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ORIGINAL ARTICLE

CHEMOPREVENTIVE POTENTIAL OF GRAMINE AGAINST 7, 12-DIMETHYLBENZ [A] ANTHRACENE INDUCED HAMSTER BUCCAL POUCH CARCINOGENESIS

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ABSTRACT

Aim of the present study was undertaken to evaluate the chemopreventive effects of Gramine on 7,12 Dimethyl benz(a)anthracene (DMBA) induced hamster buccal pouch carcinogenesis by monitoring biochemical markers (lipid peroxidation, enzymatic and non-enzymatic antioxidants, phase I and phase II detoxification enzymes). Male Syrian hamsters were divided into seven groups of six hamsters 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-VI animals were painted with 0.5% DMBA and oral administration of Gramine at different doses (20, 40, 80, 160mg/kg). Group VII animals were received Gramine (160 mg/kg) only and served as drug control. After16th week hamsters were sacrificed, and plasma, erythrocyte and tissues were harvested and analyzed. The results showed that oral administration of Gramine at a dose of 80mg/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 Gramine is probably due to its anti-lipid peroxidative, antioxidant potential and retrieve effect and detoxifying potential during DMBA-induced hamster buccal pouch carcinogenesis.

Keywords: Oral cancer, DMBA, Lipid peroxidation, Antioxidant, Chemoprevention

1.INTRODUCTION

Oral Squamous Cell Carcinoma (OSCC) is one of the fifth most common forms of all cancers and affects more than 5,00,000 new cases each year worldwide(Warnakulasuriya, 2010). In South East Asia 62% of the aggregate instances of oral cancers are accounted from developing countries (Shool Rohit, 2013). Especially the Indian subcontinent oral cancer is accounting for 40-50% of all cancers (Gaur *et al*., 2011). A number of aetiologic factors have been implicated in the development of oral SCCs, such as tobacco smoking, chewing and alcohol abuse (Johnson *et al*., 2001.). Nevertheless, 7,12-dimethylbenz(a)anthracene (DMBA) fit in with class of aromatic polycyclic hydrocarbons, which are byproducts of the ignition of tobacco and other natural substances(lee *et al*., 2002). This agent may induce generation of free radicals and oxidative stress through the production of superoxide, hydrogen peroxide and nitric oxide in cells (Ames and Gold, 1991). Interactions of free radicals with DNA can lead to the formation of single-strand or double-strand breaks, formation of apurinic and apyrimidinic site and DNA-protein and DNA-DNA cross links and adduct formation (Kontek *et al*., 2013; Blair, 2008).

Chemoprevention offers a novel methodology to control the rate of oral tumor, an paramount contributor of disease morbidity and mortality in the Indian subcontinent (Suresh *et al*., 2010). Plant items and their dynamic standards have pulled in the center of the late consideration as putative chemopreventive because of their easy availability, non-toxic and anticarcinogenic properties through scavenging oxygen free radicals and enhancing antioxidant levels (Johnson, 1997).

Gramine, a natural indole alkaloid, has been isolated from different raw plants (Semenov and Granik, 2004), which exhibits wide pharmaceutical activities similar to Ephedrine such as relaxation of bronchial smooth muscle, vasorelaxation, blood pressure elevation, relief of bronchitis nephritis and bronchial asthma (Iwata *et al*., 2001). Gramine also assumes critical roles for the metabolic function of

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amino acid in living organism. It generally utilized as a pharmaceutical lead framework for constructing different biologically active indole-containing compounds (Katritzky *et al*., 2009) including heteroauxin, 5-hydroxytryptamine (5-HT), and L-tryptophan. However, to the best of our knowledge no scientific studies has been undertaken to evaluate chemopreventive effect of Gramine against oral cancer. Therefore, we determine the antioxidant and anti-carcinogenic potential of Gramine as dose dependent manner during 7,12 dimethylbenz[a]anthracene (DMBA)-induced hamster buccal pouch carcinogenesis.

2.MATERIALS AND METHODS

Chemicals

7,12-Dimethylbenz(a)anthracene (DMBA), Gramine, reduced glutathione, reduced nicotinamide adenine dinucleotide (NADH), 1,1',3,3'-tetramethoxypropane, were obtained from Sigma-Aldrich Chemicals Pvt. Ltd., Bangalore, India. All other chemicals were commercially available and analytical grade.

Animals

Male golden Syrian hamsters, eight to ten weeks old, weighing 80-120g were purchased from National Institute of Nutrition, Hyderabad and maintained in the Central Animal House, Rajah Muthiah Medical College and Hospital, Annamalai University. 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 12:12hr. The animals were provided with standard pellet diet (Amrut Laboratory Animal Feed, Mysore Feeds Limited, Bangalore, India) and water *ad libitum*. Hamsters were maintained in accordance (Proposal No. 946) with the guidelines of ethical committee for animal care of Annamalai University (Register number 160/1999/CPCSEA) in accordance with Indian National Law on animal care and use.

