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Protective and Prophylactic Role of Brassicaceae Vegetables Extracts on N-nitrosodiethylamine Induced Initiation of Hepatocellular Carcinoma in Rat Liver

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Research Article

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ABSTRACT

Nitrosamine compounds are known hepatic carcinogens. In the metabolism of nitrosamines, such as diethylnitrosamine (DEN), there is evidence of the formation of reactive oxygen species (ROS) resulting in oxidative stress, which may be one of the factors in the etiology of cancer. The formation of ROS may alter the antioxidant system, through measuring essential and non-essential amino acids, the study was undertaken to investigate the Protective and Prophylactic role of ethanolic extracts of *Brassica oleracea* or *botrytis* (200 mg of plant extracts/kg body weight) on diethylnitrosamine (DEN) (1 µl/100 g b.wt.) in rats, induced initiation of hepatocellular carcinoma in rat liver, Brassicaceae vegetables are also recognized as a rich source of nutrients such as vitamins, minerals, amino acids (for example, L-alanine, L-aspartic acid, L-glutamic acid, L-glutamine, L-histidine, L-methionine, L-phenylalanine, L-threonine, L-tryptophan, and L-valine) Brassicaceae vegetables have been reported to have anticancer and antioxidant properties.

INTRODUCTION

Nitroso compounds and their precursors in the environment, in certain occupational settings, in diet and also due to the use of tobacco products, cosmetics, pharmaceutical products as well as their endogenous formation in the human body from dietary components, may be potential risk factors in cancer, Nitrosamines, such as, *N*-nitrosodiethylamine (NDEA), one of the most important environmental carcinogen of this class, has been suggested to cause the generation of reactive oxygen species (ROS) resulting in oxidative stress and cellular injury [1]. As liver is the main site of NDEA metabolism, the production of ROS in liver may be responsible for its carcinogenic effects.

Plant and animal foods, in addition to supplying essential nutrients for the mankind also possess a variety of bioactive substances like phenols, flavonoids, carotenes and organosulphur compounds having anti proliferative activities [2]. Nearly 30-40% of cancers are directly linked to improper diet and related factors [3]. Epidemiological studies indicated positive association between intake of fruits and vegetables and reduced mortality from common cancers, heart and other degenerative diseases [4].

Brassicaceae plants are considered as one of the most popular vegetables consumed all over the world and considered to be a good source of bioactive phytochemicals [5]. Additionally, *Brassica* species and varieties are increasingly becoming a research model in plant science, as a consequence of the importance of their primary and secondary metabolites. Brassicaceae vegetables have been reported to have anticancer and antioxidant properties [6]. Broccoli (*Brassica oleracea* L. var. *italica*) belongs to the family Brassicaceae having leaves characterized by more divided and petiolate. The main head consists of clusters of fully differentiated flower buds which are less densely arranged with longer peduncles. Sprouting forms of broccoli bear many small flowers heads. It is an annual herb reaching 400 mm during vegetative stage and 1-2 m at the end of flowering [7].

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Amino acids, a class of biologically active compounds present in food and beverages, are important for human nutrition and affect the quality of foods [8]. Glutathione (γ-L-glutamyl-L-cysteinylglycine, GSH) is a major antioxidant acting as a free radical scavenger that protects the cell from reactive oxygen species (ROS). Sulfur amino acids (SAAs), such as methionine and cysteine, play a critical role in the maintenance of health. GSH depletion as well as alterations of SAA metabolism is linked to a host of disease states including liver cirrhosis [9].

Phytochemicals, such as carbohydrates (sucrose and glucose), amino acids, phenolics (phenylpropanoids and flavonoids) and glucosinolates, these phytochemicals have diverse applications due to their antimicrobial [10], antioxidant [11] and anticarcinogenic properties activities [12]. A natural component of *Brassica* vegetables has an interesting anticarcinogenic potential, acting via different metabolomic and hormonal pathways [13]. It reduces the incidence of tumours in reproductive organs [14].

Flavonoids are present in most plant tissues and often in vacuoles, the major flavonoids in *Brassica* spp. are myricetin and quercetin [15]. The flavonoid composition of different edible Brassica species pak choi (*Brassica campestris*), broccoli (*Brassica oleracea*), and cauliflower (*Brassica oleracea*), turnip tops (Brassica rapa) and tronchuda cabbage (Brassica oleracea) have been reported [16,17].

