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Biochemical Studies on the Cytoprotective Efficacy of Geraniol in Benzo(a)pyrene Induced Experimental Lung Cancer

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ABSTRACT

Isoprenoids are one of the largest groups of natural products comprising numerous compounds with important roles in physiological and pathological processes. High intake of fruits and vegetables has proven to show protective action against different cancer types. Diet-derived isoprenoids represent promising cancer therapeutic agents. Monoterpenes such as Geraniol (GOH), found in essential oils of citrus fruits, cherry, mint, and herbs, are non-nutritive dietary micro constituents mainly responsible for the distinctive fragrance of many plants; have anticancer activities. In the present study the cytoprotective efficacy of geraniol, an acyclic monoterpene alcohol was evaluated in Benzo(a)pyrene [B(a)P] induced lung cancer in male Swiss Albino mice. Level of antioxidants such as superoxide dismutase (SOD), catalase (CAT), Glutathione peroxidase (GPx), Reduced Glutathione (GSH), Vitamin C, Vitamin E, glycoproteins and Lung marker enzymes were studied. Oral administration of Geraniol (150 mg/kg body weight) helped in maintaining the cellular redox status of the animals which plays an important role in cellular function there by increasing the efficiency of antioxidant defense system and reducing the adverse effects of cancer.

Key Words: Antioxidants, Isoprenoids, Benzo(a)Pyrene, Geraniol, lung cancer.

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INTRODUCTION

Lung cancer is the leading cause of cancer deaths in both men and women. It is a disease in which the cells of lung tissues grow uncontrollably and form tumors it occurs most commonly between the ages of 45 and 70. Although lung cancer can be divided into many subtypes, the most important distinction is between SCLC and non-small cell lung carcinoma (NSCLC). Only 1 in 10 lifetime smokers will develop lung cancer. Lung cancer is initiated by activation of oncogenes or inactivation of tumor suppressor genes.[1] Susceptibility to lung cancer may be modulated by host-specific factors including differences in carcinogen metabolism and detoxification, DNA repair, cell cycle control, cell signaling, apoptosis, and inflammation pathways. Tobacco smoke contains over 60 established carcinogens. Among the constituents of smoke the polycyclic aromatic hydrocarbons (PAHs) such as benzo(a)pyrene, play a major role in lung carcinogenesis.[2] Benzo(a)pyrene is metabolized to (T)-B[a]P-r-7,t-8-dihydrodiol-t-9,10-epoxide (BPDE), the ultimate carcinogen. BPDE isomers then bind to the exocyclic nitrogen of deoxyguanosine in DNA via trans-addition of the C-10 position in the epoxide molecule. This adduct may also cause activation of protooncogenes. [3]

Isoprenoids or the so called terpenoids form the most numerous class of natural compounds with a total number of structures exceeding 50,000. Lower terpenoids (monoterpenes and sesquiterpenes) are volatile compounds of the scents and fragrances of aromatic plants. These monoterpenoids are frequently chemically modified after their initial biosynthesis to give them highly specific biological activities. Acyclic monoterpene geraniol (GOH) is a colourless liquid with a flowery rose-like odour. It is found in almost all the essential oils and is an important constituent of essential oil of palmrosa, ginger, lemon, lime, lavender, nutmeg, orange, rose etc. Experimental studies demonstrated several pharmacological activities including antioxidant and anticancer potential of GOH.[4] Geraniol exerted anti-tumor activity against various cancer cells both in vitro and in vivo.[5, 6, 7] It has also been reported that GOH exhibited potent insecticidal, antimicrobial, anti-inflammatory, and anti-proliferative effects.[8] Profound studies have demonstrated the free radical scavenging properties of GOH. But no studies are currently available on the protective effect of GOH against lung carcinogenesis. The present study is an effort to analyze the cytoprotective efficacy of GOH on B (a) P induced lung cancer in male Swiss Albino mice.

MATERIALS AND METHODS

Chemicals

Benzo(a)pyrene and Geraniol was purchased from sigma chemical company St. Louis, MO, USA. All other chemicals used were of analytical grade.

