Protective Effects of [6]-Paradol on Histological Lesions and Immunohistochemical Gene Expression in DMBA Induced Hamster Buccal Pouch Carcinogenesis

Arokia Vijayaanand Mariadoss¹, Suresh Kathiresan¹*, Rajasekar Muthusamy¹, Sivakumar Kathiresan²

Abstract

Background: The search for naturally occurring agents in routinely consumed foods that may inhibit cancer development is of high priority. [6]-Paradol is a pungent phenolic bioactive component from ginger with well-documented health promoting antioxidant, antimutagenic, antigenotoxic and anti-inflammatory properties. However, anticarcinogenic effects have yet to be fully explored. The objectives of the present study were therefore to assess protective effects against 7,12-dimethylbenz(a)anthracene (DMBA) induced buccal pouch carcinogenesis in male golden Syrian hamsters. Methods: Oral squamous cell carcinomas developed in the left buccal pouch of hamsters on painting with 0.5% of DMBA, three times in a week. To assess the apoptotic associated gene expressing potential of [6]-paradol, it was orally administered to DMBA treated hamsters on alternate days from DMBA painting for 14 weeks. Results: We observed 100% tumor formation with marked levels of neoplastic changes and altered the expression of apoptotic associated gene (p53, bcl-2, caspase-3 and TNF-α) was observed in the DMBA alone painted hamsters as compared to control hamsters. Oral administration of [6]-paradol at a dose of 30 mg/kg b.wt to DMBA treated animals on alternative days for 14 weeks significantly reduced the neoplastic changes and improved the status of apoptosis associated gene expression. Conclusion: These observations confirmed that [6]-paradol acts as a tumor suppressing agent against DMBA induced oral carcinogenesis. We also conclude that [6]-paradol also effectively enhances apoptosis- associated gene expression in DMBA treated animals.

Keywords: [6]-paradol - apoptosis – DMBA – hamster - oral squamous cell carcinoma

Introduction

Oral cancer is the sixth most prevalent cancer worldwide, and accounts for up to 40% of all malignancies in parts of India and Southeast Asia. In these regions, oral cancer constitutes approximately 17% of all male cancers and 10.5% of all female cancers, making it the most common cancer in males and the third most common cancer in females (Jemal et al., 1999). Data from the National Cancer Registry Project of the Indian Council of Medical Research (ICMR) also confirm that oral cancer is common in India (Takiar et al., 2010). Epidemiological research and the scientific literature have demonstrated that the use of tobacco and alcohol as well as human papilloma virus infection (HPV) are the predominant causative factors for oral carcinoma, which arises through a multistep process of cumulative genetic alterations leading to a loss of cell cycle regulation (Marur et al., 2010; Smith et al., 2010).

The tumor suppressor gene p53, play a vital role in the protection of DNA damage and other forms of physiological stress primarily by inducing cell cycle arrest or apoptosis represents the guardian of human genome and it is one of the most studied molecular markers in oral cancer (Kim et al., 2011). The tumor suppressing activity of p53 is lost in various types of human carcinoma because of its mutational events or interactions with other proteins (Shirkoohi et al., 2012). Several studies has been proposed that the close interaction between p53 and bcl-2 proteins during carcinogenesis (Mitselou et al., 2011). bcl-2 family proteins are the central regulators of mitochondria dependent cell death pathway, functioning as either anti or pro-apoptotic factors (Leibowitz and Yu, 2010). The bcl-2 gene, encoding a 25 kD protein located both in the inner mitochondrial membrane and in the cell cytosol, has been shown to prolong cell survival by inhibiting the apoptosis and may promote tumor development. Over expression of bcl-2 gene has been reported in haematopoetic malignancies and several solid tumors including oral cancer (Hasnan et al., 2012).

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These family proteins can regulate the various caspases activation through the regulation of cytochrome C release, which is inhibited by death antagonists, and promoted by death agonists (Martinou and Youle, 2011).

