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CHEMO PREVENTIVE EFFECTS OF ACTINOBACTERIAL STRAIN SNI5 EXTRACT ON N-NITROSODIETHYLAMINE INDUCED CANCER (COLON) ACTIVITY IN MICE

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ABSTRACT

The present study is designed to investigate the preventive effects of actinobacterial extract on colon carcinogenesis in NDEA induced mice tumor. Twenty four mice were divided into four groups of six animals each. Animals (Group I) proceed as the control without any treatment. Animals (Group II) were intraperitonially injected with N-nitrosodiethylamine at three doses at an interval for ten days so as to induce colon cancer. Animals (Group III) were orally administrated of actinobacterial strain SNI5 extract. Animals (Group IV) were administered orally with actinobacterial extract and N-nitrosodiethylamine. The present study demonstrated that the actinobacterial extract effectively prevented the formation of preneoplastic foci in mice when given along with the NDEA treatment i.e. during the inhibition phase and often the NDEA treatment until the end of the experiment. Therefore, the present investigation demonstrated that the actinobacterial extract SNI5 seems to be a potential, and a promising chemopreventive agent.

KEYWORDS: **Actinobacteria, colon carcinogenesis, nitrosodiethylamine, mice, chemopreventive.**

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INTRODUCTION

Colorectal cancer is a malignant tumor recognized as the third most common cancer worldwide with high morbidity and mortality[1]. Hence, new chemo-preventive and chemo-therapeutic approaches are required to reduce its mortality. Chemoprevention of colon cancer appears to be a very realistic possibility because various intermediate stages have been identified preceding the development of malignant colonic tumors[2]. It has already been proved from various experiments that chemo preventive agents, by virtue of their anti-oxidant nature, have curable properties for anti-inflammatory, anti-proliferative and apoptosis inducing activity and act at various levels including molecular, cellular, tissue and organ levels to interfere with carcinogens. Considering the potential of chemotherapy in controlling the colon cancer, different groups are working on chemicals or radicals of different origin to treat and control the disease. In this respect, actinobacteria have been proved to have chemosynthetic property by producing antitumor and anticancer substances[3]. Recently, marine actinobacteria have been identified as the potential source for isolating new anticancer compounds which were not described earlier from any other source. Actinobacteria are high G + C content, Gram-positive bacteria with an unparalleled ability to produce diverse secondary metabolites[4]. They hold a prominent position as targets in screening programs due to their diversity and their proven ability to produce novel metabolites and other molecules of pharmaceutical importance[5]. However, there are only limited studies on marine actinobacteria and their anticancer potentials. Though, there are several biological organisms whose extracts were reported to have anticancer activity against different cell lines, they failed to exhibit such activity when they were tested *in vivo*. In our previous study confirmed the actinobacterial extract of the strain SNI5 which showed potential activity *in vitro* (Data not shown) and the strain was identified as *Streptomyces* sp. (GU320191) using 16S rDNA analysis. Thus, the present study is designed to investigate the preventive effects of actinobacterial extract SNI5 strain on colon carcinogenesis in NDEA induced mice tumor.

MATERIALS AND METHODS

Actinobacterial extract

A loopful culture of actinobacterial strain SNI5 was inoculated in 50 ml of ISP 2 broth and kept for 7 days in the shaker. After the 7th day, the culture was centrifuged at 10, 000 rpm for 15 minutes at 4°C. The equal volume of ethyl acetate was added to the collected supernatants and subjected to vigorous shaking for an hour using a separating funnel. Then, the separating funnel was kept undisturbed for 5 minutes; the aqueous phase was collected in a beaker and subjected to concentration through evaporation in a vacuum desiccator. After evaporation, the concentrated residue of 2 mg was dissolved in 2ml of Hank's buffer and filtered using Millipore filter (20µm). This filtered extract was used for the *in vivo* experiment with the concentration of 62.5µg, which showed promising activity in cell lines studies (data not shown).

Experimental animals, carcinogen and chemicals

In vivo experiments were carried out after obtaining the necessary clearance from the Ethical Committee (Clearance No. 589 dated 15.12.2008) of the Annamalai University. A total of 25 Swiss albino male mice (each weighing approximately 25g) was obtained from the Central Animal House, Rajah Muthiah Medical College and Hospital, Annamalai University. The animals were maintained at 14: 10 light / dark cycle at room temperature. They were fed with standard pellet diet and tap water. N-nitrosodiethylamine (NDEA) (Sigma chemicals, USA) which causes oxidative stress to the animal and it was used to induce cancer in the mice[6] and all other reagents used were of analytical grade.

Experimental design

The animals were randomly divided into four groups of six animals each,

a) Animals in group I was considered as control without any treatment.
b) Animals in group II were intraperitonially injected with N-

nitrosodiethylamine (NDEA) (5µl/g of body weight at 1ppm/gram body weight) with 10% DMSO in the left flank of the animals (3 doses at an interval of 10 days) so as to induce colon cancer in the animals.

c) Group III animals were administered orally with only actinobacterial extract with a dose of 8.6µl/g of animal body weight per day.

d) Animals in group IV were treated with N-nitrosodiethylamine (NDEA) as in group II; In addition, the animals were administered orally with actinobacterial extract with a dose of 8.6µl/g animal body weight per day.

After an incubation period of 45 days, the mice were sacrificed and the blood samples were collected from experiments. For histological study, experimented animal tissues were used.

