AMERICAN JOURNAL OF PHARMTECH RESEARCH

Journal home page: http://www.ajptr.com/

Effect of Acetone Root Extract of Canthium parviflorum Lam. in N-Nitroso N-Methyl Urea Induced Mammary Carcinoma in Female Sprague-Dawley Rats

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ABSTRACT

The objective of the study was to evaluate the anticancer activity of Acetone root extract of *Canthium parviflorum Lam.* (ARECP) in N-Nitroso N-methyl urea (NMU) induced mammary carcinoma in female Sprague-Dawley rats. Thirty virgin female Sprague-Dawley rats of 55 day old were divided into five groups (normal control, disease control, ARECP 200 mg/kg, ARECP 400 mg/kg, tamoxifen10 mg/kg) of six animals each. The mammary carcinoma was induced by two intraperitoneal doses of N-Nitroso N-methyl urea (NMU) (50mg/kg/body weight each) in 0.9% Nacl (maintained at 4^0C) at 55 day of age and 4 week after the prior administration. The drug ARECP was administered in two doses, 200 mg/kg, and 400 mg/kg orally and compared with the oral dose of tamoxifen (10 mg/kg). ARECP 200 mg/kg, 400 mg/kg, and tamoxifen10 mg/kg decreased the tumor incidences by 33.33%, 50%, and 83.33% and the total number of tumors in group by 33.33%, 60% and 93.33% respectively, when compared to the disease control. ARECP 200 mg/kg, 400 mg/kg, and tamoxifen 10 mg/kg decreased the average tumor burden by 65.06%, 92.16% and 95.72% and average tumor volume by 62.25%, 85.18%, and 93.73% respectively, when compared to disease control group. The results concluded that ARECP prevent the mammary carcinogenesis induced by N-Nitroso N-methyl urea in female Sprague-Dawley rats.

Keywords: Mammary Carcinoma, *Canthium parviflorum*, tamoxifen, N-Nitroso N-methyl urea.

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Received 17 July 2016, Accepted 23 July 2016

Please cite this article as: Milton JD *et al.*, Effect of Acetone Root Extract of Canthium parviflorum Lam. in N-Nitroso N-Methyl Urea Induced Mammary Carcinoma in Female Sprague-Dawley Rats. American Journal of PharmTech Research 2016.

INTRODUCTION

Mammary gland cancer is the leading cause of morbidity and mortality in females all-over the world, and is the second most common type of cancer after lung cancer and the fifth most common cause of cancer death.[1, 2] In India 70000 new cases of breast cancer and 35000 deaths due to this cancer are reported every year.[2] Various active compounds derived from medicinal plants have been assessed for their efficacy and tolerability in the treatment of human breast cancer. Some of these plant species, have well recognized anticancer activity in breast and other human malignancies, and several isolated pure compounds and their semisynthetic derivatives have been evaluated in clinical trials and marketed.[3] Cancer is one of the aliments which cannot be completely subdued by chemotherapy. The chemotherapeutic agents though effective against various types of tumor are not totally free from side effects.[4] Therefore there is a need for the development of pharmaceuticals that have anticancer potential, which are cost effective and safe without serious adverse effects. In the present study, we have evaluated the effect of acetone root extract of *Canthium parviflorum* (ARECP) on N-Nitroso N-methyl urea induced mammary carcinogenesis in virgin female Sprague-Dawley rats.

MATERIALS AND METHOD

Plant Collection

The whole plant *Canthium parviflorum Lam.* was collected from Neyyoor, Kanyakumari District of Tamilnadu, South India during the month of October.

Preparation of *Canthium parviflorum* root extracts

The root parts were separated carefully and dried in shade and ground well to fine power by using mechanical blender. Then it was extracted sequentially with Ethyl acetate, Chloroform, Acetone, Ethanol and Methanol using soxhlet apparatus. The extracts were filtered and concentrated using rotary vacuum evaporator and lyophilized to give a dry extract.

Chemicals and drugs

Drugs

Acetone root extract of *Canthium parviflorum* in two doses (200mg/kg & 400mg/kg). A gift sample of Tamoxifen citrate was obtained from the Torrent Pharmaceuticals, Ahmadabad, India.

