

## In Vivo Evaluation of Antigenotoxic and Anti-Inflammatory Potential of *Turbinaria conoides* (J. Agardh) Kuetz.

Arumugam P<sup>1\*</sup>, Murugan M<sup>2</sup>, Ramar M<sup>1</sup> and Murugan K<sup>1</sup>

<sup>1</sup>Department of Zoology, School of Life Science, Bharathiar University, Coimbatore, Tamil Nadu, India

<sup>2</sup>Department of Microbial Technology, School of Biological Science, Madurai Kamaraj University, Madurai, Tamil Nadu, India

\*Corresponding author: Ponnann Arumugam, Department of Zoology, School of Life Science, Bharathiar University, Coimbatore-641 046, Tamil Nadu, India, Tel: +914222428491; Fax: +914222422387; E-mail: [ponnannarumugam@gmail.com](mailto:ponnannarumugam@gmail.com)

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### Abstract

Genetic alteration occurred in human being due to the exposure various hazardous substances which are leads to array of physiological, biochemical and pathological changes such as enzyme activation, release of mediators, tissue breakdown and inflammation. *Turbinaria conoides*, marine brown algae commonly found in the Gulf of Mannar, Southeast coast of Tamil Nadu, India was evaluated for its antigenotoxic and anti-inflammatory potential in mice and rat model. Known genotoxin, 4-NQO was used to induce micronuclei in mice bone marrow cells and estimated by micronuclei assay. Acute inflammatory study was carried out with carrageenan induced paw edema in rats, acetic acid induced writhing and tail immersion methods in mice. 4-NQO enhanced the frequency of MnPCEs was about 4.1-fold over the control value,  $16.0 \pm 0.71$  MnPCEs/2500 PCEs. Ethyl acetate fraction of *T. conoides* along with 4-NQO treated group significantly reduced the frequency of MnPCEs ranging from about 52 to 18%. The reduction of MnPCEs by *T. conoides* was greatest at 450 mg/kg bw (about 72%) and lowest at 75 mg/kg bw (about 20%). The increased paw edema in rat was measured at time interval of 1, 2, 4 and 8 hours. In both indomethacin and ethyl acetate fraction of *T. conoides* were observed to be decrease the paw volume significantly based on the dose along with time interval. Hence, writhes and tail immersion methods were also found to be dose dependent and their effective dose (450 mg/kg) observed to be comparable to aspirin standard.

**Keywords:** *Turbinaria conoides*; Ethyl acetate fraction; 4-NQO; Antigenotoxicity; Anti-inflammatory

### Introduction

Human populations are under threat due to the exposure of various endogenous and environmental agents like pesticides, metals, polycyclic aromatic hydrocarbons (PAHs), solvents, and alkylating agents along with therapeutic compounds including antitumor and antibiotics [1]. As a result, the cellular macromolecules get damaged including DNA and leads to genetic alteration, endothelial dysfunction, gastrointestinal dysfunction, tissue injury and inflammation. Inflammation is a main precursor for the following diseases such as rheumatoid arthritis, atherosclerosis, inflammatory bowel diseases, pleuritis and nephritis. There are conventional drugs used to ameliorate those diseases. However, it is too expensive, offer only temporary relief and often elicits undesirable side effects. Therefore, inhibition of genotoxicity and their subsequent inflammatory events might be remedy for treating those diseases through marine resources [2]. Many drugs from marine source have potential to eradicate the hazardous substance induced oxidative stress by scavenging the free radicals which are known to possess antioxidant, antigenotoxic, anti-inflammatory and antinociceptive properties [3].