Histolological study

As the liver is the main site of NDEA metabolism[7] as it was intended to study the colon cancer, in the present study, liver and colon tissues were sectioned and fixed in periodic acid-Lysine-Paraformaldehyde and embedded in paraplast. Small sections of liver and colon tissues were stained with Haematoxylin and eosin stain and with the High Iron Diamine Alcian Blue method[8] at pH 2.5. Further, microscopical observation of histological changes in the tissues of the experimental mice was carried out and photomicrographs were taken.

Antioxidant assays

Lipid peroxide levels (LPO) in plasma were determined by the method of Yagi[9] using thiobarbituric acid (TBA) as standard and the level of plasma has been expressed in terms of malondialdehyde (MDA) as nmoles/dl plasma. The enzymatic antioxidant properties like Superoxide dismutase (SOD) was estimated based on the change in optical density (480 nm) during the oxidation of epinephrine to adrenochrome transition by the dismutase enzyme[10]. Inhibition of epinephrine 50% to adenchrome of the enzyme was taken as one unit of enzyme and results has been expressed as U/mg of Hb. Catalase (CAT) enzyme activity has been assayed by following the method of Sinha[11] and the activity is expressed as µmoles of H_2O_2 liberated /min/mg protein. Glutathione peroxidase (GPx) was assayed in the hemolysate according to the method of Rotruck *et al.*[12] and the enzyme activity has been expressed as U/mg of Hb. Glutathione S-transferase (GST) activity was estimated by following the method of Habig *et al.*[13] and the value has been expressed as U/mg of Hb. The non-enzymatic antioxidant properties like reduced glutathione[14] (GSH), total thiols[15], Vitamin A[16], Vitamin C[17] and Vitamin E[18] were estimated and the values have been expressed as µmoles/dl and the respective standards were used for each assay. All the data were subjected to analysis of similarity (ANOSIM) and the group means were compared by ANOSIM range test for differences among treated animal groups and among the antioxidant assays in multivariate data sets that was analogous to analysis of variance in univariate statistics. The results were considered statistically significant if the R value was 1.0.

RESULTS

NDEA (5µl/g of body weight) induced a well developed colon carcinoma in the mice tested. The actinobacterial extract significantly controlled the incidences of cancer induced by N-nitrosodiethylamine (NDEA) in the liver and colon regions of the mice, which has been as revealed by the histological evidences in the experimental animals (Figs. 1 & 2). Histology Histological examination of liver tissue under light microscope was done to observe the effect of NDEA on the structural integrity of the cells. Fig. 1a shows the normal histological appearance of liver cells. The extract SNI5 alone treated animals also showed normal architecture of the liver (Fig. 1c). The liver tissue of NDEA treated animals showed acute inflammatory cells with fragmented, presence of binucleated nucleus due to increase in mitotic activity and vacuolated cytoplasmic structures (Fig. 1b). In the animals treated with the actinobacterial extract of SNI5 along with NDEA, mild lesions were observed (Fig. 1d) along with regeneration of cells. This finding indicates that there was recovery in the liver cells, with the actinobacterial extract treatment.

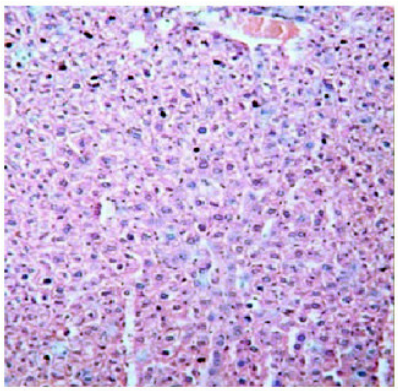

Fig.1a. H & E stained section of liver control mice.

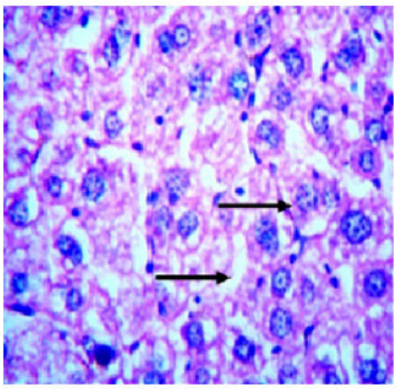

Fig.1b. H & E stained section of liver of NDEA treated mice showing fragmented and vacuolated cytoplasm.

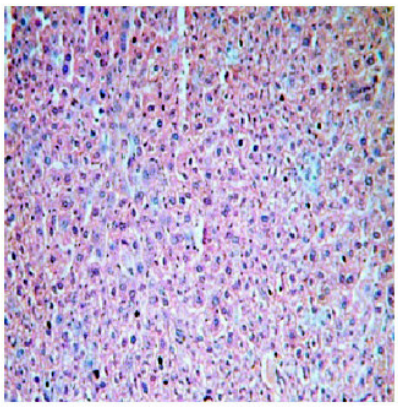

Fig.1c. H & E stained section of liver of actinobacterial extract (SNI 5) treated mice showing normal hepatic architecture.

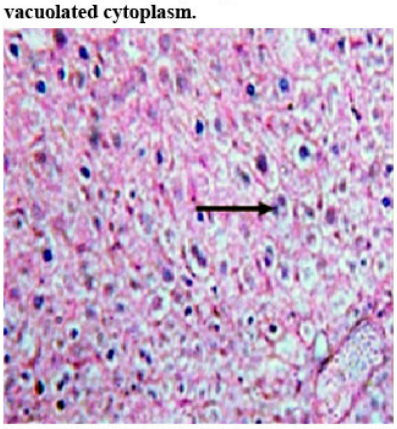

Fig.1d. H & E stained section of liver of NDEA and actinobacterial extract (SNI 5) treated mice showing little necrosis.

