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Protective effect of sinapic acid against 7,12 dimethyl benz(a)anthracene induced hamster buccal pouch carcinogenesis

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A B S T R A C T

The aim of the present study was to evaluate the free radical scavenging and antioxidants in DMBA induced hamster buccal pouch carcinogenesis in golden Syrian hamsters. A total of 32 animals were divided into 4 groups of 8 animals each. Group I served as control and received normal saline only. Group II animals were painted with 0.5% DMBA with liquid paraffin three times a week for 16 weeks on their left buccal pouches. Groups III animals were painted with DMBA and treated with sinapic acid (50mg/kg). Group IV animals were received sinapic acid only. After16th week hamsters were sacrificed, and plasma, erythrocyte and tissues were harvested and analyzed. The results showed that oral administration of Sinapic acid at a dose of 50mg/kg body weight (bw) to DMBA treated hamsters significantly the recovered lipid peroxidation and improved antioxidants as well as modulating effects on phase I and phase II detoxification enzymes. Therefore, chemopreventive potential of Sinapic acid is probably due to its antioxidant potential and retrieve effect and detoxifying potential during DMBA-induced hamster buccal pouch carcinogenesis.

Introduction

Oral cancer is a serious and developing issue in various parts of the world, is the sixth most common cancer on the planet, and 3 – 4% of all diseases in western nations (Ferlay *et al*., 2004). Various etiologic factors have been implicated in the improvement of oral SCCs, for example, tobacco smoking, biting and alcohol abuse (Johnson *et al*., 2001).

7,12-dimethylbenz(a)anthracene (DMBA), a class of aromatic polycyclic hydrocarbons, which are repercussions of the ignition of tobacco and other natural substances(lee *et al*., 2002). This agent may prompt generation of free radicals and oxidative stress through the creation of superoxide, hydrogen peroxide and nitric oxide in cells

(Ames and Gold, 1991). Reactive oxygen species (ROS) participate in a variety of chemical reactions with biomolecules leading to a pathological condition known as oxidative stress (Cooke *et al*., 2003). Oxidative stress is initiated by free radicals like hydroxyl, peroxyl and superoxide radicals, which become stable through electron pairing with biological macromolecules such as proteins, lipids and DNA (Burton and Jauniaux, 2011). Oxidative stresses have implicated in the pathology of several human diseases, including cancer, atherosclerosis, malaria, and rheumatoid arthritis and neurodegenerative diseases. The roles of antioxidants are to neutralize the excess of free radicals, to protect the cells against their toxic effects and to contribute to disease prevention (Ganie *et al*., 2011). Vitamins C and E satisfy various natural exercises, including safe incitement, immune stimulation, scavenging free radicals and modifying the metabolic initiation of cancer-causing agents (Van Poppel and Vanden Berg, 1997). Vitamin E is a powerful oxygen radical scavenger that shields cells from cancer-causing chemicals by inhibiting LPO and free radical mediated consequences.

Recent investigations have shown that the antioxidant properties of plants could be correlated with oxidative stress defense, different human diseases and aging process. Keeping in view of the demand for developing natural antioxidants drugs, the present study was aimed to investigate its antioxidant, free radical scavenging activity using various antioxidants assays system. However, to the best of our knowledge no scientific studies has been undertaken to evaluate chemopreventive effect of sinapic acid against oral cancer. Therefore, we determine the free radical scavenging activity, antioxidant and anticarcinogenic potential of sinapic acid during 7,12 dimethylbenz[a]anthracene (DMBA)-induced hamster buccal pouch carcinogenesis.

Materials and Methods

Chemicals

Sinapic acid, DMBA, reduced glutathione (GSH), 2, 2'-dipyridyl, 2, 4-dinitro phenylhydrazine (DNPH), 5, 5'-dithiobis-2-nitrobenzoic acid (DTNB), 2, 2'-diphenyl-1-picrylhydrazyl radical (DPPH), 2, 2'-azinobis (3-ethylbenzothiazoline-6-sulfonic acid) radical (ABTS) were obtained from Sigma Chemical Co. (St. Louis, MO, USA). All other chemicals were obtained from S.D. Fine chemicals Mumbai, India and were of analytical grade.

