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RESEARCH ARTICLE

PROTECTIVE ROLE OF [6]-PARADOL ON DMBA INDUCED GENOTOXICITY IN MALE GOLDEN SYRIAN HAMSTERS

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ABSTRACT

The present study designed to investigate the anti-genotoxic and detoxifying effects of [6]-paradol on 7, 12-dimethylbenz[a]anthracene (DMBA) induced genotoxicity in male golden Syrian hamsters. DMBA, a well known site specific carcinogen was given by intra peritoneal (i.p.) injection at the dose of 30 mg/kg b.wt. to induce acute toxicity in hamsters. A significant induction of chromosomal aberration, high frequency of micronucleus and altered levels of detoxifying agents status were recorded in DMBA treated hamsters. Oral administration of [6]-paradol at a dose of 30 mg/kg b.wt, through the gastric intubations for 5 days prior to DMBA treatment. However, the oral pre-treatment of [6]-paradol significantly improved chromosomal abberation, micronucleus frequency as well as improved the statuse of detoxification enzyme were recorded. Anti-genotoxic capacity of [6]-paradol probably due to presence of antioxidant principle and enhance the detoxifying enzyme activities.

INTRODUCTION

Genotoxicity, a branch of toxicology is developed to identify the elements or compounds present in the environment having the potential to cause mutation by damaging the DNA. Genotoxic substances are known to be potentially mutagenic or carcinogenic, specifically those capable of causing genetic mutation and contributing to the development of tumors (Cimino, 2006). Typical genotoxins like aromatic amines are believed to cause mutations because they are nucleophilic and form strong covalent bonds with DNA resulting in the formation of aromatic amine-DNA adducts, preventing accurate replication (Pavanello, 2003). The genotoxicity tests have been used mainly for prediction of carcinogenicity. DMBA a polycyclic aromatic hydrocarbon extensively used as a mutagenic agent in several in vivo and in vitro mutation assay studies, its carcinogenic and cytotoxic effects are manifested only after activation (Gao, 2005). Mutagenic agents are known to cause chromosomal aberrations with generation of micronuclei. This fact has been exploited to study chromosomal damage in response to radiation exposure, chemotherapy drugs and other mutagens (Joseph and Gadhia, 2000). The *in vitro* chromosome aberration assay in mammalian cells has been widely used in genotoxicity testing for many decades and provides information on genetic damage that may be associated with adverse health outcomes (Aardema *et al.,* 1998). Micronuclei are cytoplasmic chromatin masses with the appearance of small nuclei that arise from chromosome lagging at anaphase or from acentric chromosomal fragments (Holland *et al.,* 2008). The most frequently used genotoxicity screening test in mammals is the micronucleus test, which provides a simple and rapid quantifiable measure of recent DNA injury that result from when acentric fragments or whole chromosomes are left behind the main nucleus at telophase (Sasaki *et al*., 1997). The comet assay, also known as the single cell gel electrophoresis (SCGE) is a rapid, simple, visual and sensitive technique for measuring DNA damage in mammalian cells. It is technique for detecting a wide range of genotoxic insults including single and double strand DNA breaks, alkali labile sites, single strand breaks associated with incomplete repair, and DNA–DNA or DNA–protein cross-links (Tan and Spivack, 2009). Most of the tissues and organs in human body are well equipped with diverse functions, detoxification by drug metabolizing enzymes including phase I, phase II metabolizing enzymes. The role of phase I and II enzymes in carcinogen metabolism was corroborated in numerous experiments and reviewed convincingly (King *et al*.. 1999). Hence, the idea of impact on carcinogen bioactivity through suppression of phase I and induction of phase II reactions; gained interest as a possible role for the prevention of genotoxicity strategy (Crampsie *et al*., 2011). During the course of xenobiotic metabolism, phase I enzymes predominantly (CYP^{450}) metabolize the xenobiotics to more reactive electrophilic moieties, which in turn are detoxified by phase II enzymes (Sheweita and Tilmisany, 2013).