Dietary fibre is composed of non-starch polysaccharides [18] and is an important constituent in Brassicaceae vegetables, contributing to prevent colon cancer [19]. In white cabbage (*B. oleracea* var. *capitata*) dietary fibre represents one-third of the total carbohydrate content, the other two-third being low-molecular weight carbohydrates, including glucos, uronic acid, arabinose , and galactose [20]. Various fractions of *Brassica oleracea* extract might offer a natural key in hypolipidemic and hepatoprotective activity [21]. There is ever-increasing evidence that a higher consumption of *Brassica* vegetables, for example, broccoli, cabbage, kale, mustard greens, Brussels sprouts, and cauliflower, reduces the risk of several types of cancer [22]. The anticarcinogenic effect of these vegetables has been attributed to decomposition products of glucosinolates, indoles, and iso-thiocyanates, phytoalexins, and other antioxidants [23,24]. Thus, the aim of the present work is to investigate the levels of essential and non-essential amino acids against hepatocellular carcinoma.

MATERIALS AND METHODS

Chemicals

All chemicals used were of high analytical grade, products of Sigma (US), Merk (Germany) and BDH (England).

Animals

Female Wister strain albino rats (120-150 g) were obtained from the animal house, National Research Centre, Cairo, Egypt and maintained on stock commercial pellet diet (El- Kahira Company for Oil and Soap) and water ad-libitum.

Plant material

Leaves of *Brassica oleracea* Var. (Family: Brassicaceae), sprouts of *Brassica botrytis* L. (Family: Brassicaceae) were natively collected from Egyptian country and freshly extracted. Voucher specimens (BOV, BBL, DCL and CZL-2010) were deposited at Therapeutic Chemistry Dept. National Research Center, Cairo, Egypt as references.

Plant Extraction

Plant materials were extracted in a Soxhlet apparatus using 95% ethanol for 72 h [25]. Solvent removal was carried out under vacuum for drying at 40°C. The dried residues were stored at 4°C till used.

Doses and route of administration

All treated animals were orally received (200 mg of plant extracts/kg body weight) by stomach gavage daily for one month, while prophylactic groups were orally received (200 mg of plant extracts/kg body weight) daily for three and two weeks [26]. Diethyl nitrosamine (DEN) (Sigma) was injected intraperitoneally in a dose of 1 µl (diluted 1:100 with 0.15 mol/l sterile NaCl)/100 g body weight for 7 consecutive days [27].

Experimental design

Sixty female rats were divided into ten groups of six rats each. Group (1) served as normal healthy control rats, groups (2,3) were normal healthy rats received *Brassica oleracea* or botrytis daily for one month, group 4 intraperitoneally injected with diluted diethylnitrosamine daily for seven days and sacrificed after three weeks of the last injection, groups (5,6) served as the treated groups, where it received diethylnitrosamine for one week, left free for three weeks followed by administration of *Brassica oleracea* or *botrytis* extracts respectively for one month. Groups (7,8) served as prophylactic groups, where it received the plant extracts for three weeks followed by diethylnitrosamine for one week and sacrificed after three weeks. Groups (9,10) served as prophylactic groups where it received each plant extracts for two weeks followed by diethylnitrosamine for one week and sacrificed after three weeks of the last. Anesthetic procedures and handling with animals complied with the ethical guidelines of Medical Ethical Committee of the National Research Centre in Egypt.

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Amino acid assay

The amino acid composition of mouse liver was determined by the HPLC-Pico-Tag method according to Cohen et al. [28] using a standard amino acid (Sigma, USA). The samples were hydrolyzed with 6 M HCl in a vacuum sealed tube at 110°C under nitrogen atmosphere for 24 hours. The composition was measured by using Eppendorph LC 3000 Amino Acid Analyzer (Eppendorf-Biotronic, Hamburg, Germany). The amino acids were quantified by comparing peak areas with corresponding amino acid standard solutions using the Spectra Physics Data System Program; the data were expressed as µg/mg protein.

Statistical analysis

The results of biochemical analysis were analyzed using one-way analysis of variance followed by Co-stat computer program. Values of less than $P \leq 0.05$ were regarded as statistically significant.

RESULTS

Essential free amino acid

Threonine, valine, isoleucine and leucine highly elevated in nitrosamine group with different percent change as shown in Table 1 and Figure 1 with +57.7 +114.9, +156.77 and +185.8% respectively. These percent enhanced after treated with *olerace* & *botrytis* extracts for three weeks. Also, enhancement in *olerace* and *botrytis* prophylactic groups for two and three groups where their value nearest to control as clear in Table 1 and Figure 1a.