Chemicals

N-Nitroso-N-methyl urea (NMU) (Sigma-Aldrich Chemicals Pvt. Ltd., Bangalore, India). Carboxy Methyl Cellulose (CMC) (Loba Chemie Pvt. Ltd., Mumbai). 37-40% formaldehyde (Nice

Chemicals Pvt. Ltd., Mumbai). Sodium phosphate monobasic (Loba Chemie Pvt. Ltd., Mumbai). Sodium phosphate dibasic (Loba Chemie Pvt. Ltd., Mumbai).

Experimental design[5,6]

A total of 30 virgin female Sprague-Dawley rats of 55 days old were randomly assigned to one of the five experimental groups, each group containing 6 animals. Group 1 animals received vehicle (0.9% sodium chloride (NaCl) intraperitoneally (i.p) and 1% W/V carboxy methyl cellulose (CMC) orally and served as normal controls. Rats in groups 2, 3, 4 and 5 were induced mammary cancer by two intraperitoneal doses of N-Nitroso-N-methyl urea (NMU) (50 mg/kg/body weight each) in 0.9% NaCl (maintained at 4°C)[7] at 55 days of age and 4 weeks after the prior administration. Group 2 rats received 1% W/V CMC orally and served as disease controls. Treatment groups 3, 4 and 5 rats were orally administered with ARECP 200 mg/kg,[8] 400 mg/kg,[8] and tamoxifen 10 mg/kg [9] suspended in 1%W/V CMC respectively. Treatment with ARECP, tamoxifen and 1%W/V CMC started 1 week before and continued upto 8 weeks after the first NMU administration.

The first administration of NMU was considered as day1. Animals were weighed and palpated twice in a week for tumors for a period of 122 days. The time of appearance of the first tumor (latency period) was recorded for individual animals in each group. After 122 days, all the animals were sacrificed by decapitation and observed for the presence of mammary tumors. Relative organ weights of liver, uterus, ovary and adrenal gland were noted. Tumor incidence, tumor multiplicity (mean ± SEM) and the total number of tumors in each group were recorded. Wet weight and diameter of individual excised tumor was measured. Tumor volume was calculated using the formula $4/3\ \pi r^3$, where 'r' is half the average diameter.

The study protocol was approved by Institutional Animal Ethical Committee (IAEC), Swamy Vivekanandha College of Pharmacy (proposal No. SVCP/IAEC/Ph.D/020/Feb/2012) and conducted in accordance with guidelines set by the CPCSEA (Committee for the Purpose of Control and Supervision of Experiment on Animals), India.

Statistical Analysis

The values are expressed as mean ± SEM. One - Way Analysis of Variance (ANOVA) followed by Dunnett's multiple comparison tests were carried out by using Graph-Pad Prism software, version 6; $P<0.05$ was considered as significant.

RESULTS AND DISCUSSION

Anticancer effects of ARECP in mammary carcinogenesis in female Sprague-Dawley rats are tabulated in Table 1 to Table 3. In ARECP 200 mg/kg, 400 mg/kg, and tamoxifen 10 mg/kg, the percentage of rats with tumors were decreased by 33.33%, 50%, and 83.33% respectively. The tumor latency period was increased in drug treated groups when compared to cancer control group. Total number of tumor, tumor multiplicity, increased tumor burden, and average tumor volume were decreased in treated group when compared to cancer control group.

Table 1: Effect of ARECP on tumor incidence rate, % of rats with tumors during necropsy and tumor latency period

Treatment	Tumor incidence rate	% of rats with tumors	Tumor latency period in days (Mean ± SEM)
Control (0.9% Nacl i.p + 1% W/V CMC orally)	0/6	0	0
Disease control (Two i.p dose of NMU (50mg/kg/body weight each) in 0.9% Nacl + 1% W/V CMC orally)	6/6	100	85.00 ± 1.770
Two i.p dose of NMU (50mg/kg/body weight each) in 0.9% Nacl + ARECP (200mg/kg) in 1% W/V CMC orally	4/6	66.67 (-33.33%)	89.50 ± 1.323(+5.29%)
Two i.p dose of NMU (50mg/kg/body weight each) in 0.9% Nacl + ARECP(400mg/kg) in 1% W/V CMC orally	3/6	50 (-50%)	96.33 ± 0.882(+13.32%)
Two i.p dose of NMU (50mg/kg/body weight each) in 0.9% Nacl + Tamoxifen (10mg/kg) in 1% W/V CMC orally	1/6	16.67 (-83.33%)	103 ± 0 (+21.17%)

n=6; The values are expressed as mean ± SEM.