*Turbinaria conoides* (J. Agardh) Kuetz. brown algae coming under the order of Fucales and belongs to the family Sargassaceae. Traditionally, it is being used as a remedy for the children's fever, as a fertilizer, insect repellent, pesticide and bactericidal [4]. *T. conoides* possessed important phytochemicals such as fucosterol, sulfated polysaccharides fucoidan, neutral glucan, guluronic and alginic acid [5]. Apart, it contained digestible proteins along with mineral salts (K, Ca, and Fe) and polyunsaturated fatty acids with wealthy source of dietary fiber. Rich source of iodine shows play an immense role in enhancing the food quality and biochemical homeostasis [6,7]. *T. conoides* and *T. ornata* collected from Gulf of Mannar of Southeastern coast of India were also reported to have the antioxidant activity due to the presence of total phenolic content in ethyl acetate fraction [8]. Sodium alginate was one of the bioactive compounds present in brown

seaweeds which exhibit various biological effects like removal of heavy metal, antitumor and anti-inflammatory property [9]. Most marine plants having biologically active constituents are resided mainly in the polar fraction. Moreover, brown alga being used as a traditional medicine which is essentially required a scientific validation through experimentally and clinically to find out their efficacy. Thus, the present study was undertaken to determine the antigenotoxic and anti-inflammatory activities of ethyl acetate fraction of ethanol extract of *T. conoides* in animal model.

### Materials and Methods

#### Chemicals

Indomethacin, Giemsa stain, May-Grunwald stain, Aspirin and 4-nitroquinoline-1-oxide (4-NQO) were obtained from Sigma-Aldrich, USA. Phosphate buffered saline, acetic acid and DMSO were obtained from Hi-media, Mumbai, India. All solvents used in this study were of analytical grade.

#### Seaweed collection and preparation of solvent fractions

The brown seaweed of *T. conoides* was collected from intertidal zone of Mandapam, Gulf of Mannar, South-east coast of India. The algae samples (1 kg) were washed with fresh tap water and followed by distilled water to remove salt and other debris along with necrotic parts. Samples were made into small pieces, shade dried and then powdered using a mixer grinder. The powdered sample was stored in a polyethylene bag at room temperature. The shade dried powder 250 g was immersed in a 2 L of two conical flasks with each 1.25 L of 95% ethanol and left for 24 h under constant stirring. After the 24 h, transfer from conical flask to Soxhlet apparatus and extracts was collected in bottom flask at 40°C. This was repeated twice with 2.5 L of 95% ethanol. Final volume of 4.5 ml ethanol extract was brought out into 25 g (10%) with rotary evaporator [10]. The ethanol extract was transfer into a separating funnel and partitioned between distilled water and hexane with 1:6 ratio. This mixture was thoroughly mixed for 15 minutes and the hexane fraction (HF) was collected 1.4 L after 1 h incubation. Similarly, aqueous layer 250 ml was further fractionated

with dichloromethane (DMF) and ethyl acetate (EAF). All the fractions were concentrated in rotary evaporator. The yield of the fractions was 20%, 16%, and 52% respectively. The aqueous fraction (AF) was lyophilized and obtained 12% of yield. Among fraction of *T. conoides*, EAF of was used *in-vivo* experiment based on their *in-vitro* antioxidant potential.

### Experimental animals

Both sexes of *Swiss albino* mice (20-25 g) and *Wistar albino* rat (120-180 g) were obtained from King Institute, Chennai, India. The animals were housed in standard environmental conditions (12 h light/12 h dark; 22 ± 2°C) for one week prior to the experiments to acclimatize to the laboratory conditions. They were allowed free access to tap water and pelleted rodent diet. The animal care and experimental protocols were accordance with the Guidelines of the Committee for Control and Supervision of Experiments on Animals (CPCSEA), India.

### Experimental design for antigenotoxic potential of *T. conoides*

Either sex of *Swiss albino* mice was divided into six groups with five mice each. The concentrated ethyl acetate fraction (EAF) was dissolved in 15% DMSO and administered by oral gavage to the mice for 5 consecutive days. Control group 1 fed with 15% DMSO by orally. Group 2 received 450 mg/kg bw of EAF. Group 3 received with 7.5 mg/kg bw of 4-NQO by intraperitoneally. Groups 4, 5 and 6 received respective test doses 75, 150, and 450 mg/kg bw of EAF for five consecutive days plus 4-NQO (7.5 mg/kg bw; i.p.) on sixth day. After 24 hours of 4-NQO treatment, all the mice were sacrificed by cervical dislocation. The bone marrow cells were flushed out into phosphate buffered saline (PBS) and prepared for the micronucleus assay based on the protocol reported in our previous publication [11]. For the micronucleus assay, each animal (experimental/control), 2,500 polychromatic erythrocytes (with or without micronuclei) and a corresponding number of normal chromatic erythrocytes (NCEs) were scored under a light microscope.

