**In vivo anti-tumor efficacy of Croton oblongifolius in DMBA induced mammary tumor in rats**


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**ABSTRACT**

The present study was designed to evaluate anti-tumor activity of the methanolic extract of stem bark of *Croton oblongifolius* in Sprague Dawley rats. The tumor was induced in rats by DMBA given orally and intramammarily and challenging with plant extract. After obtaining suitable mass of tumor, the extract was gavaged to rats @ 200, 500 and 800 mg/kg which showed reduction in mammary tumor volume in dose-dependent manner, which was supported by histopathological observations of the treatment groups.

**Key words:** Anti-tumor activity, *Croton oblongifolius*, Histopathology.

**INTRODUCTION**

A mammary tumor is a tumor originating in the mammary gland which is a common finding in older female dogs and cats that are not spayed. In rats, most mammary tumors are benign fibroadenomas (Greenacre, 2004). Mammary tumors are the third most common neoplasia in cats (Viste *et al*., 2002). Mammary tumor also has been reported in rabbits, cows, horses and even in sea lions (Munson and Moresco, 2007). The International Agency for Research on Cancer has estimated the incidence of mortality and prevalence from major types of cancer at national level. For 184 countries of the world revealed that there were 14.1 million new cancer cases, 8.2 million cancer deaths, and 32.6 million people living with cancer (within 5 years of diagnosis) in 2012 worldwide. By 2030, it is projected that there will be 26 million new cancer cases and 17 million cancer deaths per year (Thun *et al*., 2009).

India is the largest producer of medicinal plants and is rightly called the “Botanical Garden of the World”. These medicinal plants are easily available, cheaper and possess lesser toxicity as compared to the modern drugs. Secondary metabolites are of special interest like polyphenols, terpenes and alkaloids have been reported to possess anti-mutagenic and anti-cancer properties. There are at least 2,50,000 species of plants out of which more than 1000 plants have been found to possess marked anticancer properties (Umadevi *et al*., 2013).

*C. oblongifolius* popularly known as Vumme Marada Gida in Kannada or Chukka in Hindi is widely distributed in India. It is used in gastric ulcer, the bark is used to treat dyspepsia and the roots to treat dysentery (Ngamrojnavanich *et al*., 2003). Barks and roots are purgative and chologogue and in remittent fever. It is externally applied to sprains, bruises and rheumatic swelling. The leaves are used as tonic, the flowers against flat worms, the fruits to treat dysmenorrhoea, the seeds as purgative (Sommit *et al*., 2003). It is applied externally to the hepatic region in chronic hepatitis (Ahmed *et al*., 2002).

Anazetti *et al.* (2003) reported that *C. oblongifolius* stem bark contained trans-dehydrocrotonin (DCTN) and crotonin (CTN) which induced apoptosis and cell differentiation.

A new furoclerodane, croblongifolin, isolated from the stem bark of *C. oblongifolius* showed a marked cytotoxicity against various human tumor cell lines including HEP-G2, SW620, CHAGO, KATO3 and BT474 (Roenqsumran *et al*., 2002).

However there was no *in vivo* study on methanolic extract of *C. oblongifolius* stem bark against chemically induced mammary tumor in rats. So the present study was focused on *in vivo* anticancer property of stem bark of *C. oblongifolius*.

**MATERIALS AND METHODS**

**Plant material:** Bark of *C. oblongifolius* was collected from Talaguppa, Sagara *Taluk* of Shivamogga District, Karnataka State. Bark were dried for a period of 40 days and kept in air tight container until preparation of extract.

Powdered stem bark of *C. oblongifolius* (100 g) was mixed in 1000 ml of methanol and kept for 5 days. The

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contents were periodically shaken using an orbital shaker. After 5 days, contents were filtered through Buchner’s funnel and it was further concentrated by Rotary Flash Evaporator at 39-40°C till the solvent got completely evaporated and extract settled down to bottom. The residual methanol from the extract was evaporated after keeping the extract in a vacuum oven at 60°C at the pressure of 25 psi.