The ginger (*Zingiber officinalae*, Roscoe, Zingiberaceae) is one of the most frequently consumed dietary substances in the world (Mills and Bone 2000). It contains about 1–3% volatile oils, which give the plant its distinctive aroma, and from 1 to 2.5 % pungent principles are considered a nonvolatile oily liquid consisting of homologous phenolic compounds. [6]-paradol is a pungent phenolic compound isolated from the seeds of grains of paradise as well as from the rhizome of ginger; It is a major pungent principle and exhibits diverse pharmacological activities (Lee and Surh, 1998). The evidence of pharmacological role for [6]-paradol comes from both epidemiological and experimental investigations. However, no scientific reports were available on the literature for anti-genotoxic and detoxifying activity of [6]-paradol in DMBA induced genotoxicity. Hence, In the present attempt; we examined the anti-genotoxic and detoxifying activity of [6]-paradol during DMBA induced genotixicity.

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MATERIALS AND METHODS

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.

Animals

Male golden Syrian hamsters 8–10 weeks old, weighing 80–120g 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 maintained under controlled conditions of temperature and humidity with a 12-hour light/dark cycle.

Isolation of (6)-paradol

[6]-paradol was extracted and isolated from *Zingiber officinale* root powder using the method of Locksley *et al*., (1972). Crude extract was prepared with hexane and further fractionation was carried out using hexane and ethyl acetate (9:1). The fractions were subjected to column chromatography (silica gel). The final product [6]-paradol was isolated as a waxy crystalline substance. The identity of isolated [6]-paradol determined by HPLC and NMR. The yield and purity of the isolated [6]-paradol were found 0.11% and>90%, respectively. For experimental studies,[6]-paradol was first dissolved in 0.5% Dimethyl sulfoxide (DMSO).

Experimental protocol

A total number of 24 hamsters were categorized into 4 equal groups. Group 1 hamsters served as untreated control. Groups 3 animals were pre-treated with 30mg/kg b.wt of [6]-paradol for 5 days. At the end of the 5th day, groups 2 and 3 animals were intraperitonealy injected with DMBA (30mg/kg b.wt. Single dose) after 2 hours administration of the [6]-paradol. Groups 4 hamsters received only [6]-paradol for 5 days. All the animals sacrificed at the 6th day by cervical dislocation for the assessment of chromosomal aberrations, frequency of micronucleated polychromatic erythrocytes (MnPCEs), comet assay and detoxification enzyme.

Clastogenic assay

The frequency of bone marrow MnPCEs was carried out according to the method of Schmid, (1975). Assessment of chromosomal aberration in bone marrow was carried out according to the procedure of Kilian *et al*., (1977). The alkaline comet assay was performed as described by Singh *et al*., (1988).

Biochemical estimations

Cytochrome p450 and cytochrome b5 activity was measured by the method of Omura and Sato, (1964). The activity of DT-diaphorase was determined by the method of Lind *et al*., (1967). Glutathione reductase (GR), Glutathione S-transferase **(**GST) and G-glutamyl transpeptidase (GGT) activity were determined by according to the method of Carlberg and Mannervik, (1975), Habig *et al*., (1974) and Fiala *et al*., (1972), respectively.

Statistical analysis

Values are expressed as mean ± SD. Statistical comparisons were performed by one-way analysis of variance (ANOVA) followed by Duncan's multiple range test (DMRT). The values were considered statistically significant if the p-value was less than 0.05.