### Carrageenan induced paw edema in rats

*Wistar albino* rats were divided into five groups with each 5 rats. Control (15% DMSO), three test doses of EAF (75, 150, and 450 mg/kg) and indomethacin as a positive control (10 mg/kg in distilled water) were administrated orally. After 1 hour, 0.1 mL of 1% solution of freshly prepared carrageenan in normal saline was injected in the sub plantar surface of the right hind paw of the rats. The paw volume was measured (as cm) before and after injection of carrageenan at the time periods of 1, 2, 4 and 8 hours by mercury displacement Plethysmograph. The degree of acute inflammation was expressed as centimeter with % inhibition of paw volume by standard and EAF of *T. conoides* over the control value [12].

### Acetic acid induced writhing and tail immersion method in mice

The writhing and tail immersion method was carried in mice per the method of Ferreira et al. [13], Kumar and Shankar [14] respectively. *Swiss albino* mice were divided into five groups with each five mice. Control group (15% DMSO), three test doses (75, 150, and 450 mg/kg of EAF) and Aspirin (100 mg/kg) as a positive control were administrated orally. All the test doses and standard drug were fed one hour before of chemical stimulus. The writhes were induced by i.p. injection of 1% acetic acid (v/v, 10 mL/kg) in all the groups except control. After ten minutes, the number of writhe was recorded over a period of ten minutes. A writhe is indicated by abdominal constriction and full extension of hind limb. The tail flick response was measured after administration of test and standard drugs at the period of 60,

120 and 180 minutes. Tail flick latency difference or mean increase in latency after drug administration was used to indicate the analgesia produced by test and standard drug.

### Statistical analysis

Results were presented as mean ± standard deviation for five animals of each group. Statistical analyses were performed by one-way ANOVA using SPSS Software Version 16.0. The Student-Neuman-Keuls (SNK) test was applied to assess for differences among the groups. Values of P ≤ 0.05 were considered to be significant.

### Results

The antigenotoxic potential of ethyl acetate fraction of *T. conoides* against 4-NQO induced micronucleated polychromatic erythrocytes (MnPCes) in mice bone marrow cells were evaluated and presented in Figure 1. The well-known genotoxin, 4-NQO enhanced the frequency of MnPCes was about 4.1-fold over the control value, 16.0 ± 0.71 MnPCes/2500 PCEs. Ethyl acetate fraction alone treated group showed no changes in the frequency of MnPCes (17.0 ± 0.10, P>0.05). However, pretreatment of ethyl acetate fraction of *T. conoides* along with 4-NQO was observed to be significantly reduced the frequency of MnPCes ranging from about 52 to 18%. The reduction was greatest at 450 mg/kg bw (about 72%) and lowest at 75 mg/kg bw (about 20%). Hence, dose 150 mg/kg bw was also effectively reduced the frequency of the MnPCes (about 57%) induced by 4-NQO.

Anti-inflammatory potential of ethyl acetate fraction of *T. conoides* was evaluated against carrageenan induced paw edema in *Wistar albino* rats. The increased paw edema in rat was measured at time interval of 1, 2, 4 and 8 hours (Table 1). Paw edema in the control group was observed to be enhanced about 0.74 ± 0.10 to 0.44 ± 0.05 at time interval of 1-8 hrs. In both indomethacin and ethyl acetate fraction of *T. conoides* were observed to be decrease the paw edema significantly based on the dose along with time interval. The reduction rate of paw edema by indomethacin was about 49-64% with respect to the control group. However, ethyl acetate fraction of *T. conoides* was also effectively reducing the carrageenan induced paw edema. At the dose of 150 mg/kg bw, the reduction of the paw edema was observed