Caspase, a family of intracellular cysteine protease, is known to play a pivotal role in the inhibition and execution of apoptosis induced by various stimuli (Prunet et al., 2005). Among the ten different types of caspases in mammalian cells, caspase-3 may serve as a general mediator of apoptosis through mitochondrial dependent and independent apoptotic pathways (Porter and Janicke, 1999). Caspase-3 is a pro-apoptotic protease, which induces intracellular processes leading to cell death (Fraser and Evan, 1996). Over expression of anti-apoptotic proteins with down regulation of pro apoptotic proteins and caspases has been documented in malignancies (Coutiñas and Strasser, 2003). Keum et al., reported that elevated levels of proenzyme caspase-3 was present in untreated tumor cells, and active caspase-3 gradually increased after [6]-dehydroparadol treatment, suggesting that this synthetic vanilloid induces apoptosis through a caspase-3-dependent mechanism (Keum et al., 2002).

Tumor necrosis factor-alpha (TNF-α), a pro-inflammatory cytokine, it has multiple well recognized biological activity in many physiological process such as cell differentiation, proliferation, apoptosis, energy metabolism and immune systems (Elliott et al., 1994). It is also involved in the pathogenesis of multiple chronic inflammatory disorders, and associated with metabolic diseases such as cancer, obesity, diabetes, dislipidemia and atherosclerosis (Muller-Ladner et al., 2009). TNF-α is one of the main mediators of inflammation, it has been linked to many types of tumorogenesis including oral cancer, it has been described as a powerful anti-cancer effectors cytokine produced by immune cells such as macrophages and lymphocytes. It is also induces mitochondrial dysfunction by its downstream consequences, resulting in further increase in oxidative stress, cytochrome C release, caspase 3 activity (Kischkel et al., 2000).

A synthetic chemopreventive agent in current use for the treatment of oral cancer produces serious side effects. Hence, the novel researches are needed to identify new bioactive compounds with low toxic or side effects than the chemical drugs (Tanaka, 1997). Numerous studies have been suggested that bioactive compounds from medicinal plants could serve as alternatives to synthetic anti-cancer agents (Satoh et al., 1993; Ramirez-Mares et al., 2004). Ginger (Zingiber officinale, Roscoe) has been used worldwide not only as food but also as medicine (White, 2007). The rhizomes of ginger contain number of bioactive compounds, which can be obtained from gingerol by successive dehydration and hydrogenation reactions. In particular, [6]-paradol, possess interesting pharmacological and physiological activities, including anti-inflammatory, anti-hepatotoxic and cardiotonic effects (Thomson et al., 2002).

[6]-paradol is known to have antimicrobial and analgesic activities and exerts cytotoxic and anti-proliferative effects against the KB oral squamous carcinoma cell line (Keum et al., 2002). Previous experimental studies in our laboratory demonstrated the anti-oxidative and anti-lipidperoxidative effects of [6]-paradol during DMBA induced hamster buccal pouch carcinogenesis (Suresh et al., 2010). However, the effects of [6]-paradol on the gene expression of p53, bcl-2, caspase 3 and TNF-α during DMBA-induced carcinogenesis in male golden Syrian hamsters have not previously been reported. Therefore, this study was designed to investigate the ability of [6]-paradol to modulate expression of the apoptosis related proteins which are implicated in DMBA induced hamster buccal pouch carcinogenesis, including p53, bcl-2 caspase-3 and TNF-α.

Materials and Methods

Chemicals

7,12-dimethylbenz(a)anthracene, xylene, haematoxylin, analytical grade methanol, monoclonal antibodies for p53, bcl-2, Caspase-3 and TNF-α were purchased from Sigma-Aldrich Chemical Company (St. Louis, MO). The anti-mouse horseradish-peroxidase conjugated secondary antibodies were obtained from Biogenex, San Ramon, CA. All other fine chemicals used were analytical grade.

Animals

Male Syrian hamsters (80±120 g), were obtained from National Institute of Nutrition, Hyderabad, India. Animals were housed in groups at constant temperature (23±2°C), and a light/dark (12 h/12 h) cycle. The animals were allowed to free access to food (VRK Nutritional Solutions, Maharashtra, India) and water (ad libitum) throughout the experimental period. The experiments were designed and conducted in accordance with the institutional guidelines (Register number I(6)/0/1999/CPCSEA).

