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RESEARCH

Anti Genotoxic Effect of Mosinone-A on 7, 12-Dimethyl Benz[a] Anthracene Induced Genotoxicity in Male Golden Syrian Hamsters

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Abstract The present study was aimed to evaluate the antigenotoxic effect of Mosinone-A on 7,12-dimethylbenz [a]anthracene induced genotoxicity. The frequency of micronucleated polychromatic erythrocytes [MnPCEs], chromosomal aberrations [CA], DNA damage (comet assay) as cytogenetic markers and the status of lipid peroxidation byproducts, antioxidants and phase II detoxification agents were used as biochemical markers to assess the antigenotoxic effect of Mosinone-A on DMBA induced genotoxicity. A single intraperitoneal injection of DMBA (30 mg/kg b.wt) to golden Syrian hamsters, resulted in marked elevation in the frequency of MnPCEs, aberrations in the chromosomal structure were found in bone marrow and DNA damage (comet assay) was found in blood cells and altered level of lipid peroxidation, antioxidants, and phase II detoxification agents. Oral pretreatment of Mosinone-A (2 mg/kg b.wt) for 5 days to DMBA treated animals significantly reduced the frequency of MnPCEs, chromosomal abnormalities such as chromosomal break, gap, minute, fragment, DNA damage and reversed the status of biochemical variables. Our results thus demonstrated the antigenotoxic effect of Mosinone-A on DMBA induced genotoxicity in male golden Syrian hamsters.

Keywords Chromosomal aberrations · Comet assay · Genotoxicity · DMBA · Micronuclei · Mosinone-A

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Abbreviation

DMBA	7, 12-dimethylbenz[a]anthracene
MnPCEs	Micronucleated polychromatic erythrocytes
NCEs	Normochromatic erythrocytes
PCEs	Polychromatic erythrocytes
CA	Chromosomal aberrations
CAT	Catalase
GPx	Glutathione peroxidase
SOD	Superoxide dismutase
GR	Glutathione reductase
GST	Glutathione-S-transferase
GSH	Reduced glutathione
TBARS	Thiobarbituric acid reactive substances

Introduction

Cytogenetics is the study of inheritance in relation to the structure and function of chromosomes [1]. Genotoxicity is characterised by genetic instability, which has often been examined at the single base mutation level, caused by both genetic and environmental factors, also evidenced by gross chromosomal abnormalities. These abnormalities include altered DNA repair capacity, segregation of DNA, DNA damage with normal replication process and abnormal cell division [2, 3]. A variety of genotoxic related markers such as metaphase chromosomal aberrations, micronuclei and comet parameters (% tail DNA, tail length, tail movement and olive tail movement in alkaline version) as a rapid, simple and reliable markers for evaluating DNA damage in mammalian cells [4].

Agents which induce genotoxic abnormalities can be assessed by determination of chromosomal aberration and frequency of micronucleated polychromatic erythrocytes

(MnPCEs) [5]. Micronuclei are formed by the action of genotoxic agent which induce chromosome breaks or agent that causes damage to the spindle apparatus [6]. A high frequency of MnPCEs is associated with pathogenesis of several diseases including diabetes, cancer and neoplastic transformation [7]. Increased micronuclei frequency and alteration of chromosomal structure in the bone marrow compartment helps to assess the risk of cancer in human population [8]. Several studies reported that detect and migration of DNA fragment in single cell electrophoresis (Comet assay) during the DMBA induced genotoxicity [9].

7, 12-dimethylbenz[a]anthracene (DMBA), a polycyclic aromatic hydrocarbon, potent organ and specific carcinogen, is widely used to induce genotoxicity in experimental animals [10]. On metabolic activation, DMBA is converted into its ultimate carcinogen dihydrodiol epoxide, mediates genotoxicity by inducing mutation and over production of reactive oxygen species (ROS) [11]. Increased micronucleus frequency and marked chromosomal abnormalities were reported in DMBA induced genotoxicity [12].