Table 2: Effect of ARECP on tumor frequency and tumor multiplicity

Treatment	Total no. of tumors (%)	Tumor multiplicity (Mean ± SEM)
Control (0.9% Nacl i.p + 1% W/V CMC orally)	0	0
Disease control (Two i.p dose of NMU (50mg/kg/body weight each) in 0.9% Nacl + 1% W/V CMC orally)	15	2.500 ± 0.428**
Two i.p dose of NMU (50mg/kg/body weight each) in 0.9% Nacl + ARECP (200mg/kg) in 1% W/V CMC orally	10 (-33.33%)	1.667 ± 0.667
Two i.p dose of NMU (50mg/kg/body weight each) in 0.9% Nacl + ARECP(400mg/kg) in 1% W/V CMC orally	6 (-60%)	1.000 ± 0.516
Two i.p dose of NMU (50mg/kg/body weight each) in 0.9% Nacl + Tamoxifen (10mg/kg) in 1% W/V CMC orally	1 (-93.33%)	0.167 ± 0.167

n=6; The values are expressed as mean ± SEM; ** P<0.01 Vs control group, (one way ANOVA followed by Dunnett's multiple comparison Test).

Table 3: Effect of ARECP on average tumor burden and average tumor volume

Treatment	Average tumor burden in grams/animal (Mean ± SEM)	Average tumor volume in cm^3 (Mean ± SEM)
Control (0.9% Nacl i.p + 1% W/V CMC orally)	0.00	0.00
Disease control (Two i.p dose of NMU (50mg/kg/body weight each) in 0.9% Nacl + 1% W/V CMC orally)	4.98 ± 0.884**	7.020 ± 0.779***
Two i.p dose of NMU (50mg/kg/body weight each) in 0.9% Nacl + ARECP (200mg/kg) in 1% W/V CMC orally	1.74 ± 0.746 (-65.06%)	2.650 ± 1.108 (-62.25%)
Two i.p dose of NMU (50mg/kg/body weight each) in 0.9% Nacl + ARECP(400mg/kg) in 1% W/V CMC orally	0.39 ± 0.204 (-92.16%) $^{\$\$}$	1.040 ± 0.535$^{\$\$\$}$ (-85.18%)
Two i.p dose of NMU (50mg/kg/body weight each) in 0.9% Nacl + Tamoxifen (10mg/kg) in 1% W/V CMC orally	0.21 ± 0.213 (-95.72%) $^{\$\$}$	0.440 ± 0.440$^{\$\$\$}$ (-93.73%)

n=6; The values are expressed as mean ± SEM; ** P<0.01, ***P<0.001 Vs control group, $^{\$\$}$ P<0.01, $^{\$\$\$}$ P<0.001 Vs disease control group (one way ANOVA followed by Dunnett's multiple comparison Test). No macroscopic changes were observed in the selected organs (liver, uterus, ovary, adrenal gland) due to ARECP and tamoxifen administration. The body weight gain and the relative organ weights of liver, uterus, ovary and adrenal gland didn't showed any significant difference (Table 4).

Table 4: Effect of ARECP on relative organ (liver, uterus, ovary and adrenal gland) weights (Mean ± SEM)

Treatment	Liver (wt. in g /100 g bodyweight)	Uterus (wt. in mg/100 g bodyweight)	Ovary (wt. in mg/100 g body weight)	Adrenal gland (wt. in mg/100g body weight)
Control (0.9% Nacl i.p + 1% W/V CMC orally)	2.48 ± 0.092	216 ± 3.856	53 ± 1.633	21.50± 1.057
Disease control (Two i.p dose of NMU (50mg/kg/body weight each) in 0.9% Nacl + 1% W/V CMC orally)	2.31 ± 0.065	219.33 ± 7.177	55.50 ± 1.118	21.83 ± 0.872
Two i.p dose of NMU (50mg/kg/body weight each) in 0.9% Nacl + ARECP (200mg/kg) in 1% W/V CMC orally	2.55 ± 0.067	202.50 ± 5.679	53.66 ± 1.476	21.33 ± 0.843
Two i.p dose of NMU (50mg/kg/body weight each) in 0.9% Nacl + ARECP(400mg/kg) in 1% W/V CMC orally	2.38 ± 0.067	218.33± 2.603	52.33 ± 2.028	20.33 ± 1.085
Two i.p dose of NMU (50mg/kg/body weight each) in 0.9% Nacl + Tamoxifen (10mg/kg) in 1% W/V CMC orally	2.33 ± 0.079	218.83 ± 3.390	55.66 ± 1.229	19.83 ± 1.014