Animals

Male golden Syrian hamsters, eight to ten weeks old, weighing 80-120g were purchased from National Institute of Nutrition, Hyderabad. The animals were housed in polypropylene cages at room temperature (27±2°C) with relative humidity (55±5%) in an experimental room, the LD (light: dark) cycle is almost 12:12h. The animals were provided with standard pellet diet (M/s Kamdhenu Ltd., Bangaluru,) and water ad libitum.. The recommendations of Institutional Animal Ethical Committee (Committee for the purpose of control and supervision of experiment on animals (Regn. no 1416/a/11/CPCSEA, Muthayammal College of Arts and Science, Rasipurasm, India) for the care and use of laboratory animals were strictly followed throughout the study.

Experimental design

A total number of 32 hamsters were divided into 4 groups of 8 animals each. Group I animals were served as untreated control.

Animals in groups II were induced oral carcinogenesis by painting with DMBA in liquid paraffin three times a week for 14 weeks. Group II received no other treatment. . Groups III animals were painted with DMBA and treated with sinapic acid (50mg/kg). Group VI animals were orally administered with Sinapic acid alone throughout the experimental period. The experiment was terminated at the end of 16th week all animals were sacrificed and samples were collected.

Biochemical assays

The reduced glutathione level was determined by the method of Beutler and Kelly (1963). The level of vitamin C was determined by the method of Omaye *et al*. (1979). Vitamin E in plasma and erythrocyte was estimated by the method of Palan *et al*. (1991) as well as in buccal mucosa was estimated by the method of Desai, (1984). Thiobarbituric Acid Reactive Substances (TBARS) in erythrocytes were estimated for method of Donnan (1950) and TBARS in plasma were assayed by the method of Yagi (1987) and in tissue was estimated by Ohkawa *et al*., (1979). Superoxide dismutase activity in plasma, erythrocyte and buccal mucosa was assayed by the method of Kakkar *et al*. (1984). The activity of catalase was assayed by the method of Sinha (1972). Cytochrome P450 and b 5 in liver and buccal mucosa tissues microsomes were estimated by the method of Omura and Sato, (1964).

Statistical Analysis

The data were expressed as mean ± S.D with 8 animals in each group. Values were analysed using SPSS/15.0 software. Hypothesis testing methods were included with analysis of variance (ANOVA) followed by least significance difference (LSD). P values of > 0.05 were considered statistically significant.

Results and Discussion

Figure 1 Shows enzymatic antioxidants such as SOD, CAT and GPx levels were decreased in plasma and erythrocytes whereas buccal tissue GPx levels were increased in DMBA treated animals. Oral administration of Sinapic acid (50 mg/kg bw) to DMBA painted animals were significantly restored the levels of SOD, CAT and GPx when compared to control animals. Animals were treated with Sinapic acid showed no significant differences were observed compared to control animals.

Figure 2 Shows plasma and erythrocyte GSH, Vit-C and Vit-E levels were decreased and simultaneously increased in buccal tissue in DMBA painted animals. Oral administration of Sinapic acid (50 mg/kg bw) to DMBA painted animals were more effective and significantly brought back the levels of GSH, Vit-C and Vit-E when compared to control animals. Animals treated with Sinapic acid alone showed no significant differences were observed compared to control animals.

Figure 3 Shows the status of phase I (cytochrome p_{450}) and phase II (GST and GR) detoxification enzymes in the buccal mucosa and liver of control and experimental hamsters in each group. The phase I enzymes activity were significantly increased in buccal and liver tissue whereas phase II enzymes were increased in buccal mucosa and simultaneously decreased in liver tissue in tumor bearing hamsters as compared to control hamsters. Oral administration of sinapic acid (50mg/kg) to hamsters treated with DMBA significantly recover the status of phase I and phase II detoxification enzymes. Animals treated