RESULTS

Table 1 shows the frequency of MnPCEs in control and experimental animals in each group. All groups (groups 2–4) treated with DMBA showed a high frequency of MnPCEs when compared with control animals. The frequency of MnPCEs was significantly reduced in DMBA treated animals and pre-treated with [6]-paradol (30 mg/kg b.wt). Oral administration of [6]-paradol alone displayed no significant differences in MnPCEs when compared with control animals. Table 2 and Figure 1 shows the frequency of CAs in control and experimental animals in each group. The DMBA treated animals showed a high frequency of CAs (structural aberrations, chromatic gaps, chromosomal gaps, chromatic breaks, chromosomal breaks, fragments, and minutes) when compared with control animals. The chromosomal abnormalities were significantly reduced in DMBA treated animals and pre-treated with oral administration of 30 mg/kg b.wt of [6]-paradol. Oral administration of [6]-paradol alone displayed no significant differences in chromosomal abnormalities when compared with control animals.

Table 1. The effects of [6]-paradol on DMBA induced micronuclei formation of control and experimental animals in each group of experimental design

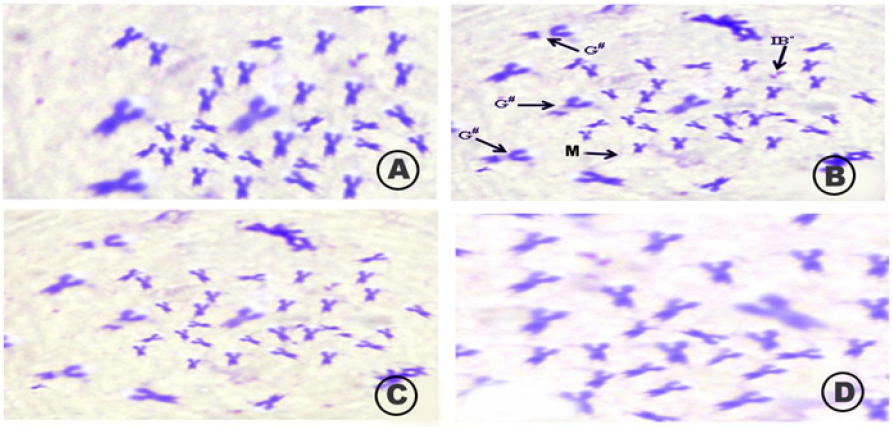
Treatment	MnPCEs/2500 PCEs	PCEs/NCEs	%PCEs
Control	6.23 ±0.47 ^{a}	1.02.±0.09^{a}	52.14%^{a}
DMBA alone	69.47±5.87^{b}	0.81±0.07^{b}	38.48% ^{b}
DMBA+ [6]-paradol (30mg/kg b.wt)	41.12±3.98^{c}	0.93±0.09^{c}	42.86%^{c}
[6]-paradol alone (30mg/kg b.wt)	6.01±0.39^{a}	1.04± 0.08^{a}	49.55%^{a}

Values are expressed as mean ± SD; (2500 PCEs were scored per animal). Values not sharing a common superscript letter in the same column differ significantly at $P < 0.05$. (DMRT). * percentage polychromatic erythrocytes were calculated as follows: [PCEs+NCEs] 10

Table 2. The effects of [6]-paradol on DMBA induced chromosomal aberrations of control and experimental animals in each group of experimental design