Induction of oral squamous cell carcinoma

Oral squamous cell carcinoma was developed in the left buccal pouch of male Syrian golden hamsters by painting with 0.5% DMBA (No: 4 brush) in liquid paraffin three times a week for 16 weeks. The animals were given standard pellet diet and water *ad libitum*.

Experimental design

A total of 42 hamsters were randomized into seven groups of six hamsters in each. Group I hamsters served as control and received normal saline only. Group II hamsters were painted with 0.5% DMBA in liquid paraffin three times a week on their left buccal pouches. Groups III-VI hamsters were received Gramine at a doses of 20, 40, 80 and160mg/kg bw/day, starting one week before exposure to the carcinogen and continued on days alternate to DMBA painting, until the scarification of the hamsters. Group VII hamsters received oral administration of Gramine alone (180 mg/kg bw) throughout the experimental period. At the end of 16^{th}week and all hamsters were sacrificed by cervical dislocation.

Processing of blood samples

Blood samples were collected into heparinized tubes. The plasma was separated by centrifugation at 3000rpm for 15 mins. After plasma separation, the erythrocyte membrane was prepared by the method of Dodge *et al*., (1968) modified by Quist (1980).

Preparation of Tissue Homogenate

Tissue samples from animals were washed with ice cold saline and dried between folds of filter paper, weighed and homogenized using appropriate buffer [appropriate buffer of concerned parameter (TBARS-0.025M Tris-Hcl buffer, pH 7.5; GSH and GPx- 0.4 M phosphate buffer, pH - 7.0; SOD - 0.025 M sodium pyrophosphate buffer, pH 8.3; CAT - 0.01 M phosphate buffer, pH 7.0)] in an All-glass homogenizer with teflon pestle. The homogenate was centrifuged at 1500rpm for 5 minutes and the supernatant was then used for the biochemical estimations.

Biochemical parameters and methods

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). 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).

Cytochrome P_{450} and b_5 in liver and buccal mucosa tissues microsomes was estimated by the method of Omura and Sato, (1964). The activity of GST was assayed by the method of Habig *et al*. (1974). The activity of GGT was assayed by the method of Fiala *et al*. (1977). Glutathione reductase activity was assayed by the method of Carlberg and Mannervik,(1985).

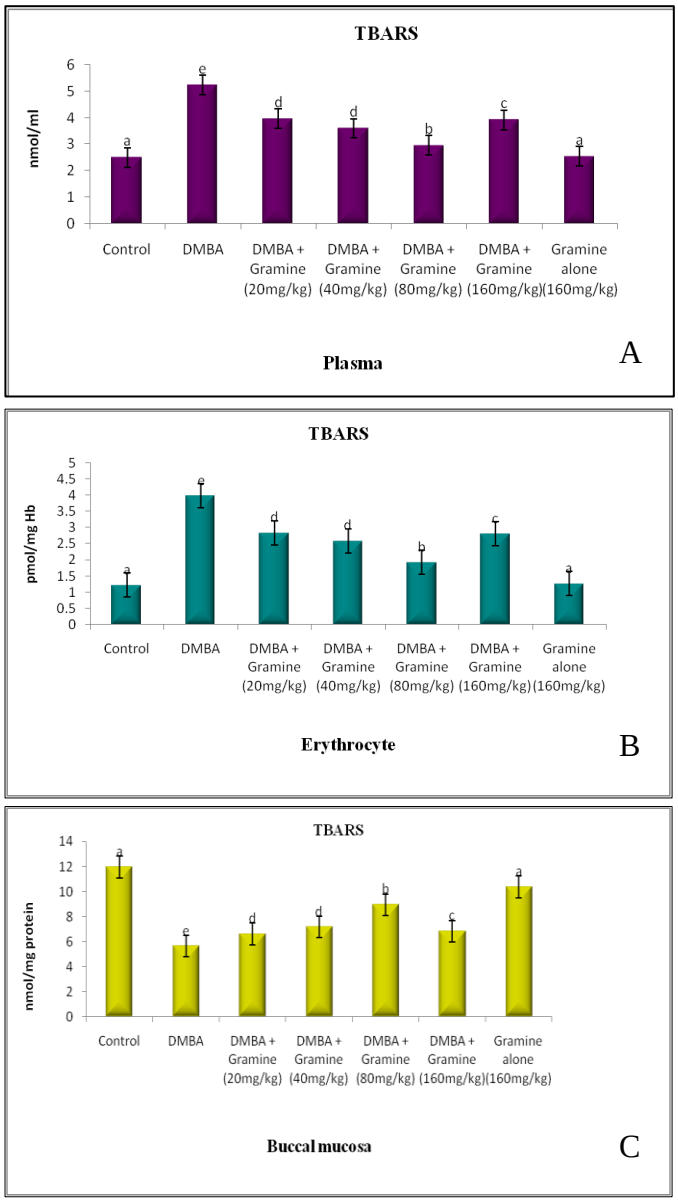
Statistical Analysis

The data were expressed as mean ± S.D with six 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.

