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Research Article

Chemopreventive potential of Geraniol on 4-Nitroquinoline-1-oxide induced oral carcinogenesis in rats

Arumugam Madankumar, Subramaniyan Jayakumar, Selvamani Asokkumar, Subramanian Raghunandhakumar, Chandrashekar Naveenkumar, Thiruvengadam Devaki*

Department of Biochemistry, University of Madras, Guindy Campus, Chennai-600 025, Tamil Nadu, India

ABSTRACT

The present study was aimed to investigate the chemopreventive potential of geraniol against 4Nitroquinoline-1-oxide induced oral carcinogenesis. Geraniol (GOH) and other monoterpenes found in essential oil of fruits and herbs have been suggested to represent a new class of agents for cancer chemoprevention. As a first step in clarifying, the chemopreventive potential of GOH we have analyzed its effect on tumor incidence, lipid peroxidation and level of antioxidants such as superoxide dismutase (SOD), catalase (CAT), Glutathione-S-transferase (GST), Glutathione peroxidase (Gpx) and Reduced Glutathione (GSH). Administration of geraniol (25mg/100g b.w.) effectively suppressed 4-NQO induced oral carcinogenesis. The results of the present study suggest that GOH may exert its chemopreventive effect by modulating lipid peroxidation and enhancing the level of antioxidant enzymes of the tumor bearing animals.

Keywords: Antioxidants; Chemoprevention; Geraniol; 4-Nitroquinoline-1-oxide; Oral cancer.

INTRODUCTION

Oral cancer accounts for one third of the various cancers affecting the human body. It is one of the ten leading cancers in the world (Sankaranarayanan, 1990), and shows marked geographic differences in occurrence. In India the disease presents a major health problem with 15-70% of all cancers diagnosed being found in the oral cavity (Ko, 1995). Several factors have been considered responsible for the development of oral cancer. The use of tobacco, ill-fitting dentures, poor oral hygiene, syphilis, inadequate diet, malnutrition and chronic irritation from rough or broken teeth have been shown to be more frequent in oral cancer patients (Naseem Shah, 1989). However, among these factors use of tobacco stands first, with cigarette smokers at 4-7 times higher risk of developing the disease compared to non-smokers (Ko, 1995). The prevalence of the disease in diverse parts of the world reflects different forms and extents of exposure to these etiological agents (Hsieh, 2001).

4NQO is a water soluble carcinogen and it is known to induce multistep carcinogenesis in rats (Takashi, 1992). The chronic administration of 4NQO in drinking water stimulates rat tongue carcinogenesis similar to its human counterpart (Hendler, 1996) and serves as a good experimental model to investigate oral carcinogenesis (Srinivasan, 2006). 4NQO exerts potent intracellular oxidative stress and its metabolites bind to the DNA predominantly at guanine residues. These insults appear similar to damage imposed by other carcinogen present in tobacco, which is the major risk factor for oral cancer (Deepak kanojia, 2006).

Oxygen free radicals are natural physiological products; they are also responsible for the production of reactive oxygen species (ROS). Many studies have proved that ROS causes enormous cellular damage by modifying the bio molecules and impairing cellular function (khandrika, 2009). Excessive intracellular levels of ROS, as well as a defective antioxidant system, can give rise to pathological conditions including inflammation, atherosclerosis, angiogenesis, aging, and cancer (Behrend, 2003; Apel, 2004). These ROS are responsible for oxidation of tissues leading to lipid peroxidation and tissue damage (Bergamini, 2004). They are also responsible for the oxidation of bases in cellular DNA leading to multiplication of cells resulting in cancer (Fridorich, 1986). The modern approach to chemoprevention of cancer deals with identification of biologic modifiers that function as specific scavenger of ROS, and thereby reduce free radical induced oxidative stress, and restores a balanced antioxidant defense system (Crohns, 2009).

Recent studies have shown that a number of dietary monoterpenes posses antitumor activities and the ability to prevent the formation and/or progression of cancer. Therefore these components are considered as a new class of chemopreventive agents (kelloff, 1996).

