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Research Article

PHARMACOLOGICAL EVALUATION OF CINNAMALDEHYDE AGAINST DMBA-INDUCED SKIN CANCER IN ALBINO MICE

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ABSTRACT

Objectives: The primary objective of this study was to assess the pharmacological activity of Cinnamaldehyde (CA) obtained from cinnamon bark in 7, 12-Dimethylbenz antharacene (DMBA)-induced skin cancer in albino mice and to assess the important cell antioxidant enzymes levels which play an important role in cancer.

Methods: Cinnamon bark was collected to obtain CA phytochemical screening of extract was performed. Then pharmacological screening of extract was done in albino mice with skin cancer, after 12 weeks of observation, the animal was sacrificed, the skin samples were collected and various parameters were assessed and at the end histopathological studies were performed. Further statistical methods were applied to analyze the results.

Results: The results revealed that CA produces significant increase in cell anti-oxidant enzymes such as superoxide dismutase, catalase, and glutathione peroxidase content. It is also reported that it has inhibited the activity of lactate dehydrogenase and levels of lipid peroxidation at the same time which are responsible for cell damage.

Conclusion: The present study shows that CA obtained from cinnamon bark consist of significant anti-cancer activity against DMBA-induced skin cancer in albino mice.

Keywords: Cutaneous basal cell carcinoma, Cinnamaldehyde, Antioxidants, DMBA.

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INTRODUCTION

Skin cancer is the major threat for mankind affects about nine in 100,000 people annually and it accounts for about 2% of all cancers. The survival rate for all cancer patients is about 42%, but varies greatly depending on the type of cancer. Males are more likely to contract the disease than females, and whites more than other racial or ethnic groups [1].

Literature survey on herbal drugs has shown significant anti-cancer activity which has not shown any remarkable side effect [2]. The several herbal formulation have derived from the Ayurveda, traditional system of Indian medicine and its additional system of drugs, yet to be scientifically validated that they need exhibited pharmacological action against cancer. Only less number of scientific data of traditional medicines is available for the treatment of skin cancer [3].Thus, the knowledge provided in this research will help the researchers for the development of alternative methods instead of chemotherapy and oral anti-cancer drugs for the treatment of skin-related cancer which can minimize the complication [4].

Cinnamaldehyde (CA) is an active constituent of cinnamon which provides cinnamon its flavor and odor. CA is commonly used in perfume industry and also for flavoring food items such as ice creams and beverages. CA has antipyretic activity, astringent activity, antimicrobial activity, anti-inflammatory activity, hepatoprotective activity, antibacterial, and cytotoxic effects [5]. Cinnamon is one of the oldest herbal plants; its various parts are used as a spice and traditional medicine. CA is the main component of cinnamon bark extract and produces its distinct cinnamon odor and flavor [6]. The action of cinnamon is introduced on cell structure and membrane functions, proteins, and enzymes, or other essential processes involved in biosynthesis. Cinnamon is capable of altering the lipid profile of the microbial cell wall. Consequently, tracking biochemical alterations during treatment of the biological system by this antibacterial agent can be used to find specific biomarkers or pathway mechanisms [7].

METHODS

Plant collection

Fresh bark oil of *cinnamom* was purchased from authenticate supplier, Sainath Aromas International, Kharibaowli, New Delhi.

As per literature review, the essential oils of Cinnamon species have CA as major component. This is resembled for cytotoxic activity, to check the presence of these components sample of essential oils sent for FTIR.

Drugs: Acetohexamide 250 mg/kg, CA 200 mg/kg

Chemicals: 12-dimethyl benzanthracene (DMBA7), Croton oil, 5, 5'-dithiobis-2-nitrobenzoic acid (DTNB), glutathione (GSH), superoxide dismutase (SOD), catalase, and NADPH (nicotinamide adenine dinucleotide phosphate, pyrogallol, and thiobarbituric acid (TBA)) were procured from Sigma Chemicals, Bengaluru, India. All the other chemicals used were of analytical grade.

Solvents

Normal Saline, (carboxy methyl cellulose).