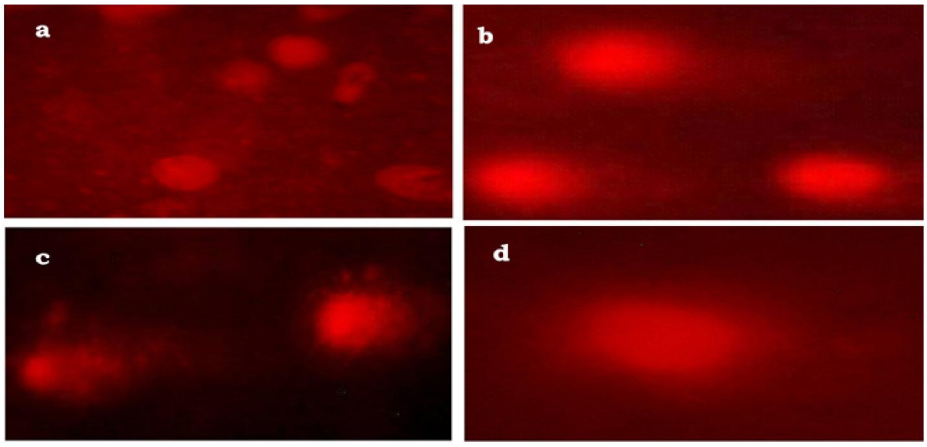
Treatment	Miotic index (%)	Chromosomal aberration hamster^{-1}					Total aberration hamster^{-1}	Abnormal metaphase rat^{-1}
		G	B'	B''	F	M		
Control	51.91±.52^{a}	0.52±.05^{a}	1.94 ± .01^{a}	0^{a}	1.85± .17^{a}	0.39±.02^{a}	2.22 ± .22^{a}	1.92±.18^{a}
DMBA alone	19.82±.18^{b}	13.24± .13^{b}	5.19 ± .50^{b}	2.84± .19^{b}	6.36± .59^{b}	2.97± .21^{b}	18.23 ±.18^{b}	18.17±.82^{b}
DMBA+ [6]-Paradol (30mg/kg b.wt)	44.87± .41^{c}	0.49 ± .04^{c}	2.29 ± .09^{c}	1.41± .01^{c}	2.17± .02^{c}	0.81±.08^{c}	3.83 ±.31^{c}	1.49 ±.13^{c}
[6]-paradol alone (30mg/kg b.wt)	52.74± .49^{a}	0.54 ± .05^{a}	1.93 ± .02^{a}	0^{a}	1.81±.08 ^{a}	1.31±.03^{a}	2.17 ±.24^{a}	1.98 ±.18^{a}

Values are expressed as mean ± SD; (n = 6; 2500 PCEs were scored per animal). Values not sharing a common superscript letter in the same column differ significantly at $P < 0.05$. (DMRT). G-Gap, B'-Break, B''-iscchromatid break, F-Fragment, M-Minute. A miotic index was calculated by analyzing 1000 cells/ animals (for a total of 6000 cells /treatment) and percentage of the miotic cells calculated for each treatment group. B-Frequency per 100 cells. Each chromosomal aberration has been counted by analyzing 100 cells/animals (6 animals/group for a total of 600 cell/treatment) and the means ± SD were calculated per treatment group.*Gaps were not included in total chromosomal aberration.



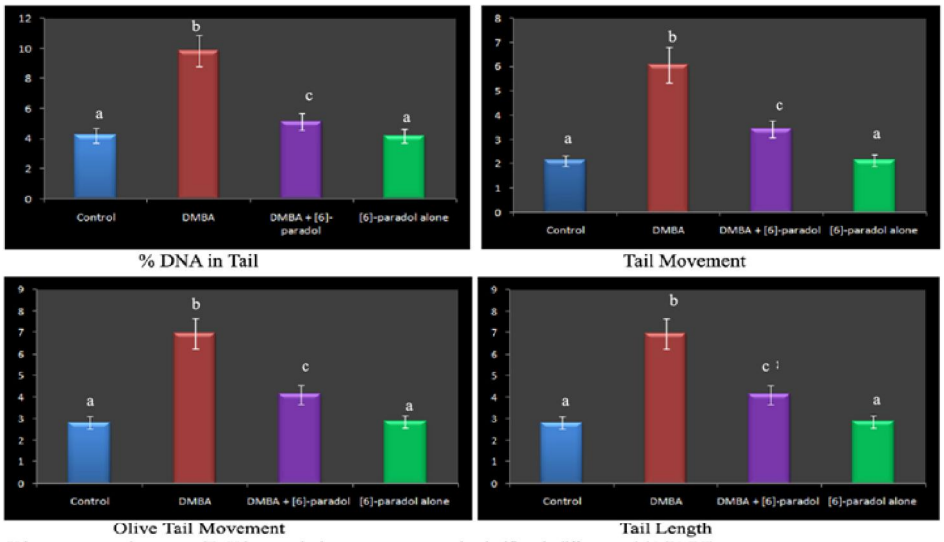
A & D : No chromosomal aberration in control and [6]-paradol alone treated hamsters.
B : Increased chromosomal abnormalaties in DMBA treated hamsters [G-chromosomal Gap, M-Minute, B-Chromatid Break].
C : Significantly decreased chromosomal abnormality in DMBA + [6]-paradol treated hamsters. (**40x Magnification**).

