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RESEARCH ARTICLE **OPEN ACCESS**

PROTECTIVE EFFECT OF MOSINONE-A ON BIOMEMBRANE INTEGRITY IN 7, 12 DIMETHYL BENZ [A] ANTHRACENE (DMBA) INDUCED HAMSTER BUCCAL POUCH CARCINOGENESIS

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ABSTRACT

The present investigation the protective effect of Mosinone-A on cellular integrity by measuring the status of lipid profile, osmotic fragility and membrane bound enzyme activity in 7, 12-Dimethylbenz (a) antharacene (DMBA) induced oral carcinogenesis. A total number of 24 golden Syrian hamsters were randomized into 4 groups of 6 animals in each. Group I animals were served as untreated control. Groups II and III animals were painted with 0.5% DMBA in liquid paraffin three times per week for 14 weeks on the left buccal pouches. Group III animals were orally administered with Mosinone-A (2 mg kg^{-1} b.wt) starting one week before the exposure to the carcinogen and continued on days alternate to DMBA painting, until the sacrification of the animals. Group IV animals were received Mosinone-A alone throughout the experimental period. We observed altered levels of lipids and membrane bound enzyme activity in DMBA painted hamster. Oral administration of Mosinone-A 2mg/kg b.wt significantly prevented the tumor formation as well as normalized the above said biochemical variables in DMBA painted hamster. Mosinone-A has potent efficacy to exert their anticarcinogenic effect by modulating the status of lipid, integrity of cell membrane in DMBA induced hamster buccal pouch carcinogenesis

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INTRODUCTION

Oral squamous cell carcinoma (OSCC), is the fifth most malignancy worldwide, is a major cause of morbidity and mortality[1]. betel nut chewing with tobacco is identified as the risk factor for the high oral cancer incidence in India[2]. Heavy alcohol consumption is also associated with more than 70-80% of oral cancer. Heavy alcohol consumption is also associated with elevated oral cancer risk [3]. DMBA, potent organ and site specific carcinogen is commonly used to induce buccal pouch carcinogenesis in hamsters [4] which is the excellent animal model for for studying oral carcinogenesis [5].

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Lipids are major cell membrane components essential for various biological function including cell growth and division of normal and malignant tissue [6]. The membrane bound enzymes such as Na^+/K^+-ATPase, Mg^{2+}-ATPase and Ca^{2+}ATPase are is the intrinsic membrane protein responsible for the transport of sodium/potassium, magnesium and calcium ions across the cell membranes at the expense of ATP by hydrolysis [7] Osmotic fragility reflects the structural and geometrical changes of red blood cells have been found to be altered in various pathological conditions. The integrity of the RBCs may be determined by measuring the changes in erythrocyte osmotic fragility [8]. Abnormalities in the levels of lipids and altered cholesterol phospholipids molar ratio in the cell membranes have been implicated as an important aspect of malignant transformation [9]. Chemoprevention a recent novel approach in experimental oncology, Deals with inhibition or

suppression of the tumor formation by using natural or synthetic entities [11]. Profound articles evidently proved chemopreventive agents exhibited anticarcinogenic and antimutagenic effects[10] Mosinone-A is one of the novel mono-tetrahydrofuran ring acetogenin, from the bark of *Annona squamosa*, viewing cytotoxic selectivities for the human pancreatic carcinoma cell line [11]. However, no scientific report is available in literature about the study. Therefore, the present investigation was undertaken to study the effects of Mosinone-A on lipid profile, membrane-bound enzymes and osmotic fragility in hamster buccal pouch carcinogenesis.