Isolation of [6]-paradol

[6]-paradol was isolated from Zingiber officinale root extract by using the method of Locksley et al. (1972). The extract was prepared with hexane and its fractionation was carried out using hexane and ethyl acetate (9:1, v/v). 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 was determined by LC-MS and NMR. Its identification was confirmed by comparison to the reference [6]-paradol, which was purchased from Betapharma (Shanghai) Co., Ltd., China. The yield and purity of the isolated [6]-paradol were found to be 0.11% and > 90%, respectively. For experimental studies, [6]-paradol was dissolved in 0.5% dimethyl sulfoxide (DMSO).

Experimental design

The male golden Syrian hamsters were divided into 4 groups of 10 each. Group I, serve as an untreated control. The Group II and Group III were painted with 0.5% DMBA in liquid paraffin three times a week for 14 weeks on the left buccal pouches using (No. 4 brush) to induce the buccal pouch carcinogenesis. The Group II received no other treatment. Group III animals were orally administered [6]-paradol (30 mg/kg b.wt; dissolved in
0.5% DMSO) starting one week before the exposure to the carcinogen and continuing on alternate days of the DMBA painting until the animals were sacrificed. However, Group IV was orally administrated with [6]-paradol to exclude any toxic effects.

Sample collections
At the end of the experimental period, the hamsters were sacrificed by cervical decapitation under anesthetic conditions (Ketamin 30 mg/kg, i.p.). The control and experimental animal buccal tissues were immediately removed, washed using ice-cold phosphate buffer solution (pH 7.4), and then the buccal tissues were divided for assessment of histological and immunohistochemical studies.

Tumor study
The left sides of the control and experimental animal’s buccal pouch was excised and flattened on a transparency. Visible tumors in the oral cavity were counted and the diameter of each tumor was measured with a vernier caliper.

Histopathological evaluation
Histological slides were prepared by according to the method of Ramos Vara, (2005). The buccal pouches of the hamsters were fixed in 10% buffered formaldehyde and dehydrated in rising concentrations of ethanol and embedded in paraffin. 5 µm thickness of tissue sections were mounted on frosted glass slides and dried for overnight. The sections were then deparaffinized with xylene, rehydrated with alcohol and water. The rehydrated sections were stained using H&E and viewed under the microscope 40X magnifications (Olympus BX51 microscope, Tokyo, Japan).

Immunohistochemical evaluation
To examine the expression patterns and inter correlation of apoptosis related molecular markers of p53, bcl-2, caspase-3 and TNF-α immunohistochemical staining were prepared by according to the method of Cotran et al. (1994). 5µm paraffin embedded tissue sections were dewaxed in xylene and hydrated with graded ethanol. The pretreatment was performed in tris citrate buffer (pH 6.0) at 98°C for 40 min. After the pretreatment the slides were stained by horseradish peroxidase (HRP) polymer standard method. The primary antibodies of anti-mouse p53, anti-mouse bcl-2, anti-mouse caspase-3 and anti-mouse TNF-α were diluted 1:100 and incubated at room temperature in a humidity chamber for 30 min. All the reactions were performed manually at room temperature. Immunolabelling was visualized by inhibition in 3,3-Diaminobenzidine (DAB) solution used as the chromogenic substrate and the tissue sections were counterstained with hematoxylin and permentently mounted.

Statistical analysis
The data were expressed as Mean±Standard Deviation (SD). Statistical analysis on the data for tumor incidence, tumor volume, and tumor burden were performed by one-way analysis of variance followed by Duncan’s Multiple Range Test. The percentage of positive cells in immunohistochemical was scored according to the method of Nakagawa et al. (1994) as follows: **=strong staining, more than 50% of cells were stained; *= moderate staining, between 20 and 50% of cells were stained; -= weak staining, between 1 and 20% of cells were stained; 0= negative, less than 1% of cell staining. The statistical data were analyzed by using the number of positively stained cells using Chi-square ($\chi^2$) test. The results were considered statistically significant if the p values were less than 0.05.

Results
In Table 1, the application of 0.5% DMBA thrice a week for 14 weeks fashioned the buccal pouch carcinogenesis in hamsters. The tumor incidence, tumor volume and tumor burden were extensively higher in DMBA treated hamsters, when compared to control hamsters. Treatment with [6]-paradol at a dose of 20 mg/kg b.wt effectively suppressed the visible oral tumor incidence, tumor volume, and decreased tumor burden (p<0.05). Hamsters treated with [6]-paradol alone there is no significant changes were observed.

