Anti cancer and Antioxidant Effect of *Acanthophora spicifera* against EAC induced carcinoma in mice

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**ABSTRACT**

*Acanthophora spicifera* is important seaweed in folklore remedies as well as food supplement in Gulf of Mannar, coastal region of Tamil Nadu. In the present study, the anticancer and anti-oxidant activities of the alcoholic extract of *A. spicifera* have been evaluated. The anti-tumour effect of the extract at the dose of 100 and 200 mg/kg oral route was carried out in mice treated with EAC cancerous cell lines. 5 Fluorouracil (5FU) at the dose of 20 mg/kg given intraperitoneally to EAC treated mice serving as standard drug control. After 21 days of test and standard drug administration, anti-cancer effect was assessed by measuring tumor volume, tumor weight, mean survival day (MSD) and various haematological parameters. In addition, anti-oxidant status of the liver tissue was assessed. The ethanol extract of *A. spicifera* exhibits significant anti-cancer effect by significantly reducing tumor volume and weight with mean survival day (MSD) in EAC cell lines treated cancerous mice. The extract also showed anti-oxidant effect by significantly increasing endogenous anti-oxidant and decreasing oxidative stress marker in liver homogenates. The results of these study indicated that ethanol extract of *A. spicifera* possess anti-tumour and anti-oxidant activity and the beneficial effect may be due to the presence of bioactive components like flavonoids, terpenoids and tannins.

**Key words:** Migrain, Antiserotonergic activity, Abrus precatorius, sumatriptan.

**INTRODUCTION**

Free radical and reactive oxygen species (ROS) are by products produced in body due to various physiological and biochemical processes. Generally, most of these free radicals generated from cellular metabolism are scavenged by endogenous defence system such as superoxide dismutase, Catalase and Peroxidase–glutathione system (Govindarajan et al., 1992). However, in many cases, such as in unhealthy physical condition, ageing, or under stress environments, the endogenous antioxidants are either exhausted or insufficient to scavenge these free radicals. Imbalance of these free radicals can cause oxidative damage to biomolecules like lipids, proteins and DNA (Halliwell, 1994; Niki, 1997). These reactive oxygen species (ROS) has been implicated in oxidative stress, cell death and cell transformation. ROS have been shown to be involved in many diseases such as cancer, ageing, heart disease, and neurodegenerative disorders (Harman, 1994; Simonian and Coyle, 1996). Among this, Cancer is a complex disease characterized by proliferation (uncontrolled cell division), cell transformation, and cap of apoptosis, invasion, angiogenesis and metastasis. Cancerous cells are to produce reactive oxygen species (ROS) and their in/onflammatory mediators. ROS may cause DNA mutation, which may be followed by oncogenes activation and down regulation of tumour suppressor genes (Ames, 1983; Loft and Poulsen, 1996). By the activity of ROS, scavenging system gets altered in tumour cells (Trachootham et al., 2009). Over the past decades, seaweeds or their extracts have been shown to produce a variety of compounds and some of them have been reported to possess biological activity of potential medicinal values (Heo et al., 2005). The identification and exploitation of potent anticancer molecules from the marine environment such as marine algae has generated great interest in recent years. Extensive screening of marine microalgae has led to the isolation and chemical determination of over 15,000 compounds, including fatty acids, sterols, phenolic compounds, terpenes, enzymes, polysaccharides, alkaloids, flavonoids (Mohamed et al., 2012). More recent reports revealed that marine algae possess rich sources of antioxidant compounds with potential free radical scavenging activity as in *Halimeda tuna* (Ananthi et al., 2008) and *Acanthophora spicifera* (Vasanthi et al., 2006). In this study *Acanthophora spicifera* (Vahl) Borgesen (Ceramiales: Rhodophyta) are commonly known as spiny seaweed, is widely distributed throughout the tropic and subtropics throughout the Gulf of Mannar, Rameswaram. (Umanaheswara Rao, 1970). To the date, research on biologically active substances from this species is rather limited or not been clearly established (Ahira et al, 1968; Prakash et al, 1989; Wahidulla et al, 1986, Wang et al, 1998). Therefore the aim of the present study was to evaluate anticancer and antioxidant activity of *Acanthophora spicifera*, a red algae in suitable experimental conditions.