Oxidative stress, an imbalance between oxidants and antioxidants, plays an important role in the pathogenesis of several diseases including cancer [13]. Over production of reactive oxygen species (ROS) due to oxidative stress during metabolic activation of DMBA to diol epoxide cause oxidative damage to the structure and functions of macromolecules DNA, proteins and lipids [14]. Previous studies were reported that elevated levels of TBARS in liver and plasma of DMBA treated hamsters could therefore be due to over production of reactive oxygen species [15]. An imbalance between antioxidants (Plasma, liver) and cytogenetic markers includes Micronuclei and chromosomal aberration (bone marrow) were reported in carcinogen induced genotoxicity [16]. Screening the levels of detoxification agents in liver homogenate during carcinogen induced genotoxicity can also assess the antigenotoxic potential of Mosinone-A. An altered level of detoxification agents such as GST, GR and GSH in the liver homogenate indicates that disturbance of detoxification process by the carcinogen, DMBA [17].

Chemoprevention is a good and promising approach to control, inhibit or suppress tumor formation using naturally occurring or synthetic chemicals [18]. Several studies reported that medicinal plants and their products exhibit chemopreventive and chemotherapeutic effects in animals [19]. It has been suggested that medicinal plants possess anticarcinogenic and antimutagenic activities are considered novel chemopreventive agents through the variety of mechanisms such as inhibition of genotoxic effects, induction of apoptosis, alteration of signal transduction, modulation of antioxidant activities, scavenging free radicals, and enhancing the activities of detoxification enzymes [20].

Annona squamosa (annonaceae) is commonly known as custard apple, a native of West Indies and is now cultivated throughout India. Chavan et al. reported that *Annona squamosa* possess several medicinal properties and used in folkoric medicine for the treatment of degenerative diseases including cancer [21]. Annonaceous acetogenins constitute a series of natural products isolated exclusively from annonaceae species that are widely distributed in tropical and sub-tropical regions [22]. Most annonaceous acetogenins are potentialy bioactive, but the mode of action of these compounds was unknown. Londerhausen et al. reported that Mosinone-A act to inhibit complex I mitochondrial oxidative phosphorylation with an activity several times greater than that of rotenone [23]. Mosinone-A (Fig. 1) is one of the novel mono-tetrahydrofuran ring acetogenin, from the bark of *Annona squamosa*, showing cytotoxic selectivities for the human pancreatic carcinoma cell line [24]. However, no scientific reports were available on literature about anti genotoxic effect of Mosinone-A on DMBA induced genotoxicity in male golden Syrian hamsters. Thus, in the present study we investigated the antigenotoxic effect of Mosinone-A on DMBA induced genotoxicity in male golden Syrian hamsters.

Materials and Methods

Animals

Male golden Syrian hamsters, 8–10 weeks old, weighing 80–120 g were purchased from National Institute of Nutrition, Hyderabad, India and maintained in Central Animal House, Rajah Muthiah Medical College and Hospital, Annamalai University. The animals were housed in polypropylene cages and provided standard pellet diet and water ad libitum. The animals were maintained under controlled conditions of temperature and humidity with a 12 h light/dark cycle.