Animals

Healthy male Swiss Albino Mice (6-8 weeks old) were used throughout the study. The animals were purchased from Central Animal House Facility, Dr.ALM PG IBMS, University of Madras, Taramani, and Chennai-600113 and were maintained in a controlled environmental condition of temperature and humidity on alternatively 12 h light/dark cycles. All animals were fed standard pellet diet (Gold Mohor rat feed, Ms. Hindustan Lever Ltd., Mumbai) and water ad libitum. The experimental designs were approved by the institutional animal ethical committee. (IAEC No.02/025/09).

Experimental design

The animals were divided into four groups of six animals each.

Group I (control) -Animals received corn oil (vehicle) orally for 4 weeks.

Group II (B(a)P) -Animals received B(a)P (50 mg/kg body weight dissolved in corn oil) orally twice a week for four successive weeks to induce lung cancer.

Group III (B(a)P + GOH) - B(a)P treated animals were treated with GOH (150 mg/kg body weight dissolved in corn oil) orally on alternate days for a period of two weeks.

Group IV (GOH alone) - Animals were treated with GOH alone (as in group III) for two weeks.

After the experimental period the animals were sacrificed by cervical decapitation and lung tissues were immediately excised, weighed, and processed for homogenization with motor driven Teflon coated homogenizer in ice-cold 0.1 M Tris-HCl buffer pH 7.4 to get 10% homogenate. The lung tissue homogenate was used for the following biochemical estimations.

Biochemical analysis

Total protein was estimated by the method of Lowry et al.[9].The activity of superoxide dismutase (SOD),[10] catalase (CAT), [11] Glutathione peroxidase (GPx),[12] Glutathione reductase (GR), [13] reduced glutathione (GSH), [14] .Lipid peroxides (LPO) was estimated by the method of Ohkawa et al., [15] ,Vitamin C [16] and vitamin E [17].The marker enzyme, γ – glutamyl transpeptidase (GGT) was estimated according to the method of Orlowski and Meister [18] modified by Rosalki and Rao, the aryl hydrocarbon hydroxylase (AHH) was estimated by Mildred et al., [19] and the activity of lactate dehydrogenase (LDH) was assayed by the method of King.[20]

To the weighed amount of the defatted tissue, 2ml of 4N HCl was added and the mixture was refluxed at 100 degree Celsius for 4 hours in a test tube with suitable marble lids. The hydrolysate was neutralized with sodium hydroxide. Aliquots of the neutralized samples were

taken for the analysis. Hexose level was estimated by the method of Neibes, [21] hexosamine by the method of Wagner, [22] and for Sialic acid a weighed amount of defatted tissue was hydrolyzed with 1.0 ml of 0.1 N sulphuric acids at 80 degree Celsius for 60 minutes to release sialic acid bound to the proteins. The solution was then neutralized with sodium hydroxide. Sialic acid was estimated by the method of Warren.[23]

Statistical analysis

Results are expressed as mean ± standard deviation (S.D). Statistical comparisons were performed by one-way analysis of variance (ANOVA), followed by Duncan's multiple Range Test (DMRT).The results were considered statistically significant if the p values were 0.05 or less.

RESULTS AND DISCUSSION

Figure1 (a) and 1(b) shows the effect of GOH on the activities of enzymic antioxidants in the lung of control and experimental animals. The activities of SOD and GR were found to be significantly ($p<0.001$) decreased in cancer induced group (G-II) when compared with the control group (G-I). On administration of GOH (G-III), there found to be a significant ($p<0.05$) increase in these enzyme activities. There found to be no significant difference in the enzymic activities between the control animals and the control treated with GOH (G-IV).

