Bulletin of Environment, Pharmacology and Life Sciences
Bull. Env. Pharmacol. Life Sci., Vol 2 (11) October 2013: 100-106

Online ISSN 2277-1808
Journal's URL:http://www.bepls.com

ORIGINAL ARTICLE

Histopathological study on Inhibitory effects of *Brassica oleracea* L. Var. Italica (Broccoli) Ethanolic extract on 4-Nitroquinoline-1-oxid -induced Pre-neoplastic changes in the Rat

Jamal Agha Mohammadi[1], Daryoush Mohajeri[*2], Ramin Kaffashi Elahi[3]
[1]Dept. of Veterinary Medicine, Faculty of Veterinary Medicine, Tabriz Branch, Islamic Azad University, Tabriz, Iran
[2]Department of Pathobiology, Faculty of Veterinary Medicine, Tabriz Branch, Islamic Azad University, Tabriz, Iran
[3]Department of Clinical Sciences, Faculty of Veterinary Medicine, Tabriz Branch, Islamic Azad University, Tabriz, Iran

ABSTRACT
Oral squamous cell carcinoma is one of the most common cancers in the world. Novel treatment is best studied in animal models that mimic the same clinical features as human squamous cell carcinomas. For this end, 48 male Wistar rats were randomly allocated into four groups of 12 animals each. Group 1 served as control and was given the basal diet and tap water without 4-NQO. Rats from Groups 2 to 4 assigned to receive 30 ppm 4-NQO in drinking water for 12 weeks. When the feeding of 4-NQO was started the rats of groups 3 and 4, received broccoli extract at a dose of 200 and 300 mg/kg respectively, 3 times per week. At the end of experiment, the rats were euthanized and the tongue was removed. Tissue specimens collected from tongues and 5 µm thick microscopic sections were prepared through hematoxylin-eosin staining method. Histological evaluations for carcinogenesis were performed for tongues epithelial tissue. The incidences of tongue lesions were compared among the groups. There were no pathological alterations in control rats. Premalignant lesions appeared after 12 weeks of the last application of 4NQO. Administration of broccoli extract at both doses during the experiment caused a significant reduction in the frequency of tongue preneoplasms. The incidences of tongue severe dysplasia in the high dose group was significantly smaller than the low dose group ($p<0.05$). These findings suggest that broccoli, is effective in inhibiting the development of oral mucosa neoplasms induced by 4-NQO.
Key words: *Brassica oleracea L., 4-Nitroquinoline-1-oxid, Oral carcinogenesis, Rat*

Received 29/08/2013 Accepted 04/10/2013

INTRODUCTION

Oral cancer accounts for almost 275,000 new cases worldwide each year [1]. The main risk factors for developing oral cancer are tobacco smoking and consumption of alcohol [2]. Continuation of smoking during treatment can lead to increased morbidity and mortality [3]. Primary treatment is associated with high morbidity and function loss of the organ, while up to 50% die due to recurrence [4]. Present-day treatment, surgery, radiotherapy or chemotherapy or a combination of these three, has had notwithstanding the improvements, no significant improvement in survival [5]. However, there has not been any kind of long-term treatments reporting success in preventing second primary cancers. New therapies can be investigated both *in vitro* and *in vivo*. The drawback of *in vitro* laboratory study is the dissimilarity between the cell culture and the physiological processes giving misleading results. Several animal models for oral squamous cell carcinoma are used including hamster, rat and mouse models, with each model having its own advantages and disadvantages [6, 7].

In a large number of epidemiological data on the relationships between diet and cancer, a protective effect of the consumption of vegetables and fruits on various forms of cancer including oral cancer is found [8-10].

In fact, several natural compounds present in fruits and vegetables are reported to inhibit chemical carcinogenesis [11-16]. Thus, vegetables and fruits are rich sources for chemopreventive agents against certain cancers [15,17,18]. Recent studies indicate that some flavonoids in daily diets have an inhibitory effect on oral carcinogenesis [13,19,20, 15, 21, 22].

Cruciferous vegetables, belong to family cruciferae in particular of the Brassica genus such as broccoli, cauliflower, kale and brussels sprouts, have significant cancer preventive effects, as shown in epidemiological and animal carcinogenesis studies [23]. They contain substantial quantities of isothiocyanates (mostly in the form of their glucosinolate precursors) some of which (e.g., sulforaphane or 4-methyl sulfinyl butyl isothiocyanate) are very potent inducers of phase 2 enzymes [24]. *Brassica oleracea* L. var. italica (broccoli) is a good source of health promoting compounds since it also contains a variety of polyphenolics [25]. The cancer protective properties of broccoli consumption are most likely mediated through bioactive compounds that induce a variety of physiologic functions including acting as direct or indirect antioxidants, regulating enzymes and controlling apoptosis and the cell cycle [26]. Broccoli also contains other protective constituents like beta-carotene, vitamin C and vitamin E, which can help to reduce reactive oxygen species level and prevent cancers [27]. Broccoli showed its antioxidant and cytoprotective efficacy against many diseases such as parkinson's disease [28], breast cancer [29], bladder cancer [30], prostate cancer [31], lung cancer [32], renal cancer [33], hepatic cancer [34], skin cancer [35], brain injury [36] and cholesterol [37].