Preliminary phytochemical studies

The pharmacological action of crude drug was determined by the nature of its constituents [8]. Thus, the plant species may be considered as a biosynthetic laboratory for the chemical compounds, for example, carbohydrates, proteins, and fats that are utilized as a food by humans and animals and for compounds such as alkaloids, flavonoids, and glycosides which exert definite physiological effects [9]. These

compounds are responsible for the desired therapeutic properties. To obtain these pharmacological effects, the plant materials are used as such in their crude form or extracted with suitable solvents to take out the desired components and the resulting principle being employed as therapeutic agents [10]. It is necessary to evaluate the nature of extract before evaluating the biological activity of same. We have selected such extracts which contain large number of chemical constituents. Hence, for this purpose, we have to go for phytochemical tests to evaluate the chemical nature of extracts qualitatively.

Screening methods for anti-cancer activity of CA

Animal selection

Swiss albino mice (23–25 g, 6–8 weeks) were obtained from Sainath agency, Hyderabad, India and these mice were housed in cages. Animals were acclimatized before the experiment. The animals were kept in polypropylene cages, housed in climate controlled facility at standard laboratory conditions, at temperature 24±2°C, humidity 55±5%, and with a photoperiod of 12 h light/12 h dark cycle. They were provided with water ad libitum and all time access to the pellet diet available commercially available from Sainath agency, Hyderabad, India [11]. The experiment was performed according to the experimental protocol provided by the Institutional Animal Ethical Committee (Protocol approval no. CPCSEA/2017-01/4) that confirms to the guidelines of Committee for the Purpose of control and supervision of experiments on animals (CPCSEA), Ministry of Environment, Government of India, India.

Dose selection

It was observed from preliminary toxicity studies that the animals were safe for maximum dose of 2000 mg/kg body weight [12]. However, there were few changes in the behavioral responses such as alertness, touch response, and restlessness. 1/10th of maximum tolerated dose, that is, 200 mg/kg body weight was chosen for the study.

Swiss albino mice weighing 20–25 g were used for this activity. All the mice were selected randomly and grouped into five (Groups I, II, III, and IV) each containing six animals. All the mice were numbered group-wise and individually.

Group I (Control group)

It served as healthy control consisted of healthy animals will receive Milli-Q water (10 mL/kg body weight a normal diet and tap water *ad libitum* daily.

Group II (DMBA/Croton oil applied)

In this group, the animals received topical applications of DMBA at an interval of 72 h at a dose of 0.05 g/kg in acetone (100 µL/mouse). Starting from the 8 days after first DMBA application, croton oil (1% w/v) in acetone (100 µL/mouse) was applied twice in a week for a total of 12 weeks.

Group III (Test-I)

The animals in this group were applied with DMBA/Croton oil similar to Group II following concomitant treatment with CA administered orally using intragastric gavage at 100 mg/kg, respectively, for 15 days before the first DMBA application and continued up to 12 weeks after DMBA application.

Group 1V (Test-II)

The animals in this group were applied with DMBA/Croton oil similar to DMBA/Croton oil-treated group along with topical application of CA at a dose of 100 mg/kg prepared with 100 µL of 0.05% Tween-80 for 15 days before the first DMBA application and continued for 12 weeks after DMBA application.

During the 12-week period, the animals were observed each day for the appearance of skin papillomas and the tumor volume was recorded. At the end of 12 weeks, all the mice were sacrificed and the skin samples were collected after 16 weeks, the mice were euthanized, and the dorsal skin will be removed for histopathology and blood will be taken for biochemical analysis (Figs 1-3 and Tables 1-4).