**MATERIALS AND METHODS**

**Marine algae collection**

The red algae *Acanthophora spicifera* (Family: Rhodomelaceae, Ceramiales) was collected from Mandapam, during the month of March 2008 from Rameshwaram coast, Tamil Nadu, India. It is identified and authenticated by Dr. Krishnamurthy, Institute of algology, Anna nagar, Chennai. The voucher specimen (VCP/09-345) was deposited in the Department of Pharmcognosy, Vels college of Pharmacy, Chennai 117.

**Extraction of Acanthophora spicifera**

Dried, pulverized *A. spicifera* (1 kg) was extracted with 5 liters of ethanol using soxhlet apparatus for 24 hrs. The extract was filtered, and the filtrate was evaporated by rotary vacuum evaporator and sample was freeze dried for further use. The percentage yield of ethanol extract was found to be 20.22% w/w. The ethanolic extract was subjected to qualitative chemical test and thin layer chromatography studies (Kokate, 1994; Harborne, 1998).

**Chemicals and drugs**

The chemicals used were 5-Fluorouracil (Ranbaxy Laboratories, Ltd., India),...
Coboxy methyl cellulose (CMC) (Ranbaxy Laboratories, Ltd., India), Adrena-
line bi-tartrate (Scisco chemicals, Mumbai), Thiobarbituric acid (Scisco chemi-
cals, Mumbai), Elman reagent (SRL Chemicals, Mumbai) and all other chemi-
cals and reagents used were purely of analytical grade.

Animals
Swiss male albino mice weighing around 20-22g were used for present inves-
tigation. Animals were obtained from the central animal house, Vels College of
Pharmacy, Chennai. Mice were grouped and housed in poly acrylic cages
(n=6) and maintained in standard laboratory conditions under the tempera-
ture 25 ± 2°C dark/light cycle. They were allowed free access to standard
dry pelit diet (Hindustan Lever, Kolkata, India) and water ad libitum. The
mice were acclimatized into a laboratory conditions for 7 days before the
experiment. All procedures described were reviewed and approved by the
Institutional Animal Ethical Committee.

Acute Toxicity Study
Toxicity study - up and down procedure was carried out as per the guidelines
set by Organization for Economic Co-operation and Development (OECD).
Oral toxicity study was done according to OECD guidelines 423. In this
experiment two groups of wistar rats (n=3) were used. Animals were fasted
over night with water ad libitum and foods were withheld for 3-4 hrs after
oral administration of the extracts. One group of animals were treated with
starting dose of the Acanthophora spicifera extract of 2000mg/kg b.wt orally.
Another group of rats were treated with normal saline. Observation includes
mortality and clinical signs, which includes changes in skin, fur, eyes and
mucous membranes. The gross behaviors like body positions, locomotion,
rearing, tremors, gait was observed. The effect of Acanthophora spicifera on
passivity, grip strength, pain response, stereotypy, vocalization, righting
reflex, body weight and water intake was observed (Lipnic et al., 1995).

Transplantation of tumour
Ehrlich ascites carcinoma (EAC) cells were obtained through the courtesy of
Amala Cancer Research Centre, Thrissur, Kerala. The EAC cells were main-
tained in vivo in Swiss albino mice by intraperitoneal inoculation of 2 × 10^6
cells per mouse. From the peritoneal cavity of the mice, the EAC cells were
aspirated, washed with saline and were given intraperitoneally to develop
ascitic tumor (Rajkapoor et al, 2004).

Animal grouping and Drug treatment
Animals were divided into five groups (n=6)
Group-I was served as normal control treated with saline control (5 ml/kg
i.p.)
Group-II was served as EAC treated control group
Group-III was served as EAC tumour control administered with 5 FU (20
mg/kg, i.p.)
Group-IV was served as EAC treated tumour control treated with alcoholic
extract of Acanthophora spicifera (100 mg/kg/p.o)
Group-V was served as EAC treated tumour control treated with alcoholic
extract of Acanthophora spicifera (200 mg/kg/p.o)
The standard 5-FU and the test drug Acanthophora spicifera extract were
administered after 72 hrs of tumour inoculation. All the drugs were adminis-
tered for 21 days continuously and the following parameters were evaluated
periodically.

Tumor volume, tumour weight and Mean survival Day
The ascetic fluid was collected from the peritoneal cavity. The volume was
measured by taking it in a graduated centrifuge tube. The tumor weight was
measured by taking the weight of the mice before and after the collection of
the ascetic fluid (Balà et al., 2010).

The Mean survival day were calculated by animals survived from the date of
tumor inoculation to the date at which the animal death occurs. Mean
survival day is calculated by using the formula given below.