3.RESULTS

Figure 1A,B&C Shows the levels of plasma and erythrocyte TBARS were increased and buccal tissue TBARS were decreased in DMBA treated animals. Oral administration of Gramine (80 mg/kg bw) to DMBA painted animals were significantly recoverd the TBARS level near to normal when compared to control animals. Animals treated with Gramine alone showed no significant differences were observed compared to control animals.

Figure 1. Changes in the levels of Thiobarbituric acid reactive substances (TBARS) in plasma, erythrocyte and buccal mucosa of control and experimental animals



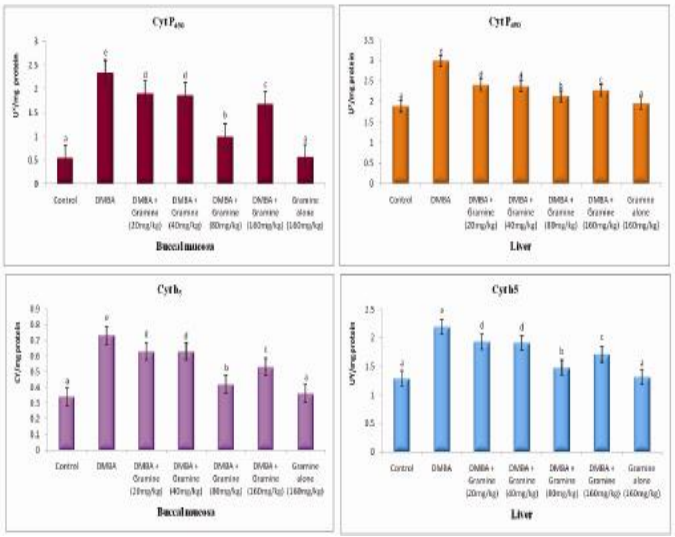
Values are expressed as the mean ± SD for 6 hamsters in each group. Values that are not sharing a common superscript letters in the same column differ significantly at p<0.05(DMRT).

Figure 2 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 Gramine (80 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 Gramine showed no significant differences were observed compared to control animals.

Figure 3 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 Gramine (80 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 Gramine alone showed no significant differences were observed compared to control animals.

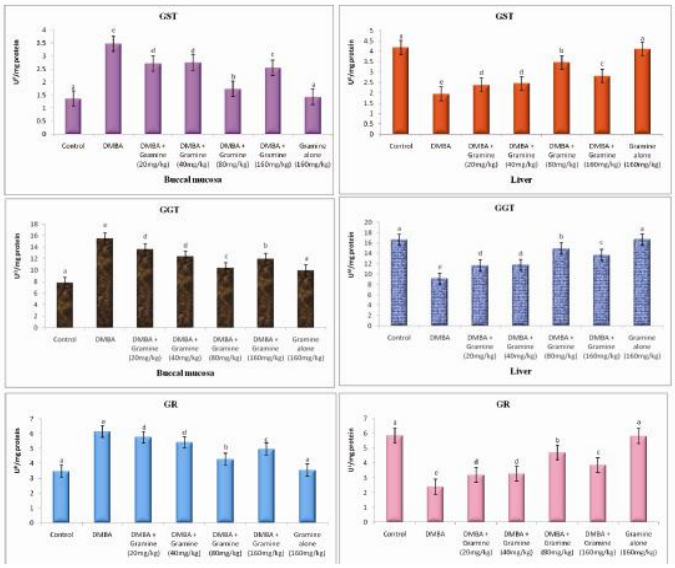
Figures 4 and 5 Shows the status of phase I (cytochromes P450 and b5) and phase II (GST, GGT and GR) detoxification enzymes in the buccal mucosa and liver of control and experimental hamsters in each group. The phase I enzymes activity were significantly increased in buccal and liver tissue whereas phase 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 Gramine (80mg/kg) to hamsters treated with DMBA significantly recover the status of phase I and phase II detoxification enzymes. Animals treated with Gramine alone showed no significant differences were observed compared to control animals.

Figure 4. Changes in the activities of Phase I enzymes (Cyt P_{450} and b_5) in buccal mucosa and liver of control and experimental animals