Figure 1

Histological changes in liver tissues of control and experimental mice.

Intestine and colon are two important areas of the gastrointestinal tract where a number of activities occur; most of the digestion and absorption occur in the intestine while in the colon, many materials which remain as undigested forms can be released and they may become carcinogens. In the present study, we examined the histology of colon tissue under light microscope showed that the effect of NDEA on mucosal layer. Fig. 2a shows the normal histological appearance of colonic mucosal cells. The actinobacterial extract SNI5 alone treated animals also showed similar architecture of the normal cells (Fig. 2c). The colon tissue from NDEA treated animals showed intermediate adenoma stage along with dysplasia formation, epithelial stratification, and structural abnormality (Fig. 2b) in the mucosal layer. The animals treated with actinobacterial extract SNI5 along with NDEA showed early adenoma stage (Fig. 2d) along with regeneration of the mucosal cells. This could be due to the administration of actinobacterial extract of SNI5 which probably defends the free radical formation.

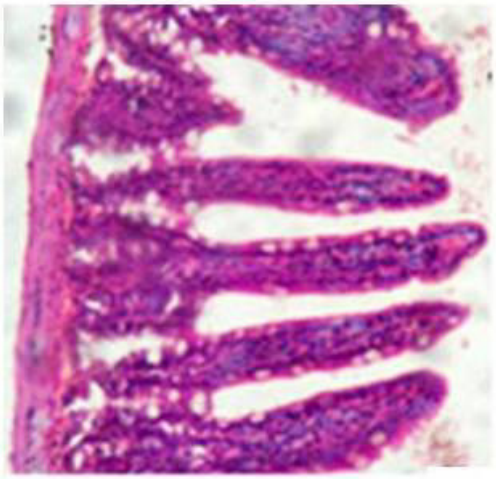

Fig.2a. H&E stained normal colonic mucosa of mice.

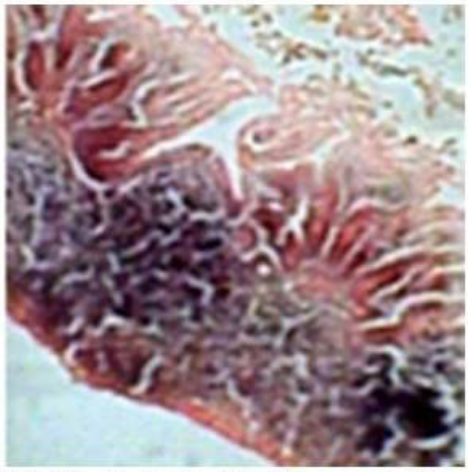

Fig.2b. H&E stained NDEA treated mice showing intermediate adenoma stage as well as dysplasia formation in mucosal layer.

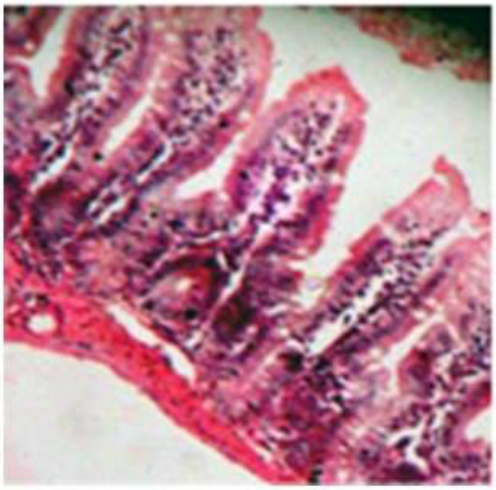

Fig.2c. H&E stained actinobacterial extract (SNI 5) treated mice, showing colonic mucosa.

Fig.2d. H&E stained NDEA and actinobacterial extract (SNI 5) treated mice, showing early adenoma stage.

Figure 2
Microscopic, histopathological observations in colon of mice in different experimental groups.

Antioxidant properties

The plasma obtained from the sacrificed mice was showed at 0.468 nmoles/dl in the control and it was 0.401 nmoles/dl in the actinobacterial extract administrated mice. Whereas, NDEA induced mice showed 0.981µmoles/dl and the actinobacterial extract along with NDEA induced mice showed 0.581 µmoles/dl (Fig. 3).

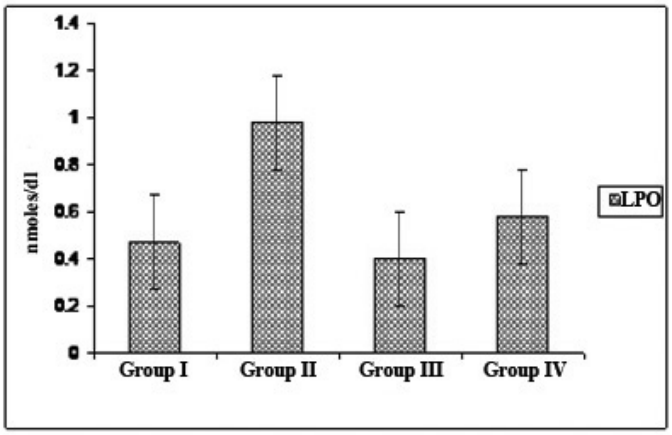

Figure 3
Lipid peroxidase activity recorded in different experimental groups.