Table 1a: Essential amino acid fractions (Threonine, Valine, Isoleucine, Leucine and Phenylalanine) in different groups in hepatic rats

Amino acid fractions	Control	Control- treated		+ve – 3wk	+ve -treated					
		Olerace	Botrytis		+ve – 3wk Olerace treat	+ve – 3wk Botrytis treat	3 wk Olerace-prophylactic	3 wk Botrytis prophylactic	2 wk Olerace-prophylactic	2 wk Botrytis prophylactic
	G1	G2	G3	G4	G5	G6	G7	G8	G9	G10
	Mean ± SD	Mean ± SD	Mean ± SD	Mean ± SD	Mean ± SD	Mean ± SD	Mean ± SD	Mean ± SD	Mean ± SD	Mean ± SD
Threonine	4.07 ± 0.69^{bc}	2.49 ± 0.39^{d}	2.52 ± 0.58^{d}	6.42 ± 0.72^{a}	2.47 ± 0.79^{d}	3.29 ± 0.67^{cd}	2.98 ± 0.72^{d}	4.67 ± 0.85^{b}	3.03 ± 0.92^{d}	4.37 ± 0.43^{b}
Valine	5.57 ± 0.71^{d}	3.63 ± 0.97^{e}	3.38 ± 0.49^{e}	11.97 ± 1.15^{b}	7.86 ± 0.91^{c}	8.96 ± 1.07^{c}	8.87 ± 1.05^{c}	7.73 ± 0.83^{c}	14.99 ± 1.15^{a}	10.62 ± 1.46^{b}
Isoleucine	3.84 ± 0.59^{de}	2.16 ± 0.43^{f}	2.05 ± 0.45^{f}	9.86 ± 1.22^{a}	3.50 ± 0.52^{e}	4.31 ± 0.95^{de}	5.81 ± 0.64^{bc}	6.98 ± 1.25^{b}	4.93 ± 0.89^{cd}	5.58 ± 0.84^{c}
Leucine	4.87 ± 0.47^{ef}	3.39 ± 0.46^{g}	4.21 ± 0.55^{fg}	13.92 ± 1.29^{a}	6.72 ± 0.73^{d}	6.12 ± 1.08^{de}	10.87 ± 1.3^{2b}	9.21 ± 1.39^{c}	6.73 ± 0.63^{d}	10.48 ± 0.67^{bc}

- Data are expressed as means ± SD of six rats in each group.
- Free amino acid fractions are expressed in µg /ml protein.
- P is level of significance, where $P < 0.05$ is significant (*)
- Unshared superscript letters between groups are the significance. All values at $p< 0.0001$.
- Statistical analysis is carried out using one way analysis of variance (ANOVA) using CoStat computer program.

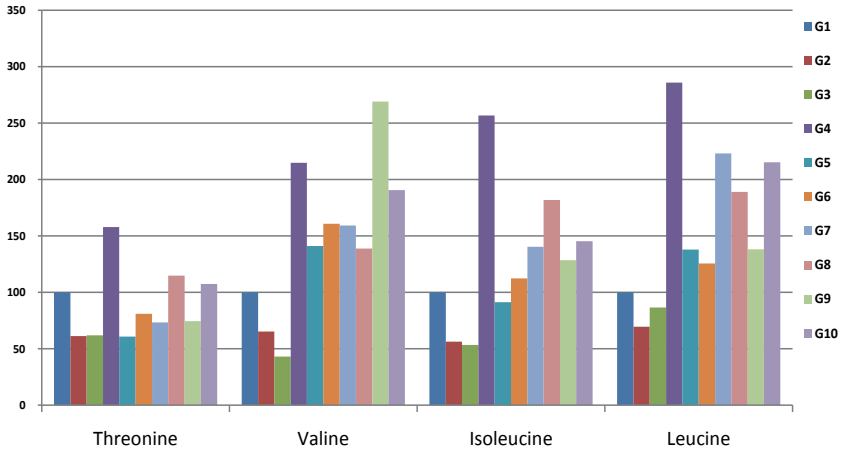

Figure 1a: Percent change of different essential amino acids [a] (Threonine, valine, isoleucine and leucine).

