Research Article

Anti-inflammatory activity of catechol derivatives on buccal tumor cell line TR146 *in vitro*

Gokul G. L.[1], V. Vishnupriya[1], R. Gayathri[1], G. Dhanasekaran[2], K. Muthu[3], R. Sundaram[1]*

ABSTRACT

Background: Squamous cell carcinoma (SCC) is the most prevalent cancer of the oral cavity and the fifth most prevalent of all malignancies in males. Many researchers have attempted to develop new treatments that will improve the prognosis of SCC patients. **Aim:** The aim of this study was to test the anti-inflammatory potential of catechol derivatives I and II against oral SCC cells. **Materials and Methods:** The structurally similar two catechol derivatives I and II were analyzed for activity against the buccal tumor cells TR 146 cell line at various concentrations 1, 100, 250, 500 and 750 ng/ml and then incubated for 24 h. **Results:** Results showed that the levels of NO in the human buccal cancer cell line TR146 was significantly decreased in a dose dependent manner upon treatment with catechol derivative I & II at different concentrations (1,100,250,500 and 750ng) when compare to untreated control TR146 cells. **Conclusion:** Among the catechol derivatives, catechol derivative I was found to be more effective than the catechol derivative II. This may be due to the reason of positioning the functional group in their structures.

KEY WORDS: Buccal tumor cells, Catechol derivatives, Inflammatory cytokines, Nitric oxide

INTRODUCTION

Squamous cell carcinoma (SCC) is the most prevalent cancer of the oral cavity and one of the most frequent cancers in the world. Annually, approximately 350,000 new cases of oral and oropharyngeal SCCs are diagnosed worldwide.[1] Following the use of cisplatin chemotherapy, many changes have been associated with the multidrug-resistance phenotype of tumor cells.[2] Cancer chemotherapy is limited by the development of drug resistance by cancer cells and the adverse effects of antitumor drugs. The search for novel antitumor agents that circumvent these limitations has turned to natural plants.[3]

Semecarpus anacardium belongs to the family Anacardiaceae, grown in sub-Himalayan, tropical and central parts of India. Seeds of *S. anacardium* have been used in the Indian traditional system of medicines (Ayurveda and Siddha) either alone or as an ingredient of many polyherbal formulations for treating various ailments. Ayurveda describes the *S. anacardium* as a potent drug for arthritis, leprosy helminthic infection, and venereal disorders.[4-6] *S. anacardium* seeds have already been subjected to broad investigations and isolation of number of compounds including tetrahydroamentoflavone,[7] semicarpoflavonone[8] jeediflavanone,[9] galluflavonone,[10] nallaflavonone,[11] semecarpetin,[12] and anacardioflavonone.[13] Moreover, there are a number of catechol derivatives that have been reported in some other *Semecarpus* species.[14] However, we report these compounds for the 1st time in this species. Catechol derivatives are one of the phytoconstituents of *S. anacardium* seeds that have been isolated using thin-layer and column chromatography and tested their anti-inflammatory potential on buccal cancer cell TR146 *in vitro*, as there is no report on this aspect.

Access this article online	
Website: jprsolutions.info	ISSN: 0975-7619

MATERIALS AND METHODS

Chemicals

Dulbecco's Modified Eagle Medium (DMEM), Trypsin-EDTA, fetal bovine serum (FBS), phosphate-buffered saline (PBS), Antibiotic-Antimycotic solution, 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide and dimethyl sulfoxide, and propidium iodide were procured from HiMedia Laboratories, Mumbai, India.

[1]Department of Biochemistry, Saveetha Dental College and Hospital, Saveetha Institute of Medical and Technical Sciences, Saveetha University, Chennai, Tamil Nadu, India, [2]Department of Zoology, University of Madras, Guindy Campus, Chennai, Tamil Nadu, India, [3]Department of Chemistry, Manonmaniam Sundaranar University, Tirunelveli, Tamil Nadu, India

***Corresponding authors:** R. Sundaram, Department of Biochemistry, Saveetha Dental College and Hospital, Saveetha Institute of Medical and Technical Sciences, Saveetha University, Chennai, Tamil Nadu, India Phone: +91-9087333046. E-mail: rsundaram77@yahoo.co.in.