* Corresponding Author
Email: biochemtdt@gmail.com
Contact: +91-9942110146 Fax: 91-44-22352494
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Geraniol (GOH) an acyclic dietary monoterpene found in lemon (Carnesecchi, 2001), lemongrass and aromatic herb oil exerts antitumor activity against various cells both in vivo and in vitro (Mo, 2004). The antitumor potential of GOH against murine leukemia, hepatoma, and melanoma cells has been previously documented (Shoff, 1991). But there are no studies, available on the potential effects of GOH against oral carcinogenesis. Therefore the present study was designed to carry out a systematic investigation of the protective role of GOH against 4NQO induced oral carcinoma by estimating the level of lipid peroxidation and antioxidant activity.

MATERIALS & METHODS

Reagents and chemicals

4NQO and Geraniol were purchased from Sigma Aldrich chemicals Pvt ltd (Bangalore, India). All other chemicals used were of analytical grade.

Animals

Wistar strain male albino rats, 8 weeks old (140- 150g) were purchased from TANUVAS, Chennai, India. The animals were housed four per cage in a room with controlled temperature and humidity with 12 h light: dark cycles. All the animals were given a standard rat feed (Hindustan liver ltd, Bangalore) and tap water. The experimental designs were approved by the institutional animal ethical committee of University of Madras. (IAEC No. 02/019/2010).

Experimental design

The animals were divided into four groups of six animals each

Group 1 (control), Animals received corn oil (0.25ml/100g b.w.) twice a week orally for 22 weeks.

Group 2 (4NQO), Oral carcinoma was induced by administration of 50ppm 4NQO solution by drinking water for 22 weeks.

Group 3 (4NQO + GOH), Animals were treated with GOH (25mg/100g b.w. dissolved in corn oil 0.25ml/100g b.w.) twice a week orally. GOH treatment was started one week prior to the first dose of 50ppm 4NQO administration (as in group 2) for 22 weeks.

Group 4 (GOH alone), Animals were treated with GOH (25mg/100g b.w. dissolved in corn oil 0.25ml/100g b.w.) twice a week orally for 22 weeks to assess the cytotoxicity if any, induced by GOH, and rats were referred as drug control.

After the experimental period, the animals were anesthetized using ether and sacrificed by cervical decapitation. The mouth was cut opened using a surgical knife, the tongue was excised out, weighed and the tissue was homogenised in 0.1M Tris-Hcl buffer pH-7.4 and centrifuged at 3000 rpm for 10min. the supernatant was used for the biochemical studies.

Lipid peroxidation, evidenced by the formation of thiobarbituric acid reactive substance (TBARS) was assayed in tissue sample as described by Ohkawa (1974). The activity of antioxidant enzymes was assayed; superoxide dismutase (SOD) by the method of Markulund (1974), catalase (CAT) was by the method of Sinha (1972), glutathinone peroxidase (Gpx) by the method

Table 1: Effect of GOH on 4-nitroquinoline 1-oxide induced oral carcinogenesis in rats; body weight, liver weight and relative liver weight of control and experimental animals

Group No	Treatment	No of animals	Body weight	Liver weight	Relative liver weight (g/100g) b.w.
1	Control	6	286.40±1.87	3.21±0.31	1.15±0.12
2	4NQO	6	252.42±8.20[a]	4.10±0.45[a]	1.45±0.21[a]
3	4NQO+GOH	6	269.10±7.90[a,b]	3.65±0.35[a,b]	1.39±0.19[a,b]
4	GOH alone	6	285.80±1.35[b,c]	3.19±0.18[b,c]	1.14±0.06[b,c]

Each value is expressed as Mean ±SD for six animals in each group;

Statistical significance $p < 0.05$ compared with [a]group 1, [b]group 2, and [c]group 3 based on Duncan's multiple range test.