Figure 1 shows the histopathological changes (dysplasia, keratosis, hyperplasia and squamous cell carcinoma) of control and experimental hamsters of each group, which was scrutinized by a pathologist Dr. S. Prathipa, Associate Professor, Shree Ramachandra Medical College, and Research Institute, Porur, Chennai. We observed well differentiated squamous cell carcinomas with evidence of keratin formation, and severe hyperplasia, dysplasia in DMBA alone treated animals (Figure 1B). The animals treated with DMBA and [6]-paradol exhibit only mild to moderate hyperplasia and dysplasia were observed (Figure 1C). Hamsters treated with [6]-paradol alone there is no significant changes were observed compared to control hamsters (Figure 1A & 1D).

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Control (Group I)</th>
<th>DMBA (Group II)</th>
<th>DMBA+[6]-paradol (Group III)</th>
<th>[6]-paradol alone (Group IV)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tumor incidence (Oral squamous cell carcinoma)</td>
<td>0/38</td>
<td>0/38</td>
<td>0/38</td>
<td>0/38</td>
</tr>
<tr>
<td>Total number of tumors/animals</td>
<td>100%</td>
<td>100%</td>
<td>100%</td>
<td>0%</td>
</tr>
<tr>
<td>Tumor volume (mm³/animals)</td>
<td>0.8</td>
<td>299.17±47.75b</td>
<td>89.78±8.12b</td>
<td>0.8</td>
</tr>
<tr>
<td>Tumor burden (mm³/animals)</td>
<td>0.0</td>
<td>974.96±63.10b</td>
<td>68.13±6.43b</td>
<td>0.0</td>
</tr>
<tr>
<td>Keratosis</td>
<td>No change</td>
<td>Severe</td>
<td>Mild</td>
<td>No change</td>
</tr>
<tr>
<td>Hyperplasia</td>
<td>No change</td>
<td>Severe</td>
<td>Mild</td>
<td>No change</td>
</tr>
<tr>
<td>Dysplasia</td>
<td>No change</td>
<td>Severe</td>
<td>Mild</td>
<td>No change</td>
</tr>
<tr>
<td>Squamous cell carcinoma</td>
<td>No change</td>
<td>Moderately differentiated</td>
<td>(3 animals)</td>
<td>No change</td>
</tr>
</tbody>
</table>

Note: Values not sharing a common superscript letter in the same row differ significantly at p<0.05 (DMRT). Tumor volume was measured using the formula $V = \frac{4}{3} \pi r^3$, where $r$ is the three diameters (mm) of the tumor. Tumor burden was calculated by multiplying tumor volume and the number of tumors/animal indicates ( ) total number of animals bearing tumors.
Figure 1. Histological Changes of Control and Experimental Animals in Each Group (20x). A) Microphotograph of untreated control animals (Group I), picturising normal epithelium in buccal mucosa. B) Microphotograph of DMBA alone treated animals (Group II), picturising well defined squamous cell carcinoma with hyper chromatic nuclei containing epithelial and keratin pearls. C) Microphotograph of DMBA+[6]-paradol treated animals (Group III), picturising mild to moderate dysplasia and hyperplasia. D) Microphotograph of [6]-paradol alone treated animals (group IV), picturising normal epithelium in buccal mucosa

Figure 2. Immunohistochemical Expression Patterns of p53 in Buccal Mucosa of Control and Experimental Animals in Each Group (20x). A) Shows the normal expression of p53 genes in buccal tissues of control hamsters. B) Shows the well defined oral squamous cell carcinoma with significant expression of p53 in buccal tissues of DMBA treated hamsters. C) Mild expression of p53 genes in [6]-paradol and DMBA treated hamsters. D) Normal expression of p53 in buccal tissues of [6]-paradol alone treated hamsters

Figure 3. Immunohistochemical Expression Patterns of bcl-2 in Buccal Mucosa of Control and Experimental Animals in Each Group (20x). A) Normal expression of bcl-2 in buccal tissues of control hamsters. B) Shows the well defined oral squamous cell carcinoma with significant expression of bcl-2 in buccal tissues of DMBA treated hamsters. C) Mild expression of bcl-2 in [6]-paradol and DMBA treated hamsters. D) Normal expression of bcl-2 in buccal tissues of [6]-paradol alone treated hamsters