**Animals:** Young Sprague Dawley rats (35 days) were used in the present study and were allowed to acclimatize for a period of 7 days prior to commencement of experiment. The rats were maintained in standard laboratory condition and were fed with *ad libitum* of commercial pellet ration and potable water. Prior permission was obtained from the Institutional Animal Ethics Committee.

**In vivo anti-tumor study:** At the age of 45 days, the treatment group animals, which weighed 120±10 g were administered by oral gavage with 1 ml of soya oil (Fortune®: Soya Refined oil) consisting of 6 mg of DMBA (7, 12-dimethylbenz (a) anthracene, Sigma, USA) thrice at weekly interval 4 days after administration of 60 µg of estradiol valerate (Progynon®, Cadila Healthcare Ltd. India) i.m followed by 5 mg of DMBA in soya oil as last dose intramammarially (Samy et al., 2006; Pattanayak and Sunita, 2008). Animals were palpated weekly for the presence of tumor carefully. Initiation of tumor started after 85 days post administration of DMBA. The tumor size (mm) was measured using a digital Vernier calliper (Aerospace®, China) before and after the experiment. The development of tumor was also confirmed by periodical histological examination of biopsy samples. The rats having suitable tumor were randomly divided into different groups.

**Grouping of animals and dosing:** After the rats obtained suitable tumor size it was divided into 6 groups. Group I served as negative control where it was not induced with tumor and was given normal feed and water. Group II served as DMBA control where the rats were induced with tumor but didn’t receive any treatment. Group III served as standard drug control where the tumor induced rats were treated with standard anti-cancer drug cyclophosphamide (Endoxan 50®, Zydus Cadila Healthcare, India) @ 10 mg/kg. Group IV served as low dose control where the tumor induced rats were treated with methanolic extract of *C. laevigatus* as the dose rate of 200 mg/kg. Group V served as medium dose control where the tumor induced rats were gavaged with methanolic extract of *C. oblongifolius* at the dose of 500 mg/kg. Group VI served as high dose control where the tumor induced rats were gavaged with methanolic extract of *C. oblongifolius* at the dose of 800 mg/kg. The standard drug and test drug were administered for a period of 30 days. Doses were selected based on earlier studies on *C. laevigatus* (Tomy, 2013). The methanolic extract of *C. oblongifolius* was dissolved in 0.4% CMC and solution was made to 1 ml and gavaged. The rats were humanely sacrificed after 30 days of administration and the organs were subjected to histopathology. All the observations were compared with Group II and Group III and were statistically analysed.

**Determining per cent decrease in the tumor size:** The size of tumor was measured using digital Vernier callipers 30 days post administration of the standard and test drug respectively and were statistically analysed.

\[
\text{Size of tumor (mm) in DMBA control} - \frac{\text{Size of tumor (mm) in test group}}{\text{Size of tumor (mm) in DMBA control}} \times 100
\]

**Histopathology:** Organs like liver, lungs, kidney, intestine, mammary tumor were collected in 10% neutral buffered formalin for histopathology with size of five microns thickness and were stained with haematoxylin and eosin (Luna, 1968).

**RESULTS AND DISCUSSION**

**Anti-tumor activity:** The percentage of tumor inhibition in the rats administered with cyclophosphamade was 70.07%, whereas methanolic extract of *C. oblongifolius* showed 64.94% in group VI, 62.12% in group V and 29.98% in group IV. There was significant decrease (P<0.05) in tumor volume when compared to DMBA control group (Table 1). This indicated the efficacy of crude plant extract of *C. oblongifolius*. This finding is in accordance with that of Tomy (2013) who reported antitumor efficacy of methanolic extract of *C. laevigatus* and observed that the tumor volume in the mice treated with 500 and 750 mg/kg on day 30 of inoculation decreased by 58.35 and 62.02% respectively. The tumour volume had decreased by 64.78% in cyclophosphamide treated group (10 mg/kg).