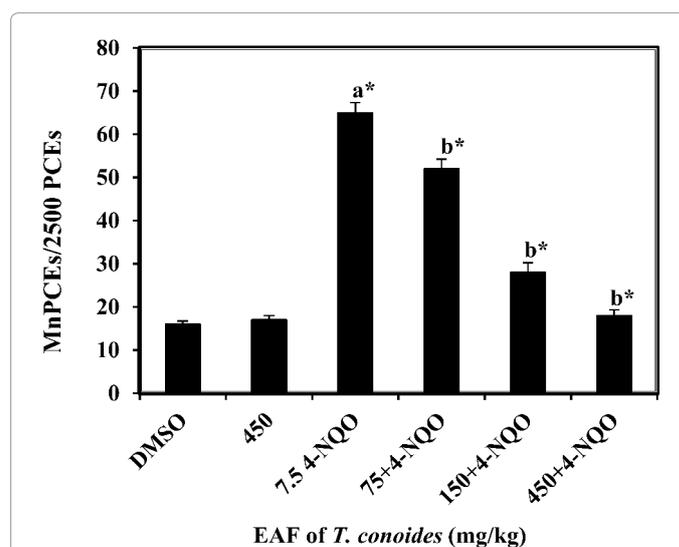
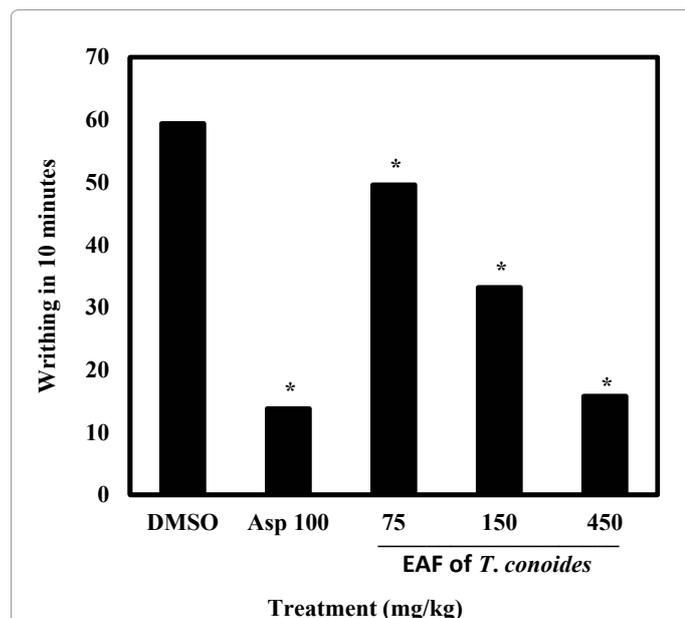


Figure 1: Antigenotoxic potential of ethyl acetate fraction of *T. conoides* on 4-NQO induced micronuclei in mice bone marrow cells (C: control; EAF: ethyl acetate fraction; \*P<0.05; Comparisons were made between control and drug treated groups).

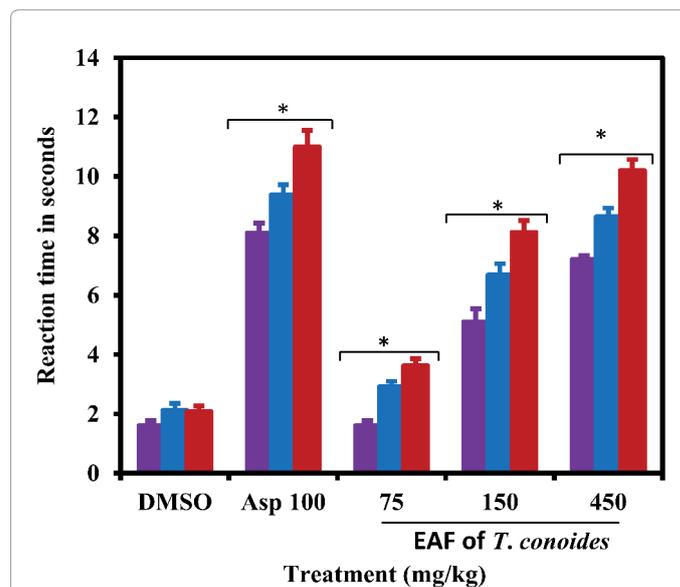
to be 32-36% at the time intervals of 1-8 hrs. The dose 75 mg/kg bw treated group showed the least reduction of paw edema about 15%. The significant reduction of paw edema by ethyl acetate fraction of *T. conoides* was found to be as equal as standard anti-inflammatory drug, indomethacin.

