

RESEARCH ARTICLE

Evaluation of antigenotoxic activity of ethanolic extract of *Calotropis procera* root in 7,12-dimethylbenz[a]anthracene induced genotoxicity in Wistar rats

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Received: August 21, 2018; Accepted: September 13, 2018

ABSTRACT

Background: Chemopreventive compounds may play an important role in cancer prevention. However, many chemopreventive agents available currently are associated with toxicity. Hence, there is a need to screen for newer compounds with chemopreventive potential. Many medicinal plants can be developed as prospective chemopreventive candidates. **Aims and Objectives:** The aim of the study was to assess the protective effect of ethanolic extract of *calotropis procera* root in 7,12-dimethylbenz[a]anthracene (DMBA) induced genotoxicity in Wistar rats by micronucleus assay. **Materials and Methods:** Wistar rats were divided into 4 groups randomly. Group 1 received distilled water (control group). Group 2 was administered DMBA (30 mg/kg body weight [BW], single dose) intraperitoneally on the 5th day of the experiment. Group 3 rats were pretreated with *C. procera* root extract (500 mg/kg BW) orally for 5 days followed by DMBA injection intraperitoneally 2 h after *C. procera* root extract on the 5th day. Group 4 rats were given *C. procera* root alone orally for 5 days. The animals were sacrificed on the 6th day, and bone marrow was harvested for the micronucleus test. The percentage of micronuclei in polychromatic erythrocytes (%MnPCE) and PCE: NCE were determined. **Results:** Group 2 rats exhibited significant ($P < 0.0001$) increase in the %MnPCEs compared to control group. Group 3 and 4 rats did not show a significant increase in %MnPCEs compared to control group. The PCE: NCE ratio is decreased in Group 2 rats compared to the control group, but it is not statistically significant. The PCE: NCE ratio does not differ significantly from the control group in Groups 3 and 4. **Conclusion:** The ethanolic extract of *C. procera* root exhibits antigenotoxic activity in DMBA induced genotoxicity in Wistar rats.


KEY WORDS: *Calotropis procera*; 7,12-Dimethylbenz[a]anthracene; Antigenotoxic Effect; Wistar Rats

INTRODUCTION

Chemoprevention of cancer is a novel approach. Many chemopreventive agents are recognized in various

epidemiological, preclinical, and clinical studies.^[1,2] However, many of these agents have produced toxic effects which have limited their use.^[3] Hence, it is essential to identify a potential chemopreventive agent with minimal toxic effects.

Medicinal plants have played a key role as a source of innumerable new drugs because of easy availability and less toxic effects. *Calotropis procera* is one such plant. It is a tropical plant belonging to Asclepiadaceae family. It grows in various parts of India.^[4] Conventionally, *C. procera* has been used for toothache, skin disease, asthma, elephantiasis, rheumatism, leprosy, etc.^[5] It is reported to possess various activities

Access this article online	
Website: www.njppp.com	Quick Response code 
DOI: 10.5455/njppp.2018.8.0929013092018	

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such as antimicrobial,^[6] immunomodulatory,^[7] antioxidant,^[8] anti-inflammatory,^[9] antimalarial,^[10] anticonvulsant,^[11] and anticancer^[12]. Phytochemical screening of this plant shows the presence of calotropursenyl acetate, calopfriedelenyl, triterpenoids, a norditerpenyl ester, calotropernyl ester oleanene, procerleanol A and B, triterpenes - calotropoleanyl ester, and cardiac glycosides - calotropin, calotropogenin, uscharin, calactin, and calotoxin.^[13]

7,12-Dimethylbenz[a]anthracene (DMBA) is a widely used site-specific cancer inducing agent.^[14] DMBA is reported to induce DNA damage and mutagenesis in various experimental models.^[15] It has been commonly used for screening of both natural and synthetic compounds for the chemopreventive property. *In vivo* micronucleus assay is one of the frequently used tests for genotoxicity screening. It detects compounds causing chromosome breaks.^[16] Genotoxicity is indicated in this assay by the increase in the frequency of micronuclei in polychromatic erythrocytes (MnPCE) and decrease in the polychromatic to normochromatic erythrocytes ratio (PCE: NCE).