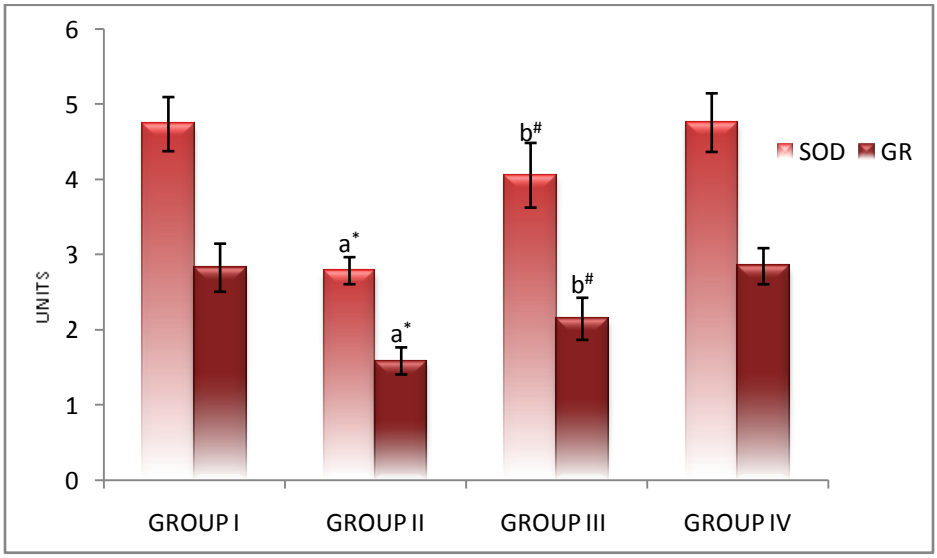

Figure 1(a): Effect of GOH on enzymic antioxidants, SOD and GR in the lung tissue of control and experimental animals

Each value is expressed as mean ± S.D. for six mice in each group.

a: Group I compared with Group II

b: Group II compared with Group III

Units: Superoxide Dismutase – one enzyme unit = amount of enzyme required to prevent 50% auto oxidation/min/mg protein; Glutathione Reductase-n moles of NADPH oxidized/min/mg protein. Statistical significance: $^{*}p<0.001$, $^{\#}p<0.05$

In Figure 1(b), the activities of CAT and GPx were found to be significantly ($p<0.001$) decreased in cancer induced group (G-II) when compared with the control group (G-I). On administration of GOH (G-III), there found to be a significant ($p<0.05$) increase in these enzyme activities. There found to be no significant difference in the enzymic activities between the control animals and the control treated with GOH (G-IV).

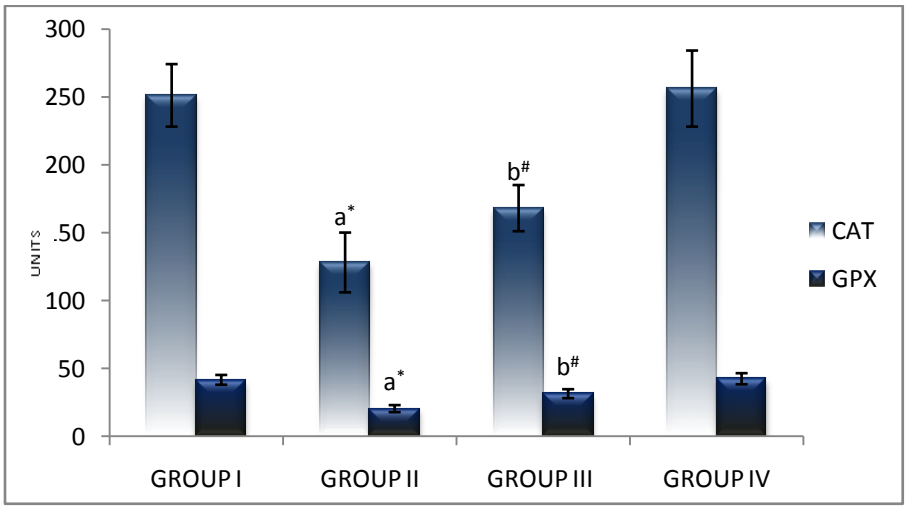

Figure 1(b): Effect of GOH on enzymic antioxidants, CAT and GPx in the lung tissue of control and experimental animals

Each value is expressed as mean ± S.D. for six mice in each group.

a: Group I compared with Group II

b: Group II compared with Group III

Units: Catalase – nmoles of H_2O_2 consumed/min/mg protein; Glutathione Peroxidase-μmoles of GSH oxidized/min/mg protein.