Figure 4. Immunohistochemical Expression Patterns of Caspase-3 in Buccal Mucosa of Control and Experimental Animals in Each Group (20x). A) Shows the normal expression of caspase-3 in buccal tissues of control hamsters. B) Application of DMBA significantly decreased the caspase-3 expression in buccal tissues of DMBA alone treated hamsters. C) Up regulation of caspase-3 expression in [6]-paradol and DMBA treated hamsters. D) Normal expression of caspase-3 in buccal tissues of [6]-paradol alone treated hamsters

Figure 3 and Table 2 confirm the immunohistochemical expression of bcl-2 in control and experimental hamsters. The bcl-2 cells were identified as diffused golden yellow colour in cytoplasmic staining. In the normal hamster buccal mucosa, bcl-2 was barely noticeable in the epithelial cells (Figure 3A). We observed a high level of bcl-2 expression in DMBA alone treated hamsters as compared to control hamsters, which were obviously up regulated in hyperplasia, dysplasia, and squamous cell carcinoma (Figure 3B). Whereas, the oral administration of [6]-paradol at a dose of 30 mg/kg b.wt significantly restored the level of bcl-2 expression (Figure 3C). Control and [6]-paradol alone treated hamsters (Figure 2A & 2C) shows a negligible bcl-2 expression, when compared to DMBA treated animals.

Apopototic cells stained with caspase-3 in buccal pouches of the hamster are illustrated in Figure 4 and Table 2. Low intensity (Down regulation) of caspase-3
The present study provides evidence that the pungent phenolic bioactive constituents of ginger namely [6]-paradol can effectively modulate the pathological changes and enhances the apoptotic related gene expression in DMBA induced oral carcinogenesis. In recent years, the number of molecular based assays has increased, but histopathology and immunohistopathology remains the gold standard for most diagnostic and therapeutic responses. Thus the present study, we evaluated the effects of [6]-paradol on pathological changes in DMBA induced buccal pouch carcinogenesis using immunohistochemical techniques.

DMBA is a potent organ and site-specific carcinogen; it is commonly used to induce buccal pouch carcinogenesis in hamsters. The developments of chemically induced carcinogenesis in a hamster model parallel the development of those in human oral mucosa (Salley, 1954). Some of these similarities include progression from a normal stratified squamous epithelium to hyperplasia and hyperkeratosis, followed by development of dysplasia, which progresses in situ and invasive carcinomas (Cruz et al., 2002). The conclusion of the pathological examination documented that DMBA treatments twisted the inflammation, severe hyperplasia, hyperkeratosis, dysplastic changes and well differentiated squamous cell carcinoma. However, mild to moderate pre-neoplastic lesions (hyperplasia, keratosis and dysplasia) were observed in DMBA and [6]-paradol treated hamsters. Together these observations clearly indicate that the oral administration of [6]-paradol has the ability to reduce the neoplastic changes DMBA induced oral carcinogenesis.

p53 plays a major role in preventing tumor development, it acts as a “molecular brake” to critically regulate the cell cycle. This DNA-binding protein has been involved in DNA repair and synthesis, cell proliferation, cell differentiation, programmed cell death, and in the maintenance of genomic stability mechanism (Rivlin et al., 2011). Rundle et al. (2000) reported that, the metabolic intermediates of DMBA mediate carcinogenic process by inducing the chronic inflammation through the over production of reactive oxygen species causes DNA damage. In these pathological conditions, p53 can arrest cell cycle progression, and allowing the DNA to be repaired or it can lead to apoptosis (Rundle et al., 2000). The results of the present study showed that down regulation of the p53 expression by the [6]-paradol treatment, which is in accordance with the previous reports where natural and dietary compounds have been shown to exert their chemopreventive property through modulating the balance between wild type p53 and mutant p53 protein expression (Bourdon, 2007). In our study, a significant increase in the p53 expression was observed in DMBA (group II) treated hamsters. Also, it was seen that decrease in expression of p53 in DMBA and [6]-paradol treated hamsters (group III). The findings of this observation conclude that the pungent phenolic bioactive principle of [6]-paradol shows the effective inhibition of DMBA

Table 2. The Intensity of Staining of p53, bcl-2, caspase-3 and TNF-α Expression in the Buccal Pouch of Control and Experimental Animals