Table 2: Effect of GOH on 4-Nitroquinoline-1-oxide induced oral carcinogenesis in rats; incidence of preneoplastic and neoplastic lesions

Group No	Treatment	No of animals	No. of animals (%)		
			Preneoplastic lesions		Carcinoma
			Hyperplasia	Dysplasia	
1	Control	6	0	0	0
2	4NQO	6	6 (100) [a]	6(100) [a]	5(88) [a]
3	4NQO+GOH	6	4(45) [a,b]	2(48) [a,b]	1(13) [a,b]
4	GOH alone	6	0	0	0

Each value is expressed as Mean ±SD for six animals in each group;

Statistical significance $p < 0.05$ compared with [a]group 1, [b]group 2, based on Duncan's multiple range test.

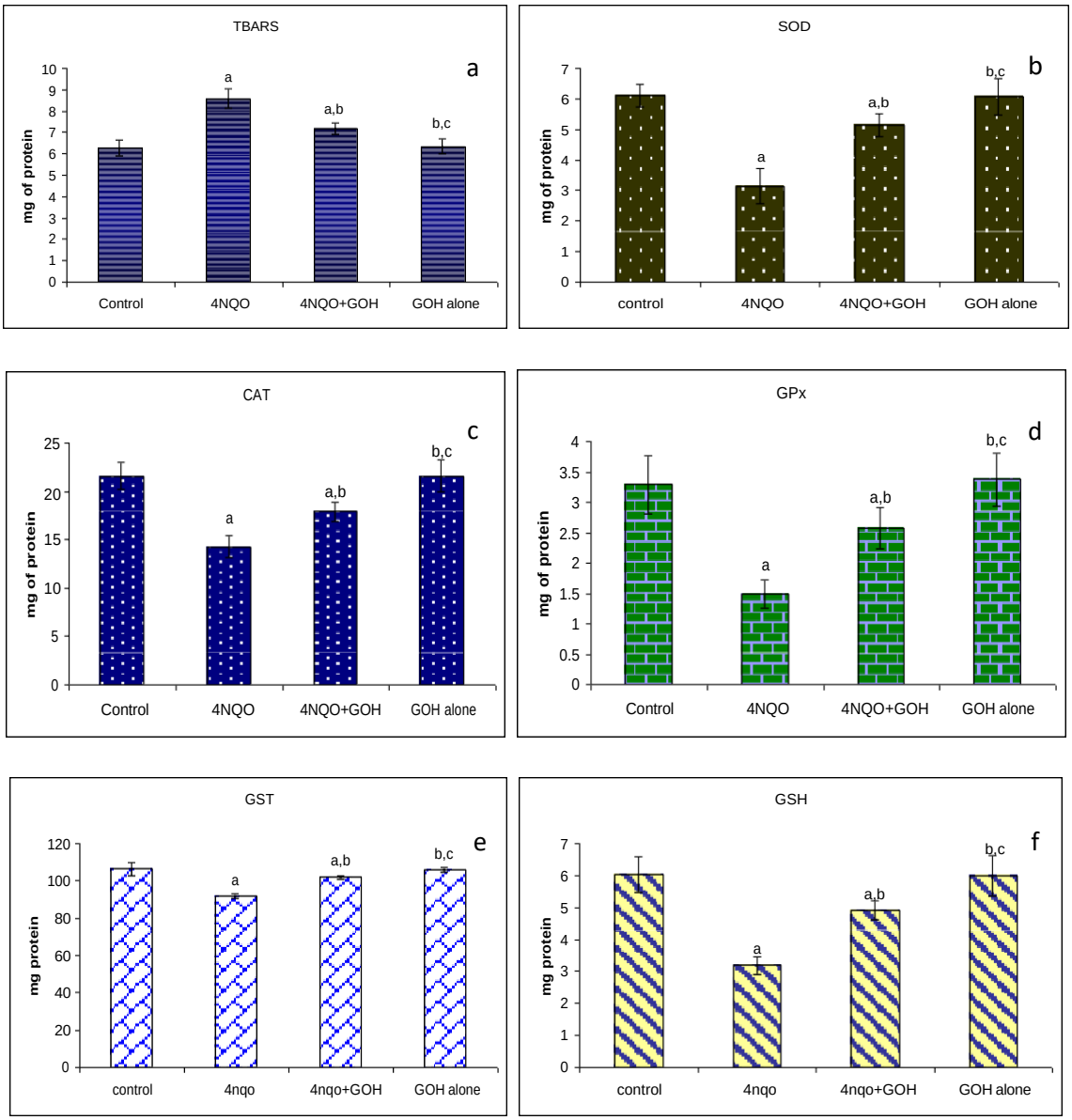

Figure 1: (a-f) shows the level of lipid peroxidation and antioxidants such as SOD, CAT, GPx, GSH and GST in the tongue of control and experimental animals

Results are expressed as mean ± S.D for six rats in each group. Statistical significance $p < 0.05$ compared with [a]group 1, [b]group 2, and [c]group 3.

of Rotruck (1973), reduced glutathione (GSH) was by the method of Ellman, (1959) and glutathione-S-Transferase (GST) by the method of Habig (1974).

Statistical analysis

The data is expressed as mean ± SD. Statistical comparisons were performed by one-way analysis of variance (ANOVA), followed by Ducan's multiple range test. The results were considered statistically significant if the p values were 0.05 or less.

RESULTS

Table 1 represents the body weight, liver weight and relative liver weight of the control and experimental rats. Body weights were noted periodically once a week until completion of the experimental period.

Control rats did not show significant change in the body weight throughout the experimental period. There was significant decrease in the body weight of the oral carcinoma bearing group 2 and 3 rats, when compared with normal control rats. GOH treated group 3 animals showed gradual increases in their body weight when compared with cancer bearing group 2 animals. There is no significant difference was observed among GOH alone treated group 4 rats and group 1 control rats. And there was significant increase in the liver weight and relative liver weight of the oral carcinoma bearing group 2 and 3 rats, when compared to normal control rats. GOH treated group 3 showed a gradual decrease in their liver weight when compared with cancer bearing group 2 animals. GOH alone treated group 4 rats were closer to the control rats.