GPx activity showed 144.18 units/mg Hb in the control mice, while 142.27 units/mg Hb activity was recorded in the actinobacterial extract treatment. The NDEA induced mice showed a GPx activity of 88.5 units/mg Hb. The activity was 100.87 units/mg Hb in the actinobacterial extract and NDEA treated mice. Levels of the antioxidants in the plasma and erythrocytes of the control and experimental groups were noted (Fig. 4). Superoxide dismutase (SOD) level was 31.06 units/mg Hb in control mice and 30.50 units/mg Hb in actinobacterial extract treated mice. Whereas in the NDEA induced mice, it was 15.06units/min. The actinobacterial extract treated NDEA induced mice registered 26.66 units/mg Hb. Glutathione-s-transferase (GST) activity was varied between 20.09 units/mg Hb in NDEA induced mice and 40 units/mg Hb in control mice. An activity of 39 units/mg Hb was found in the actinobacterial extract administrated mice. The activity was 31.5 units/mg in the actinobacterial extract with NDEA treated mice.

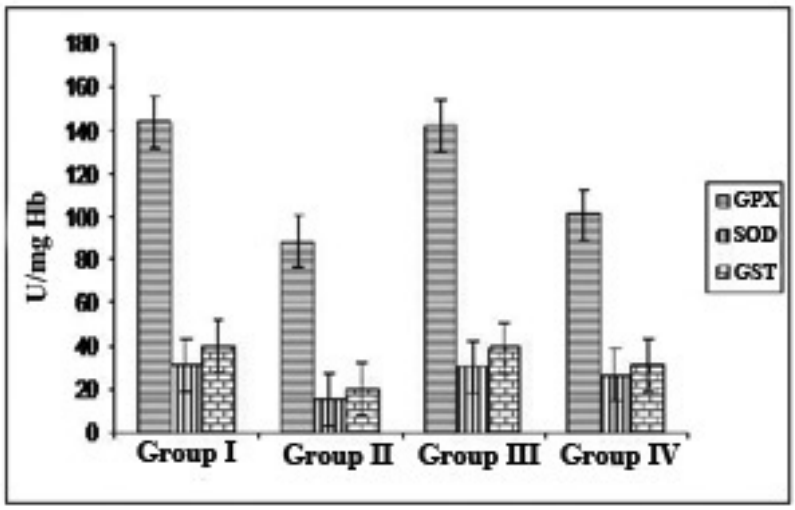

Figure 4
Enzymatic antioxidant levels recorded in different experimental groups.

Catalase activity was 0.551 µmoles of H_2O_2 liberated /min/mg protein in the control mice and 0.5 µmoles of H_2O_2 liberated/min/mg protein activity was found in the actinobacterial extract administrated mice. Whereas, NDEA induced mice showed the catalase activity of 0.1 µmoles of H_2O_2 liberated /min/mg protein and actinobacterial extract treated NDEA induced mice recorded 0.212 µmoles of H_2O_2 liberated /min/mg protein of catalase activity (Fig. 5).

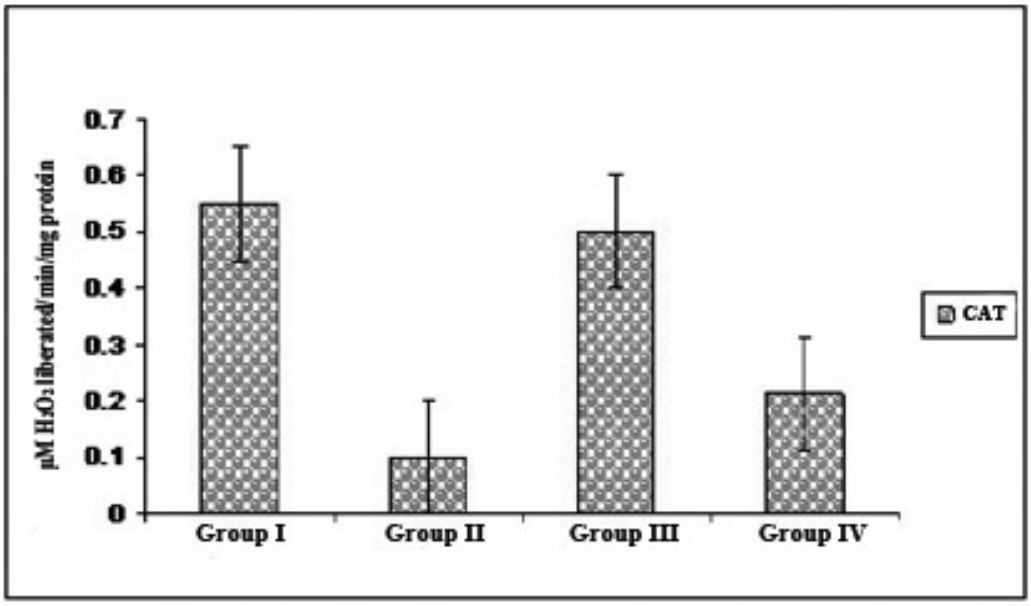

Figure 5
Catalase activity recorded in different experimental groups.