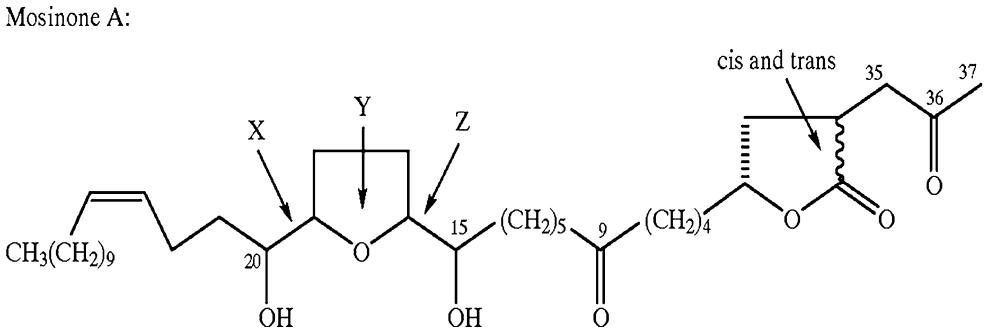
Chemicals

The carcinogen 7,12-dimethylbenz[a]anthracene (DMBA), colchicine (95% purity, soluble in water), Giemsa and May-Grunwald's stains, ethidium bromide, low melting point agarose (LMA), normal melting point agarose (NMA), Triton X-100, EDTA and sodium sarcosinate were purchased from Sigma-Aldrich Chemical Pvt. Ltd., Bangalore, India. All other chemicals used were of analytical grade.

Isolation of Mosinone-A

Mosinone-A was isolated from *Annona squamosa* bark by the method of Maclaughlin [25]. The dried and pulverized

Fig. 1 The structure of Mosinone-A



bark of *Annona squamosa* was extracted with ethanol. The residues were portioned between chloroform and water, 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. The isolated Mosinone-A was compared with the reference Mosinone-A purchased from Lock chemicals, china. The yield and purity of the isolated Mosinone-A was found to be 0.21% and >90% respectively. The isolated Mosinone-A was first dissolved in 0.5% DMSO and used for experimental studies.

Experimental Protocol

The institutional animal ethics committee, Annamalai University, Annamalainagar, approved the experimental design. A total of 24 hamsters were divided into 4 groups of 6 animals each. Group 1 hamsters were untreated control. Group 3 hamsters were pretreated with Mosinone-A (2 mg/kg b.wt) for 5 days. Groups 2 and 3 hamsters were injected with DMBA (30 mg/kg b.wt *ip* in liquid paraffin). Group 4 hamsters were pretreated with Mosinone-A (2 mg/kg b.wt) alone for 5 days and were not received DMBA. All the hamsters were sacrificed by cervical dislocation for the assessment of micronucleus frequency, chromosomal aberrations, comet assay (single cell gel electrophoresis), antioxidant status and detoxification enzymes.

Bone Marrow Micronucleus Test

Bone marrow micronucleus test was carried out according to the method of Schmid [26]. The femur bones removed from the hamsters were cleaned and the content was flushed into tube containing 1 ml of fetal calf serum and was centrifuged at 500 g for 10 min. The obtained pellet was suspended with few drops of fresh serum and slides were prepared and air-dried for 18 h. After drying, the slides were stained with May-Grunwald stain followed by Giemsa stain. The frequency of MnPCEs in each group was calculated by scoring 2,500 polychromatic erythrocytes (PCEs) per hamsters.

Assessment of Chromosomal Aberrations

Assessment of chromosomal aberrations in bone marrow was carried out according to the procedure of Kilian et al. [27] The femur bones were removed from hamsters injected intraperitonealy with 0.1% colchicine (1 ml/100 g bw) 90 min before sacrificing the animals. The bone marrow contents were flushed into 5 ml of physiological saline and centrifuged at 500 g for 5 min. The sediments obtained were resuspended in 6 ml of hypotonic KCl (0.075) and incubated at 37°C for 25 min. The obtained pellets were then fixed using methanol: acetic acid (3:1) fixative and stained with the Giemsa stain. A total of 100 well-spread metaphase cells were scored or each animal and structural chromosomal aberrations were observed and recorded.

Alkaline Single Cell Gel Electrophoresis (Comet) Assay

The comet assay was carried out according to the protocol described by Singh et al. [28]. With minor modification, hamsters were decapitated and 2 ml of blood was collected in an eppendorff tube containing 100 µl of 10% EDTA, the blood samples were stored in a dark box at 4°C until use. In brief, frosted microscopic slides were covered with 200 µl of 1% normal melting agarose (NMA) in PBS at 45°C and were kept at 4°C for 10 min to allow the agarose to solidify the second layer of 200 µl of 0.5% low melting point agarose (LMA) containing approximately 7 µl blood was added. After solidification for 15 min on ice, the slides were placed in the chilled lysing solution containing 2.5 M Nacl, EDTA, 100 mM Tris–HCl at P^H10 and 1% DMSO, 1% Triton X- 100, and 1% sodium sarcosinate for 1 h at 4°C. The slides were removed from the lysing solution and were