In this study, the preventive efficacy of broccoli ethanolic extract (EEB) was evaluated in a 4-nitroquinoline 1-oxide (4-NQO)-induced oral carcinogenesis model. 4-NQO was used as a carcinogen because it is known to produce a spectrum of preneoplastic and neoplastic lesions in the oral cavity [19, 38]. Of particular interest is that oral lesions induced in rats by oral administration of 4-NQO in drinking water have been reported to be similar to lesions in human [39, 40].

MATERIALS AND METHODS

Animals

The study was conducted in male Wistar rats, aged 10 weeks, with an average weight of 220 g, provided by Central Animal House Facility of Drug Applied Research Center-Tabriz University of Medical Science. A prior approval was obtained from the Animal Ethics Committee of Islamic Azad University of Tabriz (IAUT) for the study protocol. The animals were maintained under the standard conditions of humidity, temperature (25±2^{oC}) and light (12 h light/12 h dark), and fed with commercial pellet diet and water ad *libitum*.

Plant extract and Chemicals

Plant material, *Brassica oleracea* L. var. italica, (broccoli) was purchased from the local market, Tabriz, Iran and authenticated by the Pharmacognosy Department of Tabriz University of Medical Science. Ethanolic extract of broccoli (EEB) was prepared by soxhlet method using 500 ml ethanol (95%) for 100 g (dry weight) of plant material. Extract was concentrated in water bath to semisolid form. The yield of extract was 19.80%. Chemical profile of broccoli extract has been described by various researchers and main identified constituents are glucocynolates, tocopherols, carotenoids, polyphenolics, etc. [56]..

4-Nitroquinoline-1-oxid (4NQO) obtained from Sigma Aldrich Co., St. Louis, MO, USA).

Study design

48 male Wistar rats were randomly allocated into four groups (1-4) of 12 animals each. Group 1 served as control and was given the basal diet and tap water without 4-NQO. Rats from Groups 2 to 4 assigned to receive 30 ppm 4-NQO in drinking water for 12 weeks. When the feeding of 4-NQO was started the rats of groups 3 and 4, received EEB at a dose of 200 and 300 mg/kg/b.w. in intraperitoneal rout [57] respectively, 3 times per week.

At the end of experiment, the mean body weights of the rats were calculated in order to evaluate the potential toxicities on body weights of rats. The animals were euthanized and the tongues were removed. Tissue specimens collected from tongues, were fixed in 10% buffered formalin, embedded in paraffin and 5 µm thick microscopic sections were prepared through hematoxylin-eosin staining method. Histological evaluations for carcinogenesis were performed for tongues. Epithelial lesions of the tongue were diagnosed according the criteria described by Baonczy and Csiba [41], Kramer et al. [42] and Schoop et al [6].

Statistical analysis

Statistical analysis on the incidence of lesions was performed using Fisher's exact probability test or Chi-square test and the data including body weight were compared by ANOVA test and Tukey post-hoc test. The results were considered statistically significant if the P value was 0.05 or less.

RESULTS

The mean body weights at the end of the study are indicated in Table 1. The mean body weights of rats in group 2, 3 and 4 were significantly lower than that of group 1 (p<0.05). There were no significant differences among the groups 2-4 from this view point. In the present study, after 12 weeks treatment, hyperplasia and three types of dysplasia (mild, moderate and severe dysplasia), that are considered to be

preneoplastic lesions for oral cancer were present in the tongue of rats in group 2 through 4, but not in rats of group 1. Almost all rats in group 2 had hyperplasia and all types of dysplasia. Tongues in group 4 did not show severe dysplastic changes although at the same time hyperplasia without atypia and mild to moderate dysplastic changes were detected. These incidences were significantly smaller than those of group 2, with statistical differences between groups 2 and groups 1, 4 and 3 (p<0.001, p<0.01 and p<0.05, respectively). The frequencies of hyperplasia and dysplasia in group 3 were significantly lower than in group 2 (p<0.05). The incidence of moderate dysplasia in rats of group 4 was significantly smaller than of group 3 (P<0.05). However, only two rats from group 4 (given EEB 300 mg/kg during 4-NQO administration) had moderate dysplastic changes. The incidences of such lesions are listed in Table 2 and Table 3.