The non-enzymatic antioxidant property like Vitamin A activity noticed in the plasma of mice was as follows: 4.27 µmoles/dl in group I; 4.09 µmoles/dl in group III; 2.5741 µmoles/dl in group II and 3.5765 µmoles/dl in group IV. The vitamin C activity noticed in the blood cells of the mice was 1.28 µmoles/dl in group I; and 1.115 µmoles/dl in group III. Whereas, it was 0.189 µmoles/dl in group II and 0.987 µmoles/dl in group IV. Level of Vitamin E of plasma of mice was 6.27 µmoles/dl in group I and 5.09 µmoles/dl in group III. Whereas, group II showed 1.9741 µmoles/dl and in group IV, it was 4.965 µmoles/dl. Reduced glutathione activity (GSH) of blood in mice was 3.985 µmoles/dl in group I and 3.905 µmoles/dl in group III. Group II showed 0.987 µmoles/dl activity and group IV revealed 1.018 µmoles/dl activity. The total thiol activity of blood in mice was 6.066 µmoles/dl in group I and 5.985 µmoles/dl in group III. Whereas group II showed 3.765 µmoles/dl activity and an activity of 5.009 µmoles/dl was noticed in group IV (Fig. 6).

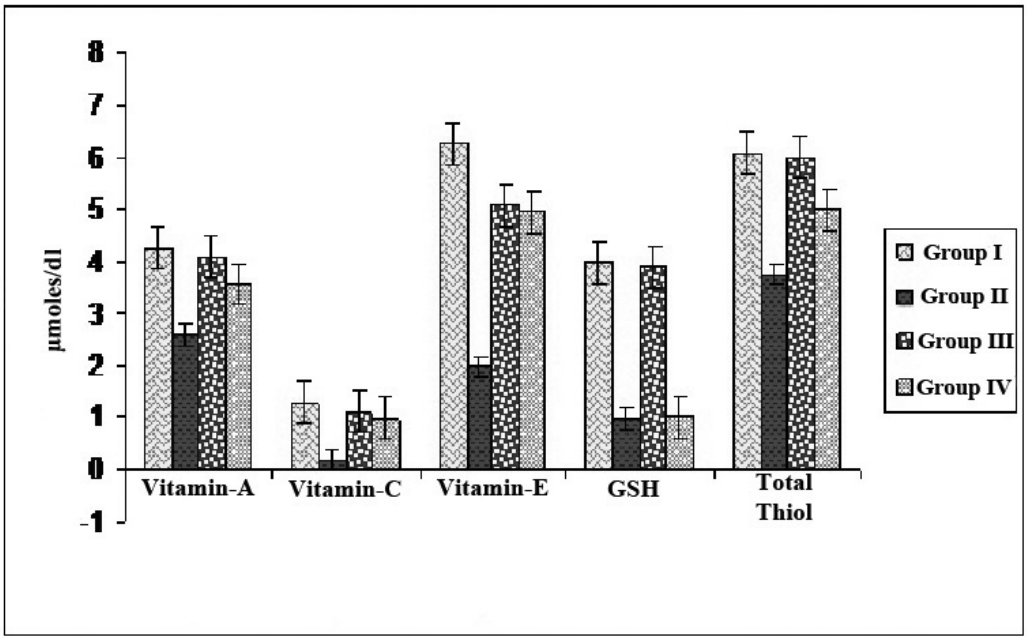

Figure 6
Levels of non-enzymatic antioxidants recorded in different experimental groups.

ANOSIM analyses were made to find out the differences between control and treated animal groups and among antioxidant parameters. For that, treated animal groups and antioxidant parameters were pooled separately and the results obtained are illustrated in histograms (Fig 7 and 8). The 'R' values for each combination and significance levels are presented. These indicated differences between all groups. The 'R' also shown in histogram -0.4 to 1.0 and -0.3 to 1.0 ranges respectively. The R values for the all the combination was outside, i.e., range of 1.0. It showed the significant differences between the groups during the experimental period. For the treatment groups, the R value was found to vary from -0.4 to 1. The global R value was 1 and it fell quite away from the 95% histogram showing heterogeneity among control and the treatment groups ($p < 0.01$). Control differed significantly with ($R = 1$ $p < 0.05$) all the other treated animal groups. Same way the differences among the treatment group were also significant as the R value was 1 in all this instances ($p < 0.05$). In the same way, all the antioxidant parameters also differed significantly each other ($R = 1$ $p < 0.05$).

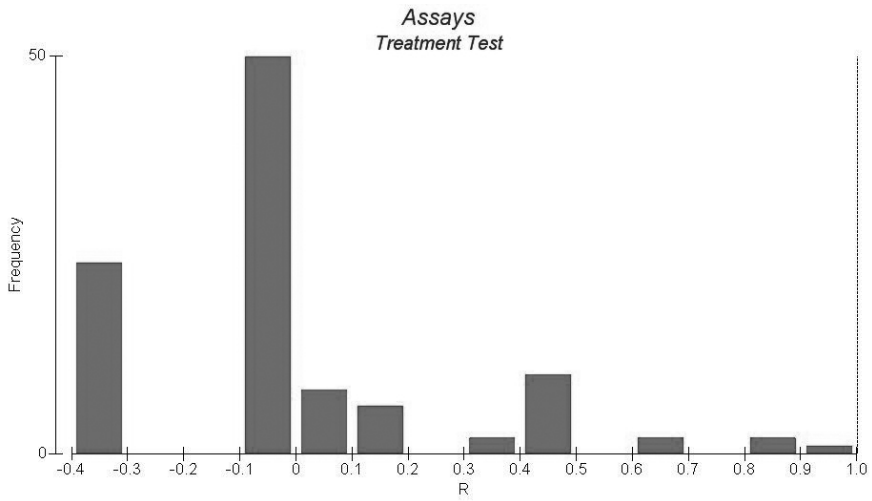

Figure 7
Stimulated variations of 'R' the among the treatment group animals.