**Gross pathology:** On gross pathology, it was observed that the methanolic extract of *C. oblongifolius* had potent anti tumor activity. The results were obtained in percent decrease

<table>
<thead>
<tr>
<th>Group</th>
<th>Group details</th>
<th>Tumor volume (mm)</th>
<th>% decrease in tumor volume</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group I</td>
<td>Negative Control</td>
<td>0.00</td>
<td>-</td>
</tr>
<tr>
<td>Group II</td>
<td>DMBA control</td>
<td>41.39±0.58*</td>
<td>70.07</td>
</tr>
<tr>
<td>Group III</td>
<td>Cyclophosphamide @ 10 mg/kg</td>
<td>12.39±0.01</td>
<td>70.07</td>
</tr>
<tr>
<td>Group IV</td>
<td>Plant extract @ 200 mg/kg</td>
<td>28.98±0.28*</td>
<td>29.98</td>
</tr>
<tr>
<td>Group V</td>
<td>Plant extract @ 500 mg/kg</td>
<td>15.68±0.09*</td>
<td>62.12</td>
</tr>
<tr>
<td>Group VI</td>
<td>Plant extract @ 800 mg/kg</td>
<td>14.51±0.17*</td>
<td>64.94</td>
</tr>
</tbody>
</table>
and also by histopathology and were compared to DMBA control and the activities were in dose dependent manner (Table 1 and Fig 1). This finding was in accordance with the findings of Santhosh Kumar (2014) who had also reported the similar changes who evaluated in vivo efficacy of anticancer property of Astracanthus longifolia in rats. **Histopathology:** Anti-tumor activity was further confirmed by histopathology. The mammary gland of rats treated with DMBA alone showed tumour was microscopically characterized by proliferation of epithelial cells arranged in tubulopapillary pattern (Fig 2). This is in support with findings of Barros et al. (2004) who reported that the neoplasms were adenocarcinomas and papillary carcinoma, which consisted of proliferating epithelial cells.

Liver showed multifocal areas of necrosis with infiltration of mononuclear cells were observed. Also there were areas of cholestasis. The Kupffer cells were prominent and increased in number with brownish black coloured pigments. This is in accordance with the findings of Santhosh Kumar (2014) who had also reported the similar degenerative changes in liver of Sprague Dwaley rats treated with DMBA.

In Group III rats, there was induction of tubulopapillary adenocarcinoma and lipid rich adenocarcinoma. Treatment with cyclophosphamide induced loss of neoplastic cells with apparent degeneration and necrosis and a massive fibroplasias (Fig 3).

All the other organs were comparable with that of normal microscopically.

Group IV rats there was induction of tubule papillary adenocarcinoma. Treatment with *C. oblongifolius* at the dose of 200 mg/kg resulted in large multifocal areas of necrosis of neoplastic tissue and mild fibroplasias.

Group V rats there was induction of lipid rich adenocarcinoma. Treatment with *C. oblongifolius* at the dose of 500 mg/kg resulted in large multifocal area of liquefactive necrosis of the neoplastic tissue.

Liver showed congestion and mild degree of vacuolar degeneration. Except liver, all the other organs viz. intestine, kidney, lung, heart, brain, spleen and testis showed normal appearance.

Group VI rats revealed induction of tubule papillary adenocarcinoma. Supplementation of extract at the dose of 800 mg/kg resulted in diffuse areas of liquefactive necrosis of neoplastic tissue. Fibroplasia was minimum and the necrotic changes were maximum with extensive loss of tumor tissue in higher dose (Fig 4).

Liver displayed vacuolar degeneration with multifocal necrosis infiltrated with lymphocytes. Also it was observed to have very mild fibrotic change.

Except liver, all other organs viz. intestine, kidney, lung, heart, brain, spleen and testis showed normal appearance.
These histological changes were almost similar to the effect of standard mammary cancer drug cyclophosphamide which showed extensive necrosis of neoplastic tissue which is supported by Dhanya et al. (2011) who observed similar changes in cyclophosphamide treated group. The present study revealed the anti-tumor efficacy of the plant *C. oblongifolius* in rats which may be a tool to find the novel remedy to treat the cancer after isolating the active principle.

**REFERENCES**