Statistical significance: $^{*}p<0.001$, $^{\#}p<0.05$

Our present study is designed with a well-known terpenoid GOH found in commonly used herbs.[24] B(a)P, a well-identified environmental carcinogen is known to produce enormous amounts of free radicals and these free radicals and non-radical oxidizing species are highly reactive, toxic and mutagenic. Antioxidant enzymes are the main scavengers of free radicals and function as the inhibitors at both initiation and promotion or transformation stages of carcinogenesis.[25] The antioxidant enzymes SOD, CAT and GPx play an important role as protective enzymes against reactive oxygen species in tissues and also comprise the cellular

antioxidant defense system.[26] Antioxidant status has been suggested as a useful tool in estimating the risk of oxidative damage induced carcinogenesis. Enzymatic antioxidants like SOD, CAT, and GPx synergistically scavenge reactive oxygen species (ROS) and prevent LPO. Our study shows a reduction in the activities of the enzymic antioxidants SOD, CAT and GPx in lung cancer bearing animals. On terpenoidal treatment, the activities of these enzymes inclined to near normal. The effect of reactive oxygen is balanced by the antioxidant action of non-enzymatic antioxidants, as well as by antioxidant enzymes. Such antioxidant defenses are extremely important as they respect the direct removal of free radicals (pro oxidants), thus providing maximal protection for biological sites. The most efficient enzymatic antioxidants involve SOD, CAT and GPx.[27] Non enzymatic anti-oxidants involve Vitamin C, Vitamin E and GSH.[28]

Figure 2 represents the effect of GOH on the levels of non-enzymic antioxidants such as GSH, vitamin E and vitamin C in the lung of control and experimental animals. The levels of these antioxidants were found to be significantly ($p<0.001$) decreased in cancer bearing group (G-II) when compared with the control group (G-I). On administration of GOH (G-III), there found to be a significant ($p<0.05$) increase in the levels of these antioxidants when compared with cancer bearing animals. There found to be no significant difference in the antioxidant levels between the control animals (G-I) and drug control animals treated with GOH (G-IV).

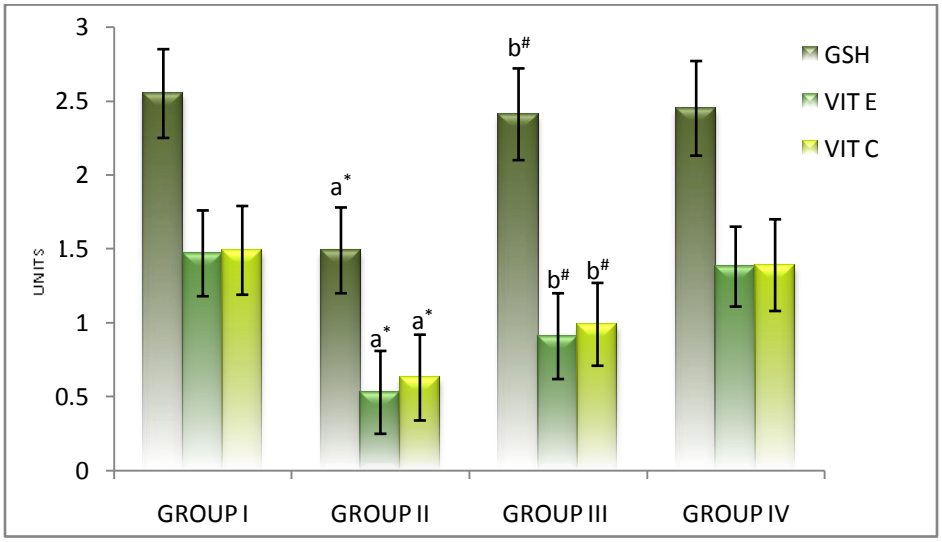

Figure 2: Effect of GOH on non-enzymic antioxidants in the lung tissue of control and experimental animals

Each value is expressed as mean ± S.D. for six mice in each group.

a: Group I compared with Group II

b: Group II compared with Group III

Units: GSH - µmol/g tissue; Vitamin-E - µg/g tissue; Vitamin-C -µg/g tissue.

