous vegetables contain sulforophane, which has anticancer properties. The anticancer effects of cruciferous vegetables may attribute to organic sulfur compounds (diallyl disulfide) and isothiocyanates, which had the ability to modulate expression/activity of antioxidative and phase 2 drug-metabolizing enzymes and scavenging free radicals (Antosiewicz et al., 2008).

The cancer-preventive effect of broccoli may be due to its content of sulforaphane (Zhang et al., 1992). The chemoprotective effect of sulforaphane may to be due its ability to behave as an inducer of phase II detoxification enzymes (Prestera and Talalay, 1995). Sulforaphane was also shown to inhibit the CYP2EI isoenzyme of the cytochrome P450, thus emerging as an inhibitor of phase I enzymes (Barcelo et al., 1996). Nishikawa et al., (2009) also concluded that isothiocyanates sulforaphane presented in broccoli has inhibitory effects on tumor cell growth in vitro and in vivo. In addition to broccoli was reported to provide moderate antioxidant capacity, likely attributed to tocopherols and flavonoids (Plumb et al., 1997). However, Cindy and John, (2003) revealed that selenium-enriched broccoli activates certain pro-apoptotic genes linked to p53, NFκB and stress signal pathways in response to “danger signals” such as tumorigenesis.

The protective effect of red cabbage against HCC that reported in this study agreed with the finding of Fekadu et al., (2003) who mentioned that that the chemoprotective properties of red cabbage involve inhibition of the formation as well as development of preneoplastic lesions in liver. One of the most important mechanisms of chemoprotection is induction of phase II enzymes, which detoxify DNA-reactive metabolites and thereby inhibit the formation of initiated cells (De Flora and Ramel, 1990). Another possible mechanism of protection is the inhibition of enzymes which are involved in the activation of heterocyclic aromatic amines (Rauscher et al., 1998). The effect of red cabbage may be attributed to the prevalence of anthocyanins in its extract. In addition, Wu and Prior, (2005) reported that several highly conjugated anthocyanins were identified in red cabbage with potential antioxidant activities. However, Hagiwara et al., (2002) indicated that color extracted from red cabbage shown to inhibit adenoma and carcinoma formation in rats initiated with and subsequently fed a diet containing the heterocyclic amine. Kassie et al., (2003) also reported that administration of red cabbage extract to rats resulted in chemoprevention of liver and colon cancers induced by heterocyclic amine. On the other hand, Lynn et al., (2006) showed that breakdown products of glucosinolates such as isothiocyanates in red cabbage are responsible for the chemopreventive properties of cruciferous vegetables.

5. Conclusion:

This study concluded that broccoli and red cabbage have a protective effect against hepatocellular carcinoma in rats, therefore this study recommends increased dietary intake of broccoli and red cabbage may be beneficial for patients with liver cancer as a preventative measures.

6. References:

[1] Aherne, S. A. and O'Brien, N. M., 2002. Dietary flavonols: chemistry, food content, and metabolism. Nutrit. 18, 75–8 1.

[2] Antosiewicz, J., Ziolkowski, W., Kar, S., Powolny, A. A. and Singh, S. V., 2008. Role of reactive oxygen intermediates in cellular responses to dietary cancer chemopreventive agents. Planta Med. 74 (13), 1570-1579.

[3] Bansal, A. K.; Bansal, M.; Soni, G and Bhatnagar, D., 2005. Protective role of Vitamin E pre-treatment on N-nitrosodiethylamine induced oxidative stress in rat liver. Chemico-Biological Interactions. 156, 101-111.

[4] Barcelo, S., Gardiner, J. M., Gescher, A. and Chipman, J. K., 1996. CYP2E1-mediated mechanism of anti-genotoxicity of the broccoli constituent sulforaphane, Carcinogenesis, 17 (2), 277–282.

[5] Block, G., Patterson, B. and Subar, A., 1992. Fruits, vegetables and cancer prevention: A review of the epidemiologic evidence. Nutri. Cancer, 18, 1-9.

[6] Beutler, E. D. and Kelly, B. M., 1963. Improved method for determination of blood glutathione. J. Lab. Clin. Med. 61, 882-888.

[7] Boitier, E., Boudia, M. M., Guillouzo, C. G., Defer, N. Picot, I. C., Leroux, J. P. and Marsac, C., 1995. Impairment of the mitochondrial respiratory chain activity in diethylnitrosamine induced rat hepatoma: possible involvement of oxygen free radicals. Cancer Res. 55 (14), 3028–3035.