Microscopically, no histological changes in tongue base epithelia were observed in the control group (Figure 1a). In spite of severe epithelial hyperplasia of tongue, evidences of severe dysplasia in tongue base epithelia as a preneoplastic change was identified in group 2 (4-NQO alone) (Figure 1b). Loss of polarity of epithelium and development a number of small cell nests in spinous layer with primary keratin pearl formation were found in rats with severe dysplasia (Figure 2a). Moreover, formations of severe exophytic hyperplasia in epithelial tissue of tongue tip were found in group 2 (Figure 2b). In addition to moderate to severe hyperplasia and hyperkeratosis throughout the whole thickness of tongue epithelia, moderate to severe dysplasia of tongue base epithelia was also found in group 3 (4-NQO +200 mg/kg EEB), (Figure 1c). The Mild to moderate histological changes including hyperplasia, and hyperkeratosis with thickened spinous cell layer was evidenced after 12 weeks treatment in tongue base epithelia of group 4 (4-NQO +300 mg/kg EEB). In this group, tongue base epithelial dysplasia was mainly found in mild to moderate forms (Figure 1d).

Table 1- Mean body weights in experimental groups

Group	Treatment	Mean Weight
1	Control	224.38±15.52[a]
2	4-NQO alone	193.67±10.15[b]
3	4-NQO+ EEB 200 mg/kg	201.33±12.63[b]
4	4-NQO+ EEB 300 mg/kg	207.56±14.28[b]

Different superscripts shows significant differences by ANOVA test (followed by Duncan posttest) (P<0.05)

Table 2- Incidence of tongue preneoplastic changes of rats given 4-NQO plus EEB

Group	Treatment	No. of rats		
		Normal	Hyperplasia	Dysplasia
1	Control	12/12[a]	0/12[a]	0/12[a]
2	4-NQO alone	0/12	12/12	10/12
3	4-NQO+ EEB 200 mg/kg	2/12[c]	10/12[c]	7/12[c]
4	4-NQO+ EEB 300 mg/kg	4/12[b]	8/12[b]	5/12[b]

Different superscript shows significantly different from group 2 by Fisher's exact probability test ([a] P<0.001, [b] P<0.01 and [c] P<0.05).

Table 3- Incidence of tongue dysplasia in rats given 4-NQO plus EEB

Group	Treatment	No. of rats with dysplasia	No. of rats		
			Mild dysplasia	Moderate dysplasia	Severe dysplasia
1	Control	0/12	0/12[a]	0/12[a]	0/12[a]
2	4-NQO alone	10/12	2/12	4/12	4/12
3	4-NQO+ EEB 200 mg/kg	7/12	2/12	3/12[c]	2/12[b]
4	4-NQO+ EEB 300 mg/kg	5/12	3/12 [b]	2/12[b]	0/12[a]

Different superscript shows significantly from group 2 by Fisher's exact probability test ([a] P<0.001, [b] P<0.01 and [c] P<0.05).