Anti-nociceptive of ethyl acetate fraction of *T. conoides* was performed in mice by following chemical and thermal methods. In chemical method, acetic acid induced abdominal writhes showed about  $59.40 \pm 1.86$  in the control group over the period of 10 minutes (Figure 2). In treated groups, the number of abdominal writhes were significantly ( $P < 0.05$ ) inhibited by standard drug, aspirin ( $13.80 \pm 2.25$ ) as well as ethyl acetate fraction of *T. conoides* ( $15.83 \pm 0.86$ ). Writhes inhibited by aspirin was about 77% over the control where as in ethyl acetate fraction of *T. conoides* was about 73.4% observed at the maximum dose 450 mg/kg bw. At the dose of 150 mg/kg bw, the writhes inhibition was about 44% over the control. In contrast, the writhes inhibition was found to be lower at the dose of 75 mg/kg bw ( $P < 0.05$ ) which has shown about 17% over the control. The potential of ethyl acetate fraction of *T. conoides* on writhes was found to be significantly dose dependent.

The analgesic potential of ethyl acetate fraction of *T. conoides* was evaluated using tail immersion method in mice. The tail flick response was measured after administration of test and standard drugs at the time period of 1, 2 and 3 hrs. The pain response in the control group showed decrease reaction time  $2.08 \pm 0.18$ - $1.60 \pm 0.17$  at time period of 1-3 hrs (Figure 3). Standard drug, aspirin significantly enhanced the reaction time in the range of  $8.10 \pm 0.33$ - $11.00 \pm 0.18$  by reducing the pain response. The reduction of pain response seems to be better than control and ethyl acetate fraction treated groups. Among the ethyl acetate fraction tested dose, the highest enhanced reaction time was observed at 450 mg/kg bw ( $10.20 \pm 0.37$  at 3 h) and least at the dose 75 mg/kg bw ( $3.62 \pm 0.24$  at 3 h). At the dose of 150 mg/kg bw, the enhanced reaction time by reducing pain response observed to be  $5.12 \pm 0.45$ - $8.12 \pm 0.39$  which was better than the control ( $P < 0.05$ ). Ethyl acetate



**Figure 2:** Anti-analgesic potential of ethyl acetate fraction of *T. conoides* on acetic acid induced writhing in mice (C: control; Asp: aspirin; \* $P < 0.05$ ; Comparisons were made between control and drug treated groups).



**Figure 3:** Anti-analgesic potential of ethyl acetate fraction of *T. conoides* on tail flick latency method in mice (C: control; Asp: aspirin; \* $P < 0.05$ ; Comparisons were made between control and drug treated groups).

fraction of *T. conoides* was significantly ( $P < 0.05$ ) reduce the pain response by the increase of reaction time with dose dependent manner.

## Discussion

The antigenotoxicity of ethyl acetate fraction of *T. conoides* was evaluated against 4-NQO induced chromosome damage in mice bone marrow cells. Genotoxicity of 4-NQO occurred through its metabolite 4-hydroxyaminoquinoline 1-oxide (Ac-4-HAQO) which is readily interacts with DNA and forming adducts at the N2, C8 and of N6 position [15]. As consequence, the helical structure of the DNA changed and turns to micronuclei/chromosomal breakage [16]. In the present study, 4-NQO enhanced the frequency of MnPCEs by about 4.1 fold over the control group (16.0 MnPCEs/2500 PCEs; Figure 1). Pretreatment with ethyl acetate fraction of *T. conoides* effectively decreased the 4-NQO enhanced MnPCE frequency about 20-72% depending on dose tested (Figure 1). Moreover, the reduction of MnPCE by the highest dose of ethyl acetate fraction of *T. conoides* was well corresponded with their control value. The maximum reduction observed about 72% at dose 450 mg/kg bw which was 3.6-fold better than that of their lower dose, 75 mg/kg bw. In addition, at dose 150 mg/kg bw also showed 2.9 fold of activity over the dose at 75 mg/kg bw. The reduction of 4-NQO enhanced MnPCE frequency was significant ( $P < 0.05$ ) and dose dependent.