Received on: 27-12-2018; Revised on: 21-01-2019; Accepted on: 18-02-2019

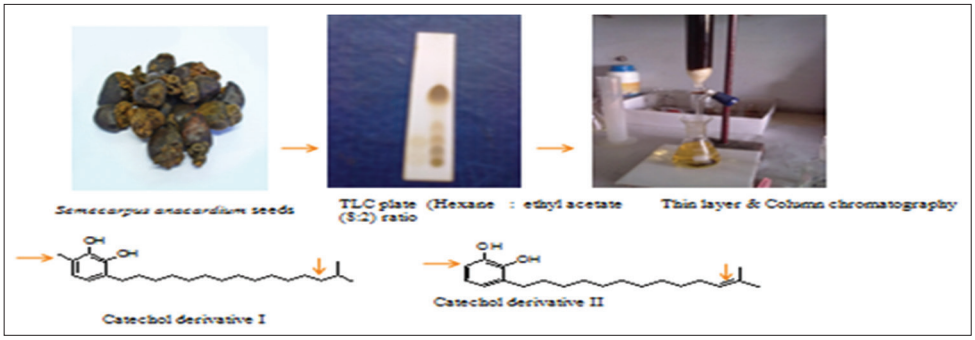

Figure 1: Structures of catechol derivatives I and II

Plant Material

S. anacardium seeds were purchased from K.R. Vasan Traditional and Herbal Medicine shop, Parris, Chennai, Tamil Nadu, India. The identity of the plant was confirmed by Professor Ramabn, plant taxonomist, Centre for Advanced Studies in Botany, University of Madras, and voucher specimen (MUCASB-H105) was deposited in the department herbarium.

Isolation and Characterization of Catechol Derivatives from *S. anacardium*

Catechol derivatives I and II were isolated and characterized from *S. anacardium* seeds, and their structures were confirmed by ^{1}H,^{13}C-NMR, IR, and HRMS.[15] The structures of the catechol derivatives I and II are given in Figure 1.

Cell Culture

Human buccal tumor cells TR146 lines were procured from the National Centre for Cell Science, Pune, India. The cells were grown in T25 culture flask containing DMEM supplemented with 10% FBS, and the flask was placed at 37°C in a humidified incubator with 5% CO_2. When the cells reached 70–80% confluent, the spent medium was discarded and the monolayer was rinsed with PBS. Trypsin-EDTA solution was added and placed in incubator for 2 min. After incubation, 5 ml of growth medium was added to the flask and mixed gently. Then, it was transferred into a 15 ml falcon tube and centrifuged at 1000 rpm for 5 min. The supernatant was carefully aspirated and the pellet was gently resuspended in 2 ml of growth medium. The cells were diluted with the appropriate volume of growth medium and aliquoted to new culture flask at the density of $2 \times 10^3/cm^2$ and kept back to the controlled environment for large-scale production. The morphology of buccal tumor cells TR146 is given in Figure 2.

Cell Line Cultivation and Preparation for Assays

TR146 cells were plated in 6-well plates with DMEM medium containing 10% FBS. The cells were incubated for 24 h under 5% CO_2 and 95% O_2 at 37°C. The medium was removed and washed with PBS, and fresh serum-free medium was added and kept

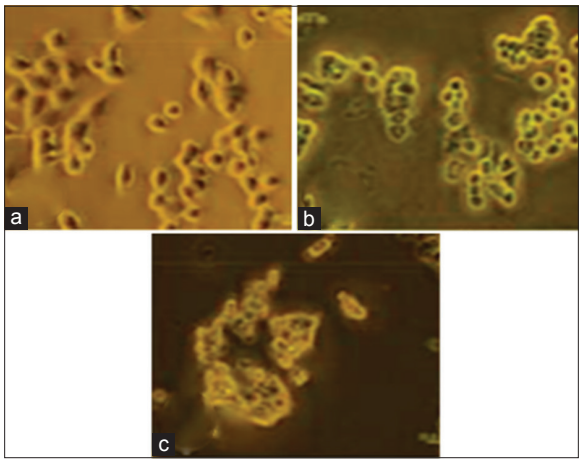

Figure 2: Morphology of TR146 cell. (a) Control cells (b) Minimum concentration (c) Maximum concentration