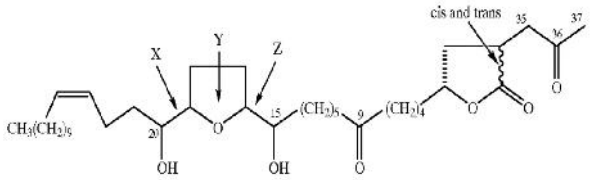

Fig. 1. Structure of Mosinone-A ($C_{37}H_{64}O_7$)

MATERIAL AND METHODS

Animals: Eight to ten weeks old male golden Syrian hamsters, weighing 80-120g were purchased from National Institute of Nutrition, Hyderabad, India and were maintained in the Central Animal House, Rajah Muthaiah Medical College and Hospital, Annamalai University. The animals were housed in polypropylene cages and provided with a standard pellet diet and water *ad libitum*. The animals were maintained under controlled conditions of temperature and humidity with a 12h light /dark cycle.

Chemicals: The carcinogen, 7, 12-dimethylbenz[a]anthracene (DMBA) was obtained from Sigma-Aldrich Chemical Pvt. Ltd. Bangalore, India. All other chemicals used were of analytical grade, marketed by Himedia laboratories, Bangalore and Sisco Research Laboratories Pvt, Ltd, Mumbai, India.

Isolation of Mosinone-A: Mosinone-A was isolated from *Annona squamosa* bark using the method of Maclaughlin [12]. The dried and pulverized bark of *Annona squamosa* was extracted with ethanol. The residues were portioned between chloroform and water, and further portioned between 90% methanol and hexane to get hexane soluble residues. The hexane soluble residue was subjected into column chromatography over silica gel using hexane and chloroform followed by chloroform and methanol solvent system. The resulting fractions were combined on the basis of HPTLC analysis. Then, the combined fractions were run into column chromatography to get the final product of Mosinone-A, a whity waxy solid substance. The identity of isolated Mosinone-A was done by LC-MS and NMR. Its identy was confirmed by comparism to the reference Mosinone-A, which was purchased from Lock chemicals Ltd China. The yield and purity of the isolated Mosinone-A were found to be 0.21% and >90% respectively. For experimental studies Mosinone-A was first dissolved in 0.5% dimethyl sulfoxide (DMSO).

Experimental protocol: A total of 24 golden Syrian hamsters were randomized into 4 groups of 6 animals each. The first group served as the control group while the last 3 groups served as the experimental groups. Group 1animals were served as untreated control. Groups 2 animals were painted with 0.5% DMBA in liquid paraffin three times per week for 14 weeks on the left buccal pouches (No:4 brush). Group 2 animals received no other treatment. Group 3 animals were orally administered with Mosinone-A (2 mg kg^{-1} b.wt) starting one week before the exposure to the carcinogen and continued on days alternate to DMBA painting, until the sacrification of the animals. Group 4 animals were received Mosinone-A alone throughout the experimental period.

Biochemical Estimation: Lipid extraction from the tissues was done by the method of Folch et al [13]. The level of total cholesterol parekh and Jung [14] The estimation of phospholipids was done by the method of Zilversmit and Davies [15] Free fatty acids and triglycerides were measured by the methods of Falholf et al [16] and Foster and Dunn [17] respectively. Osmotic fragility was determined by the method of parpart et al [18] and mean corpuscular fragility (MCF) was calculated by recording the saline concentration, which would have in 50% hemolysis

RESULTS

Table 1 to 3 shows the levels of lipids profile (total cholesterol, phospholipids and free fatty acid) in plasma, erythrocyte membrane and buccal tissue of control and experimental animals in each group. The levels of total cholesterol and FFA were increased whereas the Phospholipids and triglycerides levels were decreased in tumor bearing animals (Group 2). Oral administration of Mosinone-A 2 mg per kg b.wt siginificantly revert back the above mentioned lipid abnormalities in DMBA treated animal. Mosinone-A alone showed (group4) no significant differences in the levels of lipid as compared to control animals. Table 2 Shows the activities of membrane bound enzymes (ATPase) in erythrocyte membrane and plasma, of control and experimental animal in each group. The decrease in the Na^+/K^+ ATPase, Mg^{2+}-ATPase and Ca^{2+}-ATPase activity in cancer bearing hamsters when compared with control animals. Oral administration of Mosinone-A normalized the altered levels of membrane bound enzymes in animals treated with Mosinone-A alone showed (group4) no significant differences in the levels of membrane bound enzymes as compared to control animals.