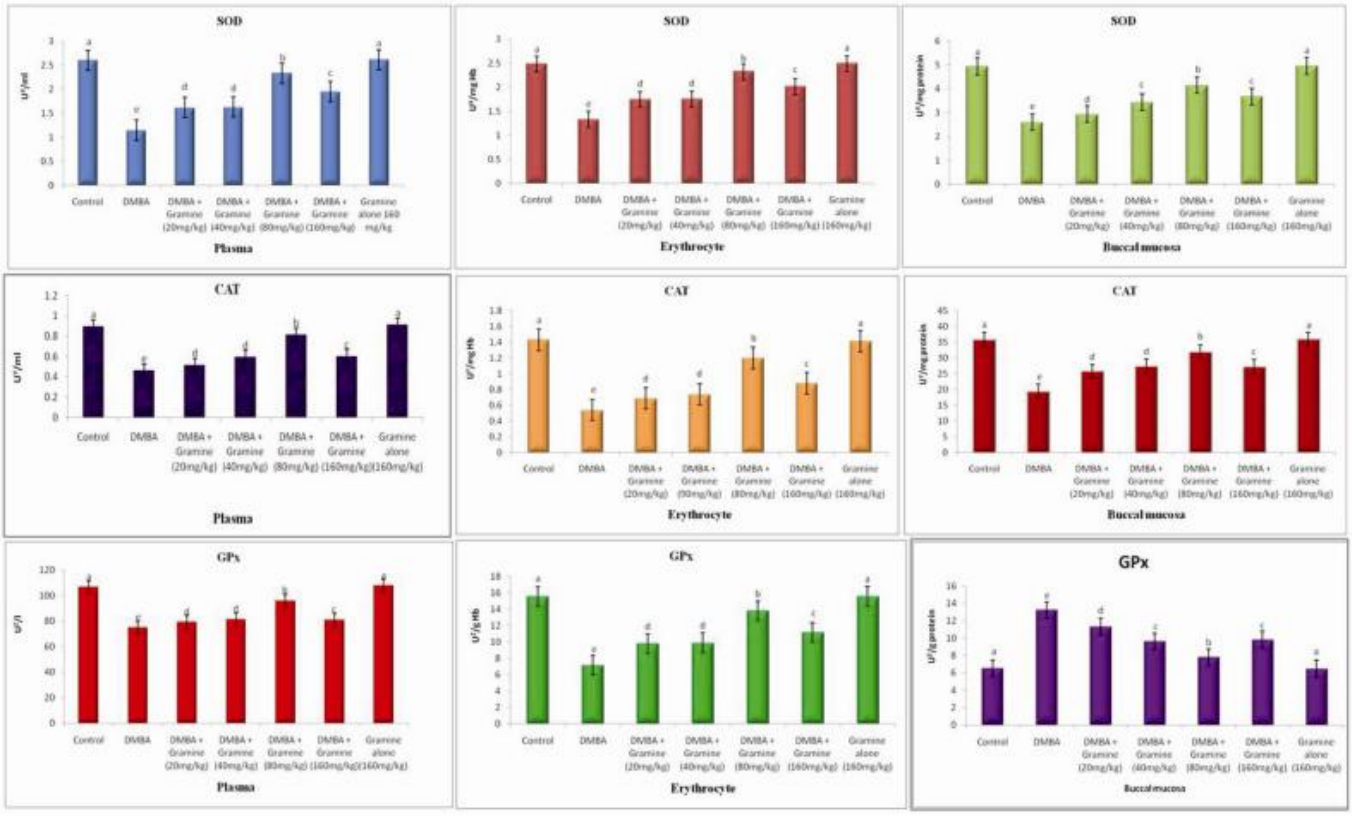
Values are expressed as the mean ± SD for 6 hamsters in each group. Values that are not sharing a common superscript letters in the same column differ significantly at p<0.05(DMRT). **X** - Micromoles of cytochrome P_{450}. **Y** - Micromoles of cytochrome b_5

Figure 5. Changes in the activities of Phase II enzymes (GST, GGT and GR) in buccal mucosa and liver of control and experimental animals