in incubator for 1 h. After starvation, the cells were treated with catechol derivatives I and II at different concentrations (1, 100, 250, 500, and 750 ng/ml) and then incubated for 24 h.The untreated TR146 cells were maintained as control for 24 h. After incubation, the cells were washed twice with PBS (pH 7.2) and treated with 0.25% Trypsin, 2 mM EDTA in PBS for 10 min. The cell suspension was centrifuged for 10 min in a centrifuge (600 rpm). Cell pellets were then lysed in 50 mM phosphate buffer (pH 7.0), followed by sonication for 2 min on ice. The mixture was then centrifuged for 10 min at 10,000 × g and the supernatant was assayed for NO assay.

Estimation of NO Production

The production of NO was determined by quantification of nitrite concentrations in the medium by the Griess reaction by Jung *et al.*[16] Briefly, 100 µl of cell culture medium (without phenol red) was mixed with an equal volume of Griess reagent (1% [w/v] sulfanilamide and 0.1% [w/v] N-[1-naphthyl]ethylenediamine dihydrochloride in 0.1 N HCl), incubated at room temperature for 10 min, and absorbed at 540 nm using a microplate reader. A standard curve of different concentrations of sodium nitrite was generated to calculate the nitrite accumulated in the supernatant.

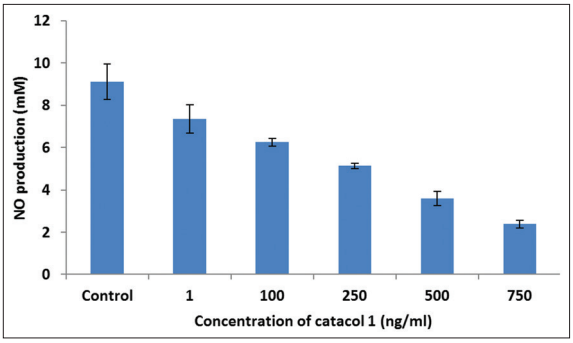

Figure 3: Effect of catechol derivatives I on NO production in control and buccal tumor cells. (The data are expressed as mean ± standard deviation of triplicate samples)

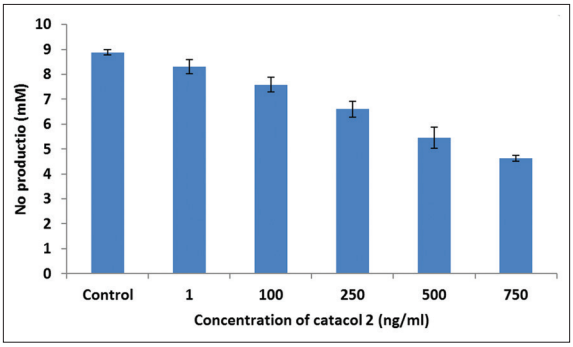

Figure 4: Effect of catechol derivatives II on NO production in control and buccal tumor cells. (The data are expressed as mean ± standard deviation of triplicate samples)

Statistical Analysis

All data were analyzed with SPSS 17 student software. Hypothesis testing methods included one-way analysis of variance followed by least significant difference. All values are means of three replicates. The values are expressed as mean ± SD, and the results were considered to be statistically significant if $P < 0.05$.

RESULTS AND DISCUSSION

Production of NO in Control and Catechol Derivative I and II 24 h Treated Buccal Tumor Cells

In this study, we have investigated *in vitro* anti-inflammatory effects of catchol derivatives I and II individually on the human buccal cancer cell lines (TR146) to elucidate the baseline levels of NO in buccal cancer cells. Results showed that the levels of NO in the human buccal cancer cell line TR146 was significantly decreased in a dose-dependent manner on treatment with catechol derivatives I and II at different concentrations (1, 100, 250, 500 and 750 ng) when compare to untreated control TR146 cells. Among the catechol derivatives, catechol derivative 1 was found to be more effective than the catechol derivative II. This may be due to the reason of positioning the functional group in their structures. These reactions are reversible. The continuations of this previous study has proved that the levels of lipid peroxidation (LPO) was significantly increased in a dose-dependent manner in the catechol derivative-treated buccal cancer cells, whereas the levels of antioxidant enzymes (superoxide dismutase) were significantly decreased. Therefore, the elevated levels of LPO and declined levels of antioxidant enzymes may be responsible for these declined levels of NO [Figures 3 and 4].