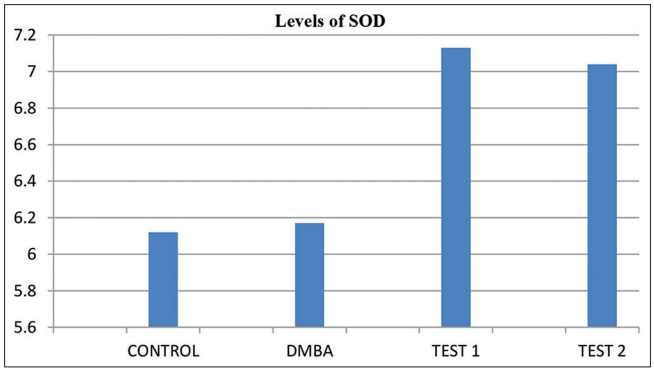

Fig. 1: Effect of cinnamaldehyde on superoxide dismutase

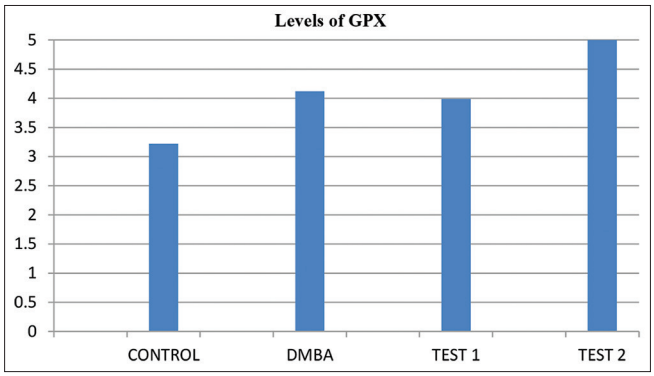

Fig. 2: Effect of cinnamaldehyde on glutathione

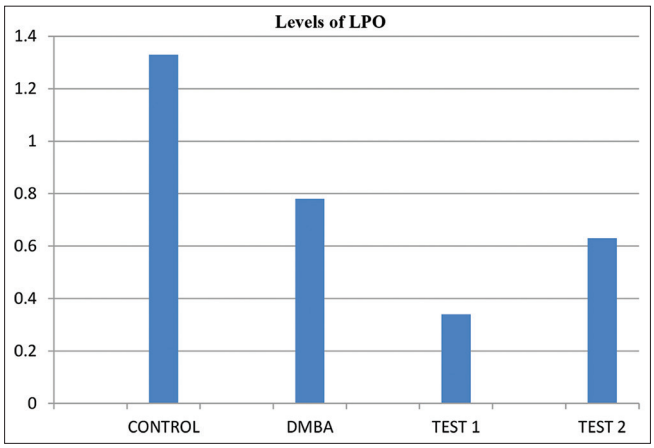

Fig. 3: Effect of cinnamaldehyde on lipid peroxidase

Assessment of parameters

Oxidative stress biomarkers

On the final day of the experiment, all the animals were euthanized by cervical dislocation. The dorsal skin was removed immediately and held in ice-cold saline (0.9% NaCl), followed by removal of extraneous material. The skin was then weighed and blotted dry. A 10% of tissue homogenate of skin was prepared in 0.15 M Tris-KCl (pH 7.4), and then centrifuged at 12,000 rpm for 15 min. For biochemical estimation, supernatant was used on the same day. Protein content will be measured by the Bradford method, 10 using bovine serum albumin as the standard [13].

Table 1: Effect of drugs on body weight of albino mice

Groups	Initial body weight	Final body weight
Control	29.2±1.24	28.2±2.63
DMBA	29.2±0.17	24.8±1.25
Test 1	30.1±2.01	28.2±1.20
Test 2	24.2±1.45	22±1.82

DMBA: 7, 12-Dimethylbenz antharacene

Table 2: Effect of Cinnamaldehyde on superoxide dismutase

Groups	SOD
Control	6.12±0.24
DMBA	6.17±4.09
Test 1	7.13±2.1
Test 2	7.04±1.1

DMBA: 7, 12-Dimethylbenz antharacene, SOD: Superoxide dismutase

Table 3: Effect of cinnamaldehyde on glutathione peroxidase

Group	GPX
Control	3.22±0.33
DMBA	4.12±0.5
Test 1	3.99±0.18
Test 2	5.0±0.31

DMBA: 7, 12-Dimethylbenz antharacene, GPX: Glutathione peroxidase

Table 4: Effect of cinnamaldehyde on lipid peroxidase

Groups	LPO
Control	1.72±0.13
DMBA	2.14±1.2
Test 1	1.24±0.12
Test 2	1.18±1.2

DMBA: 7, 12-Dimethylbenz antharacene, LPO: Lipid peroxidation

Lipid peroxidation (LPO) assay

LPO will be estimated by measuring the formation of malondialdehyde. A mixture of 0.1 mL tissue lysate and 1.9 mL of 0.1 M sodium phosphate buffer (pH 7.4) will be incubated at 37°C for 1 h. After precipitation with 5% trichloroacetic acid, the incubation mixture will be centrifuged (2300× g for 15 min at room temperature) and the supernatant will be collected. Next, 1.0 mL of 1% TBA will be added to the supernatant and placed in boiling water for 15 min. After cooling to room temperature, the absorbance of the mixture will be taken at 532 nm and expressed in nmol malondialdehyde per hour/mg protein using a molar extinction coefficient of 1.56×10^5/M/cm [10,14].