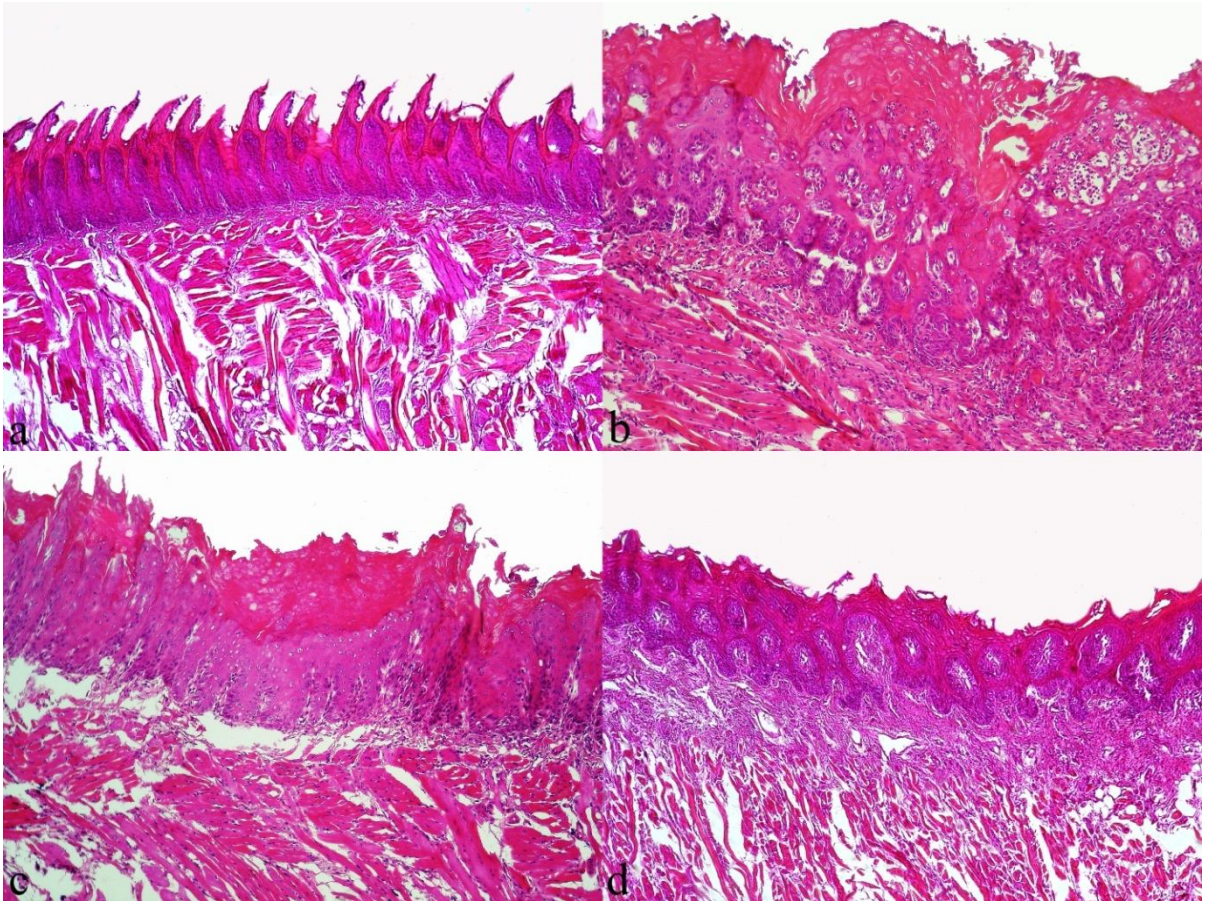

Figure 1- Histologic appearance of tongue epithelium of rats 12 weeks after starting the experiment (H&E staining, Original magnification ×40). (**a:** control group**)** Normal tongue with no histological changes. (**b:**4-NQO**)** Severe dysplasia and hyperkeratosis. (**c:** 4-NQO+200 mg/kg EEB**)** Moderate dysplasia and hyperkeratosis. (**d:** 4-NQO+300 mg/kg EEB**)** Mild dysplasia and hyperkeratosis.

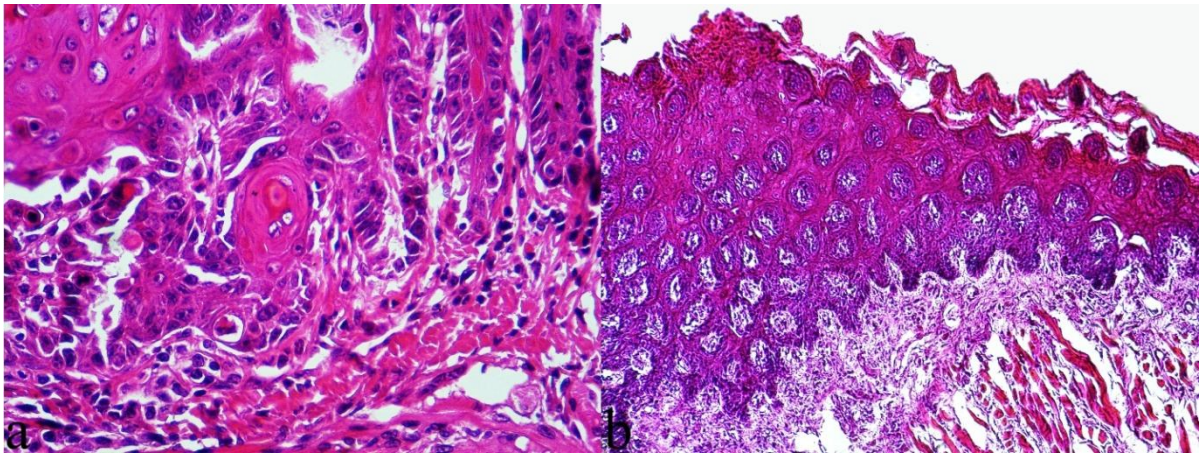

Figure 2- Histologic appearance of tongue epithelium of rat 12 weeks after starting the treatment with 4-NQO (H&E staining). **a:** Loss of polarity of epithelium and development a number of small cell nests in spinous layer with primary keratin pearl formation (Original magnification ×250). **b:** Development of exophytic hyperplasia in epithelial tissue of tongue tip (Original magnification ×10).