Osmotic fragility curves for control and experimental animals in each group of experimental design are shown in figure 2 The fragility curve of tumor bearing animals was shifted to the right for the control hamsters. The mean corpuscular fragility was also significantly higher in tumor bearing animals as compared to control. Treatment of tumor bearing animals with Mosinone-A at a dose of 2 mg kg^{-1} b.wt shifted the curve to the left of cancer animals Mean Corpuscular Fragility (MCF) values did not differ significantly in hamster treated with Mosinone-A alone as compared to control animals.

DISCUSSION

Lipids take part in an important role in biological functions which comprise membrane composition and regulation, energy metabolism and signal transduction [20]. The exact mechanism by which Lipids have been creating to be involved in many cancer including oral cancer. Several studies have reported that shown an inverse association between blood lipid profiles and different cancers [21]. Researchers have been reported that the level of plasma/serum lipids with different cancers. Many scientists have been reorted that the exact mechanism by which

Table 1. The levels of lipid profile in plasma of control and experimental animals in each group

Groups	Treatment	Total cholesterol (mg/dl)	Phospholipids (mg/dl)	Free fatty acid (mg/dl)
1	Control	91.5 ± 9.21[a]	131.13 ± 13.1[a]	7.48 ± 0.75[a]
2	DMBA	135.06 ± 13.42[b]	78.00 ± 8.00[b]	16.8 ± 1.72[b]
3	DMBA+Mosinone-A(2 mg/ kg b.wt)	113.93 ± 11.30[c]	105.63 ± 10.4[c]	10.50± 1.00[c]
4	Mosinone-A alone(2 mg/ kg b.wt)	90.30 ± 9.13[a]	30.46 ± 12.4[a]	7.40 ± 0.74[a]

Values are expressed as mean ± SD. Values not sharing a common superscript significantly differ at $p < 0.05$ (DMRT).

Table 2. The levels of Lipids profile in erythrocytes of control and experimental animals in each group

Groups	Treatment	Total cholesterol (mg/dl)	Phospho lipids (mg/dl)	Free fatty acid (mg/dl)
1	Control	110.00±10.14[a]	244.50 ±25.26[a]	8.43±0.81[a]
2	DMBA	156. 84±16.5[b]	182.16±17.96[b]	16.85±1.72[b]
3	DMBA+Mosinone-A (2 mg/ kg b.wt)	130.15±12.98[c]	213.28±20.45[c]	10.50±1.23[c]
4	Mosinone-A alone(2 mg/ kg b.wt)	108.83±10.48[a]	243.14±25.12[a]	8.41±0.85[a]

Values are expressed as mean ± SD. Values not sharing a common superscript significantly differ at $p < 0.05$ (DMRT).

Table 3. The levels of Lipids profile in buccal tissue of control and experimental animals in each groups.

Groups	Treatment	Total cholesterol (mg/dl)	Phospholipids (mg/dl)	Free fatty acid (mg/dl)
1	Control	3.89 ± 0.39[a]	14.08 ± 1.36[a]	14.08 ± 1.47[a]
2	DMBA	7.71 ± 0.78[b]	6.05 ± 0.67[b]	6.54 ± 0.67[b]
3	DMBA+Mosinone-A (2 mg/ kg b.wt)	5.66 ± 0.59[c]	10.56 ± 1.16[c]	11.25 ± 1.18[c]
4	Mosinone-A alone(2 mg/ kg b.wt)	3.99 ± 0.40[a]	13.31 ± 1.34[a]	14.85 ± 1.48[a]

Values are expressed as mean ± SD. Values not sharing a common superscript significantly differ at $p < 0.05$ (DMRT).