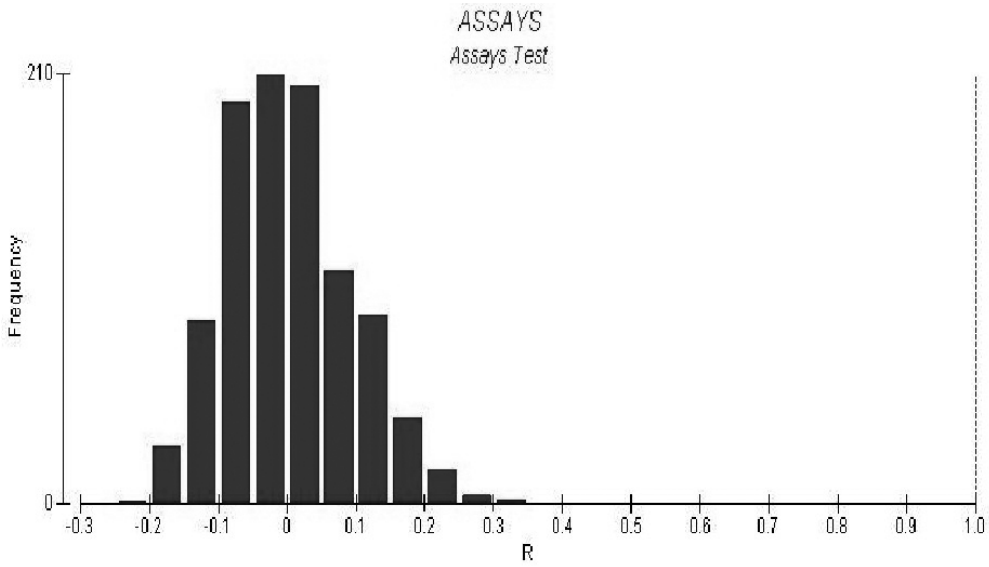

Figure 8
Stimulated variations of 'R' the antioxidant assays in treated animals.

DISCUSSION

The implications of reactive oxygen species (ROS) in carcinogenic nitrosamines toxic hepatic injury was well documented[19,20]. In the present study, increased level of lipid peroxidation (LPO) was observed in the plasma cells of NDEA induced mice. This trend has revealed the fact that N-nitrosodiethylamine administered in three doses might have generated free radicals. In the metabolism of nitrosamines, such as *N*-nitrosodiethylamine (NDEA), there is evidence for the formation of reactive oxygen species (ROS), resulting in oxidative stress, which is one of the factors in the etiology of cancer[7]. Lipid peroxidation leads to destabilization and disintegration of the cell membranes, altering the activities of membrane-bound enzymes and other proteins, as well as the concomitant release of hydroperoxides and alkoperoxide radicals, which are potentially toxic[21]. Decrease in the LPO levels was noticed in the plasma of mice which were administered actinobacterial extract along with N-nitrosodiethylamine, in contrast to the group that was administered N-nitrosodiethylamine alone. These results suggest that radicals such as superoxide anion, hydroxyl radical and H_2O_2, produced in the metabolism of NDEA increase the oxidative stress, which is considerably decreased by the actinobacterial extract of the strain SNI5 in the NDEA treated mice. Primary mechanism of this effect caused by the actinobacterial extract might involve the scavenging of free radicals by the anti oxidants present in the test[7]. Moreover, nature of the extract and breed of the animal used for administration, dose administered and the duration of administration would also decide the level of the oxidative stress[22]. Present study lends support to similar studies conducted with garlic extract[23] (250 mg/ kg/body weight on alternate days), *Ceriops sp*. extract[24] (250 mg/ kg/body weight) and *Propolis* extract[22] with 15 mg/kg on alternate days. Inhibition of GPx, SOD, GST and CAT activities was found to be involved in many degenerative diseases[25]. But, the free radicals scavenging systems viz. GPx, SOD, GST and CAT which exist in all oxygen metabolizing cells can prevent the cells from the damage caused by the free radicals and provide with a repair mechanism for oxidized membrane components. In the present study, significant differences were not found between the group-I and the group-III animals, the latter received actinobacterial extract alone, with regard to enzymatic oxidant levels and it was confirmed with previous studies using cholesterol rich diet[26]. The precancerous marker enzymes are highly expressed cytoplasmic protein predominant during early carcinogenesis[27]. This higher induction of radical scavenging enzyme activity in NDEA group was markedly suppressed on combined NDEA and actinobacterial extract fed mice. Present results have also shown that the actinobacterial extract administration could have significantly counteracted the decline in the activity of antioxidant enzymes (GPx, SOD, GST and CAT) in the animals by the NDEA. Similar results were observed by Thirunavukkarasu and Sakthisekaran[28], when selenite was supplemented in cancer bearing animals which reduced the adverse changes that occurred during cancer condition and Sundaresan and Subramanian[23] found that the garlic administered rats experienced a significant reduction in lipid peroxidation with a simultaneous elevation in antioxidant levels, when cancer was initiated by NDEA. Experimental studies have demonstrated that vitamin C (ascorbic acid) can inhibit the formation of nitroso compounds both *in vivo* and *in vitro*[29]. However, deficiency of vitamins E and C would result in enhanced tissue susceptibility towards free radicals and an increased LPO *in vivo*[30]. In the present study, marked decrease in the levels of Vitamins A, E and C was noticed in N-Nitrosodimethylamine treated tumor bearing animals. Whereas, actinobacterial extract alone treated mice (group-III) showed equivalent levels of these vitamins as that of group I. Interestingly, group IV mice treated with both NDEA and actinobacterial extract showed increased vitamin concentration than group III treated with NDEA alone. This again confirming that the actinobacterial extract might have played a scavenging role in the formation of free radicals by the NDEA. Glutathione, an important cellular antioxidant, offers protection against free radicals,