DISCUSSION

Significant reductions in body weights of the rats in the treatment groups compared to control rats and no statistical difference in the body weights of rats among them with pre-neoplastic lesions at week 12, indicates that administration of EEB at these dosages have no side effects on the rats and this alterations is due to pre-neoplastic lesions induced by 4-NQO.

The results in the present study demonstrated that EEB effectively suppressed 4-NQO-induced oral carcinogenesis as revealed by reduction in the incidence of tongue epithelial dysplasia. This may suggest that such a short-term pilot study may be useful for detecting compounds possessing 'blocking [43]' chemopreventive property against oral carcinoma. Vegetable and fruits contain a variety of compounds that inhibit mutagenesis and/or carcinogenesis in laboratory animals [44]. Broccoli is rich in several potent anticancer substances such as indolesglucosinolates, beta-carotene [46]. Broccoli is known for containing isothiocyanates and indoles namely indole-3-carbinol (I3C) are phytochemicals that are well-known protectors against the development of cancer suggesting that greater intakes of these vegetables may lower the risk of several types of cancer including Bladder cancer, Prostrate cancer, Breast cancer, Non-Hodgkin's lymphoma. It also contains lutein, another phytochemical with health benefits [45]. Broccoli contains the compound glucoraphanin, leading to an anticancer compound sulforaphane [46]. Thus, it is likely that EEB is applicable for human clinical trials, although no or less toxicity of the compound should be confirmed by the bioassay.

The results of the present study indicate that administration of EEB at the dose levels of 200 and 300 mg/kg during 4-NQO-induced oral tumorigenesis could effectively suppress tumour development. Several mechanisms by which EEB exerts its anticarcinogenic action in oral carcinogenesis could be considered. Although prevention of carcinogenesis might be due to multiple mechanisms, one way of action of anticarcinogens is an enhancing effect on carcinogen detoxification systems. Fruits and vegetables that elevate tissue phase II enzyme levels in rodents can effectively block experimental carcinogenesis and increase the clearance of drugs in human [43,12, 47]. It is reported that broccoli-related compounds exert anti-carcinogenic effects through modulation of phase II enzymes [48, 49]. Broccoli contains substantial quantities of isothiocyanates (mostly in the form of their glucosinolate precursors) some of which (e.g., sulforaphane or 4-methylsulfinylbutyl isothiocyanate) are very potent inducers of phase 2 enzymes [24]. Other possible mechanism is anitioxidant properties of broccoli. The ability of antioxidants to scavenge free radicals and thereby decrease the amount of free radical damage to biological molecules like lipids and DNA may be one of their protective mechanisms [50]. We propose that the additive and synergistic antioxidant activity of phytochemical such as flavonoids, phenols, alkaloids, triterpenoids, steroids, etc, present in *Brassica oleracea* Italica are responsible for the its potent antitumor activity [51, 52, 53]. Induction of apoptosis and antiproliferative activity of broccoli may also contribute its antitumor effects. The antiproliferative activity of broccoli sprout extracts is associated with induction of apoptosis and cell cycle arrest. It has been shown that broccoli sprout extracts preferentially activate the mitochondria-mediated apoptosis pathway and arrest cells in S and M phases. The latter is associated with down-regulation of cell division cycle 25C (Cdc25C) and disruption of the mitotic spindle assembly [54]. Broccoli sprouts are an exceptionally rich source of sulforaphane [1-isothiocyanato4-(methylsulfinyl) butane], a well-known cancer chemopreventive isothiocyanate [24]. Sulforaphane has since been rigorously and extensively studied in many laboratories [55], and additional chemopreventive mechanisms have been discovered, among which the induction of apoptosis and arrest of cell cycle progression have been shown to occur without cell and tissue specificity.

In summary, administration of EEB significantly inhibited 4-NQO-induced tongue tumorigenesis, in conjunction with reduction in the frequency of dysplastic lesions. Although additional studies on dose-dependent efficacy and the mechanistic basis of inhibition should be done, the results described here indicate that EEB might be candidate for a chemopreventive agent against oral carcinogenesis.

ACKNOWLEDGMENTS

The authors would like to thank Tabriz Branch, Islamic Azad University for the financial support of this research.