There is a requisite for novel chemopreventive compounds which are easily available, have good efficacy and safety profile. Hence, this study was undertaken to assess the protective effect of ethanolic extract of *C. procera* root in DMBA induced genotoxicity in Wistar rats by micronucleus assay.

MATERIALS AND METHODS

Animals

Female Wistar rats, 7–8 weeks old, and weighing 130–140 g were selected for the present study. The animals were acquired from the central animal house, Sri Kaliswari College, Sivakasi, India. They were housed in polypropylene cages at room temperature and 12 h:12 h light-dark cycle. They were provided with standard pellet diet and water *ad libitum*. The study was conducted after obtaining approval from the Institutional Animal Ethics Committee.

Chemicals

The carcinogen DMBA, biochemical compounds - reduced glutathione (GSH), reduced nicotinamide adenine dinucleotide (NADH), colchicine, giemsa, 1,1',3,3' tetramethoxy propane, bovine serum albumin, and May-Grunwald stain were procured from Sigma-Aldrich Pvt Ltd, Bengaluru, India. Cyclophosphamide was bought from Dabur pharma Ltd, Tarapur, Thane. All chemicals were of analytical grade.

Preparation of Ethanolic Extract of *C. procera* Root

The dried roots of *C. procera* were mixed with absolute alcohol for 7 days. The extract was filtered, and the supernatant was

evaporated to dryness at room temperature. The extract was stored in a sterile bottle.

Experiment

The experimental animals were divided into 4 groups randomly. Group 1 rats received the vehicle (distilled water) and served as control group. Group 2 was administered DMBA (30 mg/kg body weight, single dose) intraperitoneally on the 5th day, of the experiment. Group 3 rats were pretreated with ethanolic extract of *C. procera* root (500 mg/kg body weight) given orally for 5 days. On the 5th day they were administered DMBA (30 mg/kg body weight) intraperitoneally 2 h after *C. procera* root extract. Group 4 rats were given an ethanolic extract of *C. procera* roots (500 mg/kg body weight) orally for 5 days. They were not injected with DMBA. All the animals were sacrificed on 6th day by cervical dislocation after overnight fasting and bone marrow was harvested.

In Vivo Micronucleus Assay

It was done according to Schmid's method.^[17-19] Bone marrow sample was collected from the femur of Wistar rats. It was mixed with fetal bovine serum and centrifuged at 500 rpm for 10 minutes. The pellet so formed was suspended in fresh fetal bovine serum and was utilized for preparing slides, which were air dried for 18 h. For staining, May-Grunwald's stain followed by Giemsa stain was used. Coding of slides was done. The incidence of MnPCE was calculated per 2500 percentage of MnPCE (%MnPCE) in every slide. The PCE: NCE ratio was noted for every 500 erythrocytes in all slides.^[20,21]

Statistical Analysis

The data were represented as mean \pm standard deviation. The data were analyzed by one-way ANOVA followed by Duncan's multiple range tests. $P < 0.05$ was considered significant. GraphPad Prism version 6.05 was used for data analysis.