Statistical significance: $^{*}p<0.001$, $^{\#}p<0.05$

Vitamin C, Vitamin E and GSH comprise the non-enzymic antioxidants, which protects the cells against the deleterious effects of free radicals. It has been reported that GOH enhances GSH almost by two – fold than other antioxidants and at the same time it decreases the levels of GSSG, the oxidized product of GSH. Since GOH possesses antioxidant property, in the present study, it could have been attributed to the maintenance of the endogenous GSH antioxidant balance against B(a)P mediated cellular oxidation which protected the GSH related enzymes and in turn inhibited the oxidation of GSH.

Figure 3 depicts the effect of GOH on the levels of lipid peroxidation in the lung of control and experimental animals. There found to be a significant ($p<0.001$) increase in the levels of lipid peroxidation in the lung of cancer bearing group II animals when compared with the control group (G-I). GOH (G-III) treatment caused a significant ($p<0.05$) decrease in their levels when compared with cancer bearing animals. However, there was found to be no significant difference in the levels of the lipid peroxidation between the control animals and drug control animals treated with GOH (G-IV).

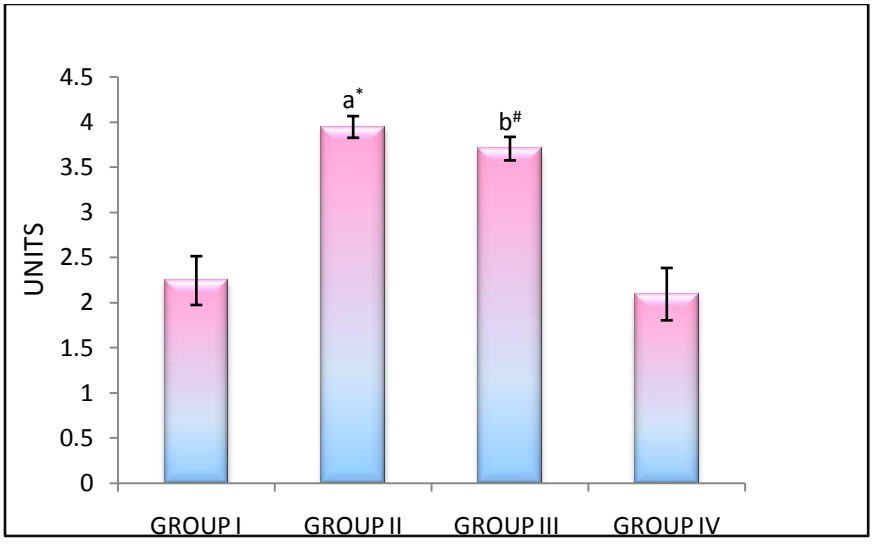

Figure 3: Effect of GOH on the level of Lipid Peroxidation in lung tissue of control and experimental animals.

Each value is expressed as mean ± S.D. for six mice in each group.

a: Group I compared with Group II

b: Group II compared with Group III

Units: n moles of MDA formed/min/mg protein.

Statistical significance: $^{*}p<0.001$, $^{\#}p<0.05$

Peroxidation of unsaturated lipids has been suspected in contributing the process of cancer development.[29] Marnett et al., (1975) revealed that through lipid peroxidation, a proximate carcinogen, 7,8 – dihydroxy 7,8 dihydrobenzo(a)pyrene is converted to dihydrodiol epoxide, which are regarded as the ultimate carcinogenic form of B(a)P. Lipid peroxidation – quinone formation seem to contribute to the carcinogenic activation of B(a)p.[30] In the present study, an increased level of lipid peroxidation was formed during lung carcinogenesis.

Figure 4 shows the effect of GOH on the levels of glycoproteins in lung of control and experimental groups. The levels of glycoproteins were found to be significantly ($p<0.001$) increased in cancer bearing group (G-II) when compared with the control group (G-I). On administration of GOH (G-III), there found to be a significant ($p<0.05$) decrease in the levels of these glycoproteins when compared with cancer bearing animals. There found to be no significant difference in the glycoprotein levels between the control animals (G-I) and drug control animals treated with GOH (G-IV).