REFERENCES

1. Parkin DM, Bray F, Ferlay J and Pisani P. 2005. Global cancer statistics, (2002). *CA Cancer J Clin*, 55(2), 74–108.
2. Boffetta P and Hashibe M. 2006. Alcohol and cancer. *Lancet Oncol*, 7(2),149–156.
3. Videtic GM, Stitt LW, Dar AR, Kocha WI, Tomiak AT, Truong PT *et al*. (2003). Continued cigarette smoking by patients receiving concurrent chemoradiotherapy for limited stage small-cell lung cancer is associated with decreased survival. *J Clin Oncol*, 21(8), 1544–1549.
4. Monnerat C, Faivre S, Temam S, Bourhis J and Raymond E. (2002). End points for new agents in induction chemotherapy for locally advanced head and neck cancers. *Ann Oncol*, 13(7), 995–1006.
5. Cooper JS, Pajak TF, Forastiere AA, Jacobs J, Campbell BH, Saxman SB *et al*. (2004). Postoperative concurrent radiotherapy and chemotherapy for high-risk squamous-cell carcinoma of the head and neck. *N Engl J Med*, 350(19), 1937–1944.
6. Schoop RA, Noteborn MH, Baatenburg de Jong RJ. (2009). A mouse model for oral squamous cell carcinoma. *J Mol Hist*, 40:177–181.