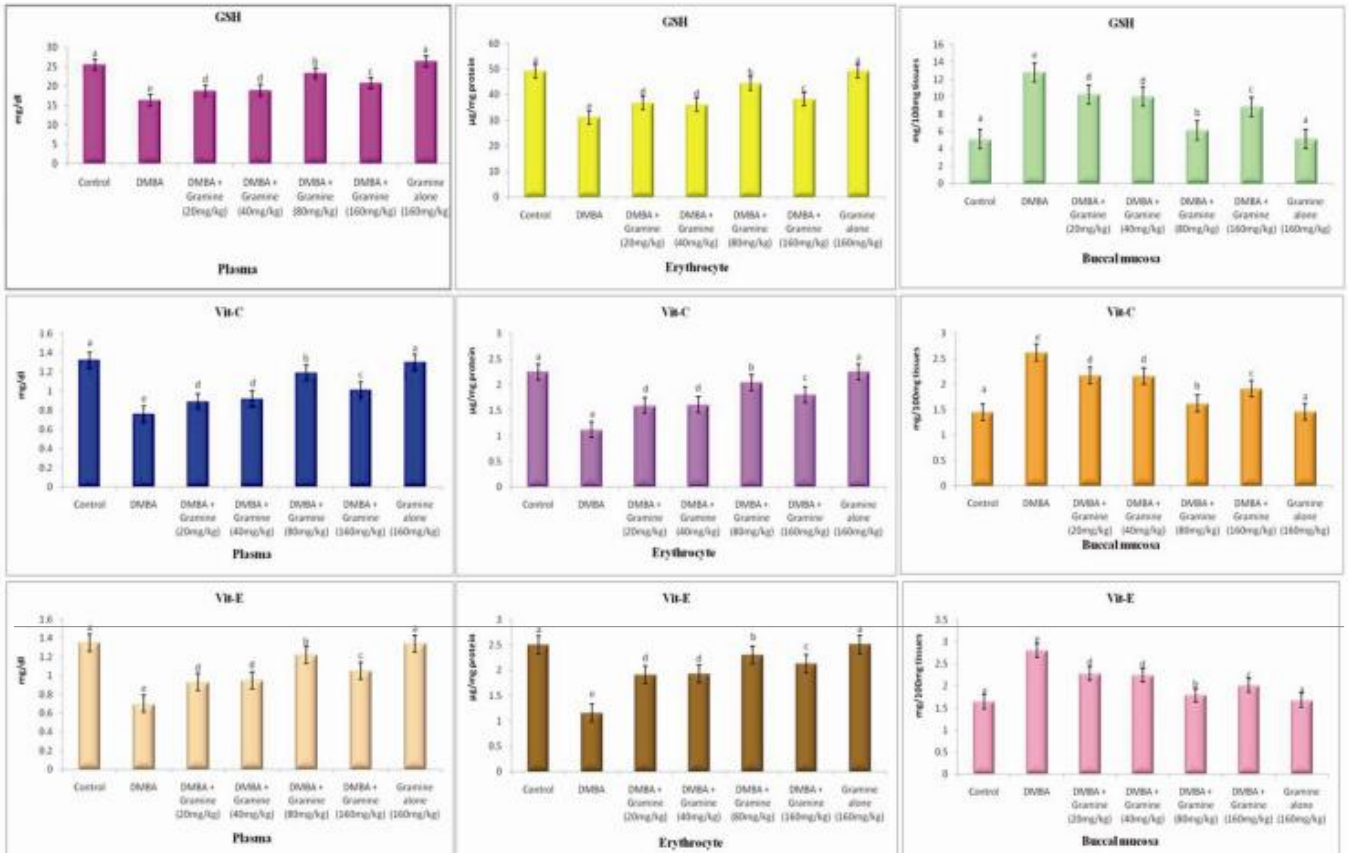
Values are expressed as the mean ± SD for 6 hamsters in each group. Values that are not sharing a common superscript letters in the same column differ significantly at p<0.05(DMRT). **P** - Micromoles of CDNB conjugated with GSH/ minute; **Q**- Micromoles of p-nitroaniline formed/ hr; **R** - Micromoles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

Figure 2. 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 3. 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.

4.DISCUSSION

In the present study, chemopreventive potential of Gramine was assessed by monitoring status of lipid peroxidation, antioxidants and phase I and II detoxification enzymes. Reactive oxygen species (ROS) generated by mitochondria or from other intracellular or extracellular sites can cause cell damage and initiate different degradation processes (Davies, and Hochstein, 1982). ROS such as superoxide anion (O_2•−), singlet oxygen ($^1O_2$), hydrogen peroxide (H_2O_2), and hydroxyl radicals (•OH), were primarily formed when mixed function oxidases system by induction of polycyclic aromatic hydrocarbons like 7,12-dimethyl benz(a)anthracene (Giri *et al*., 1995). These oxyradicals can attach covalently nucleophilic sites on cell macromolecules accordingly evoking carcinogenic reactions. Hence, DMBA-induced hamster buccal pouch carcinogenesis has been widely used as an animal model for development of chemopreventive drugs for oral cancer. Present study, topical application of DMBA to the hamster buccal pouch for 16 weeks resulted in well-developed OSCC with very mean tumor burden associated with severe hyperplasia, hyperkeratosis, and dysplasia.

The converse relationship between lipid peroxidation and the rate of cell expansion, seen in our study demonstrates that the tumor cells multiply widely when lipid peroxidation is insignificant. The thiobarbituric acid reactive substances in plasma were significantly increased whereas its level in buccal tissue was significantly reduced in DMBA treated hamsters. The tumor cells in buccal tissue showed a distinctly low level of peroxidation creating a favorable atmosphere for the proliferation of cancer cells (Schmelz *et al*., 2000). Data of the present study indicated that lipid peroxidation induced by oxidative stress caused DNA damage. Oral administration of Gramine significantly altered the lipid peroxidation status. The most significant effect of Gramine supplementation was seen at the dose of 80 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 decreased. Simultaneously increased GPx and GSH levels in buccal mucosa indicate that oxidative stress is caused by DMBA. SOD is the first antioxidant enzyme to deal with oxyradicals by accelerating the dismutation of superoxide (O_2−) to hydrogen peroxide (H_2O_2). Catalase is an enzyme that converts H_2O_2 to neutral products, O_2 and H_2O (Vennila at al., 2010). GPx will be an critical protections activated protein against oxidative harm and this, requires glutathione as a cofactor. It catalyses the oxidation of GSH to GSSG at the expense of H_2O (Soujanya *et al*., 2011). GSH is an essential cell protectant, and specifically extinguishes reactive oxygen species (Kidd, 1997). It is an essential cofactor for many enzymes that require thiol reducing equivalents, and it helps to keep redox sensitive active sites on enzymes in the necessary reduced state (Weber GF, 1999). Thus, the ROS scavenging activity of CAT, GPx and GSH is effective when it is followed by the neighbouring of SOD, which requires an additional source to inhibit oxidative stress (Weydert *et al*., 2006). Oral administration of Gramine (80mg/kg) was moderately activated SOD, CAT, GPx and strongly activated GSH to DMBA treated hamster plasma, erythrocytes and buccal mucosa. The drug alone group exhibited no significant differences when compared to the control group.