Phenylalanine, histidine, lysine, methionine and arginine also, elevated in nitrosamine group with different percent change 103.3, 77.79, 238, 180.85 and 166.79% respectively. In case of treated with olerace extract, for three weeks, phenylalanine and lysine shown 14.45% and 58.5% respectively, while histidine, methionine and arginine revealed 2.79%, 26.70% and 6.13% respectively. *Botrytis* extract treated for three weeks, shown elevation with 51.40%, 34.0% and 66.7% as compared with control group in phenylalanine, histidine and lysine respectively. In case of prophylactic groups of olerace and botrytis extracts for two and three weeks revealed a significant reduction for elevation caused by given nitrosamine with different percent, seems to control group when compared as shown in Table 1b and Figure 1 b.

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Table 1b: Essential amino acid fractions (Histidine, Lysine, Methionine and Arginine) in different groups in hepatic rats.

Amino acid fractions	Control	Control- treated		+ve – 3wk	+ve - treated					
		Olerace	Botrytis		+ve–3wk Olerace treat	+ve–3wk Botrytis treat	3 wk Olerace-prophylactic	3 wk Botrytis prophylactic	2 wk Olerace-prophylactic	2 wk Botrytis prophylactic
	G1	G2	G3	G4	G5	G6	G7	G8	G9	G10
	Mean ± SD	Mean ± SD	Mean ± SD	Mean ± SD	Mean ± SD	Mean ± SD	Mean ± SD	Mean ± SD	Mean ± SD	Mean ± SD
Phenylalanine	4.22±0.51^{de}	2.83±0.63^{f}	3.03±0.38^{ef}	8.58±0.88^{a}	4.83±0.83^{cd}	6.39±0.87^{b}	5.14±0.82^{cd}	4.08±0.75^{de}	5.91±1.16^{bc}	6.89±1.26^{b}
Histidine	6.08±0.75^{d}	3.96±0.63^{e}	3.49±0.49^{e}	10.81±1.13^{a}	5.91±0.75^{d}	8.15±0.92^{bc}	7.69±0.66^{bc}	6.95±1.35^{cd}	8.87±0.95^{b}	7.04±0.84^{cd}
Lysine	4.99±1.13^{d}	3.89±0.63^{d}	3.49±0.38^{d}	16.87±1.38^{a}	7.91±1.15^{c}	8.32±0.90^{c}	15.91±1.33^{a}	11.96±1.51^{b}	8.15±1.06^{c}	8.84±1.15^{c}
Methionine	13.89±1.52^{c}	9.73±0.80^{d}	9.02±1.22^{d}	39.01±4.19^{a}	10.18±0.96^{d}	8.94±1.60^{d}	17.79±1.19^{b}	17.32±3.47^{b}	17.27±0.70^{b}	9.97±1.22^{d}
Arginine	7.83±0.83^{bc}	4.82±0.69^{e}	4.35±0.50^{e}	20.89±2.06^{a}	7.35±0.73^{bc}	5.75±0.87^{de}	8.54±0.89^{b}	8.45±1.49^{b}	5.12±0.92^{de}	6.39±0.66^{cd}

- Data are expressed as means ± SD of six rats in each group.
- Free amino acid fractions are expressed in µg /ml protein.
- P is level of significance, where P < 0.05 is significant (*)
- Unshared superscript letters between groups are the significance. All values at p< 0.0001.
- Statistical analysis is carried out using one way analysis of variance (ANOVA) using CoStat computer program.

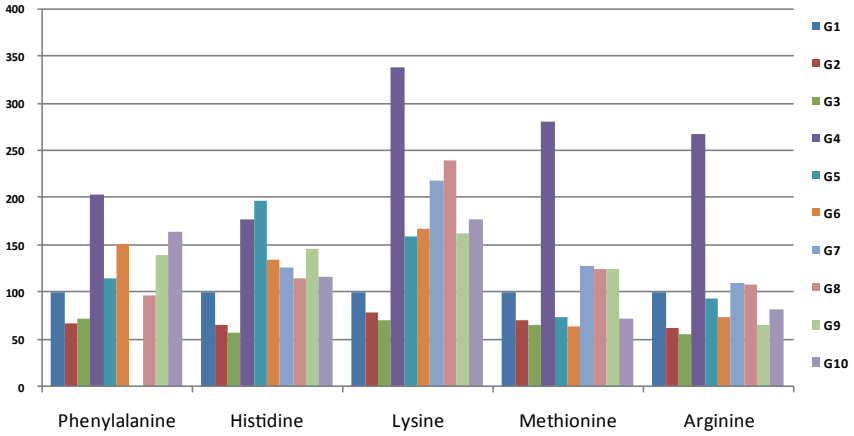

Figure 1b: Percent change of different essential amino acids [b] (Phenylalanine, histidine, lysine, methionine and arginine) in different groups in liver of rats.