Table 1 Effect of Mosinone-A on DMBA induced bone marrow micronuclei formation

Group	Parameters	MnPCEs/2500 PCEs	PCEs/NCEs	PCE (%)*
1	Control	5.17±0.50[a]	1.04±0.13[a]	50.05[a]
2	DMBA	57.6±5.69[b]	0.85±0.07[b]	42.70[b]
3	DMBA + Mosinone-A	20.8±2.18[c]	0.93±0.09[c]	45.40[c]
4	Mosinone-A alone	5.13±0.49[a]	1.03±0.12[a]	51.00[a]

Values are expressed as the mean ± SD (n=6; 2500 PCEs were scored per hamster). Values that are not sharing a common superscript letter in the same column differ significantly at $p<0.05$ (DMRT)

*Percentage of polychromatic erythrocytes was calculated as follows: [PCEs/PCEs + NCEs]_100

placed on a horizontal electrophoresis tank filled with alkaline buffer (300 mM NaoH, EDTA, and 0.2% DMSO P^H13) for 20 min. The electrophoresis was carried out at 25 V, 300 MA for 20 min. After electrophoresis, the slides were washed gently with 0.4 M Tris–HCl buffer (p^H7.4) and the slides were stained with 50 μl of ethidium bromide and visualized using a Nikon fluorescent microscope. The images were captured with high performance Nikon camera. The quantification of the DNA strand breaks of the stored images were done using the CASP software by which comet attributes could be obtained directly.

Biochemical Estimations

Blood samples were collected into heparinized tubes. Plasma was separated by centrifugation at 1,000 g for 15 min. The buffy coat was removed and the packed cells were washed three times with physiological saline. Tissue samples from animals were washed with ice-cold saline and homogenized using appropriate buffer in an all-glass homogenizer with Teflon pestle and used for biochemical estimations.

Lipid peroxidation was estimated as evidenced by the formation of thiobarbituric acid reactive substances (TBARS). TBARS in plasma was assayed by the method of Yagi [29]. Plasma was deproteinised with phosphotungstic acid and treated with thiobarbituric acid at 90°C for 1 h. The pink color formed gives a measure of TBARS, which was read at 530 nm. Tissue lipid peroxidation was done by the method of Ohkawa et al. [30]. The color formed by the reaction of thiobarbituric acid with breakdown products of lipid peroxidation was measured colorimetrically at 532 nm. Superoxide dismutase activity in plasma and liver was assayed by the method of Kakkar et al. [31], based on the 50% inhibition of formation of NADH phenazine methosulfate (PMS), nitro blue tetrazolium (NBT). The color developed was read at 520 nm. One unit of the enzyme is taken as the amount of enzyme required to give 50% inhibition of NBT reduction. The activity of glutathione peroxidase (GPx) in plasma and liver was determined using the method of Rotruck et al. [32], based on the utilization of reduced glutathione (GSH) by the enzyme. One unit of the enzyme is expressed as moles of GSH utilized per minute. The activity of catalase in plasma and liver was assayed by

Table 2 Mitotic index and frequencies of chromosomal abnormalities of control and experimental animals in each group

Group	Parameters	Mitotic index (%)	Chromosomal aberrations hamster^{-1}					Total aberration hamster^{-1}	Abnormal metaphase hamster^{-1}
			G	B’	B”	F	M		
1	Control	4.33±0.57[a]	0.39±0.05[a]	1.09±0.11[a]	0[a]	1.28±0.11[a]	0.29±0.03[a]	2.70±0.44[a]	1.44±0.15[a]
2	DMBA	1.87±0.17[b]	7.13±0.85[b]	6.48±0.71[b]	3.42±0.42[b]	6.79±0.79[b]	2.38±0.23[b]	12.5±1.24[b]	14.5±1.42[b]
3	DMBA + Mosinone-A	3.44±0.35[c]	3.85±0.34[c]	3.92±0.38[c]	1.40±0.15[c]	3.88±0.35[c]	1.54±0.15[c]	9.99±0.94[c]	6.52±0.63[c]
4	Mosinone-A alone	4.34±0.46[a]	0.40±0.04[a]	1.07±0.10[a]	0[a]	1.27±0.13[a]	0.28±0.03[a]	2.69±0.25[a]	1.43±0.16[a]