Vitamins C and E fulfill a number of biological activities, including immune stimulation, scavenging free radicals and altering the metabolic activation of carcinogens (Van Poppel and Vanden Berg, 1997). Vitamin E is a potent oxygen radical scavenger that protects cells from carcinogenic chemicals by inhibiting LPO- and free radical-mediated consequences. Vitamin C aids the metabolism of tyrosine, folic acid and tryptophan. Diminished activities of Vit-C and Vit-E in the blood and an increase in buccal tissue within DMBA treated hamsters suggests that the activities of these enzymes were impaired due to repeated aggravation by the carcinogen. A restoration of Vit-C and Vit-E following the oral administration of an oral dose of Gramine (80mg/kg) demonstrates that Gramine triggers the antioxidant effects of vitamins E and C to decrease the endogenous level of oxidative stress and this would possibly facilitate to stop disease ensuing from tissue damage caused by free radicals.

The cytochrome P450 and b5 (oxidizing phase I metabolizing enzymes) are multigene family of constitutively expressed and inducible hemoproteins with a focal part in the oxidative metabolic system of an extensive variety of endogenous and exogenous compounds including numerous cancer-causing agents and are accordingly being considered as potentials targets for tumor therapeutics (Gonzalez *et al*., 1991). DMBA requires metabolic activation by cytochrome P_{450} to form diol epoxide and other ROS that are known to increase intracellular oxidation can cause severe DNA damage, lipids and proteins and there by contribute to carcinogenesis (Lajolo C, 2010). Oral administration of Gramine on DMBA treated hamsters restored the status of phase I enzymes in the liver and buccal mucosa suggests that Gramine might have played crucial role in the detoxification of carcinogens, assisted either in the inhibition of the metabolic activation of DMBA.

The oxidized metabolites of cancer-causing xenobiotics are then detoxified by phase II metabolizing enzymes. Glutathione S-transferases (GSTs), a family of phase II detoxification enzymes, play an important role in securing cellular macromolecules from getting ambushed by genotoxic chemicals and cytotoxic chemotherapeutic agents (Reszka and Wasowicz, 2001; Coles *et al*., 2003). In this present study, the GST levels of 7,12-dimethylbenz[a]anthracene (DMBA)-treated hamster were found to be up-regulated in buccal mucosa and down-regulated in the liver when compared to the control group. These irregular GST levels are responsible for xenobiotic biotransformation. Oral administration of Gramine (80 mg/kg) to DMBA-treated hamsters resumed the GST levels. Thus, Gramine infusion was found to induce GST activities that can efficaciously eliminate xenobiotics.

Gamma-glutamyltransferase (GGT) is a membrane-bound enzyme that catalyses the hydrolytic cleavage of peptides into smaller peptides or amino acids, and it is expressed at varying levels in normal tissues (Alessandro Corti *et al*., 2010)). GGT plays a role in carcinogenesis and has been regarded as a biomarker of pre-invasive and invasive neoplasia. In particular, GGT levels are increased in oral squamous and bile duct epithelia. In the present study, GGT levels were up-regulated in buccal and down-regulated liver tissue of DMBA treated hamsters. However, when Gr was administered on DMBA treated animals, recov

activities of GGT suggest that Gramine (80mg/kg) can enhance cellular defense mechanisms against cancer. Suresh *et al*. (2010) also reported moderate activity of (6)-paradol in GGT on DMBA-induced carcinogenesis, similar to that seen in our study.

Several studies have shown that glutathione reductase (GR) is an important enzyme involved in the scavenging of reactive oxygen species. GR is well-known for its anti-oxidant function and usually used as an indicator for oxidative stress. Thus, we determined glutathione reductase activity in the hamster buccal pouch with 0.5 % of DMBA followed by treatment with different doses of Gramine. DMBA caused a severe hamster cheek pouch carcinogenesis, which increase glutathione reductase activity in buccal pouch and decrease in liver tissues. Treatment of Gramine (80mg/kg) was significantly modulates the glutathione reductase activity when compared to DMBA alone.