Estimation of glutathione peroxidase (GPX)

The GPX level was quantified using Ellman's reagent. The assay mixture contained phosphate buffer, 5, 5'-dithiobis-(2-nitrobenzoic acid), and tissue lysate. The reaction was monitored at 412 nm and the amount of GPX was expressed in terms of nmol of GPX/mg protein.

Measurement of SOD

The activity of SOD will be estimated using the method described by Kakkar *et al*. The assay mixture contained sodium pyrophosphate buffer, nitrobluetetrazolium, phenazine methosulfate, reduced nicotinamide adenine dinucleotide, and tissue lysate. One unit of SOD enzyme activity is defined as the amount of enzyme required to inhibit production of chromogen (560 nm) by 50% in 1 min under assay conditions and is expressed as specific activity in units/min/mg protein [15].

Measurement of catalase activity

Catalase activity was measured by following its ability to split hydrogen peroxide (H_2O_2) within 1 min of incubation time. The reaction was then stopped by adding dichromate/acetic acid reagent, and the remaining H_2O_2 was determined by measuring at 570 nm the chromic acetate formed by reduction of dichromate/acetic acid in the presence of H_2O_2, as described earlier. Catalase activity was expressed as μmole H_2O_2 decomposed/min/mg protein [16].

Histological evaluation

The skin tissues of mice were fixed in the buffered formalin (10%) and cut into four segments and embedded in the paraffin wax. These formalin fixed paraffin embedded sections were cut to get the serial thin sections of 4 μm thickness and subjected to the hematoxylin and eosin (H and E) staining protocol. These sections after H and E staining were examined under the light microscope (Nikon, Tokyo, Japan) and digital images were captured. The slides were evaluated by a qualified experienced pathologist masked to the experimental groups and treatments. A minimum of 10 fields for each slide were examined and scored.

Statistical analysis

The data were expressed as mean ± SEM and were analyzed by one-way analysis of variance followed by Bonferroni's multiple comparison test or Dunnette's *post hoc* test using the Graph Pad Prism software version 6.0. The criterion of statistical significance was set at ($p<0.05$).

RESULTS AND DISCUSSION

Identification and confirmation of lead component by FTIR

Cinnamon bark contains active components such as CA, eugenol, cinnamic acid, and cinnamyl acetate. Among all the other active components, CA plays a key role [17]. In this present work, the extraction of CA from the cinnamon by batch studies and purification of CA by column chromatography were performed. CA has been proven to be active against pathogenic bacteria, fungi, and viruses. The target action of cinnamon is introduced on cell structure and membrane functionality, proteins, and enzymes, or other essential processes involved in biosynthesis or energy generation.

Body weight

The average body weight of dmba induced mice was not different from control group of mice; however in the drug treated group of mice intitial increase in body weight was seen.

Measurement of SOD

Specific activity of SOD showed significant increase by both the doses of CA used in our experiment. Relative to the level in untreated control animals, the activity of SOD was enhanced by folds.

Measurement of enzyme GPX

GPX level was enhanced by both the doses of CA, GPX increased by folds ($p<0.005$)

Measurement of LPO

Significant decrease in LPO was seen in drug treated groups.

CONCLUSION

CA is the one of the constituents of the famous Indian medicinal plant "Cinnamom". It has been used in traditional system of medicine for the treatment of many disorders.[20] CA has an impressive past references of being anti-bacterial, lung cancer, anti-microbial properties, hypolipidemic activity, and anti-diabetic. The CA reported to have inhibitory effects on human immunodeficiency virus reverse transcriptase. Results of the present work with this popular plant of "Ayurvedic Medicine "showed potent inhibitory effect on skin cancer. The results revealed that CA activity was elevated significantly. The specific activities of SOD, catalase, and glutathione content were increased significantly. It was also reported to inhibit the activity of lactate dehydrogenase (LDH) and levels of lipid peroxidation at the same time. One of the major mechanisms of reducing and combating cancer risk is considered to be a selective elevation of antioxidants,

which can tackle the reactive oxygen species (ROS) and the resulting oxidative stress. As mentioned earlier, ROS have been linked with various events of mutagenesis and carcinogenesis. Thus, the increase in various antioxidant levels might help in preventing the free radical dependent process of carcinogenesis. This possibility is supported by significant decrease in the level of lipid peroxidation and LDH activity caused due to treatment of CA. It was a pilot study, further studies on activity of cinnamaldehye in cancer is needed at cellular levels.

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AUTHORS CONTRIBUTION

All authors contributed equally.

CONFLICTS OF INTEREST

The authors have no conflicts of interest to declare.

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