

IN VITRO ANTI-INFLAMMATORY ACTIVITY OF BLUE-GREEN ALGAE-GEITLERINEMA SPLENDIDUM COLLECTED FROM WESTERN GHATS, SOUTH INDIA

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Received: 15 February 2017, Revised and Accepted: 26 March 2018

ABSTRACT

Objective: *Geitlerinema splendidum* is non-edible blue-green algae available in freshwater systems. It belongs to Pseudanabaenaceae genera. In exploring the medicinal properties of algae, our present work aimed to analyze the anti-inflammatory effect of the algae *G. Splendidum*.

Methods: Anti-inflammatory activity was carried out by human red blood cell membrane stabilization assay for ethyl acetate and ethanol crude extracts of alga and found that the ethyl acetate extract has better inhibitory property than ethanol crude extract. Diclofenac was used as the standard drug.

Results: The experimental results obtained are comparable to the reference drug and are found to be dose-dependent. Even at a minimum concentration of 12.5 µg/ml, the percentage inhibition was in the range of 56.6±0.12–58.5±0.03 %, which indicated that the alga *G. splendidum* has a satisfactory anti-inflammatory activity.

Conclusion: Our present work is supposed to be the first report in exploring the anti-inflammatory effect of the algae *G. splendidum*.

Keywords: Anti-inflammatory effect, Blue-green algae, Diclofenac, *Geitlerinema splendidum*, Human red blood cell.

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INTRODUCTION

Inflammation is a local response of living tissue of our body when injured [1] which involves a multiple arrays of processes such as enzyme activation, cell migration, tissue breakdown, and repair [2]. Inflammation can either acute or chronic. Acute inflammation is the initial response of the body to harmful stimuli [3]. Chronic inflammation can lead to a number of diseases such as hay fever, periodontitis, rheumatoid arthritis, atherosclerosis, and gallbladder carcinoma [4]. Hence, the chronic inflammatory disease is one of the major health issues challenging the medicinal field. The inflammation is the first stage of any discomforts in the biological system of the human body. Nowadays, the usage of synthetic drugs is dominating the medicinal market in the treating inflammatory diseases. In particular, the nonsteroidal anti-inflammatory drugs (NSAIDs) are notable widespread classes of medicine globally for inflammation and other related diseases. These drugs are carboxylic acid-based compounds which reduce the enzyme activity and block the cyclooxygenase pathway [5,6]. However, the predominant side effects such as peptic ulcer, perforation, and bleeding associated with these drugs [7,8] make the urge of searching complementary medicine with safe efficacy. The natural products are believed as one of the reliable sources in the medicinal field [9]. In this regard, we focused our research work on the medicinal properties of blue-green algae (BGA).

Cyanobacteria, commonly known as BGA, are available worldwide in diverse habitats. Algae are members of aquatic organisms belonging to the Protista kingdom which are in different shapes and sizes. Their physical appearances may vary with respect to sea water/saltwater and freshwater systems where they are available. They can be single cellular or multi-cellular.

The single-celled BGA are usually available in freshwater only. BGA are about 70% protein content and edible. BGA as rich in nutrients act as an energy booster [10]. Studies indicated that edible BGA have a number of medicinal properties such as antiviral, antioxidant, anti-diabetic, and anti-bacterial properties [11]. One of the edible constituent of

BGA -*spiralling platensis* has found to possess notable anti-cancer activity [12-15]. According to Skulberg *et al.* [16], there are 2000 cyanobacteria species comprising 150 generics and 40 species are toxic and non edible. However, there is no strong evidence for animal and people being intoxicated by the ingestion of cyanobacteria [17]. Based on the above-mentioned literature survey, one of the non-edible algae, *Geitlerinema Splendidum* was chosen for the present study.

Our research work has been extended in identifying the medicinal properties specifically the anti-inflammatory effect of the non-edible blue-green algae - *G. splendidum* by *in vitro* mode. *G. splendidum* is belonging to Pseudanabaenaceae genera. It has a specific locality of the fresh water system and does not bloom. It has been found that *G. splendidum* has a potential inhibitory effect on acetylcholinesterase activity [18].

METHODS**Collection of the tested alga**

The alga a *G. splendidum*, is collected in the freshwater system nearby the agricultural land that is situated in Coimbatore, the Western Ghats region of South India. The collected alga was authenticated by Botanical Survey of India, Coimbatore, India, and voucher specimen was kept in the Chemistry Department of the host institution with the specimen no:17CH216. The alga was shadow dried for 40d. Then, it is powdered in the laboratory pulverizer. The powdered biomass is sieved using 250 mesh wire sieve and stored in an airtight container which is used for further experiments.

Alga description

G. splendidum is a Colony (thallus) spreading with bundles of filaments. They are in bright green to blue-green color. Trichomes are up to 1 mm long, little bent and entangled. Cells are 2–4 times longer than wide. The salient diacritic feature is the morphology of trichomes without sheaths [19]. The morphological description *G. splendidum* has not yet been clearly published, although the images and phylogeny were documented in Hasler *et al.* [20].

Preparation of crude extracts

The crude extracts were prepared by cold percolation process. An approximately weighed 1kg of the alga was defatted with petroleum ether for about 4 h. The solvent was carefully removed by rotary evaporator. Then, the algae powder was macerated with ethyl acetate and ethanol sequentially. The crude extracts were obtained after removing the solvents using rotary evaporator and were stored in airtight container.