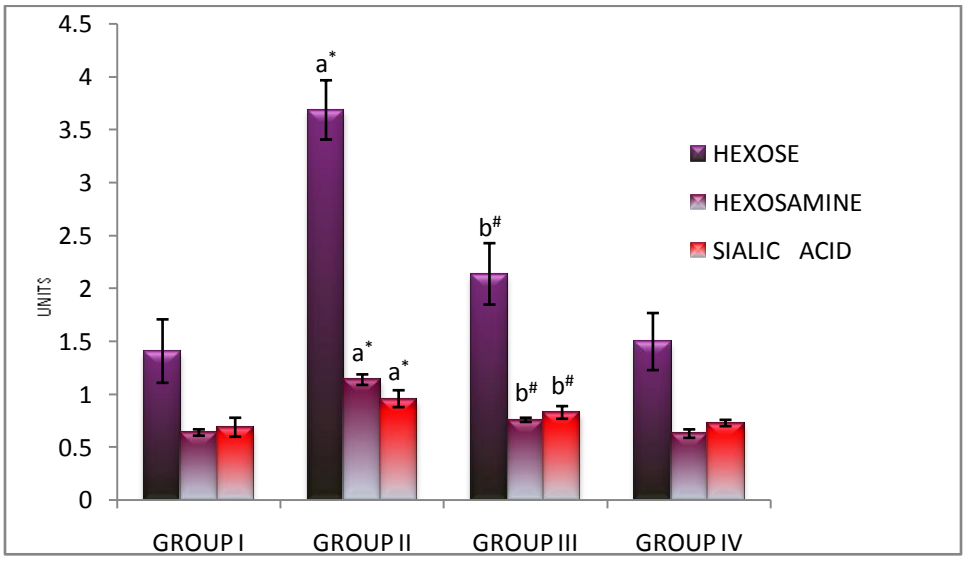

Figure 4: Effect of GOH on glycoproteins in the lung tissue of control and experimental animals

Each value is expressed as mean ± S.D. for six mice in each group.

a: Group I compared with Group II

b: Group II compared with Group III

Values are expressed as mg g^{-1} of defatted tissue.

Statistical significance: $^{*}p<0.001$, $^{\#}p<0.05$

Glycoproteins are essential for cell to cell communications which are found on the surface of all the cells and some are released in to the blood stream and other body fluids.[31] Malignant

transformation of normal cell may be accompanied by changes in the $(CH_2O)n$ of glycoprotein viz., hexose, hexosamine and sialic acid in the plasma membrane. The reduction in the levels of glycoprotein components indicates that GOH might have inhibited the synthesis of glycoproteins during malignant transformation, probably by modulating the activities of enzymes involved in glycosylation process thus decreasing the degree of lung cancer growth and controlling cell proliferation.

Figure 5 represents the effect of GOH on the levels of tissue marker enzymes in the lung of control and experimental animals. There found to be a significant ($p<0.001$) increase in the levels of lung tissue marker enzymes AHH, GGT and LDH in the lung of cancer bearing group II animals when compared with the control group (G-I). GOH (G-III) treatment caused a significant ($p<0.05$) decrease in their levels when compared with cancer bearing animals. However, there was found to be no significant difference in the levels of the enzyme between the control animals and drug control animals treated with GOH (G-IV).