7. Zhang Z, Wang Y, Yao R, Li J, Lubet RA, You M. (2006b). p53 Transgenic mice are highly susceptible to 4-nitroquinoline-1-oxide-induced oral cancer. *Mol Cancer Res*,4(6):401-10.
8. Steinmetz KA and Potter JD. (1991). Vegetables, fruits, and cancer. II. Mechanisms. *Cancer Causes Control*, 2(6), 427–442.
9. Block G, Patterson B and Subar A. (1992). Fruit, vegetables, and cancer prevention: a review of the epidemiological evidence. *Nutr Cancer*, 18, 1–29.
10. Hebert JR, Landon J and Miller DR. (1993). Consumption of meat and fruit in relation to oral and esophageal cancer: a cross-national study. *Nutr Cancer*, 19, 169–179.
11. Wattenberg L. (1993). Chemoprevention of carcinogenesis by minor nonnutrient constituents of the diet. In: Parke DV, Ioannides C and Walker R, editors. Food, Nutrition and Chemical Toxicity. London, Niigata, pp. 287–300.
12. Steinmetz KA and Potter JA. (1991). Vegetables, fruits, and cancer. *I Epidemiology Cancer Causes Control*, 2, 325–357.
13. Tanaka T. (1995). Chemoprevention of oral carcinogenesis. *Oral Oncol Eur J Cancer*, 31B, 3–15.
14. Tanaka T, Makita H, Ohnishi M, Mori H, Satoh K, Hara A, et al. (1997). Chemoprevention of 4-nitroquinoline 1-oxide-induced oralcarcinogenesis by citrus auraptene in rats. Cancer Res,57(2): 246-252.
15. Cassady JM. (1990). Natural products as a source of potential cancer chemotherapeutic and chemopreventive agents. *J Natl Prod*, 53, 23–41.
16. Hocman G. (1989). Prevention of cancer: vegetables and plants. *Comp Biochem Physiol*, 93B, 201–212.
17. Tanaka T. (1997a). Effect of diet on human carcinogenesis. *Crit Rev Oncol/Hematol*, 25, 73–95.
18. Tanaka T. (1997b). Chemoprevention of human cancer: Biology and therapy. *Crit Rev Oncol/Hematol*, 25, 139–174.
19. Tanaka T, Makita H, Ohnishi M, Hirose Y, Wang A, Mori H et al. (1994). Chemoprevention of 4-Nitroquinoline 1-oxide-induced oral carcinogenesis by dietary curcumin and hesperidin: comparison with the protective effect of carotene. Cancer *Res*, 54, 4653–4659.
20. Makita H, Tanaka T, Fujitsuka H, Tatematsu N, Satoh K, Hara A *et al*. (1996). Chemoprevention of 4-nitroquinoline 1-oxide-induced rat oral carcinogenesis by dietary flavonoids chalcone, 2-hydroxychalcone, and quercetin. *Cancer Res*, 56, 4904–2909.
21. Tanaka T, Makita H, Ohnishi M, Mori H, Satoh K, Hara A *et al*. (1997). Chemoprevention of 4nitroquinoline 1-oxide-induced oral carcinogenesis in rats by flavonoids diosmin and hesperidin, each alone, and in combination. *Cancer Res*, 57(2): 246–252.
22. Tanaka T, Kawabata K, Kakumoto M, Matsunaga K, Mori H, Murakami A, et al. (1998). Chemoprevention of 4-nitroquinoline 1-oxide-induced oral carcinogenesis by citrus auraptene in rats. *Carcinogenesis,* 19(3):425-31
23. Verhoeven DT, Verhagen H, Goldbohm RA, Van Den Brandt PA and Van Poppel G.1997. A review of mechanisms underlying anti-carcinogenecity by Brassica vegetables. *Chem Biol Interac*, 103, 79–129.
24. Fahey JW, Zhang Y and Talalay P. (1997). Broccoli sprouts: An exceptionally rich source of inducers of enzymes that protect against chemical carcinogens. *Proc Natl Acad Sci USA*, 94(19), 10367–10372.
25. Moreno CJ, Prats D, Moral R, Perez- Murcia MD, Perez-Espinosa A, Paredes C *et al*. (2006). Effects of linear alkylbenzene sulfonates (LASs) in sewage sludge amended soils on nutrient contents of broccoli plants. *Commun in Soil Sci and Plant Anal*, 37(15-20), 2605–2614.
26. Finley JW, Sigrid-Keck A, Robbins RJ and Hintze KJ. (2005). Selenium enrichment of broccoli: interactions between selenium and secondary plant compounds. *J Nutr*, 135(5), 1236–1238.
27. Chiu B and Houghton P. (2005). Investigation of common vegetables for cholinesterase inhibitory activity. Proceeding of 142nd Brit Pharma Confer; 25-28 September 2005, Manchester, United Kingdom, pp. 151.
28. Han JM, Lee YJ, Lee SY, Kim EM, Moon Y, Kim HW *et al*. (2007). Protective effect of sulforaphane against dopaminergic cell death. *J Pharmacol and Exper Therap*, 321(1), 249–256.
29. Singletary K and MacDonald C. (2000). Inhibition of benzo[a]pyrene- and 1,6-dinitropyrene-DNA adduct formation in human mammary epithelial cells by dibenzoylmethane and sulforaphane. *Cancer Lett*, 155(1), 47–54.
30. Zhang Y, Munday R, Jobson HE, Munday CM, Lister C, Wilson P *et al*. (2006a). Induction of GST and NQO1 in cultured bladder cells and in the urinary bladders of rats by an extract of broccoli (Brassica oleracea italica) sprouts. *J Agric and Food Chem*, 54 (25), 9370–9376.
31. Canene AK, Lindshield BL, Wang S, Jeffery EH, Clinton SK and Erdman JW. (2007). Combinations of tomato and broccoli enhance antitumor activity in dunning r3327-h prostate adenocarcinomas. *Cancer Res*, 67(2), 836–843.
32. Ritz SA, Wan J and Diaz-Sanchez D. (2007). Sulforaphane-stimulated phase II enzyme induction inhibits cytokine production by airway epithelial cells stimulated with diesel extract. *Am J Physiol Lung Cell and Mol Physiol*, 292, 33–39.
33. Bosetti C, Scotti L, Maso LD, Talamini, R, Montella M, Negri E *et al*. (2007). Micronutrients and the risk of renal cell cancer: A case-control study from Italy. *I J Cancer*, 120(4), 892–896.
34. Kensler TW, Chen J-G, Egner PA, Fahey JW, Jacobson LP, Stephenson KK *et al*. (2005). Effects of glucosinolate-rich broccoli sprouts on urinary levels of aflatoxin-DNA adducts and phenanthene tetraols in a randomiz ed clinical trial in He Zuo Township, Qidong, PRC. *Cancer Epidemiol Biomark and Prevent,* 14(11), 2605–2613.
35. Talalay P, Fahey JW, Healy ZR, Wehage SL, Benedict AL, Min C *et al*. Sulforaphane mobilizes cellular defenses that protect skin against damage by UV radiation. *Proc Nat Acad of Sci*, 104(44), 17500–17505.
36. Zhao J, Moore AN, Redell JB and Dash PK. (2007). Enhancing expression of Nrf2-driven genes protects the blood-brain barrier after brain injury. *The J Neurosci*, 27 (38), 10240–10248.