Anti-inflammatory study by human red blood cell (HRBC) membrane stabilization method

HRBC method was used to determine the anti-inflammatory activity of the algae as described in the literature [21,22]. The blood sample was collected from healthy volunteer who was not taking any nonsteroidal drugs two weeks before the experiment. The blood was mixed with equal volume of sterilized Elsevier solution (2% dextrose, 0.8% sodium citrate, 0.05% citric acid, and 0.42% NaCl in water). After centrifuging the reaction mixture at a rate of 3,000 rpm, the packed cells were isolated, and 10%v/v suspension was made with isosaline. The HRBC suspension was used for the estimation of anti-inflammatory property. Standard drug Diclofenac was used as the reference.

Different concentrations of the two extracts, reference sample, and control were separately mixed with 1ml of phosphate buffer; 2 ml of hyposaline and 0.5 ml of HRBC solution. All the assay mixtures were kept in incubation for a period of 30 min at 37°C and then centrifuged at 3000 rpm. This supernatant liquid was decanted, and the hemoglobin content was estimated by a spectrophotometer at 560 nm. The percentage of hemolysis was estimated by assuming the hemolysis produced in the control as 100%.

$$\text{Percentage of Protection} = 100 - (\text{OD}_{\text{sample}} - \text{OD}_{\text{control}}) \times 100$$

Statistical analysis

Triplicate of analysis was carried out for all concentrations to obtain the maximum accuracy. The values are statistically analyzed by one-way analysis of variance.

RESULTS AND DISCUSSION

Preparation of crude extract

The crude extracts of the algae were prepared, and the yields were found to be 6.3 g and 6.5 g of ethyl acetate and ethanol, respectively.

Anti-inflammatory effect

In vitro anti-inflammatory effect of the extracts was analyzed by HRBC membrane stabilization method. The inflammation is the reaction of living tissue to injury, infection or irritation. Lysosomal enzymes released during the inflammation produce a variety of disorders. The stabilization of this lysosomal membrane is important in limiting the inflammatory response. The NSAIDs exert their healing property, either by inhibiting the release of lysosomal enzymes or by stabilizing the lysosomal membranes [23].

Since HRBC membrane is analogous to lysosomal membrane components, the prevention of hypotonicity-induced HRBC membrane lyses can be taken as an *in vitro* measure of anti-inflammatory activity of Natural products [21]. The experimental data were statistically significant ($p < 0.02$).

The percentage of membrane stabilization for ethyl acetate and ethanol extracts of *G. splendidum* and Diclofenac was done at 12.5, 25, 50, and 100 µg/ml. Both the extracts are found to be effective in inhibiting the heat produced by hemolysis of HRBC at the measured concentrations ranging from 12.5 to 100 µg/ml. The results of the HRBC assay of the plant *G. splendidum* extracts were given in Table 1. It was found that maximum inhibition was 71.5±0.07% at a concentration of 100 µg/ml for ethanol extract of alga.

With the increase in concentration, the membrane hemolysis decreases, which imply the increase of stabilization of HRBC membrane. Hence,

Table 1: Percentage inhibition of the extracts of *G. splendidum*

Concentration (µg/ml)	% inhibition		
	Standard (Diclofenac)	Ethyl acetate extract	Ethanol extract
12.5	71.5±0.08	56.6±0.12	58.5±0.03
25	78.6±0.02	61.6±0.32	64.6±0.05
50	88.6±0.05	64.6±0.04	68.9±0.34
100	93.6±0.06	68.4±0.06	71.5±0.07

G. splendidum: *Geitlerinema splendidum*

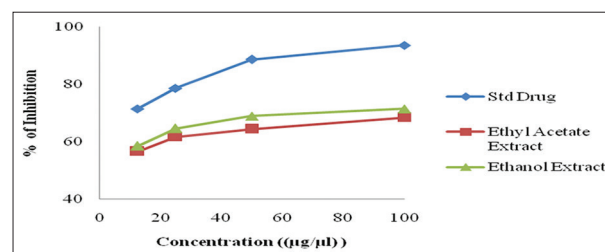


Fig. 1: Percentage inhibition versus concentrations of the extracts

the anti-inflammatory effects of the extracts are dose-dependent. Even at a minimum concentration of 12.5 µg/ml, the percentage inhibition was in the range of 56.6±0.12–58.5±0.03% for the two extracts which indicated that the alga *G. splendidum* has a significant anti-inflammatory activity and was comparable to the standard drug Diclofenac. The graph was plotted between the concentration of extracts and the percentage of inhibition for both the extracts and was shown in Fig. 1.

CONCLUSION

The experimental results demonstrated that the extracts of alga *G. splendidum* at various concentrations have a significant anti-inflammatory property. The results are dose-dependent with respect to the reference drug diclofenac. Hence, the tested alga *G. splendidum* can be an alternative biosource for anti-inflammatory agents. The anti-inflammatory effect of *G. splendidum* has not been previously reported, and our present work is supposed to be the first report providing a scientific support to the satisfactory healing property of the tested alga *G. splendidum* in the inflammatory diseases. However, the further clinical studies to elucidate the exact inhibitory mechanism and the identification of the active constituents responsible for inhibition are likely to be studied which are our future work.

CONFLICTS OF INTEREST

The authors have no conflicts of interest.

AUTHOR'S CONTRIBUTION

The first and third authors have identified the problem, analyzed literature data and carried out the experimental part. The second has compiled the entire work.

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