peroxides and toxic compounds[31]. GSH, in turn, keeps the cellular levels of the active forms of vitamins C and E elevated[32]. The deficiency of ascorbic acid, α-tocopherol and GSH in the circulation of the NDEA treated animals might have been a function of over utilization to scavenge the products of lipid peroxidation[23]. Consumption of carcinogen may be increasing the free radicals and oxidative stress in the red blood cells: organophosphate[33,34], isoproterenol[35], Sodium butyrate[36] and tobacco[37] and administration of anticancer substances would reduce such deleterious activity by increasing the vitamins and other parameters. In this direction, administration of the actinobacterial extract has significantly enhanced the level of vitamins A, C and E in the blood of tested animals. The modifying effects of actinobacterial extract SNI5 on NDEA induced tissue damage was further evidenced by histopathological observations causing prevention of pathological degenerative changes of the NDEA treatment to normalize and exhibiting marked reduction in incidence of focal preneoplastic lesions. Light micrograph, actinobacterial extract exhibited significant changes viz, enhanced cell to cell adhesion among intimate cell contacts with well defined cell boundaries resembling to normal tissue were noticed. Therefore, actinobacterial extract has proved remarkable in suppressing and/or arresting the degenerative changes, brought about by NDEA. Extract of the actinobacterial strain SNI5 tested in the present study was in the crude form and it could have contained many compounds, which may well act synergistically, and it is not possible at present to utter which compound is or compounds are responsible for the observed effects. Further studies are needed for this. However, present results suggest that the anticancer/anti oxidant property exhibited by this extract, under the experimental conditions, could be related to an overall effect of the secondary metabolites present in the extract[37, 38].

CONCLUSION

In conclusion, actinobacterial extract effectively prevented formation of preneoplastic foci in mice when given along with the NDEA treatment i.e. during the inhibition phase and often the NDEA treatment until the end of the experiment. The preventive effect of actinobacterial extract was indicated by inhibition of growth and development of preneoplastic foci. Thus, actinobacterial extract SNI5 seems to be a potential, and a promising chemopreventive agent. However, more experiments are needed for ascertaining the strain's potentiality to evolve anticancer compounds (or) drugs.

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REFERENCES

1. Haggar FA and Boushey RP, Colorectal cancer epidemiology: incidence, mortality, survival, and risk factors, Clin Colon Rectal Surg, 22: 191-197, (2009).
2. Bhattacharjee S, Rana T and Sengupta A, Inhibition of lipid peroxidation and enhancement of GST activity by cardamom and cinnamon during chemically induced colon carcinogenesis in Swiss albino mice, Asian Pac J Cancer Prev, 8: 578-82, (2007).
3. Salas JA and Mendez C, Engineering the glycosylation of natural products in actinomycetes, Trends Microbiol, 15: 219-232, (2007).
4. Das SR, Louise Ward and Burke C, Prospects of using marine actinobacteria as probiotics in aquaculture, Appl Microbiol Biot, 81: 419-429, (2008).