No. of days animals survived in test group

Mean survival day = ——— × 100
No. of days animals survived in control group

Measurement of Antioxidant / Oxidative stress markers
Animals (n=5) were sacrificed from each group. Blood samples were col-
clected transcordially for the following haematological and biochemical esti-
mations.

Biochemical parameters
After the collection of blood samples, the mice were sacrificed. Liver was
excised, rinsed in ice cold normal saline followed by ice-cold 10% KCl solu-
tion, blotted, dried and weighed. A 10% w/v liver homogenate was prepared
in ice-cold KCl solution and centrifuged at 1500rpm for 15 min at 4°C. The
supernatant thus obtained were used for the estimation of thiobarbituric
acid reactive substances (TBARS) (Okhawa et al., 1979) glutathione (GSH)
(Jollow et al, 2009), superoxide dismutase (SOD) (Magwere et al., 1997)
catalase (CAT) (Clairborne,1989), glutathione peroxidase (GPx) and glu-
thione-S-transferase (GST) (Rajkumar et al.,2010 ) and total protein (TP)
(Lowry et al., 1951)

Haematological parameters
At the end of the experimental period, next day after an overnight fasting,
blood was collected from freely flowing tail vein and used for the estimation of
haemoglobin (Hb) content, red blood cell (RBC) count, white blood cell
(WBC) count, Protein content, Packed cell volume (PVC) and differential
count of WBC was measured using standard procedures.

Statistical analysis
All the data were expressed as Mean ± SEM. All the groups were compared
by one way (ANOVA) followed by Dunnett’s post hoc test. Probability of
P< 0.05 was considered as significant.

RESULTS AND DISCUSSION
The preliminary phytochemical analysis of Acanthophora spicifera showed
that the presence of flavonoids, tannins, terpenoids and glycosides.

Effect of Acanthophora spicifera on Tumour weight, Tumour volume
and Mean survival day
Fig: 1 represents the effect of the acanthophora spicifera on tumour weight in
EAC in mice. Inoculation of EAC cell lines into mice, significantly (P <0.001)
increased the body weight of the mice as measured the indices of the tumour
mice. Oral administration of ethanolic extract of Acanthophora spicifera (100
and 200 mg/kg bd wt) significantly (P<0.001) decreased the tumour weight in
EAC treated carcinoma mice than the saline treated control tumour mice. In
addition 5-FU also significantly (P<0.001) decrease the tumour weight in
EAC inoculated mice as compared with saline treated EAC bearing tumour
mice.

Fig: 2 represent the effect of the ethanolic extract of Acanthophora spicifera
on tumour volume in EAC treated cancerous mice. Increase in tumour vol-
ume was noted in saline treated EAC mice during the 3 weeks of study
period. The tumour volume decreases significantly (P<0.01) in EAC mice
-treated with Acanthophora spicifera (100 and 200 mg/kg bd wt). The effect
was found to be dose dependent. In addition there was a significant (P<
0.001) decrease in tumour volume in 5-FU treated tumour mice than saline
-treated tumour mice.

![Graph](image)

*** represents the $P<0.001$, 5FU Vs tumour control  
** represents $P<0.01$, ASE (100 & 200 mg/kg) Vs Tumour control

**Fig: 1 Effect of Acanthophora spicifera on tumour weight in EAC treated mice**

![Graph](image)

*** $P<0.001$ represents Tumour control Vs normal control  
** $P<0.01$ represents ASE 200 mg/kg Vs tumour control

**Fig: 2. The effect of the Acanthophora spicifera on tumour volume in EAC treated cancerous mice**

**Fig: 3. Mean survival day of the tumour bearing mice treated with 5 FU and ASE (100 & 200 mg/kg)**

**Effect of Acanthophora spicifera on haemoglobin**

Mice treated with EAC showed significant decrease in ($P<0.001$) haemoglobin (Hb) content as compared with saline treated control normal mice. Interestingly the tumor bearing mice administered with high dose of ethanolic extract of Acanthophora spicifera (200mg/kg) significantly ($p<0.01$) increases the haemoglobin content as compared to that of tumour control group animals.

**Effect of extracts on WBC:**

Induction of tumor in mice had significantly ($P<0.001$) increase the WBC count as compared to that of vehicle treated control normal animal. Oral administration of Acanthophora spicifera extracts to EAC bearing mice showed significant reduction ($P<0.001$) in WBC levels as compared to that of vehicle treated control tumor mice. The effect was dose dependant and the high dose of Acanthophora spicifera (200 mg/kg/bd wt) almost reverses the WBC count towards normal.