Non-essential free amino acid

Aspartic acid, serine, glutamic acid and glycine increased in nitrosamine group with different percent change with respect to control group with 361.90, 61.17, 21.94 and 38.30% respectively. In groups treated olerace extract for three weeks these elevation was reduced with different percentage, amounting to 14.39% and 32.00%, for glutamic acid and glycine as compared to control rats , while aspartic acid and serine showed improved level reached to 224.50% and 52.2% respectively as illustrate in Table 2a and Figure 2 a. Also, in treated botrytis group aspartic acid, serine, glutamic acid and glycine showing a significant reduction for the elevation caused by nitrosamine as shown in Table 2, Figure 2b. In case of prophylactic treated rats with *olerace* and *botrytis* for two weeks, botrytis extract treated rats declared more ameliorated level than olerace as compared to normal control. In case of prophylactic groups for three weeks *botrytis* extract more enhanced than olerace extract in case of aspartic acid, serine and glutamic acid, but in case of glycine olerace extract is best than botrytis extract. Alanine, tyrosine, NH4, proline and urea showed highly significant elevation as compared to control group. These elevation was improved with a highly degree in treated olerace and botrytis extracts for three weeks, botrytis showed more enhancement than *olerace* as compared to control group. Prophylactic olerace and botrytis extracts for two weeks showed protection against nitrosamine induced toxicity with different percentage change of 228.50, 18.10, 21.30, 588.80 and 79.66%. Ln case of *olerace* while in case of *botrytis* extract showed 240.00, 44.70, 18.17, 430.80 and 71.00%, respectively as compared to control group. Also, olerace and botrytis prophylactic rats for three weeks, the two extracts showed protection against the elevation caused by nitrosamine injection , with different percentage change for alanine, tyrosine, NH4, proline and urea amino acids respectively as demonstrated in Table 2b and Figure 2b.

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Table 2a: Non-essential free amino acid fractions (Aspartic acid, Serine, Glutamic acid, Glycine and Alanine) in different groups in hepatic rats.

Amino acid fractions	Control	Control- treated		+ve – 3wk	+ve - treated					
		Olerace	Botrytis		+ve – 3wk Olerace treat	+ve – 3wk Botrytis treat	3 wk Olerace-prophylactic	3 wk Botrytis prophylactic	2 wk Olerace-prophylactic	2 wk Botrytis prophylactic
	G1	G2	G3	G4	G5	G6	G7	G8	G9	G10
	Mean ± SD	Mean ± SD	Mean ± SD	Mean ± SD	Mean ± SD	Mean ± SD	Mean ± SD	Mean ± SD	Mean ± SD	Mean ± SD
Aspartic acid	1.47±0.49^{e}	2.07±0.58^{e}	1.92±0.56^{e}	6.79±0.91^{a}	4.77±0.62^{cd}	5.82±0.87^{ab}	5.89±0.76^{ab}	5.07±0.52^{bc}	4.66±0.72^{cd}	3.87±0.65^{d}
Serine	3.58±0.57^{def}	2.48±0.38^{f}	2.39±0.25^{f}	5.77±0.78^{bc}	5.46±0.64^{bc}	7.49±0.84^{a}	3.16±0.89^{ef}	4.21±1.26^{de}	6.62±1.16^{ab}	4.57±1.00^{cd}
Glutamic acid	14.04±1.35^{b}	9.99±1.30^{de}	8.87±0.66^{e}	17.12±2.11^{a}	12.02±1.53^{bcd}	12.28±2.51^{bc}	10.93±1.02^{cde}	12.92±0.58^{bc}	12.01±1.15^{bcd}	13.97±1.45^{b}
Glycine	11.85±1.81^{b}	9.89±0.95^{cd}	10.92±0.79^{bc}	16.39±0.62^{a}	8.05±1.19^{ef}	8.38±1.37^{def}	8.66±1.09^{de}	4.85±0.67^{g}	6.69±1.16^{f}	10.62±1.77^{bc}

- Data are expressed as means ± SD of six rats in each group.
- Free amino acid fractions are expressed in µg /ml protein.
- P is level of significance, where P < 0.05 is significant (*)
- Unshared superscript letters between groups are the significance. All values at p< 0.0001.
- Statistical analysis is carried out using one way analysis of variance (ANOVA) using CoStat computer program