Values are expressed as the mean ± SD (n=6). Values that are not sharing a common superscript letter in the same column differ significantly at $p<0.05$ (DMRT). *G*-Gap, *B’* Chromatid Break, *B”* Isochromatid Break, *F* Fragment, *M* Minute. A Mitotic index has been calculated by analyzing 1,000 cells/animal (for a total of 6,000 cells/treatment) and percentage of the mitotic cells calculated for each treatment group. B–Frequency per 100 cells. Each chromosomal aberration has been counted by analyzing 100 cells/animal (6 hamsters/group, for a total of 600 cells/treatment) and the mean ± SD were calculated per treatment group. * Gaps were not included in total chromosomal aberrations

Table 3 Plasma TBARS and antioxidants in control and experimental animals in each group

Group	Parameters	TBARS (nmol/ml)	GPx (U ^{A}/L)	SOD (U^{B}/L)	CAT (U^{C}/L)
1	Control	1.65±0.18^{a}	109.4±12.0^{a}	3.52±0.45^{a}	1.10±0.12^{a}
2	DMBA	2.81±0.26^{b}	78.5±7.65^{b}	2.31±0.21^{b}	0.55±0.07^{b}
3	DMBA + Mosinone-A	2.10±0.21^{c}	98.4±9.75^{c}	3.10±0.30^{c}	0.99±0.10^{c}
4	Mosinone-A alone	1.64±0.15^{a}	107.1±11.9^{a}	3.51±0.36^{a}	1.09±0.11^{a}

Values are expressed as the mean ± SD (n=6). Values that are not sharing a common superscript letter in the same column differ significantly at $p<$ 0.05 (DMRT). *A* Micromoles of glutathione utilized/min.; *B* The amount of enzymes required to inhibit 50% nitroblue-tetrazolium (NBT) reduction; *C* Micromoles of H_20_2 utilized/s

the method of Sinha [33], based on the utilization of H_2O_2 by the enzyme. The color developed was read at 620 nm. One unit of the enzyme is expressed as μmoles of H_2O_2 utilized per min. The reduced glutathione level in plasma and liver was determined by the method of Beutler and Kelley [34]. The technique involves protein precipitation by meta-phosphoric acid and spectrophotometric assay at 412 nm of the yellow derivative obtained by the reaction of the supernatant with 5-5'dithiobis-2-nitrobenzoic acid.

The activity of glutathione-S-transferase (GST) in liver tissue homogenate was assayed by the method of Habig et al. [35]. GST activity was measured by incubate in the tissue homogenate with the substrate1-chloro-2,4-dinitrobenzene (CDNB).The absorbance was followed for 5 min at 540 nm after the reaction was started by the addition of reduced glutathione. Glutathione reductase activity in liver tissue homogenate was assayed by the method of Carlberg and Mannervik [36]. The enzyme activity was assayed by measuring the formation of reduced glutathione when the oxidized glutathione (GSSG) is reduced by reduced nicotinamide adenine dinucleotide phosphate (NADPH).

Statistical Analysis

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

Results

The frequency of MnPCEs and chromosomal aberrations in control and experimental hamsters in each group are given in Tables 1 and 2, respectively. Hamsters treated with DMBA (Groups 2 and 3) showed a high frequency of MnPCEs and chromosomal aberrations (chromosomal gap, chromatid break, chromosomal break fragment, minute) as compared to control hamsters (Group 1). However, hamsters treated with DMBA alone (Group 2) showed highest frequency of MnPCEs and chromosomal aberrations as compared to control hamsters. The frequency of MnPCEs and chromosomal abnormalities were significantly reduced in oral pretreatment of Mosinone-A to DMBA treated hamsters (Group 3). Oral pretreatment of Mosinone-A alone displayed no significant differences in MnPCEs frequency and chromosomal aberrations as compared to control hamsters.