Table 2 compares the incidence of oral neoplasm's and preneoplastic lesion between the experimental groups. In group 2, the incidence of preneoplastic and squamous cell carcinomas were 100% and 88%, respectively, whereas in group 3, the incidence of squamous cell carcinoma was 23%. No premalignant carcinoma were observed in group 1 and 4.

Figure 1(a) shows the level of lipid peroxidation as evidenced by the formation of TBARS, in tongue of control and experimental rats in each group. In group 2 rats, the lipid peroxidation levels have significantly increased ($p<0.05$) when compared with control group 1 rats. Whereas in GOH treated group 3 rats, lipid peroxidation was significantly decreased when compare to induced group 2 rats, but in GOH alone treated group 4 rats, these levels near to control rats. Figure 1 (b-f) shows the status of antioxidants in the tongue of control and experimental rats in each group. There is significant reduction in the activity of SOD, CAT, GPx, GST and GSH in oral cancer bearing group 2 rats when compared with control rats. There is significant increases in the activity of these antioxidant levels in GOH treated group 3 rats when compared to group 2 rats, there is no significant difference were observed among GOH alone treated group 4 rats and group 1 control rats.

DISCUSSION

Oral squamous cell carcinoma, one of the most common cancers in the world (Srinivasan, 2006), is the third leading cancer in Chennai, India (Shanta, 1994). Chemoprevention appears to be a logical approach in the prevention of cancer, and controlling the complex series of genetic and epigenetic events of carcinogenesis (Sporn, 2002). A large number of studies have been conducted for the assessment of efficacy of chemopreventive agents using 4NQO induced oral carcinogenesis model. The present study investigates the chemopreventive efficacy of GOH against 4NQO induced oral cancer in rats.