[8] Bradlow, H. L., Michnovicz, J., Telang, N. T. and Osbome, M. P., 1991. Effects of dietary indole-3-carbinol on oestradiol metabolism and spontaneous mammary tumors in mice. Carcinogenesis, 12, 1571-1574.

[9] Carleton, H., 1979. Histological techniques, 4th Ed, London, Oxford University Press, New York, USA, Toronto.

[10] Cindy, D. D. and John, W. F., 2003. Effect of selenium-enriched broccoli diet on differential gene expression in liver. J. of Nutri. Biochem. 14 (4), 227-231.

[11] Conner, E. M. and Grisham, M. B., 1996. Inflammation, free radicals and antioxidants. Nutrition J. 12 (4), 274–277.

[12] Dakshayani, K., Subramanian, P., Manivasagam, T., Mohamed, M. and Manoharan, S., 2005. Melatonin Modulates The Oxidant– Antioxidant Imbalance During N-Nitrosodiethylamine Induced Hepatocarcinogenesis In Rats. J. Pharm, Pharmaceut. Sci. 8(2), 316-321.

[13] De Flora, S. and Ramel, C., 1990. Classification of mechanisms of inhibitors of mutagenesis and carcinogenesis. Basic Life Sci. 52, 461–462.

[14] Devi, G. S., Prasad, M. H., Saraswathi, I., Raghu, D., Rao, D. N. and Reddy, P. P., 2000. Free radical antioxidant enzymes and lipid peroxidation in different types of lukemias .Clin Chim Acta. 293, 53-62.

[15] Draper, H. H. and Hadley, M., 1990. Malondialdehyde determination as index of lipid peroxidation. Methods Enzymol. 186, 421-431.

[16] Elizabeth, H. J. and Marcela, A., 2009. Physiological effects of broccoli consumption. Phytochemistry Reviews. 8(1), 1568-7767.

[17] Fekadu, K., Maria, U., Sylvie, R., Bettina, G., Ruud, V., Michael, K., Monika, C., Rolf, S. and Siegfried, K., 2003. Chemoprevention of 2-amino-3-methylimidazo [4,5-f]quinoline (IQ)-induced colonic and hepatic preneoplastic lesions in the F344 rat by cruciferous vegetables administered simultaneously with the carcinogen. Carcinogenesis. 24 (2), 255-261.

[18] Ferlay, J., Bray, F., Pisani, P. and Parkin, D., 2001. Cancer incidence, mortality and prevalence worldwide, IARC Scientific Publications, IARC Press, Lyon.

[19] Fong, A. T., Swanson, H. I., Dashwood, R. H., Williams, D. E., Hendricks, J. D. and Bailey, G. S., 1990. Mechanisms of anti-carcinogenesis by indole- 3-carbinol. Studies of enzyme induction, electrophile scavenging, and inhibition of aflatoxin Bl activation. Biochem. Pliannacol. 39, 19-26.

[20] Fowke, J. H., Chung, F. L., Jin, F., Qi, D., Cai, Q., Conaway, C., Cheng, J. R., Shu, X. O., Gao, Y. T. and Zheng, W., 2003. Urinary isothiocyanate levels, brassica, and human breast cancer. Cancer Res. 63, 3 980-3986.

[21] Frigo, D. E., Duong, B. N. and Melnik, L. I., 2002. Flavonoid phytochemicals regulate activator protein-1 signal transduction pathways in endometrial and kidney stable cell lines. J Nutr. 132, 1848–1853.

[22] Hagiwara, A., Yoshino, H., Ichihara, T., Kawabe, M., Taman, S., Aoki, H., Koda, T., Nakamura, M., Imaida, K., Ito, N. and Shirai, T., 2002. Prevention by natural food anthocyanins, purple sweet potato color and red cabbage color, of 2-amino-1-methyl-6-phenylimidazo [4, 5-b] pyridine (Phip)-associated colorectal carcinogenesis in rats with 1, 2-dimethylhydrazine. J of Toxicolo. Scien.. 27, 57–68.

[23] Hissin, P. L. and Hilf, R., 1976. A fluorometric method for determination of oxidized and reduced glutathione in tissues. Anal. Biochem. 74 (1), 214-226.

[24] Inas, A. E., 2005. Cancer databases in the arabe world. Ethnicity and Disease. 15 (S1), 3-5.

[25] Kaplan, A., 1984. Clin Chem the C. V. Mosby

Co. St Louis. Toronto. Princeton. Pp. 1088-1273.

[26] Kakkar, P., Das, B. and Viswanathan, P. N., 1984. A modified spectrophotometric assay of superoxide dismutase. Ind. J. Biochem. Biophyso. 21, 131-133.