CONCLUSION

In the present study, the levels of NO and proinflammatory cytokines were decreased on exposure of catechol derivatives I and II to buccal tumor cells at 24 h when compared to untreated cells. Thus, the data of the results obtained in the *in vitro* studies conducted against buccal tumor cells depicted that the catechol derivatives I and II have the significant anti-inflammatory activity against tested cell lines in a dose-dependent manner.

REFERENCES

1. Jemal A, Siegel R, Ward E, Hao Y, Xu J, Murray T, *et al.* Cancer statistics, 2008. CA Cancer J Clin 2008;58:71-96.
2. Zgurskaya HI, Nikaido H. Multidrug resistance mechanisms: Drug efflux across two membranes. Mol Microbiol 2000;37:219-25.
3. Anand P, Sundaram C, Jhurani S, Kunnumakkara AB, Aggarwal BB. Curcumin and cancer: An old-age disease with an age-old solution. Cancer Lett 2008;267:133-64.
4. Chopra RN, Chopra IC, Handa KL, Kapur LD. In: Indigenous Drugs of India. 2nd ed. Calcutta: U.N. Dhur and Sons; 1958. p. 408.
5. Chattopadhyaya MK, Khare RL. Isolation of anacardic acid from *Semecarpus anacardium* Linn. And study of its antihelmintic activity. Indian J Pharmacol 1969;32:104-5.
6. Singh D, Aggarwal A, Mathias A, Naik S. Immunomodulatory activity of *Semecarpus anacardium* extract in mononuclear cells of normal individuals and rheumatoid arthritis patients. J Ethnopharmacol 2006;108:398-406.
7. Selvam C, Jachak SM. A cyclooxygenase (COX) inhibitory biflavonoid from the seeds of *Semecarpus anacardium.* J Ethnopharmacol 2004;95:209-12.
8. Murthy SS. A biflavanone from *Semecarpus anacardium.* Phytochemistry 1983;22:1518-20.
9. Murthy SS. Confirmation of the structure of jeedi flavonone: A biflavonane from *Semecarpus anacardium.* Phytochemistry 1984;3:925-7.
10. Murthy SS. Naturally occurring biflavonoid derivatives: Galluflavanone, a new biflavonoid from *Semecarpus anacardium*. Indian J Chem 1985;24:398-402.
11. Murthy SS. New dimeric flavone from *Semecarpus anacardium*. Proc Indian Natl Sci Acad 1987;57:632-7.
12. Murthy SS. Semicarpetin: A biflavonoid from *Semecarpus anacardium.* Phytochemistry 1988;27:3020-2.
13. Murthy SS. New biflavonoid from *Semecarpus sanacardium* Linn. Clin Acta Turcica 1992;20:30.
14. Carpenter C, Sotheeswaran S, Uvais M, Sultanbawa S. 5 Methylmellein and catechol derivatives from four *Semecarpus* species. Phytochemistry 1980;19:445-7.
15. Sundarama R, Muthu K, Nagaraj S, Shanthi P, Sachdanandam P. Isolation and characterization of catechol derivatives from *Semecarpus anacardium* seeds and their antibacterial potential in *in vitro*. Biomed Prev Nutr 2014;4:177-80.
16. Jung WK, Ahn YW, Lee SH, Choi YH, Kim SK, Yea SS, *et al. Ecklonia cava* ethanolic extracts inhibit lipopolysaccharide induced cyclooxygenase-2 and inducible nitric oxide synthase expression in BV2 microglia via the MAP kinase and NF-kappaB pathways. Food Chem Toxicol 2009;47:410-7.

Source of support: Nil; Conflict of interest: None Declared