5. Ellaiah P, Ramana T, Bapi Raju KVVSN, Sujatha P and Uma Sankar A, Investigation on marine actinomycetes from Bay of Bengal near Kakinada coast of Andhra Pradesh, Asian J Microbiol Biotechnol Environ Sci, 6: 53-56, (2004).
6. IARC, Overall evaluations of carcinogenicity, in: Monographs on the Evaluation of Carcinogenic Risk of Chemicals to Humans, Supplement 7. Lyon, France: International Agency for Research on Cancer, pp 440, (1987)
7. Bansal AK, Bansal M, Soni G and Bhatnagar D, Protective role of Vitamin E pre-treatment on N-nitrosodiethylamine induced oxidative stress in rat liver, Chem Biol Interact, 156: 101-111, (2005).
8. Spicer SS, Diamine methods for differentiating mucosubstances histochemically, J Histochem Cytochem, 3: 211-234, (1965).
9. Yagi KA, Simple fluorometric assay for lipid peroxides in plasma, Biochem Med, 15: 212-216, (1976).
10. Misra HP and Fridovich I, The role of superoxide anion in the autoxidation of epinephrine and a simple assay for superoxide dismutase, J Biol Chem, 247: 3170-3175, (1972).
11. Sinha AK, Colorimetric assay of catalase, Anal Biochem, 47: 389-394, (1972).
12. Rotruck JT, Pope AL, Ganther HE, Swanson AB, Hafeman DG and Hoekstra WG, Selenium: Biochemical role as a component of glutathione peroxidase, Science, 179: 588-590, (1973).
13. Habig WH, Pabst MJ and Jakoby WB, Glutathione S-transferases: The first enzymatic step in mercapturic acid formation, J Biol Chem, 249: 7130-7139, (1974).
14. Beutler E, Duron O and Kelly BM, Improved method for the determination of blood glutathione, J Lab Cli Med, 61: 882-888, (1963).
15. Eluman GL, Tissue sulfhydryl groups, Arch Biochem Biophys, 82: 70 -77, (1959).
16. Bayfield RF and Cole ER, Colorimetric estimation of vitamin A with trichloroacetic acid, Method Enzymol, 67: 189-203, (1980).
17. Omaye ST, Turnbull JD and Sauberlich HE, Selected methods for the determination of ascorbic acid in animal cells, tissues and fluids, Method Enzymol, 62: 3-11, (1971).
18. Baker H, Frank O, de Angelis B and Feingold S, Plasma tocopherol in man at various times after ingesting free or acetylated tocopherol, Nutr Rep Int, 21: 531-536, (1980).
19. Recknagel RO and Glende Jr EA, Carbon tetrachloride hepatotoxicity-an example of lethal cleavage, CRC Criti Rev Toxicol, 2: 263-297, (1973).
20. Ravid A and Korean R, The role of reactive oxygen species in the anticancer activity of vitamin D, Anticancer Res, 164: 357-367, (2003).
21. Ohkawa H, Ohishi N and Yagi K, Reaction of linoleic acid hydroperoxide with thiobarbituric acid, J Lipid Res, 19: 1053-1057, (1978).
22. Kanbur M, Eraslan G and Silici S, Antioxidant effect of propolis against exposure to propetamphos in rats, Ecotox Environ Saf, 72: 909-915, (2009).
23. Sundaresan S and Subramanian P, S-Allylcysteine inhibits circulatory lipid peroxidation and promotes antioxidants in N-nitrosodiethylamine induced carcinogensis, Pol J Pharmacol, 55: 37-42, (2003).
24. Sitharangaboopathy N, Effects of the mangrove plant, *Ceriops decandra* on DMBA- induced Hamster buccal pouch carcinoma, Ph. D. Thesis, Annamalai University India, pp128, (2008).
25. Moron MA, Depierre JW and Mannervik B, Levels of GSH, GR and GST in rat lung and liver, Biochem Biophys Acta, 582: 67-78, (1979).
26. Mahfouz MM and Kummerov FA, Cholesterol-rich diets have different effects on lipid peroxidation, cholesterol oxides and antioxidant enzymes in rats and rabbits, J Nutr Biochem, 11: 293-302, (2000).
27. Poojari R, Gupta S, Maru G, Hade B and Bhagwat S, Chemopreventive and hepatoprotective effects of Embelin on N-Nitrosodiethylamine and carbon

tetrachloride induced preneoplasia and toxicity in rat liver, Asian Pac J Can Pre, 2: 1015-1020, (2010).
28. Thirunavukkarasu C and Sakthisekaran D, Sodium selenite, dietary micronutrient, prevents the lymphocytes DNA damage induced by N-nitrosodiethylamine and Phenobarbital promoted experimental hepatocarcinogenesis, J Cell Biochem, 88: 578-588, (2003).
29. Mirvish SS, Inhibition of the formation of carcinogenic N-nitroso compounds by ascorbic acid and other compounds. In: JH. Burchenal, and H.F. Oettgcn (eds.), Cancer Achievements Challenges, 1981, pp 557-587.
30. Poppel GV, Spanhaak S and Ockhuzen T, Effect of beta-carotene on immunological indexes in healthy male smokers, Am J Clin Nutr, 57: 402-407, (1993).
31. Vina RJ and Vina Saez ST, The physiological function of glutathione. In: J. Miguel, A.T. Quintanilla, and H. Weber (eds.), Handbook of free radicals and antioxidants in biomedicine CRC Press, Boca Raton, 1989, pp.121–132.
32. Niki E, Interaction of ascorbate and alphatocopherol, Ann NY Acad Sci, 498: 186-199, (1987).
33. Altuntas I, Delibas N, Demirci M, Kilinc I and Tamer N, The effects of methidathion on lipid peroxidation and some liver enzyme: role of vitamins E and C, Arch Toxicol, 76: 470–473, (2002).
34. Yarsan E and Cakir O, Effects of dichlorvos on peroxidation in mice on subacute and subchronic periods, Pestic Biochem Phys, 86: 106-109, (2006).
35. Suchalatha S and Devi CSS, Antioxidant activity of ethanolic extract of *Terminalia chebula* fruit against isoproternol induced oxidative stress in rats, Indian J Biochem Bio, 42: 246-249, (2005).
36. Neumann DM, Bhattacharjee PS and Hill JM, Sodium butyrate: A chemical inducer of in vivo reactivation of Herpes simplex virus type I in the ocular mouse model, J Virol, 81: 6106-6110, (2007).
37. Yu JQ, Lei JQ, Yu HD, Cai X and Zou GL, Chemical composition and antimicrobial activity of essential oil of *Scutellaria barbata*, Phytochemistry, 65: 881-884, (2004).
38. Kamala K, Karuppiah V and Sivakumar K, Comparative evaluation of in vitro antioxidant potent of the marine actinobacteria from Gulf of Mannar biosphere reserve, Int J Pharm Bio Sci 4(3): (B) 207-216, (2013).