[27] Kassie, F., Uhl, M., Rabot, S., Grasl-Kraupp, B., Verkerk, R. and Kundi, M., 2003. Chemoprevention of 2-amino-3-methylimidazo [4,5-f]quinoline (IQ)-induced colonic and hepatic preneoplastic lesions in the F344 rat by cruciferous vegetables administered simultaneously with the carcinogen. Carcinogenesis. 24, 255-261.

[28] Ketterer, B. and Meyer, D. J., 1989. Glutathione transferases: a possible role in the detoxification and repair of DNA and lipid hydroperoxides. Mutat. Res. 214 (1), 33-40.

[29] Koller, A., 1984. Clin Chem the C. V. Mosby Co. St Louis. Toronto. Princeton; Pp. 418, 13 16-11324.

[30] Kurilich, A. C., Tsau, G. J., Brown, A., Howard, L., Klein, B. P., Jeffery, E. H., Kushad, M., Wallig, M. A. and Juvik, J. A., 1999. Carotene, tocopherol, and ascorbate contents in subspecies of *Brassica oleracea*. J. Agric. Food Chem. 47, 1576–1581.

[31] Kweon, S., Park, K. A. and Choi, H., 2003. Chemopreventive effect of garlic powder diet in diethylnitrosamine-induced rat hepatocarcino-genesis. Life Sci. 73 (19), 2515– 2526.

[32] Lampe, J. W. and Peterson, S., 2002. Brassica, biotransformation and cancer risk: Genetic polymorphisms alter the preventive effects of cruciferous vegetables. Journal of Nutrition, 132, 2991–2994.

[33] Lijinsky, W., 1992. Chemistry and Biology of N-Nitroso Compounds. Cambridge: Cambridge University Press.

[34] Lynn, A., Collins, A., Fuller, Z., Hillman, K. and Ratcliffe, B., 2006. Cruciferous vegetables and colo-rectal cancer. Proc Nutr Soc. 65, 135-144.

[35] Manthey, J. A. and Guthrie, N., 2002. Antiproliferative activities of citrus flavonoids against six human cancer cell lines. J Agric Food Chem.50, 5837–5843.

[36] Michaelis, L., Schubert, M. P. and Smythe, C. V., 2008. Potentiometric Study of the Flavins. J. Biol. Chem. 116 (2), 587–607.

[37] Mittal, G., Brar, A.P. and Soni, G., 2006. Impact of hypercholesterolemia on toxicity of N - nitrosodiethylamine: biochemical and histopath-ological effects. Pharmacol Rep. 58(3), 413-419.

[38] Mohamed, N., 2002. Photochemical and biological studies on *convolvulus arvesis.* Ph D. Thesis submitted to Faculty of Science. Ain Shams University.

[39] Moon, Y. J., Wang, X. and Morris, M. E., 2006. Dietary flavonoids effects on xenobiotic and carcinogen metabolism. Toxicol in Vitro. 20, 187–210.

[40] Murray, M. and Lara, P., 2005. The encyclopedia of healing foods. Simon and Schuster Adult Publishing Group. 172.

[41] Nishikawa. M., Takeda, K., Sato, E. F., Kuroki, T. and Inoue, M., 1998. Nitric oxide regulates energy metabolism and Bcl-2 expression in intestinal epithelial cells. Am J Physiol. 274(5), 797-801.

[42] Nishikawa, T., Tsuno, N. H., Tsuchiya, T., Yoneyama, S., Yamada, J., Shuno, Y., Okaji, Y., Tanaka, J., Kitayama, J., Takahashi, K. and Nagawa, H., 2009. Sulforaphane stimulates activation of proapoptotic protein bax leading to apoptosis of endothelial progenitor cells. Ann Surg Oncol. 16(2), 534-543.

[43] Orhan, D. D., Orhan, N., Ergun, E. and Ergun, F., 2007. Hepatoprotective effect of Vitis vinifera L. leaves on carbon tetrachloride-induced acute liver damage in rats. J Ethnopharmacol. 112(1), 145-51.

[44] Pevicharova, G.T., Dimova, P. I. and Atanasova-Goranova, V. K., 1997. Effect of food products on endogenous generation of N nitrosamines in rats. Br. J. Nutr. 78(2), 325-345.

[45] Plumb, G. W., Price, K. R., Rhodes M. J. and Williamson, G., 1997. Antioxidant properties of the major polyphenolic compounds in broccoli. Free Radic Res. 27 (4), 429–435.