**Effect of Acanthophora spicifera extract on packed cell volume:**

There was a significant increase in PCV cells in EAC treated cancer group mice as compared to that of saline treated control group. It was interesting to observe that administration of Acanthophora spicifera ethanolic extract at the dose of 100 and 200mg /kg bd wt orally significantly ($P<0.001$) decreases PCV levels towards the normal in treated tumor bearing mice.

**Fig: 4 represents the blood haematological parameters (lymphocytes, neutrophils and monocytes) of control and EAC treated tumor mice treated with Acanthophora spicifera extract. Significant ($P<0.001$) increase in lymphocytes and decreases in neutrophils was observed in EAC challenged tumor bearing animal group compared to that of saline treated control group. Administration of Acanthophora spicifera significantly ($P<0.001$) increases the lymphocytes percentage as compared to that of EAC treated tumor control mice whereas the high dose level of 200mg/kg orally of Acanthophora spicifera extract exhibited significant decrease ($P<0.01$) in the neutrophil percentage of tumor bearing mice group. However no effect was observed in monocytes percentage of vehicle treated and EAC treated cancer group.**
### Table 1: Blood Haematological Parameters

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Control</th>
<th>Tumour control</th>
<th>ASE 100 mg/kg</th>
<th>ASE 200 mg/kg</th>
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<tr>
<td>Hb (g%)</td>
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<td>RBC (mill cells/cumm)</td>
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<td>WBC (mill cells/cumm)</td>
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<td>PCV (mm)</td>
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<td>Protien (mg%)</td>
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***P<0.001 represents Tumour control Vs normal control  
**P<0.01 represents ASE 200 mg/kg Vs Tumour control

### Figure 4: The effect of Acanthophora spicifera on various blood haematological parameters treated with mice bearing with EAC cell lines

***P<0.001 represents Tumour control Vs normal control  
**P<0.01 represents ASE 200 mg/kg Vs Tumour control

### Discussion

The present study highlights the in vivo antitumor effects of crude extract of Acanthophora spicifera in EAC treated mouse model. In this present investigation, the oral administration of Acanthophora spicifera extract given for 21 days has significantly decreased the tumor volume and tumor weight following the EAC inoculation in mice. The assessment of tumour volume and tumor weight was the direct parameter for assessment of tumour growth as well as anti tumour efficacy of the test drugs. The results from Mean survival day suggested that EAC treated mice shows decrease in MSD than the 5FU and Acanthophora spicifera treated EAC mice. The decreased tumour volume and tumour weight, with increased MSD in EAC bearing mice treated with Acanthophora spicifera 100 and 200 mg /kg body weight suggested the anti tumour properties of the extracts. It has been shown earlier that the decrease in life span of the tumour control was directly proportional to the propagation to the tumor growth. It has been reported by many investigators that the life span expectancy or mean survival day get increased with the decrease in tumour size (Sreelatha et al., 2011). These results clearly suggested that direct antitumor efficacy of Acanthophora spicifera in in-vivo model. It was further observed that beneficial impact of EAC inoculation on haematological parameters of the EAC treated mice in which there was decrease in RBC, increase in WBC with decrease in haemoglobin levels in tumor bearing mice. The reversal and restoration of haematological parameters like RBC, WBC and haemoglobin (Hb) levels in EAC treated mice with Acanthophora spicifera suggested that it possess the protective effect against haematopoietic system. It was interesting to observe that restoration of lymphocytes and neutrophils towards its normal suggest the influence of Acanthophora spicifera on granulocytes and agranulocytes. The involvement of Reactive oxygen species has been postulated in various degenerative disease and cancer as well (Feugang et al., 2010).

The reactive oxygen species like Superoxide (SOD), catalase (CAT), Glutathione (GSH), GPx and melonaldyaldehyde (MDA) was considered as
In our study decrease in endogenous antioxidant such as SOD,CAT with increase in lipid peroxidase products like TBRs, GST and GPx was noted in liver homogenate. This clearly indicates that the impaired endogenous antioxidant system with increased lipid peroxidase system suggest mice treated with EAC under oxidative stress. Preliminary phytochemical investigation suggests that it has flavonoids, terpenoids, tannins and glycosides. However the antitumor and antioxidant role of individual phytoconstituents yet to be explored in detail.

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REFERENCES

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