Tables 3 and 4 shows the status of TBARS and enzymatic antioxidants in plasma and liver of control and experimental hamsters in each group. The TBARS levels were increased and as well as enzymatic antioxidants status decreased in DMBA alone treated hamsters (Group 2) as compared to control hamsters (Group 1). Oral pretreatment of Mosinone-A brought back the status of TBARS and antioxidants to near normal range in DMBA treated hamsters (Group 3). No significant differences were observed between control (Group 1) and Mosinone-A alone (Group 4) treated hamsters.

Table 5 shows the levels of detoxification agents (Phase II detoxification enzymes, GST, GR and GSH) in liver of

Table 4 Liver TBARS and antioxidants in control and experimental animals in each group

Group	Parameters	TBARS (nmol/100 mg protein)	GPx (U ^{A}/g protein)	SOD (U^{B}/mg protein)	CAT (U^{C}/mg protein)
1	Control	64.4±6.15^{a}	6.87±0.83^{a}	5.65±0.67^{a}	29.3±3.21^{a}
2	DMBA	88.4±10.7^{b}	3.87±0.37^{b}	2.95±0.28^{b}	17.4±0.16^{b}
3	DMBA + Mosinone-A	77.6±8.31^{c}	5.45±0.55^{c}	3.81±0.37^{c}	23.4±2.36^{c}
4	Mosinone-A alone	63.8±6.12^{a}	6.86±0.79^{a}	5.64±0.62^{a}	28.7±3.19^{a}

Values are expressed as the mean ± SD (n=6). Values that are not sharing a common superscript letter in the same column differ significantly at $p<$ 0.05 (DMRT). *A* Micromoles of glutathione utilized/min.; *B* The amount of enzymes required to inhibit 50% nitroblue-tetrazolium (NBT) reduction; *C* Micromoles of H_2O_2 utilized/s

Table 5 Activities of detoxification agents in liver homogenate of control and experimental animals in each group

Group	Parameters	GST (nmole CDNB conjugate formed/min/mg protein)	GR (nmole NADPH oxidized/min/mg protein)	GSH (mg/g tissue)
1	Control	145.1±15.9[a]	36.4±3.99[a]	1.97±0.18[a]
2	DMBA	99.4±8.34[b]	18.5±1.83[b]	1.05±0.11[b]
3	DMBA + Mosinone-A	132.4±13.6[c]	29.3±2.83[c]	1.50±0.65[c]
4	Mosinone-A alone	144.0±15.3[a]	35.3±3.91[a]	1.96±0.17[a]

Values are expressed as the mean ± SD (n=6). Values that are not sharing a common superscript letter in the same column differ significantly at $p<0.05$ (DMRT)

control and experimental hamsters. The activities of detoxification agents were significantly decreased in DMBA alone treated hamsters (Group 2) as compared to control hamsters (Group 1). Oral pretreatment of Mosinone-A brought back the status of detoxification agents to near normal range in DMBA treated hamsters (Group 3). No significant differences