[46] Pradeep, K., Mohan, C. V., Gobianand, K. and Karthikeyan, S., 2007. Silymarin modulates the oxidant-antioxidant imbalance during diethylnitrosamine induced oxidative stress in rats. Eur J Pharmacol. 560 (2-3), 110-116.

[47] Prestera, T. and Talalay, P., 1995. Electrophile and antioxidant regulation of enzymes that detoxify carcinogens, Proc Natl Acad Sci USA, 92 (19), 8965–8969.

[48] Price, K. R., Casuscelli, F., Colquhoun, I. J. and Rhodes, M. J., 1998. Composition and content of flavonol glycosides in broccoli florets *(Brassica olearacea)* and their fate during cooking. J of the Scien of Food and Agric. 77, 468–472.

[49] Rauscher, R., Edenharder, R. and Platt, K. L., 1998. In vitro antimutagenic and *in vivo* anticlastogenic effects of carotenoids and solvent extracts from fruits and vegetables rich in carotenoids. Mutat. Res. 413, 129–142.

[50] Reeves, P. G., Nielson, F. H. and Fahmy, G. C., 1993. Reports of the American Institute of Nutrition, adhoc-wiling committee on reformulation of the AIN 93. Rodent diet. J. Nutri. 123, 1939-1951.

[51] Roy, S. E., 1970. Colorimetric determination of

serum alkaline phospohatase. Clin. Chem. 16, 43 1-432.
[52] Singh, V., Selvendiran, K., Banu, S. M., Padmavathi, R. and Sakthisekaran, D., 2004. Protective role of Apigenin on the status of lipid peroxidation and antioxidant defense against hepatocarcinogenesis in Wistar albino rats. Phytomedicine. 11, 309-314.
[53] Sinha, K. A., 1972. Colorimetric assay of catalase enzyme. Anal, Biochem. 47, 389-394.
[54] Sivaramakrishnan, V., Shilpa, P. N., Praveen, V. R. and Niranjali, D. S., 2007. Attenuation of N - nitrosodiethylamine induced hepatocellular carcinogenesis by a novel flavonol-Morin. Chemico-Biological Interactions. 171(1),79–8 8.
[55] Snedecor, G. W. and Cochran W. G., 1986. Statistical Methods. 4th Edition, Iowa State University Press. Ames, Iowa, USA, Page 91.
[56] Steinmetz, K. A. and Potter, J. D., 1991. Vegetables, fruits and cancer prevention. J. Am. Diet Assoc. 96, 1027–1039.
[57] Strauss, R. M., 1995. Hepatocellular carcinoma, clinical, diagnostic and therapeutic aspects. In: Rustgi AK, ed. Gastrointestinal Cancers. Philadelphia, Pa: Lippincott-Raven. 479-496.
[58] Sundaresan, S. and Subramanian, P., 2003. S-allylcysteine inhibits circulatory lipid peroxidation and promotes antioxidants in N - nitrosodiethylamine-induced carcinogenesis. Pol. J. Pharmacol. 55, 37–42.
[59] Vozarova, B., Stefan N., Lindsay, S. R., Saremi, A., Pratley, E. R., Bogardus, C. and Tatarnni, A. P., 2002. High alanine aminotransferase is associated with decreased hepatic insulin sensitivity and predicts the development of type 2 diabetes. Diabetes. 51, 1889-1 895.
[60] Wargovich, M. J., 1999. Nutrition and cancer: The herbal revolution.Curr Opin Clin Nutr Metab Care. 2, 421-425.
[61] Wattenberg, L.W., 1990. Chemoprevention of cancer by naturally occurring and synthetic compounds. Proc. Am. Assoc. Cancer Res. 32, 461–463.
[62] Wild, C. P. and Hall, A. J., 2000. Primary prevention of hepatocellular carcinoma in developing countries. Mutat Res. 462, 381-393.
[63] Wu, X. and Prior, R., 2005. Identification and characterization of anthocyanins by high performanceliquid chromatography electrospray ionization-tandem mass spectrometry in common foods in the United States: vegetables, nuts and grains, J Agric Food Chem. 53 (8), 3101–3113.
[64] Zhang, Y., Talalay, P., Cho, C. G. and Posner, G. H., 1992. A major inducer of anticarcinogenic protective enzymes from broccoli: isolation and elucidation of structure, Proc Natl Acad Sci USA. 89 (6), 2399–2403.

[65] Zwart, L. L., Meerman, J. N. and Commandeur, J. M., 1999. Biomarkers of free radical damage applications in experimental animals and humans. Free Radic. Biol. Med. 26 (1-2), 202–226.

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