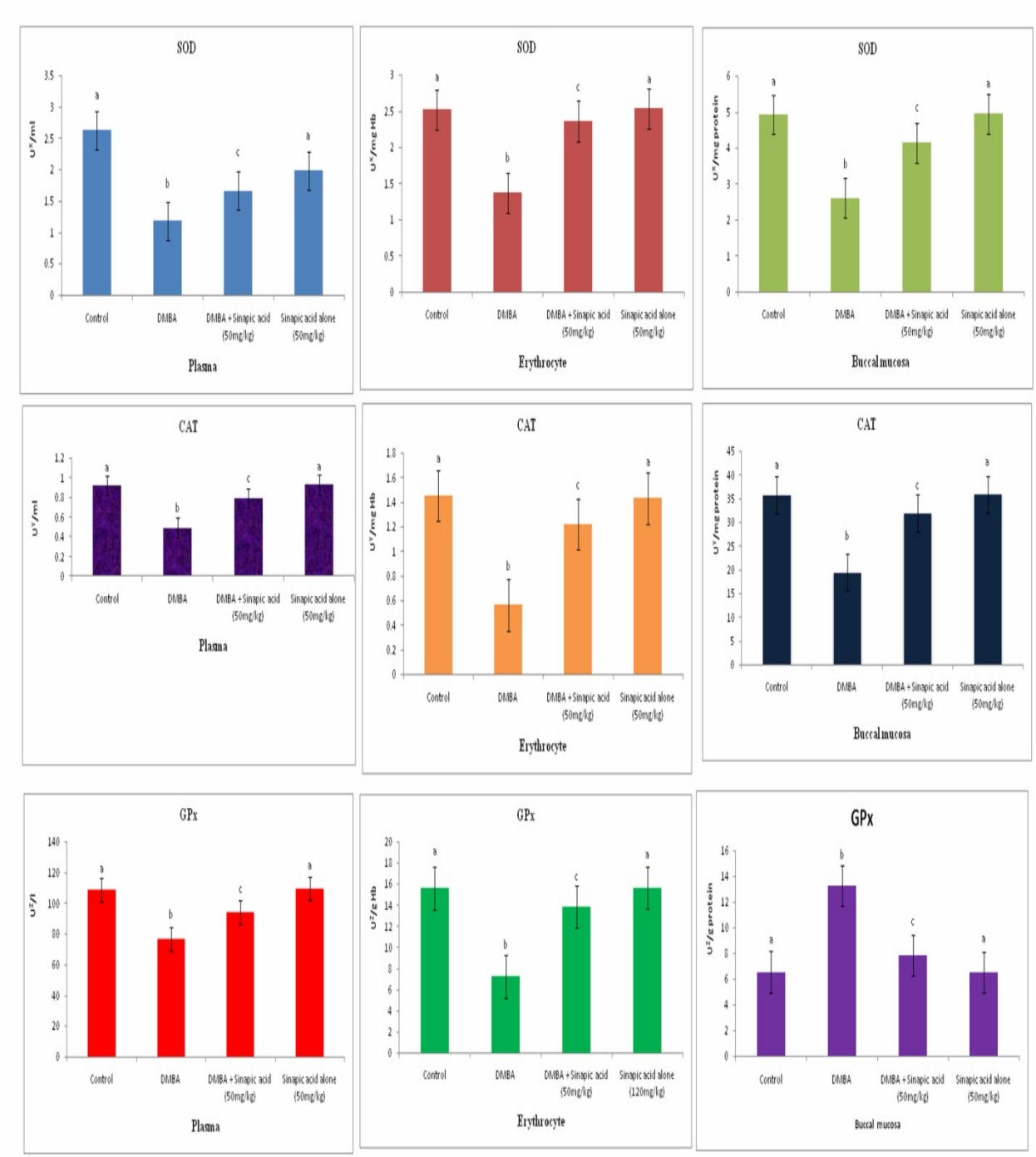
with sinapic acid alone showed no significant differences were observed compared to control animals.

In the present study, chemopreventive potential of Sinapic acid was assessed by checking status of lipid peroxidation, antioxidants and phase I detoxification proteins. Reactive oxygen species (ROS) generated by mitochondria or from other intracellular or extracellular destinations can cause cell damage and initiate diverse degradation forms (Davies, and Hochstein, 1982). ROS, for example, superoxide anion (O_2•−), singlet oxygen ($1O_2$), hydrogen peroxide (H_2O_2), and hydroxyl radicals (•OH), were primarily framed when blended capacity oxidases framework by action of polycyclic aromatic hydrocarbons like 7,12-dimethyl benz(a)anthracene (Giri *et al*., 1995). These oxyradicals can attach covalently nucleophilic locales on cell macromolecules accordingly bringing out carcinogenic reactions. Subsequently, DMBA-affected hamster buccal pouch carcinogenesis has been broadly utilized as an animal model for advancement of chemopreventive medications for oral cancer. Present study, topical application of DMBA to the hamster buccal pouch for 16 weeks brought about decently created OSCC with exceptionally mean tumor trouble associated with extreme hyperplasia, hyperkeratosis, and dysplasia.