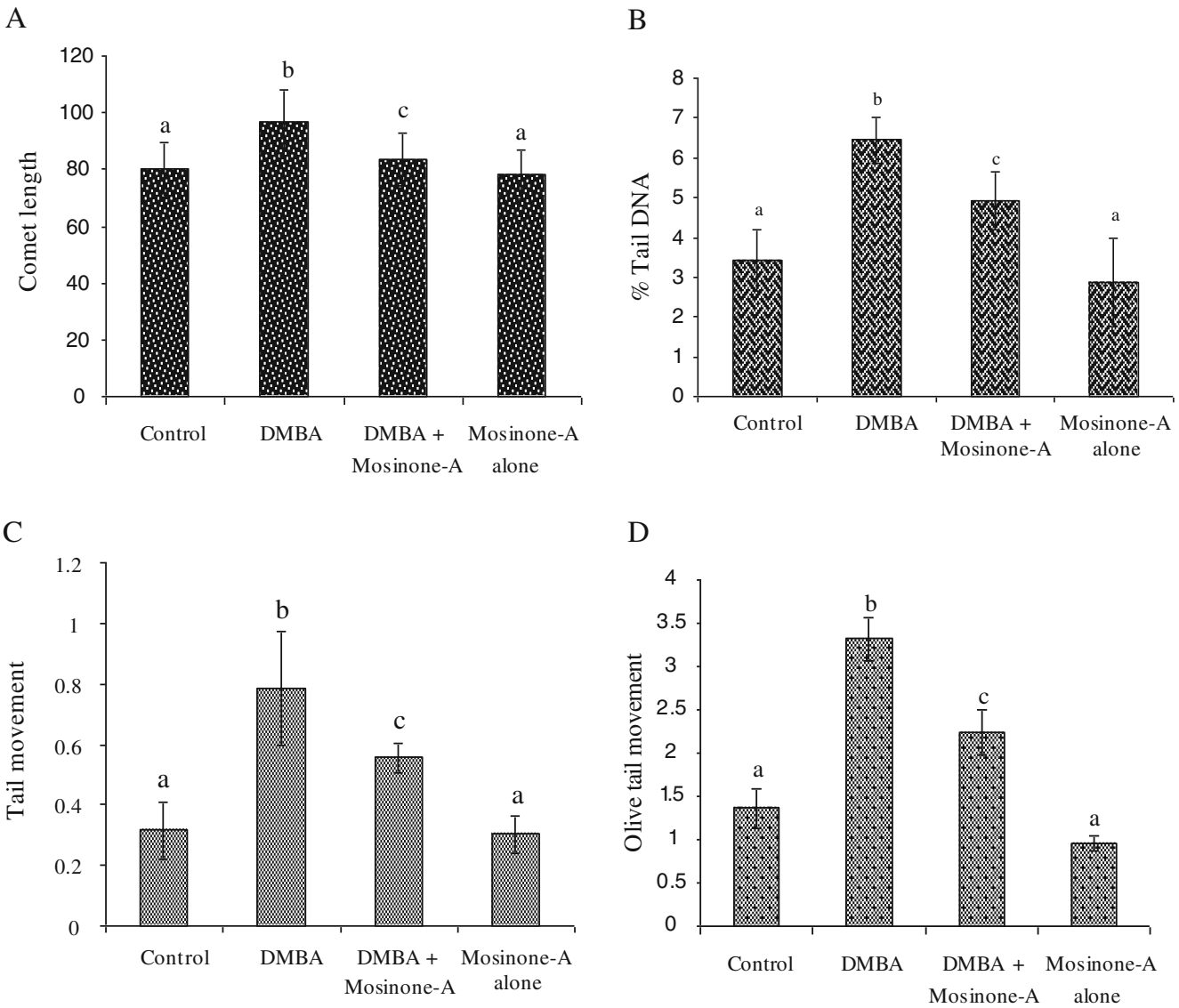


Fig. 2 Showing the effect of Mosinone-A on DMBA induced genetic damage of blood cells (Comet assay). Values are expressed as the mean ± SD (n=6). Values that are not sharing a common *superscript letter* in the same column differ significantly at $p<0.05$ (DMRT). **a** Comet length, **b**% Tail DNA, **c** Tail movement, **d** Olive tail movement

were observed between control (Group 1) and Mosinone-A alone (Group 4) treated hamsters.

Figure 2 shows the extent of DNA damage (comet length,% tail DNA, tail movement, and olive tail movement) in blood cells of control and experimental animals in each group. Increased the levels of all comet parameters (comet length,% tail DNA, tail movement, and olive tail movement) in DMBA alone treated hamsters (Group 2) as compared to control hamsters (Group 1). Oral pretreatment of Mosinone-A to DMBA treated hamsters significantly decreased the levels of comet attributes. No significant differences were observed between control (Group 1) and Mosinone-A alone treated hamsters.