5.CONCLUSION

The present study was carried out to evaluate the antitumor activity of Gramine on DMBA induced hamster buccal pouch carcinogenesis. The treatment at the dose of 80 mg/kg significantly recovers the lipid peroxidation, antioxidant and phase I and Phase II detoxification enzymes activities to near normal levels. However, the higher dose of Gramine (160mg/kg), also considerably inhibit DMBA toxicity, but it exhibited some degree of harmful effect to reduced tolerance of hamster. This indicates that on this dosage of the drugs showed increased toxicity for the host. Hence, Gramine dose 80 mg/kg/bw fixed effective dose for further studies.

6.ACKNOWLEDGEMENT

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7.REFERENCES

Alessandro Corti, Maria Franzini, Aldo Paolicchi and Alfonso Pompella. 2010. Gamma-glutamyltransferase of Cancer Cells at the Crossroads of Tumor Progression, Drug Resistance and Drug Targeting. *Anticancer Research* 30:1169-1182.

Ames, B.N, Gold, L.S. 1991. Endogenous mutagens and the causes of aging and cancer. *Mutat Res* 250: 3-16.

Beutler, E. and Kell,y BM. 1963.The effect of sodium nitrate on RBC glutathione. *Experimentia* 19: 96-97.

Blair, IA. 2008.DNA adducts with lipid peroxidation products. *J Biol Chem* 283:15545-15549.

Carlberg, I and Mannervik, B. 1985. Glutathione reductase. *Methods Enzymol*;113:484-490.

Coles, B.F. and Kadlubar, F.F. 2003.Detoxification of electrophilic compounds by glutathione S-transferase catalysis: determinants of individual response to chemical carcinogens and chemotherapeutic drugs. *Biofactors* 17:115-30.

Davies, K.J and Hochstein, P. 1982. Ubisemiquinone radicals in liver implications for a mitochondrial Q cycle *in vivo*. *Bio-chemical and Biophysical Research Communications* 107:1292-1299.

Desai, I.D. 1984. Vitamin E analysis methods for animal tissues. *Methods Enzymol* 105:138-147.

Dodge, J.F, Michell, G and Hanahan, DJ. 1968.The preparation of hemoglobin free ghosts of human red blood cells. *Arch Biochem Biophys* 110:119-130.

Donnan, S.K. 1950.The thiobarbituric acid test applied to tissues from rats treated in different ways. *J Biochem* 182:415-419.

Fiala, S., Fiala, A.E., Keller, R.W and Fiala, E.S. 1977.Gamma glutamyl transpeptidase in colon cancer induced by 1,2-dimethylhydrazine. *Arch Geschwulstforsch* 47:117-122.

Giri, U., Sharma, S.D., Abdulla, M. and Athar, M. 1995.Evidence that in situ generated reactive oxygen species act as a potent stage I tumor promoter in mouse skin. *Biochem Biophys Res Commun* 209:698-705.

Gonzalez, F.J and Gelboin, H.V. 1994.Role of human cytochromes P_{450} in the metabolic activation of chemical carcinogens and toxins. *Drug Metab Rev* 26:165-83.

Guengerich, F.P and Shimada, T. 1991.Oxidation of toxic and carcinogenic chemicals by human Cytochrome P_{450} enzymes. *Chem Res Toxicol* 4:391–407.

Habig, W.H., Pabst, M.J. and Jakoby, W.B.C. 1974.Glutathione-S-transferases: the first enzymatic step in mercapturic acid formation. *J Biol Chem* 249:7130-7139.

Iwata, S., Saito, S., Konya, K., Shizuri, Y. and Ohizumi, Y. 2001. Novel marine-derived halogen-containing Gramine analogues induce vasorelaxation in isolated rat aorta. *Eur J Pharmacol* 432:63-70.

Johnson, I.T. 1997.Plant anticarcinogens. *Eur J Cancer Prev* 6:515-517.

Johnson, N. 2001.Tobacco use and oral cancer: a global perspective. *J Dent Educ* 65:328-39.

Kakkar, P., Das, B. and Viswanathan, PN. 1984. A modified spectrophotometric assay of superoxide dismutase. *Indian J Biochem Biophys* 21:130-132.

Katritzky, A.R., Munawar, M.A, Kovacs, J. and Khelashvili, L. 2009.Synthesis of amino acid derivatives of quinolone antibiotics. *Org Biomol Chem* 7:2359-62.

Kittakoop, P., Mahidol, C. and Ruchirawat, S. 2014.Alkaloids as important scaffolds in therapeutic drugs for the treatments of cancer, tuberculosis, and smoking cessation. *Curr Top Med Chem* 14: 239-52.

Kontek, R., Kontek, B. and Grzegorczyk, K. 2013.Vitamin C modulates DNA damage induced by hydrogen peroxide in human colorectal adenocarcinoma cell lines (HT29) estimated by comet assay *in vitro*. *Arch Med Sci* 9:1006-12.