Figure 1. Photomicrographs showed the effects of [6]-paradol in DMBA induced chromosomal aberration in the bone marrow cells of golden Syrian hamsters



a & d : No DNA damage in control and [6]-paradol alone treated hamsters; b : Increased DNA damage in DMBA treated hamsters.
c: Significantly decreased DNA damage in DMBA+[6]-paradol treated hamsters.

Figure 2. Microphotograph showed the effects [6]-paradol in DMBA induced genetic demage in the bone marrow cells of golden Syrian hamsters



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

Figure 3. Photomicrographs showed the extent of DMBA mediated DNA damage in bone marrow cells (Comet assay)

Table 3. The status of Phase I and phase II detoxification agents in the liver of control and experimental animals in each group

Treatment	Phase I detoxification Enzyme		Phase II detoxification Enzyme			
	Cyt p^{450} (U^{X}/ mg protein)	Cyt b^{5} (U^{y}/mg protein)	GSH (nM/ mg protein)	GST (nM of CDNB-GSH/min/mg protein)	GR (nM of NADPH Oxidized/min/mg protein)	DT-diaphorase (U^{C} /mg protein)
Control	1.37 ± 0.17^{a}	$1.26 \pm .12^{a}$	3.88 ± 0.29^{a}	132.45 ± 12.97^{a}	30.58 ± 3.44^{a}	0.63 ± 0.06^{a}
DMBA alone	2.41 ± 0.22^{b}	$2.87 \pm .28^{b}$	5.98 ± 0.58^{b}	182.65 ± 18.35^{b}	54.42 ± 5.01^{b}	0.21 ± 0.02^{b}
DMBA + [6]-paradol (30mg/kg b.wt)	1.95 ± 0.18^{c}	$1.53 \pm .16^{c}$	4.11 ± 0.39^{c}	147.68 ± 14.12^{c}	41.99 ± 3.94^{c}	0.41 ± 0.04^{c}
[6]-paradol alone (30mg/kg b.wt)	1.38 ± 0.13^{a}	1.27 ± 0.14^{a}	3.76 ± 0.36^{a}	129.81 ± 12.68^{a}	29.22 ± 2.86^{a}	0.62 ± 0.06^{a}

Values are expressed as mean ± SD. Values not sharing a common superscript significantly differ at $p < 0.05$ (DMRT). ^{x}-Micromoles of Cyt p^{450} formed ; ^{y}-Micromoles of Cyt b^{5} formed

Figure 2 and 3 shows the extent DNA damage (comet length, % tail DNA, tail movement and olive tail moment) observed in the bone marrow of control and experimental animals in each group. Significant increase in DNA migration as determined by above said parameters were detected in DMBA alone treated animals as compared to control animals. Oral pre-treatment by [6]-paradol to DMBA alone treated animals significantly decreased the levels of comet parameters. Oral pre-treatment with [6]-paradol alone showed no significant differences in comet parameters as compared to control animals. Table 3 shows the levels of detoxification agents in liver homogenate of control and experimental animals in each group. Increases in the levels of cytp450, cyt b5, GR, GST, GGT and DT-Phorase were shown in DMBA treated animals when compared with control animals. Oral administration of [6]-paradol at a dose of 30 mg/kg bw, significantly decreased the altered levels of detoxification enzymes in DMBA treated animals. Oral administration of [6]-paradol alone displayed no significant differences in detoxification enzyme status when compared with control animals.