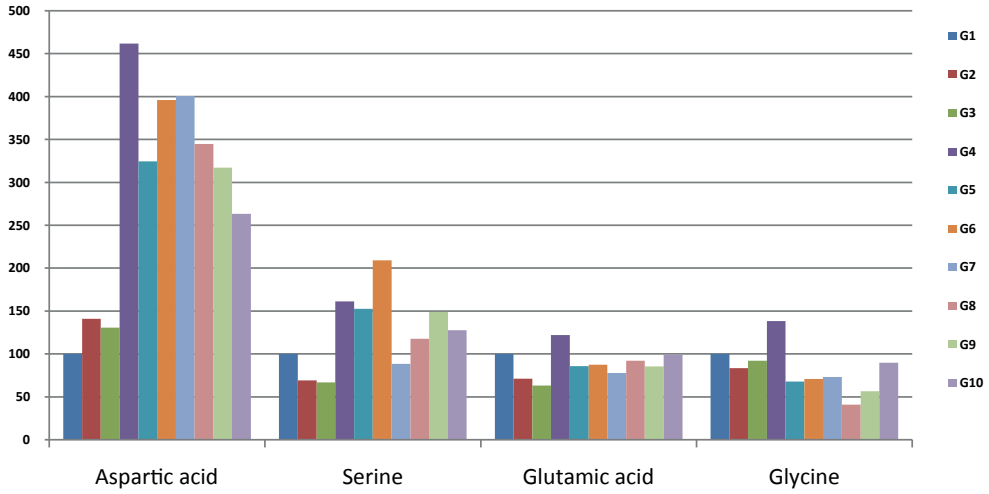

Figure 2a: Percent change of different non-essential amino acids [a] (Aspartic acid, serine, glutamic acid and glycine).

Table 2b: Non-essential free amino acid fractions (Tyrosine, NH4+, Proline and Urea) in different groups in hepatic rats.

Amino acid fractions	Control	Control -treated		+ve – 3wk	+ve - treated					
		Olerace	Botrytis		+ve – 3wk Olerace treat	+ve – 3wk Botrytis treat	3 wk Olerace-prophylactic	3 wk Botrytis prophylactic	2 wk Olerace-prophylactic	2 wk Botrytis prophylactic
	G1	G2	G3	G4	G5	G6	G7	G8	G9	G10
	Mean ± SD	Mean ± SD	Mean ± SD	Mean ± SD	Mean ± SD	Mean ± SD	Mean ± SD	Mean ± SD	Mean ± SD	Mean ± SD
Alanine	2.35± 0.65^{g}	4.17± 0.54^{ef}	3.40± 0.40^{fg}	11.02± 1.23^{a}	9.53± 1.18^{bc}	8.38± 1.28^{cd}	10.34± 0.79^{ab}	5.31± 1.06^{e}	7.72± 1.10^{d}	7.99± 1.19^{d}
Tyrosine	3.49± 1.03^{e}	3.06± 0.79^{e}	3.00± 0.41^{e}	8.99± 0.75^{a}	5.31± 0.71^{cd}	4.89± 1.04^{cd}	7.35± 0.76^{b}	5.62± 0.99^{c}	4.12± 1.33d^{e}	5.05± 1.03^{cd}
NH4+	139.1± 6.96^{b}	71.58± 1.96^{c}	62.24± 3.31^{c}	186.79± 11.5^{a}	115.4± 6.62^{b}	113.71± 2.79^{b}	117.46± 5.93^{b}	114.0± 8.0^{b}	109.48± 7.22^{b}	113.83± 2.07^{b}
Proline	13.09± 1.27^{e}	12.96± 0.73^{e}	15.15± 1.00^{e}	116.06± 4.34^{a}	104.98± 13.7^{b}	78.65± 8.19^{d}	94.48± 6.21^{c}	98.99± 7.32^{bc}	77.08± 8.67^{d}	69.49± 8.52^{d}
Urea	34.66± 2.91^{e}	22.68± 1.98^{f}	20.25± 1.51^{f}	76.03± 4.35^{a}	34.54± 4.22^{e}	41.92± 2.61^{d}	57.69± 5.45b^{c}	54.79± 4.27c	62.27± 3.02^{b}	59.28± 3.13^{bc}

- Data are expressed as means ± SD of four rats in each group.
- Free amino acid fractions are expressed in µg /ml protein.
- P is level of significance, where P < 0.05 is significant (*)
- Unshared superscript letters between groups are the significance. All values at p< 0.0001.
- Statistical analysis is carried out using one way analysis of variance (ANOVA) using CoStat computer program