<table>
<thead>
<tr>
<th>Groups</th>
<th>P53</th>
<th>bcl-2</th>
<th>Caspase-3</th>
<th>TNF-α</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>+</td>
<td>++</td>
<td>++</td>
<td>+</td>
</tr>
<tr>
<td>DMBA</td>
<td>2</td>
<td>5</td>
<td>7</td>
<td>3</td>
</tr>
<tr>
<td>DMBA+[6]-paradol</td>
<td>4.5</td>
<td>7</td>
<td>2</td>
<td>6</td>
</tr>
<tr>
<td>[6]-paradol alone</td>
<td>7</td>
<td>0</td>
<td>0</td>
<td>1</td>
</tr>
</tbody>
</table>

*+=weak, **=moderate and ***=strong

Discussion

The present study provides evidence that the pungent phenolic bioactive constituents of ginger namely [6]-paradol can effectively modulate the pathological changes and enhances the apoptotic related gene expression in DMBA induced oral carcinogenesis. In recent years, the number of molecular based assays has increased, but histopathology and immunohistopathology remains the gold standard for most diagnostic and therapeutic responses. Thus the present study, we evaluated the effects of [6]-paradol on pathological changes in DMBA induced buccal pouch carcinogenesis using immunohistochemical techniques.

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induced buccal pouch carcinogenesis, through inhibiting cell proliferation activity and inducing apoptosis as evidenced by down regulation of p53.

Several studies have proposed that close interactions among p53 and bcl-2 proteins during carcinogenesis. bcl-2 family proteins are central regulators of mitochondria dependent cell death pathway, (Leibowitz and Yu, 2010) known to play a pivotal role in the induction of caspases activation and in the regulation of apoptosis. Over-expression of bcl-2 has been reported in several tumors including breast, thyroid, lung and skin carcinomas (He et al., 2010). In oral carcinomas, over expression of bcl-2 has been reported in many experimental studies. The bcl-2 functions as a suppressor of apoptosis leading to the survival of neoplastic cells, ectopic expression of other bcl-2 family proteins, such as bax, which induces mitochondrial apoptosis with apoptosis related morphological changes, caspase activation, and subsequent substrate proteolysis (Crowe and Sinha, 2006). Recent data reported that anthocyanin rich extract from the Korean vine plant meoru inducing apoptosis with a reduction in the expression of several anti-apoptotic proteins including bcl-2 (Bishayee et al., 2011). Similar observation was found in our study. Oral administration of [6]-paradol significantly inhibited the bcl-2 expression in DMBA treated hamsters. [6]-paradol administration can also regulate the expression of anti-apoptotic protein and inducing apoptosis probably through activation of caspases cascade.

Tumor development is caused by an imbalance between cell proliferation and apoptosis. Caspase-3 is final effectors of the apoptotic programme which reflect the distinct functions such as DNA damage sensors, cell cycle checkpoints, DNA repair and the apoptotic cascade itself, among others (Bratton and Cohen, 2001). Takju et al. (2011) reported that down-regulation of caspase-3 and abnormal expression of bcl-2 leads to defective apoptosis process. There is an inverse relationship between bcl-2 and caspase-3 expression in control and experimental hamsters in each group. Munshi et al. (2001), indicated that the increase of bcl-2 expression caused the decrease of caspase-3 activity. Our results corroborate these observations. TNF-α is an activator of extrinsic pathway of apoptosis. It is a multifunctional cytokine produced mainly activated by macrophages and/or monocytes. The extrinsic apoptotic process is initiated by extracellular ligands that, upon binding to their corresponding death receptor, cause the recruitment of initiator caspase (Englaro et al., 1999). We found that the expression of TNF-α significantly increased in DMBA treated animals, due to the marked elevation of ROS during DMBA metabolism that probably leads to increase in the synthesis of TNF-α. Oral administration of [6]-paradol significantly reduced the expressions of TNF-α in DMBA induced hamster and shows its anti-inflammatory activity.

The findings of our study concludes that oral administration of [6]-paradol at a dose of 30 mg/kg b.wt significantly modulate the pathological changes as well as improve the apoptotic associated gene expression of p53, bcl-2, caspase-3 and TNF-α. However, the findings of this study are inadequate for clinical trials, further are warranted to fulfill the mechanisms of [6]-paradol against DMBA induced hamster buccal pouch carcinogenesis.

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