Lee, L.L., Lee, J.S., Waldman, S.D., Casper, R.F. and Grynpas, M.D. 2002.Polycyclic aromatic hydrocarbons present in cigarette cause bone loss in an ovariectomized rat model. *Bone* 30:917-23.

Lee, K.W., Lee, H.J., Surh, Y.J. and Lee, C.Y. 2003.Vitamin C and cancer chemoprevention. *Am J Clin Nutr* 78:1074-1078.

Ohkawa, H., Ohishi, N. and Yagi, K. 1979.Assay for lipid peroxides in animal tissues by thiobarbituric acid reaction. *Anal Biochem* 95:351–358.

Omaye, S.T., Turnbull, T.D. and Sauberlish, H.C. 1979.Selected method for the determination of ascorbic acid in animal's cells, tissues and fluids. *Methods Enzymol, Academic Press New York* 62: 3-11.

Omura, T. and Sato, R. 1964. The carbon monoxide-binding pigment of liver microsomes: Phase I evidence for its hemoprotein nature. *J Biol Chem* 239:2370-2378.

Palan, P.R., Mikhail, M.S., Basu, J. and Romney, S.L. 1991.Plasma levels of antioxidant beta-carotene and alpha-tocopherol in uterine cervix dysplasias and cancer. *Nutr Cancer* 15:13–20.

Quist, E.E. 1980. Regulation of erythrocyte membrane shape by Ca2+. *Biochem Biophys Res Commun* 92:631–637.

Reis, P.P., Waldron, L., Perez-Ordonez, B., Pintilie, M., Galloni, N.N, Xuan, Y., Cervigne, N.K, Warner, G.C, Makitie ,A.A, Simpson, C, Goldstein, D, Brown, D, Gilbert, R, Gullane, P, Irish, J, Jurisica, I, and Kamel-Reid, S. 2011. A gene signature in histologically normal surgical margins is predictive of oral carcinoma recurrence.*BMC Cancer* 437:1-11.

Reszka, E. and Wasowicz, W. 2001.Significance of genetic polymorphisms in glutathione S-transferase multigene family and lung cancer risk. *Int J Occup Med Environ Health* 14:99-113.

Rotruck, J.T, Pope, A.L, Ganther ,H.E, Swanson, A.B, Hafeman, D.G and Hoekstra, WG1973. Selenium: Biochemical role as a component of glutathione peroxidase. *Science* 179:588-590.

Semenov, B.B and Granik, V.G. 2004.Chemistry of N-(1H-indol-3-ylmethyl)-N,N-dimethylamine (Gramine): A Review. *Pharmaceutical Chemistry Journal* 38:287-310.

Shool Rohit, S, Zingade, A.P. and Kumar, M. 2013.Management of Neck in Oral Squamous Cell Carcinoma. *Indian Journal of Applied Research* 3:419-422.

Sinha, A.K. 1972.Colorimetric assay of catalase. *Anal Biochem* 47:389-394.

Slaga, T.J, Budunova, I.V, Gimenez-Conti, I.B. 1996.The mouse skin carcinogenesis model. *J Invest Dermato* 1:151-6.

Soujanya, V., Silambujanaki, P anmd Leela Krishna, V. 2011. Anticancer efficacy of *Holoptelea integrifolia*, planch. Against 7, 12-dimethyl benz(a)anthracene induced breast carcinoma in experimental rats. *Int J Pharm Pharm Sci* 3:103-106.

Suresh, K, Manoharan, S, Vijayaanand, M.A and Sugunadevi, G. 2010. Chemopreventive and antioxidant efficacy of (6)-paradol in 7,12-dimethylbenz(a)anthracene induced hamster buccal pouch carcinogenesis. *Pharmacol Rep* 62:1178-85.

Suresh, K, Sivakumar,K., Vijayaanand, M.A., Rajalingam, K. and Rajkamak,G.2010. Antilipidperoxidative and antioxidant effects of Zingiber officinale roscoe root extract in 7,12-dimethyl benz(a) anthracene induced oral carcinogenesis. Pharmacologyonline.2:689-701.

Van Poppel, G and Vanden Berg, H. 1997.Vitamins and cancer. *Cancer Letters* 114:195-202.

Vennila, R, Thirunavukkarasub, S.V and Muthumarya, J. 2010. *In-Vivo* Studies on anticancer activity of taxol isolated from an endophytic fungus pestalotiopsis pauciseta sacc. VM1. *Asian Journal of Pharmaceutical and Clinical Research* 3:11-23.

Weydert, C.J, Waugh, T.A, Ritchie, J.M, Iyer, K.S, Smith, J.L and Li, L. 2006. Over expression of manganese or copper-zinc superoxide dismutase inhibits breast cancer growth. *Free Rad Biol Med* 41:226–37.

Yagi, K. 1987.Lipid peroxides and human diseases. *Chem Phys Lipids* 45:337-351.

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