37. Murashima M, Watanabe S, Zhuo XG, Uehara M and Kurashige A. (2004). Phase 1 study of multiple biomarkers for metabolism and oxidative stress after one-week intake of broccoli sprouts. *Bio Fac*, 22, 271–275.
38. Tanaka T, Kojima T, Kawamori T, Wang A, Suzui M, Okamoto K *et al*. (1993). Inhibition of 4-nitroquinoline 1-oxide-induced rat tongue carcinogenesis by the naturally occurring plant phenolics caffeic, ellagic, chlorogenic and ferulic acids. Carcinogenesis, 14, 1321–1325.
39. Steidler NE and Reade PC. (1984). Experimental induction of oral squamous cell carcinomas in mice with 4-nitroquinolone-1-oxide. *Oral Surg*, 57, 524–532.
40. Nauta JM, van Leegoed HLLM, Witjes MJH, Nikkels PGJ, Star WM, Vermey A *et al*. (1997). Photofrinmediated photodynamic therapy of chemically-induced premalignant lesions and squamous cell carcinoma of the palatal mucosa in rats. *Int J Oral Maxillofac Surg*, 26, 223–231.
41. Banoczy J and Csiba A. (1976). Occurrence of epithelial dysplasia in oral leukoplakia. *Oral Surg*, 42, 766–774.
42. Kramer IR, Lucas RB, Pindborg JJ and Sobin LH. 1978. WHO Collaborating Centre for Oral Precancerous Lesions: definition of leukoplakia and related lesions: an aid to studies on oral precancer. *Oral Surg*, 46, 518–539.
43. Wattenberg LW. (1985). Chemoprevention of cancer. *Cancer Res*, 45, 1–8.
44. Carr BI. (1985). Chemical carcinogens and inhibitors of carcinogenesis in the human diet. *Cancer (Phil.)*, 55, 218–224
45. Sandra Bastin. (2001). Vegetables for Wellness Kentucky Broccoli. Agriculture and natural resources, Science daily.com.
46. Zhang Y, Kensler TW, Cho CG, Posner GH and Talalay P. (1994). Anticarcinogenic activities of sulforaphane and structurally related synthetic norbornyl isothiocyanates. *Proc Natl Acad Sci USA*, 91, 3147–3150.
47. Sparnins VL, Venegas PL and Wattenberg LW. (1982). Glutathione Stransferase activity: enhancement by compounds inhibiting chemical carcinogenesis and by dietary constituents*. J Natl Cancer Inst*, 68, 493–496.
48. Zhang Y, Talalay P, Cho CG and Posner GH. (1992). A major inducer of anticarcinogenic protective enzymes from broccoli: Isolation and elucidation of structure. *Proc Natl Acad Sci USA*, 89, 2399–2403.
49. Prochaska HJ, Santamaria AB and Talalay P. (1992). Rapid detection of inducers of enzymes that protect against carcinogens. *Proc Natl Acad Sci USA*, 89, 2394–2398.
50. Percival M. (1998). Antioxidants: clinical nutrition insights. Advanced Nutrition Publications, Inc., 1996, Revised.
51. Borowski J, Szajdek A, Borowska EJ, Ciska E and Zieliski H. (2008). Content of selected bioactive components and antioxidant properties of broccoli (*Brassica oleracea* L.). *Eur Food Res Technol*, 226, 459–465.
52. Gülçin I, Sat IG, Beydemir S and Küfrevioglu ÖI. (2004). Evaluation of the in vitro antioxidant properties of broccoli extracts (*Brassica oleracea* L.). *Ital J Food Sci*, 1, 17–30.
53. Piao X, Kim HY, Yokozawat LYA, Piao XS and Cho EJ. (2005). Protective effects of broccoli (Brassica oleracea) and its active components against radical-induced oxidative damage. *J Nutr Sci Vitaminol*, 51, 142–147.
54. Tang L, Zhang Y, Jobson HE, Li J, Stephenson KK, Wade KL *et al*. (2006). Potent activation of mitochondria-mediated apoptosis and arrest in S and M phases of cancer cells by a broccoli sprouts extract. *Mol Cancer Ther*, 5, 935–944.
55. Zhang Y. (2004). Cancer chemoprevention with sulforaphane, a dietary isothiocyanate. In: Bao Y, Fenwick R, editors. Phytochemicals in health and disease. New York, Marcel Dekker, pp. 121–141.
56. Eberhardt MV, Kobira K, Keck AS, Juvik JA and Jaffery E. (2005). Correlation analyses of phytochemical composition, chemical and cellular measurement of antioxidant activity of broccoli (*Brasssica oleracea* L. var. italica). *J Agri and Food Chem*, 53, 7421–7431.
57. Vadivel S and Gowry S. (1999).Antitumor Activity and Antioxidant Role of Brassica oleracea Italica against ehrlich ascites Carcinoma In Swiss Albino Mice. *Res J Pharm Biol Chem Sci*, 2(3), 275–285.

How to cite this article

Jamal Agha Mohammadi, Daryoush Mohajeri, Ramin Kaffashi Elahi. Histopathological study on Inhibitory effects of *Brassica oleracea* L. Var. Italica (Broccoli) Ethanolic extract on 4-Nitroquinoline-1-oxid -induced Pre-neoplastic changes in the Rat. Bull. Env. Pharmacol. Life Sci., Vol 2 (11) October 2013: 100-106