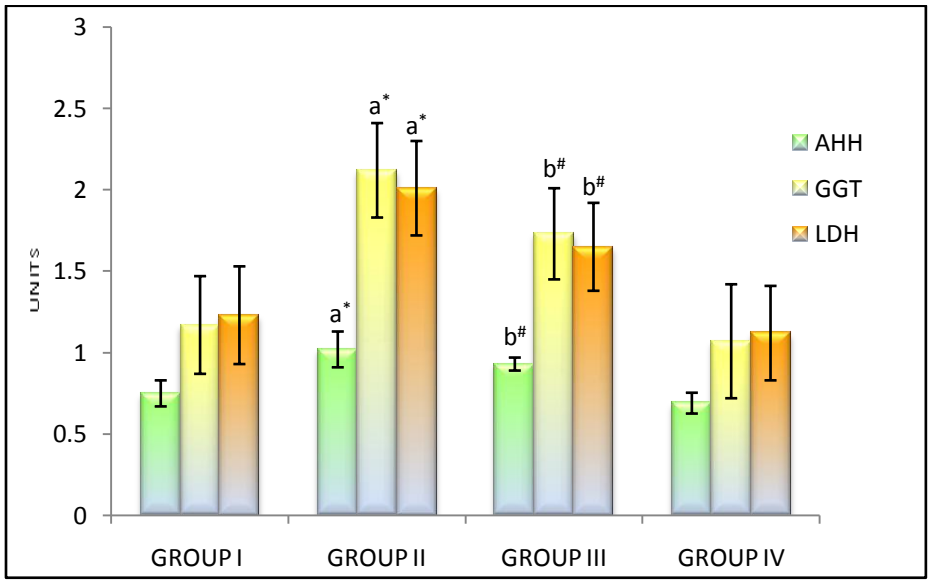

Figure 5: Effect of GOH on marker enzymes in the lung tissue of control and experimental animals

Each value is expressed as mean ± S.D. for six mice in each group.

a: Group I compared with Group II

b: Group II compared with Group III

Units: AHH – micro-moles of fluorescent phenolic metabolites formed/min/mg protein; GGT – nano-moles of p-nitroaniline formed/min/mg protein; LDH – micro-moles of pyruvate liberated/min/mg protein.

Statistical significance: $^{*}p<0.001$, $^{\#}p<0.05$

Tumor markers are biological or biochemical substances produced by tumors that are secreted into blood or body tissues in higher than normal amounts. The first tumor marker introduced into medical science was a proteinurea used by Dr Bence-Jones from a patient with multiple myeloma.The activities of marker enzymes were found to be elevated in tissues of lung carcinoma bearing animals, which could be due to the destruction of the neoplastic tissue. The abnormal variations in the marker enzymes reflect the overall change in metabolism that occurs during malignancy.[32] The marker enzymes such as AHH, ADA, GGT, and LDH are specific indicators of lung damage.[33] The increase in the activities of these enzymes may be due to the increased tumour incidence. Chen and Liu reported that AHH is a useful biomarker in the early diagnosis of lung cancer.[34] GOH treatment has strongly shown to inhibit AHH activity.

GGT is a broad specificity transferase that catalyses the transfer of gamma glutamyl groups from a large variety of peptide donors to a wide range of amino acids and peptide receptors.[35] GGT is not only useful in diagnosis but also has prognostic value in malignancies such as lung cancer and malignant melanoma. The enzyme is membrane bound and its active site is oriented on the outer surface of cell membrane. γ -GT is a cell surface enzyme that cleaves extra cellular glutathione thereby providing the increased intracellular glutathione synthesis.[36] this deviation shows the progress of carcinogenic process, since its ability correlated with growth rate, histological differentiation and survival time of the host.[37]

LDH is a tetrameric enzyme and is recognized as a potential tumor marker in assessing the progression of the proliferating malignant cells. It is a fairly sensitive marker for solid neoplasma and its activities has been found to be raised in tumor bearing animals. In the present study, significant elevation in all the above tissue marker enzymes were observed in benzo(a)pyrene-administered animals. GOH treatment brought down the levels of these marker enzymes close to normal suggesting its anti-cancer potential. The decrease in the activities of above mentioned marker enzymes on treatment with GOH suggests that terpenoids offers some protection against abnormal cell growth by changing the permeability or affecting cellular growth. This may be due to the antineoplastic property of terpenoids.

CONCLUSION

To conclude, the present study indicates that GOH plays an important role against B(a)P induced lung carcinogenesis in minimizing the free radical mediated changes in the lung tissue. The possible mechanism GOH, especially in relation to antioxidant status is inhibition of free radical formation and reduced cancer incidence. It was found that GOH treatment was effective during

the post-initiation of carcinogenesis. The increase in the activity of antioxidants and a decrease in level of marker enzymes suggest that GOH reduces the adverse effects of cancer. Also, the reduction in the levels of glycoprotein components on treatment with GOH indicates that the acyclic monoterpene alcohol has the ability to suppress malignancy by modulating cell transformation by controlling cell proliferation. The results of the present study hence clearly proves that GOH plays a very important cytoprotective role against B(a)P induced lung carcinogenesis.

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