Table 4.The activity of membrane bound enzymes in erythrocyte membrane of control and experimental hamsters in each group

Groups	Treatment	Na^+, K^+ ATPase	Ca^{2+}ATPase	Mg^{2+} ATPase
1	Control	0.77 ± 0.08[a]	0.72 ± 0.08[a]	0.74 ± 0.08[a]
2	DMBA	0.34 ± 0.04[b]	0.23 ± 0.02[b]	0.32 ± 0.04[b]
3	DMBA+Mosinone-A (2 mg/ kg b.wt)	0.55 ± 0.06[c]	0.48 ± 0.04[c]	0.57 ± 0.06[c]
4	Mosinone-A alone(2 mg/ kg b.wt)	0.76 ± 0.07[a]	0.71 ± 0.07[a]	0.75 ± 0.08[a]

Values are expressed as mean ± SD. Values not sharing a common superscript significantly differ at $p < 0.05$ (DMRT).

lipids is not clearly understood. The previous study reported that lipids might primary affect gonads and subsequent higher estradiol secretion could influence the development of malignancies [22]. In the present study, we observed an increase in the level of total cholesterol, and FFA and decrease in the level of TG and phospholipids in the plasma of DMBA treated animals as compared to control animals. Increased plasma FFA was reported in tumor bearing animals and cancer patients [23]. Elevated plasma fatty acid concentration promotes the liver in the conversion of some fatty acids into phospholipids and cholesterol. A decrease in plasma triglycerides indicates an increase in lipolysis during carcinogenesis [25]. The data obtained in the present study shows that the lipids levels were reverted back to near normal levels upon treatment with Mosinone-A.

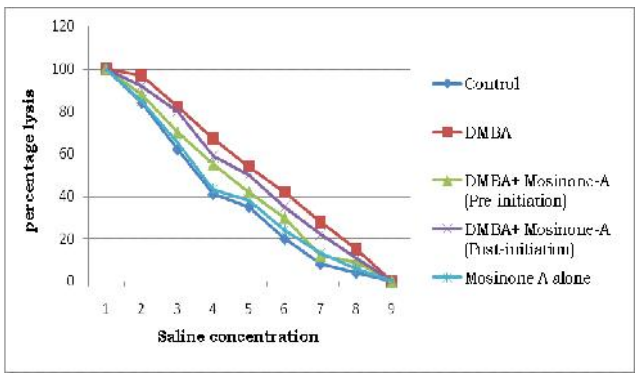

Figure 2. Effect of Mosinone-A on Osmotic fragility curve of the control and experimental animals

The defence of membrane potential is magnitude in the treatment of disease processes. The membrane bound enzymes are responsible for the transport of sodium/potassium across the cell membranes at the expense of ATP by hydrolysis [26]. The activity of ATPase in erythrocyte membrane and surrounding tissues has been found to be inhibited in tumor bearing animals. Oxidative damage to membrane bound enzymes has been assumed to be crucial for cell lysis [27].A decrease in membrane bound Na^+K^+ATPase in DMBA painted hamsters suggest that the membrane permeability was drastically altered during DMBA induced oral carcinogenesis. Oral administration of Mosinone-A significantly improved the activity of membrane bound enzyme activity in the DMBA painted hamsters. Osmotic fragility, the sensitivity to change in osmotic pressure characteristics of red blood cells, has been altered in various pathological conditions [28]. Measurement of mean corpuscular fragility of red cell membranes is useful to assess the alterations in the integrity of cell structure and function. Alteration in membrane fragility has been documented in several diseases including cancer (Kung *et al.*, 2009). Erythrocytes of tumor bearing animals were more fragile than those from control animals. Increased osmotic fragility in cancer animals could be due to increased oxidative stress in erythrocytes since over production of ROS has been implicated in the structural alterations and functions of cell membrane. Oral administration of Mosinone-A significantly improved the integrity of cell structure and function. Oral administration of Mosinone-A significantly normalized the levels of lipid profile and the activity of membrane bound enzymes and improved the osmotic fragility in the DMBA painted hamsters.

So further studies were warranted to know the mechanistic pathway of structural integrity maintenance of Mosinone-A in DMBA treated hamsters.

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Ethical Approval

"All authors hereby declare that "Principles of laboratory animal care" (NIH) were followed, as well as specific national laws where applicable. All experiments have been examined and approved by the appropriate ethics committee"

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