RESULTS

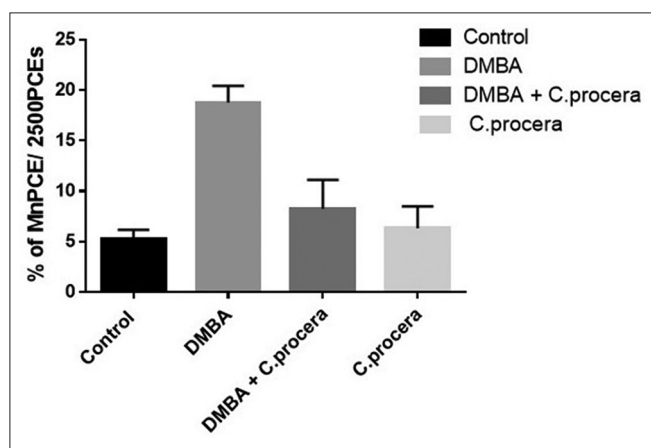
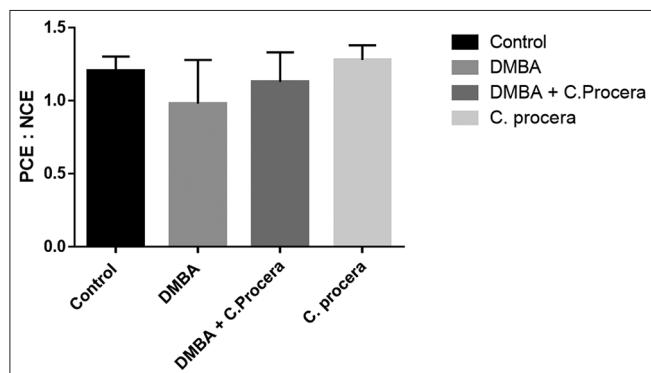
The %micronuclei per 2500 PCEs (%micronucleiPCEs) and PCE: NCE ratio in control and various test groups was statistically analyzed. The values from various experimental groups were compared with the control group. The results of *in vivo* micronucleus assay are given in Table 1.

In the current study, Wistar rats treated with DMBA alone (Group 2) exhibited significant ($P < 0.0001$) increase in the percentage of MnPCEs/2500 PCEs (%MnPCEs) compared to control group [Table 1 and Figure 1]. Group 3 rats which were pretreated with ethanolic extract of *C. procera* root before DMBA administration did not show a significant increase in

Table 1: Effect of ethanolic extract of *C. procera* root on DMBA induced bone marrow micronuclei in Wistar rats

Treatment groups	MnPCEs/2500 PCEs	PCE: NCE
Distilled water (control)	5.2±0.96	1.20±0.1
DMBA	18.75±1.67***	0.98±0.3
DMBA+C. <i>procera</i>	8.25±2.86	1.13±0.2
<i>C. procera</i>	6.31±2.18	1.28±0.1

Data expressed as mean±SD. n=6; *P < 0.05, **P < 0.01, ***P < 0.001 (compared with control). *C. procera*: *Calotropis procera*, DMBA: 7,12-dimethylbenz[a] anthracene. SD: Standard deviation, MnPCEs: Micronuclei in polychromatic erythrocytes

**Figure 1:** Percentage of micronuclei in polychromatic erythrocyte (PCE)/2500 PCEs in control and experimental groups**Figure 2:** PCE: NCE ratio in control and experimental groups

%MnPCEs compared to control group [Table 1 and Figure 1]. Group 4 rats administered with *C. procera* extract alone did not exhibit a significant change in %MnPCEs compared to control group [Table 1 and Figure 1].

The PCE:NCE ratio is decreased in rats treated with DMBA alone (Group 2) compared to the control group, but it is not statistically significant [Table 1 and Figure 2]. The PCE: NCE ratio does not differ significantly from the control group in Groups 3 and 4 [Table 1 and Figure 2].