The relationship between lipid peroxidation and the rate of cell extension, seen in our study exhibits that the tumor cells multiple broadly when lipid peroxidation is irrelevant. The thiobarbituric acid responsive substances in plasma were essentially expanded while its level in buccal tissue was fundamentally lessened in DMBA treated hamsters. The tumor cells in buccal tissue demonstrated a particularly low level of peroxidation making an ideal air for the multiplication of disease cells. Data of the present study showed that lipid peroxidation incited by oxidative stress brought about DNA harm. Oral administration of Sinapic acid altogether adjusted the lipid peroxidation and improves the status of antioxidants. The more huge impact of Sinapic acid supplementation was seen at the dosage of 50 mg/kg body weight which was comparable to control group.

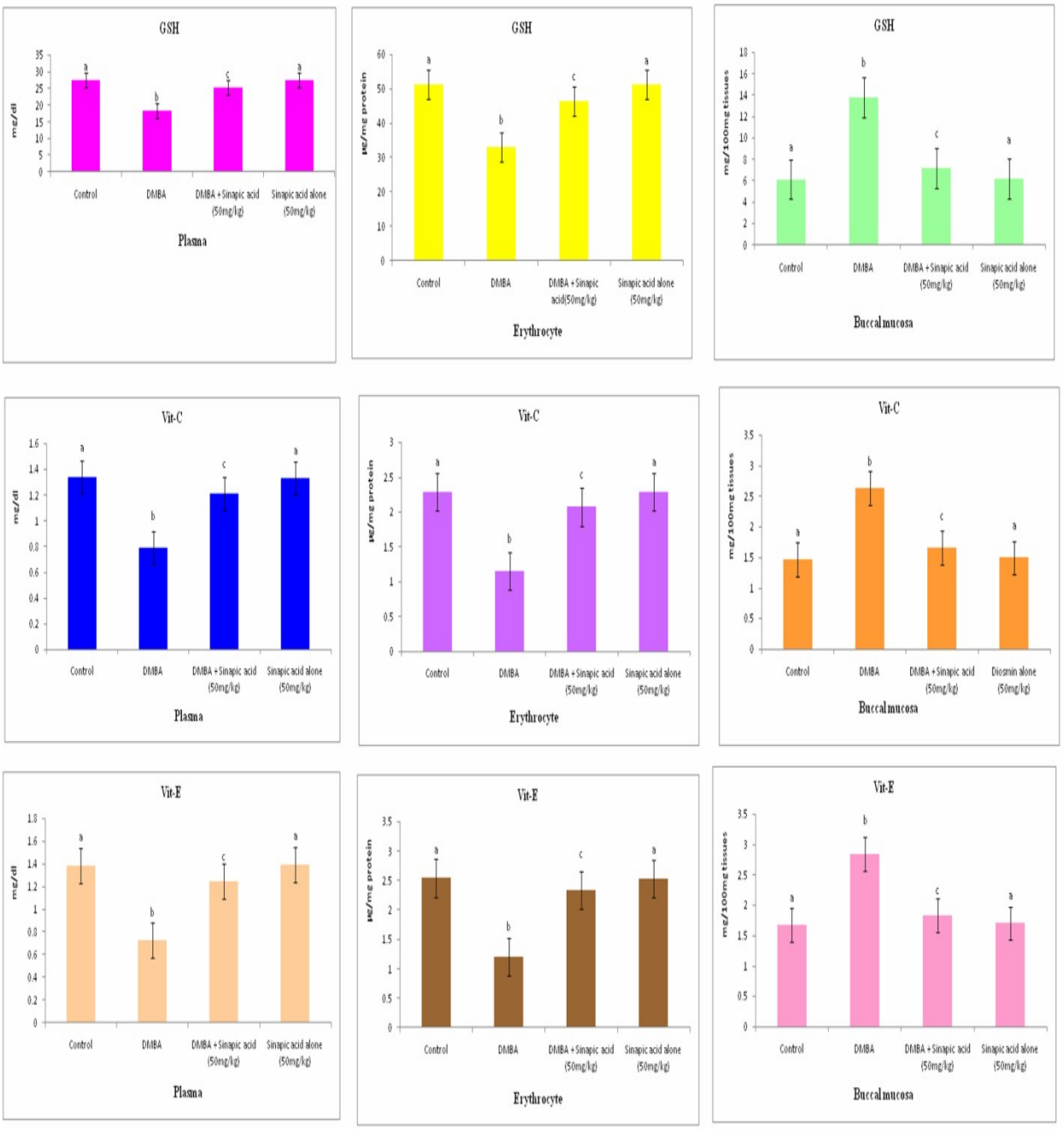
In DMBA treated animals, the level of plasma and erythrocytes SOD, CAT, GPx and GSH were diminished. At the same time expanded GPx and GSH levels in buccal mucosa show that oxidative anxiety is brought about by DMBA. SOD is the first cancer prevention protein to manage oxyradicals by quickening the dismutation of superoxide (O_2−) to hydrogen peroxide (H_2O_2). Catalase is a catalyst that changes over H_2O_2 to nonpartisan items, O_2 and H_2O (Vennila *et al*., 2010). GPx is a initiated protein against oxidative damage and this, requires glutathione as a cofactor. It catalyzes the oxidation of GSH to GSSG to the detriment of H2O (Soujanya *et al*., 2011).. Subsequently, the ROS rummaging movement of CAT, GPx and GSH are viable when it is trailed by the neighboring of SOD, which requires an extra source to repress oxidative anxiety (Weydert *et al*., 2006). Oral administration of Sinapic acid (50mg/kg) was modestly initiated SOD, CAT, GPx and emphatically enacted GSH to DMBA treated hamsters. Sinapic acid alone group showed no huge contrasts when compared to the control group. Lessened activities of Vit-C and Vit-E in the blood and an increase in buccal tissue of DMBA treated hamsters that the exercises of these catalysts were impeded because of rehashed exacerbation by the carcinogen. A reclamation of Vit-C and Vit-E taking after the oral adminstration of Sinapic acid (50mg/kg) shows that Sinapic acid triggers the antioxidant impacts at endogenous level.