Under the damaged cell/tissue condition, several inflammatory mediators such as histamine, bradykinin, serotonin, and prostaglandins are released to stimulate the inflammation and nociceptors [16]. These mediators are occupied in tissues with high content of water and plasma during arachidonic acid metabolism via cyclo-oxygenase and lipo-oxygenase enzyme pathways [17]. The first phase of inflammation begins immediately up to an hour after injection of carrageenan by the release of histamine and serotonin whereas the second phase started after one hour and up to three hours by the release of bradykinin, protease and prostaglandins [18]. Anti-inflammatory effect of indomethacin and ethyl acetate fraction of *T. conoides* were showed significantly ( $P < 0.05$ ) better than that of control (Table 1). The highest dose of ethyl acetate fraction reduced the carrageenan induced paw volume about 59% which was more comparable to the

Treatment groups	Oral dose (mg/kg bw)	Paw edema at different time intervals (h)			
		1	2	4	8
Control	DMSO	0.74 ± 0.10	0.66 ± 0.10	0.54 ± 0.08	0.44 ± 0.05
IND	20	0.38 ± 0.08 (49%)	0.30 ± 0.09 (55%)	0.26 ± 0.08 (52%)	0.16 ± 0.05 (64%)
Ethyl acetate fraction of <i>T. conoides</i>	75	0.62 ± 0.07 (16%)	0.56 ± 0.10 (15%)	0.44 ± 0.05 (19%)	0.40 ± 0.06 (9%)
	150	0.50 ± 0.09 (32%)	0.40 ± 0.09 (39%)	0.32 ± 0.07 (41%)	0.28 ± 0.10 (36%)
	450	0.40 ± 0.06 (46%)	0.32 ± 0.08 (52%)	0.28 ± 0.07 (48%)	0.18 ± 0.08 (59%)

Values expressed in centimeters (mean ± standard deviation). Percentages indicated to the change in edema size with control group. Comparisons were made between control and drug treated groups, \*P<0.05.

**Table 1:** Anti-inflammatory potential of ethyl acetate fraction of *T. conoides* on carrageenan induced paw edema in rat.

standard drug (64%). The reduction of paw volume was found to be dose dependent. The acetic acid induced abdominal writhes in mice were significantly recovered from all the tested doses of *T. conoides* (Figure 2). Indomethacin and ethyl acetate fraction of *T. conoides* might be reduced inflammation through stabilizing the lysosomal membrane. Recent report on *Turbinaria ornate* extract revealed their better anti-inflammatory and free radical scavenging property due to fucoidan like sulfated polysaccharides [19]. The reduction of writhes by the highest dose of ethyl acetate fraction was 3.8 fold over the control and more comparable to the standard drug (13.80 ± 2.25). In addition, the analgesic effect of *T. conoides* on the tail immersion-test in mice was found to be dose dependent and significantly reduced the pain response by the increase of reaction time (Figure 3). The highest dose of *T. conoides* and standard drug were showed 5-4 fold pain reduction over the time periods when compared to the control value. In both writhes and tail immersion-test were found to be significantly (P<0.05) dose dependent. *Turbinaria conoides* reported to have better antipyretic activity by restoring many hematological and biochemical parameters under toxic environment [20]. It is well known that *Turbinaria sp* possessed potential antioxidant, anti-inflammatory and anticancer properties due to rich source bioactive compounds such as fucosterol, sulfated polysaccharides fucoidan, neutral glucan, guluronic and alginic acid [2,20].

## Conclusion

Ethyl acetate fraction isolated from ethanol extract of *T. conoides* in order to understand the antigenotoxic and anti-inflammatory potential because it is being used as a traditional medicine. *In-vitro* antioxidant potential was performed and found to be highest in ethyl acetate fraction than that of hexane, dichloromethane, and aqueous fractions obtained from ethanol extract of *T. conoides*. Therefore, ethyl acetate fraction of *T. conoides* was used to evaluate the antigenotoxic and anti-inflammatory potential and found to be significantly effective and dose dependent. The potential activity might be due to the presence of synergetic bio-active compounds like steroids, phenolics, flavonoids, reducing sugars, fucosterol, sulfated polysaccharides including fucoidan, neutral glucan, guluronic and alginic acid.

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## Conflict of Interest

There was no conflict of interest.

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