n=6; The values are expressed as mean ± SEM; Differences among experimental groups were not statistically significant (one way ANOVA followed by Dunnett's multiple comparison Test).

Cancer is a major health problem in both developed and developing countries. Because of the high death rate associated with cancer and because of serious side effects of chemotherapy and radiation therapy, many cancer patients seek alternative complementary methods of treatment. Natural products have played an important role in treating and preventing human diseases such as cancer, and have been derived from various source materials, especially terrestrial plants. The importance of plants with few side effects for use as antitumor agents in modern medicine has been well recognized in recent years.[10-13] Hence, finding a new natural source with anticancer activities would aid in finding new tools for cancer therapy.[14]

In the present research, animal experimental systems are particularly useful for the study of human mammary carcinogenesis. Since the rat mammary gland shows a high susceptibility to developing neoplasms which closely mimic human breast cancer, they have been selected in comparison to other animal models.[15] Researchers reported that many plants and plant products showed anticancer activities against breast cancer using animal models.[16-18]

The *Canthium parviflorum Lam*. (syn: Plectoria parviflora) of Rubiaceae is commonly called as Carray cheddie in English, Kirma in Hindi and Mullukaarai in Tamil. It occurs in peninsular India, coramandel coast, dry plains and shrub with spreading branches, a throny shrub found throughout Indian forest and dry plains. Its leaves are simple, small, obviate, opposite with interpetiolar stipules linear and axillary spines.

The *Canthium parviflorum* plant is well known for its various medicinal properties in India. The leaves and fruits are edible. They are astringent and effective against cough and indigestion.[19] *Canthium parviflorum* leaves and roots of this plant are used as febrifuge, anthelmintic, anti diarrhoea and for leucorrhoea[20,21] decoction of flux.[22] In Ayurvedic medicine it is used as laxative and to cure gout.[23] Tribes of Orissa state in India use fruits of this plant to treat headache. Since *Canthium parviflorum* leaf is also possess wound healing property. The roots of this plant are traditionally used by the tribes of Orissa in treatment of swelling of neck. This plant is reported for its pharmacological uses as an antidysenteric and antispasdomic. Traditionally the roots and leaves are used to cure vitiated conditions of Kapha in fever and constipation. [24]

In vitro studies showed the anticancer activity of methanolic extract of *Canthium parviflorum* on DLA and Hela cell lines.[8] Pasumarthi et al, (2011)[25] reported that the methanolic leaf extract of *Canthium parviflorum* showed a potent cytotoxic activity. Milton et al, (2014)[26] reported that the acetone root extract of *Canthium parviflorum* showed maximum anticancer activity both *In vitro* and *In vivo* studies carried out in male Swiss albino mice[25,26].

In this present study we have evaluated the effect of acetone root extract of *Canthium parviflorum Lam*. [ARECP] in N-Nitroso-N-Methyl Urea [NMU] induced mammary carcinoma in female Sprague - Dawley rats. NMU is a highly specific mammary gland carcinogen and NMU induced mammary tumors model has been widely used to evaluate chemo-preventive and therapeutic agents for breast cancer in human.[27-29]

The results showed that the drug ARECP decreases the incidence rate and increases the tumor latency period. The decreases in the incidence rate and increases in the latency period revealed that the drug ARECP prevent the NMU induced mammary carcinogenesis.

The results also showed that there is no changes in macroscopical characters and relative weight of selected organ after 122 days of treatment when compare to control group. This results revealed that the drug ARECP is not causing any toxicity to the selected organs, our results were concordant with the result of previous authors.[30]

CONCLUSION

Our results concluded that ARECP suppressed the development of NMU induced mammary carcinogenesis in female Sprague-Dawley rats. Further investigations will be required to isolate the specific constituents responsible for the anticancer activity and their safety and mechanism of action.

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