Weight loss is one of the most frequent adverse systemic effects of malignancy (Dewys, 1980). A decline in food intake relative to energy expenditure is the fundamental physiological derangement resulting in cancer associated weight loss (Mulligan, 1991; Pain, 1984). Such weight loss was controlled in GOH treated group 3 animals which showed gradual increase in body weight compared to group 2 animals during the study period. This could be attributed to antineoplastic property of the drug.

Many studies reveal that nodules are the precursors of cancer and that the severity of the disease may correlate with the size and number of nodules (Bull, 2000). The significantly reduced incidence and delayed onset of tumor as observed by the reduced morphological changes in the GOH treated group 3 animals compared to group 2 animals further establishes the chemopreventive potential of GOH against 4NQO induced oral cancer.

Lipid peroxidation plays a key role in the initiation and progression of carcinogenesis (Diplock, 1994). It is an important cause of cell membrane damage since it has been shown that lipid peroxidation degrades poly unsaturated fatty acids of the cell membrane with consequent disruption of membrane integrity (Niki, 1987). The present study demonstrates that in group 3 animals GOH treatment was able to effectively control lipid peroxidation compared to group 2 animals.

Reactive oxygen species (ROS) are constantly generated and eliminated in the biological system, and play important roles in a variety of normal biochemical functions and abnormal pathological processes (Buechter, 1988). Growing evidence suggests that cancer cells exhibit increased intrinsic ROS stress. Oxygen derived species such as superoxide radical, hydrogen peroxide, singlet oxygen and hydroxyl radical are well known to be cytotoxic and have been implicated in the etiology of cancer (Halliwell, 1999). Carcinogens may also partly exert their effect by generating reactive oxygen species (ROS) during their metabolism. Antioxidants play an important role in protecting the cells from oxidative damage (Manokaran, 2005). Antioxidant enzymes scavenge intermediates of oxygen reduction process and provide the primary defence against cytotoxic oxygen radicals. Therefore in the present study the effect of GOH treatment on the activities of antioxidants enzymes like SOD, CAT, Gpx, GSH, and GST were studied. SOD is the only enzyme that disturbs superoxide radical and is present in all cells (Gunasekaran, 2010). Decreased SOD activity had been reported in various cancerous conditions (Van driel, 1997). The present study is also showing decreased activity of SOD in cancer bearing group 2 animals, where as the GOH treated group 3 animals show increased activity of SOD.

CAT thought to be the first line of defence against oxidative damage caused by hydrogen peroxide, protects the cell from highly reactive hydroxyl radicals (Daisy glory, 2011). Several reports have cited decreased activities of SOD and CAT in various carcinogenic conditions (Floyd, 1982). In the present study group 2 animals showed decreased CAT activity, which could be due to the utilization of this enzyme in the removal of highly produced hydrogen peroxide radicals induced by 4NQO administration, where as the GOH treated group 3 animals showed significantly increased activity of CAT.

GSH along with Gpx is involved in detoxification of hydrogen peroxide (Gunasekaran, 2010). Glutathione is an important non protein thiol and in conjugation with Gpx and GST it plays an important role in protecting cells against cytotoxic and carcinogenic chemicals by scavenging reactive oxygen species (Meister, 1994). Decreased expression of GSH, as well as Gpx and GST has been reported in malignancies (Saroja, 1999). In

the present study also decreased level of these enzymes was observed in oral cancer bearing group 2 animals, whereas in GOH treated group 3 animals, the activity of these enzymes is comparatively increased. The GOH alone treated group 4 animals used as drug control were similar to the group 1 control animals interms of body weight, levels of lipid peroxidation and antioxidant status which suggests that GOH may not have any cytotoxic effect.

CONCLUSION

To conclude, the present study shows that GOH is effective in controlling lipid peroxidation and ROS production by enhancing the activity of cellular antioxidants, there by protecting cells and tissues from the cytotoxic effect of carcinogens. The anticancer effect of GOH is further attributed to the antineoplastic potential of the compound evident from its ability to control the number and spread of tumor. Thus the results suggest that Geraniol could be a potential chemopreventive against oral carcinogenesis.

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