DISCUSSION

In the present study, we observed the phenolic bioactive compound [6]-paradol has been found to a potential anti-genotoxic agent in DMBA induced genotoxicity. Elevated levels of chromosomal aberrations and formation of micronuclei are usually considered to derive from unrepaired or misrepaired DNA lesions induced by exogenous or endogenous exposure of DNA damaging agents like DMBA (Khan *et al.,* 2009). Oral pre-treatment of [6]-paradol significantly reduced the frequency of chromosomal aberrations and MNPCEs in DMBA induced genotoxicity. The possible mechanism for antigenotoxic effect of [6]-paradol include improving the defense mechanism to neutralize the toxic effects generated by DMBA. Over the last decade, in vivo alkaline comet assay, besides gaining widespread use in various areas, has emerged as a standard tool in the pharmaceutical industry for assessing the safety of new drugs and, increasingly, as a means of evaluating genotoxicity testing. In the present study, we have observed abnormal comet parameters (% DNA in tail, tail movement, Olive tail movement and tail length) in DMBA induced genotoxicity. A slight increase in total damaged cells, olive tail movement and tail length were scored in DMBA alone treated animals. Baskaran *et al*. (2012) reported that abnormal Comet parameters as well as protective effect of phytochemical in experimental animals were observed. Our results corroborate this observation. Oral pre-treatment of [6]-paradol at a dose of 30 mg/kg b.wt. to DMBA treated hamsters significantly reduced the comet parameters. The activities of phase I and II detoxification enzymes levels were increased in hamsters treated with DMBA alone, which indicates that liver detoxification cascade was stimulated to detoxify the carcinogenic metabolite of DMBA. Cytochrome p450 isoenzymes, are necessary to begin the conversion of metabolize lipophilic carcinogens compounds to more water soluble metabolites, which are then acted upon by phase II enzymes to promote their polarity and assisting in their excretion (Poon *et al*., 2012). In our study, oral pre-treatment of [6]-paradol was significantly reduced the hepatic phase I enzymes, comparatively with the control groups. This might be due to inhibitory action of [6]-paradol on Phase I enzymes or blocking actions on bioactivation process or decrease in formation of ultimate carcinogenic moiety. Phase II enzymes play an important role in the detoxification of activated carcinogens by eliminating the reactive intermediates from cellular environment. There are persuasive evidences to support the induction of glutathione S-transferase and protection against a wide spectrum of cytotoxic, mutagenic and carcinogenic chemicals (Yoshimasa *et al*., 2000). GR, another major antioxidant enzyme that catalyzes the NADPH-dependent reduction of glutathione disulfide to glutathione, thus maintaining GSH levels in the cell (Xu *et al*., 2005). DT-diaphorase is generally induced coordinately with other phase II detoxifying enzymes. Induction of DT-diaphorase has been evaluated as a means for determining the potency of many anticarcinogenic substances, it catalyzes the two-electron reduction of quinine to hydroquinone without the formation of semiquinone radical intermediates, thus prevents cells from the cytotoxicity (Begleiter *et al*., 1977). Previous studies in our laboratory reported that Mosinone-A inducing phase II detoxification enzymes, as well as the ability to reduce phase I carcinogen activating enzyme in DMBA treated hamsters (Sugunadevi *et al*., 2012). Our findings correlate with this observation. The results of the present study suggest that [6]-paradol exhibits both anti-genotoxic potential as well as replacing phase I and phase II enzyme systems.

Conclusion

The findings of the present investigation confirm the anti-mutagenic and detoxifying effects of [6]-paradol on DMBA induced cytotoxic damage. The possible mechanisms behind the anti-genotoxicity mechanism of [6]-paradol might be act as a direct inhibition and suppression of toxic metabolites of DMBA. The molecular mechanisms of anti-mutagenesis of [6]-paradol must be explored further to understand its chemopreventive effects.

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