DISCUSSION

In the current study, the antigenotoxic activity of ethanolic extract of *C. procera* root was assessed using DMBA as the inducer of genotoxicity in female Wistar rats. Genotoxicity was evaluated by *in vivo* micronucleus assay. DMBA is a widely used site-specific cancer inducing agent. It is reported to damage DNA and cause mutagenesis in various experimental models. *In vivo* micronucleus assay is one of the commonly used tests for genotoxicity screening. It detects compound causing chromosome breaks. Different parts of *C. procera* were reported to possess anticancer activities in traditional medicine.^[22,23] Hence, this study was undertaken to evaluate the protective effect of ethanolic extract of *C. procera* root in DMBA induced genotoxicity in Wistar rats by micronucleus assay. In micronucleus assay, the percentage of MnPCEs/2500 PCEs (%MnPCEs) and PCE: NCE ratio was estimated in control and various test groups. In this assay, an increase in the %MnPCE in the test group when compared with a control group is the indicator of genotoxicity. Inhibition of bone marrow cell multiplication by the test compound indicates its cytotoxicity. Hence, a decrease in PCE: NCE ratio gives an estimate of cytotoxicity of the compound. In our study, a statistically significant increase in the %MnPCEs was observed in DMBA treated rats (Group 2) compared to control rats. Pretreatment with ethanolic extract of *C. procera* root before DMBA administration (Group 3) did not exhibit a significant increase in %MnPCEs compared to control group. Group 4 rats which were treated with *C. procera* extract alone did not show a significant change in %MnPCEs compared to control group. In this study, it was observed that there was a decrease in the PCE: NCE ratio in Group 2 rats treated with DMBA alone compared to the control group, but it was not statistically significant. The PCE: NCE ratio did not differ significantly from the control group in Groups 3 and 4. These findings indicate that DMBA induces genotoxicity in Wistar rats and pretreatment with *C. procera* root extract provides protection against DMBA induced genotoxicity.

Several studies have confirmed the potential of DMBA to induce genotoxicity in experimental animals.^[24-26] Our study has demonstrated that *C. procera* root extract possesses antigenotoxic activity. This is similar to the conclusion of a study by Choedon *et al.*, who reported that *C. procera* latex has chemopreventive activity and is cytotoxic to several cancer cell lines.^[27] Mathur *et al.* reported that root extract of *C. procera* has antitumor activity in Hep 2 cell lines.^[28] According to Abdel-Samad MF stem extracts of *C. procera* have *in vivo* and *in vitro* antiproliferative activity against cancer cells.^[29] In contrast to the findings of our study, Abdel-Samad and Hassanane have reported the genotoxic and cytotoxic activity of Uscharin, a glycoside from latex of *C. procera*.^[30] In a study by Qari, latex of *C. procera* was found to be mutagenic.^[31] Nidhi *et al.* also found that *C. procera* is genotoxic to human chromosomes in peripheral blood culture *in vitro* along with bovine liver homogenate.^[32] These

differences may be due to different methods and different parts of calotropis plant used for the studies. The possible mechanism of the antigenotoxic activity of *C. procera* root extract is not clear. Mathur *et al.* have proposed that *C. procera* root extract exerts antiproliferative activity in Hep 2 cells by inducing apoptosis and disrupting cell cycle.^[28] It is reported that *C. procera* has free radical scavenging property.^[33,34] This may contribute to the antigenotoxic potential of *C. procera*.

The strength of this study is that DMBA is commonly used inducer of genotoxicity in various experimental models with reproducible results and the *in vivo* micronucleus assay is one of the genotoxicity tests recommended by regulatory agencies for genotoxicity screening. Both of these are extensively utilized for screening of compounds with antigenotoxic potential. The limitation of this study is that it was carried out in a limited number of animals.

These findings suggest that *C. procera* root extract has a protective effect against DMBA induced genotoxicity in Wistar rats.

CONCLUSION

Based on the findings of our study, we can conclude that ethanolic extract of *C. procera* root possesses antigenotoxic activity in DMBA induced genotoxicity in Wistar rats. However, further studies are required to elucidate the active compound and the exact mechanism of the antigenotoxic potential of *C. procera*.

ACKNOWLEDGMENTS

We thank Dr. Kolanjiappan Kaliyaperumal for his guidance and Kavitha T G for her help and support.

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How to cite this article: Dayana K, Manasa MR. Evaluation of antigenotoxic activity of ethanolic extract of *Calotropis procera* root in 7,12-dimethylbenz[a]anthracene induced genotoxicity in Wistar rats. *Natl J Physiol Pharm Pharmacol* 2018;8(12):1617-1621.

Source of Support: Nil, **Conflict of Interest:** None declared.