Figure.1 Changes the enzymatic antioxidants (SOD, CAT and GPx) activities in plasma, erythrocyte and buccal mucosa of control and experimental animals



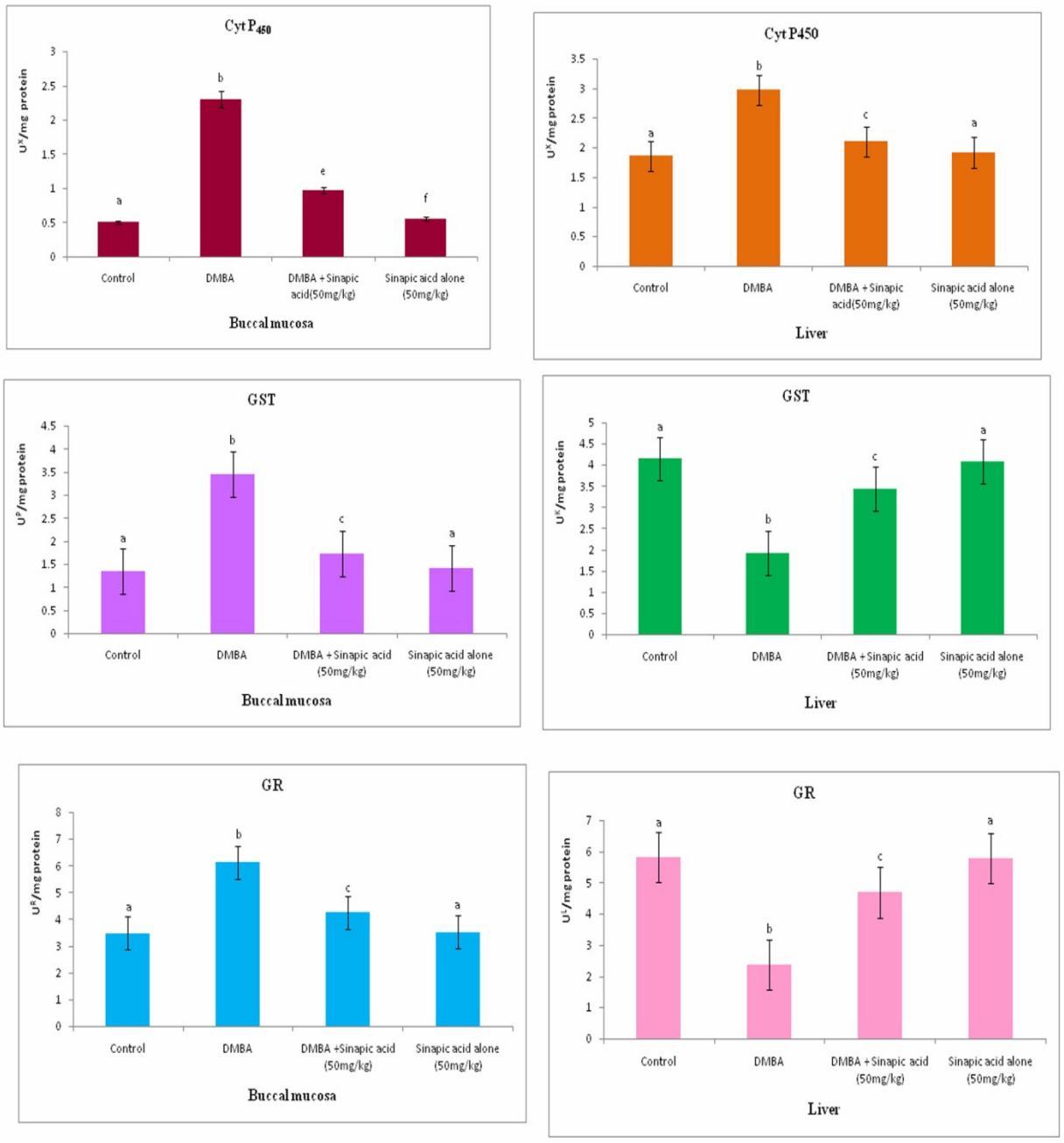
Values are expressed as the mean ± SD for 6 hamsters in each group. Values that are not sharing a common superscript letters in the same column differ significantly at $p<0.05$(DMRT). X - The amount of enzyme required to inhibit 50% NBT reduction. Y - Micromoles of H_2O_2. Z - Micromoles of glutathione utilized/min utilized/sec.