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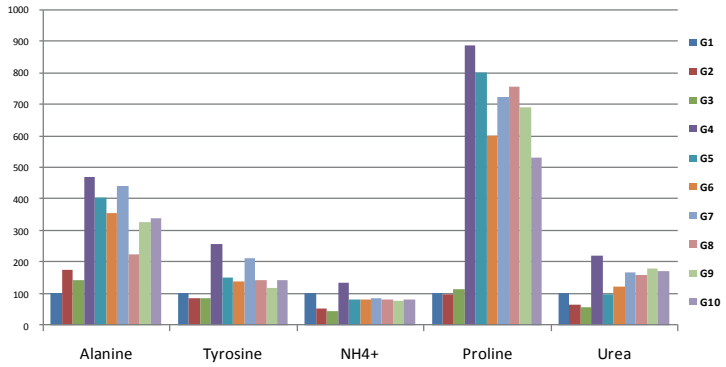

Figure 2b: Percent change of different non-essential amino acids [[b] (Alanine, tyrosine, NH4, proline and urea) in different groups in liver of rats.

DISCUSSION

Many recent studies have tried to find out whether cancer-specific amino acid exists. Alanine and glycine have been demonstrated to be released from Walker carcinoma 256 and glycine from hepatoma 7777 cells [29]. Glutamine has been declared to be an important respiratory fuel in breast cancers [30]. Free tryptophan, glutamic acid and ornithine still increased in breast cancer patients. Taurine, which is involved in swelling activated transport, has been considered to play an important part in the regulation of the cell cycle clock of human cervical cancer cells [31].

Malnutrition is a common cause of morbidity in cancer patients and may lead to serious complications affecting their quality of life. Malnourished cancer patients are in a hyper metabolic state with increased lipolysis and fatty acid oxidation, gluconeogenesis and whole-body protein catabolism. The metabolism of protein and amino acids in cancer patients is closely linked to glucose metabolism and is regulated by a number of the same hormones and their metabolites [32]. Phytochemical analysis revealed the presence of flavonoids, glycosides, saponins, steroids, terpenoids and alkaloids [33].

Enhancing the phytonutrient content of plant foods through selective breeding or genetic improvement is a powerful tool for dietary disease prevention. However, most, if not all, of these bioactive compounds confer a bitter, acid, or astringent taste to the food which is rejected by most consumers. Moreover, in the past, some of these compounds have even viewed as plant-based toxins and, as a result, the food industry routinely removes these compounds from plant foods through selective breeding and a variety of debittering processes [17]. Brassica vegetables represent a major part of the human diet all over the world providing nutritionally significant constituents, such as phenolic compounds, vitamins, fibres, soluble sugars, minerals, fat, and carotenoids. Cruciferous vegetables are a source of some very promising chemopreventive dietary constituents, which may protect against free radical damage and LDL oxidation implicated in the pathogenesis of cardiovascular diseases, as well as DNA damage and cancer [34].

It is a well-known fact that the carotenes, tocopherols, vitamin C, have the potential to prevent and treat malignant and degenerative diseases [35]. Broccoli (*Brassica oleracea*) extracts are protective against reactive oxygen species (ROS) presumably due to the presence of vitamin C, quercetin, kaempferol, lutein, zeaxanthin, α-tocopherol, ϒ-tocopherol, and β-carotene [36].

Significantly depressed levels of eight amino acids including both essential amino acids (EAA) (leucine, methionine, threonine and valine) and Non-Essential Amino Acids (NEAA) (alanine, glycine, proline, and serine) in other cancer patients receiving chemotherapy [37]. The present study is in contrast to Lai et al [37], where significant elevated levels of these amino acids including both EAA (leucine, methionine, threonine and valine) and NEAA (alanine, glycine, proline and serine) a marker of cancer hepatic cell were detected.

Glucosinolates are one of the most important groups of Brassicaceae metabolites derived from amino acid biosynthesis (e.g. methionine, tryptophan, phenylalanine etc. the flavour and odour of Brassica vegetables are typically related to their glucosinolate content [20].