Discussion

In the present study, we have investigated the antigenotoxic potential of Mosinone-A during DMBA induced genotoxicity. DMBA, an aryl hydrocarbon, used in mutagenesis and experimental carcinogenesis generates excess reactive oxygen species and induce lipid peroxidation, resulting different types of diseases including cancer [37]. Several studies have demonstrated the DNA damage and mutagenic effects of DMBA in experimental genotoxicity [38]. Elevated MnPCEs frequency and chromosome structural abnormalities were observed in topical application of DMBA with hamsters [39]. Dietary intake of chemopreventive agents has been suggested as an effective strategy for minimizing the toxic effects of genotoxins and carcinogens [40]. Profound studies on chemoprevention of human pancreatic and prostate carcinoma cell line offers Mosinone-A as chemopreventive agent due to its diverse pharmacological properties [41]. It has been pointed out Mosinone-A has played a role in the induction of cellular differentiation, apoptosis, and inhibition of cell proliferation and modification of cell cycle progression [42].

The carcinogenic potential of chemical carcinogen and mutagens have been investigated in experimental animal models using the frequency of micronucleated polychromatic erythrocytes, chromosomal abnormalities and DNA damage as principal cytogenetic parameters [43]. The genotoxic effect of DMBA and protective effect of Mosinone-A in the bone marrow were tested by using the MnPCEs frequency and ratio of PCEs/NCEs mitotic index and DNA damage (comet assay) in the blood cells [44]. Elevated MnPCEs frequency and percentage of chromosomal aberration were shown during DMBA induced genotoxicity. The comet assay (single cell gel electrophoresis) detects DNA damage such as strand breaks, alkali-labile sites and incomplete excision repair [45]. It has been used to investigate the antigenotoxic effect of Mosinone-A in DMBA induced genotoxicity. The size and shape of DNA within the comet have been correlated with the extent of DNA damage. Tail movement is a virtual measure calculated by the computerized image analysis system considering both the length of DNA migration in the comet tail and the tail intensity [46]. Our results corroborate these observations. Oral pretreatment of Mosinone-A (2 mg/kg b.wt) significantly reversed the frequency of MnPCEs and percentage of chromosomal aberrations in DMBA treated hamsters, which indicates its potent antigenotoxic potential of Mosinone-A in DMBA induced genotoxicity.

Human body has an array of antioxidant defense mechanisms to combat the harmful effects of reactive oxygen species generated by genotoxic agents [47]. ROS mediated oxidative stress has been implicated in the pathogenesis of several diseases including oral cancer and intracellular modifier of DNA [48]. Previous studies reported that increased the levels of lipid peroxidation byproducts (TBARS) in DMBA induced genotoxicity and decreased the activities of antioxidant enzymes to confirm oxidative stress in DMBA alone treated hamsters. Oral pretreatment of Mosinone-A significantly brought back the status of TBARS and antioxidant enzymes to near normal range in DMBA treated animals which suggest that Mosinone-A has potent antilipid peroxidative potential during genotoxicity.

Chemopreventive agents convert DNA damaging entities into excretable metabolites through the induction of detoxification agents [49]. Schawartz and Shklar pointed out that any agents, which induce the activity of glutathione-S-transferase has significant chemopreventive potential during genotoxicity [50]. Decreased activities of phase II detoxification agents were reported in DMBA treated hamsters. Oral administration of Mosinone-A to DMBA treated animals brought back the status of phase- II detoxification agents to near normal range.

Our results implies that Mosinone-A has maintained the status of detoxification agents during DMBA induced genotoxicity by enhancing the antioxidant defence mechanism to neutralize the toxic effects of reactive oxygen species generated by carcinogen, DMBA. The present study thus demonstrated the antigenotoxic, antilipidperoxidative antioxidative and modulating effects on detoxification enzymes in DMBA induced genotoxicity.

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