Figure.2 Changes the non-enzymatic antioxidants (GSH, Vit-C and Vit-E) activities in plasma, erythrocyte and buccal mucosa of control and experimental animals



Values are expressed as the mean ± SD for 6 hamsters in each group. Values that are not sharing a common superscript letters in the same column differ significantly at $p<0.05$(DMRT). X - The amount of enzyme required to inhibit 50% NBT reduction. Y - Micromoles of H_2O_2. Z - Micromoles of glutathione utilized/min utilized/sec.

Figure.3 Changes in the activities of Phase I (Cytochrome p450) and Phase II enzymes (GST and GR) in buccal mucosa and liver of control and experimental animals



Values are expressed as the mean ± SD for 6 hamsters in each group. Values that are not sharing a common superscript letters in the same column differ significantly at $p<0.05$(DMRT). X - Micromoles of cytochrome P450, P - Micromoles of CDNB conjugated with GSH/ minute; Q Micromoles of p-nitroaniline formed/ hr; R - Micromoles of NADPH oxidized/hr.

The cytochrome P_{450} (oxidizing phase I metabolizing enzymes) are multigene group of constitutively expressed and inducible hemoproteins with a central part in the oxidative metabolic system (Kirton *et al*., 2005). DMBA requires metabolic actuation by to structure diol epoxide and different ROS that are known to increase intracellular oxidation can cause severe DNA damage, lipids and proteins (Gao *et al*., 2005). Oral administration of Sinapic acid on DMBA treated hamsters restored the status of phase I enzymes in the liver and buccal mucosa suggests that Sinapic acid may have assumed critical part in the detoxification of carcinogens, assisted either in the hindrance of the metabolic activation of DMBA.

The oxidized metabolites of cancer causing xenobiotics are detoxified by phase II metabolizing enzymes. Glutathione S-transferases (GSTs), glutathione reductase (GR) a family of phase II detoxification enzymes, play an important part in securing cellular macromolecules from genotoxic chemicals and cytotoxic agents (Reszka and Wasowicz, 2001; Coles *et al*., 2003). In this present study, the GST and GR levels of 7,12-dimethylbenz[a]anthracene (DMBA)-treated hamster were discovered to be up-regulated in buccal mucosa and down-regulated in the liver when compared to the control . These irregular GST and GR levels are in charge of xenobiotic biotransformation. Oral administration of Sinapic acid (50 mg/kg) to DMBA-treated hamsters resumed these enzymes level. Accordingly, Sinapic acid implantation was found to induce GST and GR activities that can efficaciously eliminate xenobiotics. In conclusion of present study, treatment at the dose of 50 mg/kg significantly recovers the antioxidant and phase I and Phase II detoxification enzymes activities to near normal levels. This indicates that Sinapic acid at a dose 50 mg/kg/bw fixed effective dose for further studies.

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