Previous study from our laboratory focused on the *in vivo* acute toxic effects of NDEA on liver histology, antioxidant status of rat liver showed marked changes in the hepatic architecture which could be explained on the basis of DEN treatment manifested its toxic effects through the generation of ROS as well as elevation of malondialdehyde and conjugated dienes, which caused deleterious effects on the membranous components of hepatocytes. Liver sections of DEN rats' prophylactic with *Brassica Oleracea, Brassica botrytis*, showed enlarged hepatocytes with vacuoles. The recovery of necrosis due to the treatment of plant extracts may be due to diminution of oxidative stress and free radicals elevation, which are indicative of hepatic injury. Carcinogenic rats recorded drastic changes in all parameters under investigation. Plants therapy recorded more potent effect than prophylactic action [38]. These are at least partly responsible for their benefits for human health including anti-carcinogenic, cholesterol-reducing, and other pharmacological effects [39].

Levels of different amino acids are affected with diethylnitrosamine. Threonine balances the protein level in the body and promotes the immune system. It aids in the synthesis of glycine and serine, two amino acids that help in the production of collagen, elastin and muscle tissue. It also, speeds up wound healing after injury by boosting the immune system. Threonine, in combination with the amino acids aspartic acid and methionine, helps liver digest fat and fatty acids, a process that reduces the accumulation of fat in the liver. An accumulation of fat negatively affects the functions of liver [40]. Histidine is important for the synthesis of red and white blood cells. Alanine, removes toxic substances released from the breakdown of muscle proteins during intensive exercise. Glutamine and aspartic acid aid the functioning of all cells, RNA and DNA (the carriers of genetic code). Additional benefit of aspartic acid is the protection of the liver from damages that can be caused by excess ammonia in the bloodstream. Serine aids in the synthesis of proteins in immune system. Proline plays a role in intracellular signaling. In the present study, levels of all of these amino acids increased in all groups as compared to the control group, the concentration of free amino acids in liver tissues can be a sensitive tool for determining the effect of antigen in control and infected animals [41]. In our study, *Brassica oleracea* or *botrytis* extracts ameliorating metabolic disorders caused with diethylnitrosamine. Our study also demonstrated that initiation of hepatic cancer, caused increased amino acid demand, and is accompanied by a reduction in the amino acid availability post therapeutic or prophylactic treatments with brassicaceae vegetables extracts. The amino acids profile where characteristic of hepatic cancer cell. The amino acid profiles in other compartments can be informative as well [40].

Variability in the amino acids requirement may be associated with individual differences in the efficiency of amino acids utilization or with differences in the ratio of lipid to protein deposition. Moehn et al. [42] found that a lower rate of inevitable Lysine oxidation would lead, as was observed in the current experiment, to a greater slope for the decrease in indicator oxidation when Lysine intake was below the requirement. Although, lower rates of inevitable Lysine catabolism have been associated with greater rates of maximum protein deposition in the current study Lysine highly elevated as a result of diethyl nitrosamine induction , this disturbance was ameliorated in therapeutic and prophylactic treated rats with *Brassica oleracea* or *botrytis* extracts.

Natural products seem to work in a tightly regulated manner wherein they switch their roles either towards protective or therapeutic side depending upon either the amount of the drug being used or upon the cellular phenotype [43]. In the present study, prophylactic or treatment of carcinogenic rats with ethanolic extracts of *Brassica oleracea* or *botrytis* recorded overall, to date, the most promising anticarcinogenic dietary compounds have been detected in cruciferous vegetables and further elucidation of their protective mechanisms and the identification of other active constituents may contribute to the development of highly health supporting *Brassica* varieties [44]. Cancer cells are hypermutable and may result in amino acid changes in certain protein sequences [37].

From the results, it would seem logical to predict that consumption of *Brassicaceae* vegetables could prevent the ailments caused by food-borne pathogens; bioactive compounds from *broccoli* (*Brassica oleracea* L. var. *italica*) have scope for the possible use in food industries to stay away from food borne pathogens. Diets provided amino acids (such as, L-alanine, L-aspartic acid, L-glutamic acid, L-glutamine, L-histidine, L-methionine, L-phenylalanine, L-threonine, L-tryptophan, and L-valine) as Brassicaceae vegetables which reported to have anticancer and antioxidant properties.

CONCLUSIONS

Supplementation of these natural antioxidants through a balanced diet could be much more effective and economical than supplementation of an individual antioxidant, in protecting the body against various oxidative stresses, the administration of *Brassica oleracea* or *botrytis* extracts cause rapid induction of Nitrosamine groups as well as hepatoprotective effect in rats. Thus, in the light of our pharmacological research the study of various fractions of *Brassica oleracea* or *botrytis* extracts might offer a natural key in hepatoprotective activity. Further chemical and pharmacological investigations are in progress to elucidate in detail the active principles and the exact mechanism of actions.

Declaration of interest

The authors report no declarations of interest.

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