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IN VIVO ANTI-INFLAMMATORY ACTIVITY OF ETHYL ACETATE EXTRACT DERIVED FROM MARINE *STREPTOMYCES CARPATICUS*

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Keywords:

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ABSTRACT: Anti-inflammatory activity of the SC-EA extract of the marine *Streptomyces carpaticus* was studied in Wistar rats using acute and chronic inflammatory models like carrageenan induced paw edema and cotton pellet induced granuloma models respectively. The acute toxicity of SC-EA extract was tested on adult Wistar rats. The maximum dose of SC-EA extract at 2000mg/kg does not show any sign of visual changes in physical behaviour, protein level and enzyme activities of AST, ALT, and ALP after 14 days of administration in rats. The SC-EA extract of 50mg/kg and100 mg/kg, p.o. demonstrated the inhibition of paw edema to 42.18% and 47.08% respectively and standard diclofenac showed 46.78% inhibition after 4 h of carrageenan treatment. In cotton pellet induced granuloma, maximum inhibition was accounted with SC-EA extract 58.3% at a dose of 100mg/kg and the suppression of inflammation was comparable to Diclofenac (30mg/kg), which reduced the weight of cotton pellet granuloma by 56.5%. The hydroxyproline which is a major component of the protein collagen was also estimated in SC-EA extract treated rats. This was measured as 17.3µg/mg with SC-EA extract at 100mg, whereas Diclofenac group showed 15.60µg/mg tissue.

INTODUCTION: Most of the biological activities on our planet were borne from Oceans. Large number of these biologically active compounds with varying degrees of action, such as anti-tumor, anti-cancer, anti-inflammatory, anti-proliferative, cytotoxic, photo protective, as well as antibiotic and antifouling properties, has been isolated from marine sources. These bioactive substances, otherwise known as metabolites are profoundly used as anti-inflammatory compounds.

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Marine actinomycetes are virtually unlimited sources of novel secondary metabolites with many therapeutically applications and occupies a prominent position due to their adaptability to the marine environment, diversity and proven ability to produce novel pharmaceuticals.

Among the actinomycetes *Streptomyces* are considered to be economically important group of organisms and they are the dynamic source for wide range of biologically active compounds [1]. The secondary metabolites from *Streptomyces* sp. have been proved to possess the anti-inflammatory activity. Cyclomarin A produced from *Salinispora arenicola* CNS-205 [2] showed effective anti-inflammatory and antiproliferative activities in both *in vivo* and *in vitro* assays.

Salinamides A-E isolated from *Streptomyces* sp. CNB-091 exhibited potent topical anti-inflammatory activity [3]. Inflammation is considered as a primary nonspecific immune response in which the body reacts to any kind of infection, irritation or injury [4]. The primary cause of inflammation is a pure mechanical stress, including blunt trauma [5, 6], foreign bodies [7, 8], vibrations [9, 10] and chronic pressure of low intensity [11, 12]. Inflammatory responses take place in different phases each mediated by different mechanisms.

The acute transient phase is illustrated by local vasodilation and increased capillary permeability. Infiltration of leukocytes and phagocytic cells occurs in the sub chronic phase and finally in the chronic proliferative phase, tissue degeneration and fibrosis occurs [13]. However this chronic inflammation is associated with a number of diseases, such as rheumatoid arthritis, atherosclerosis, heart disease, Alzheimer, and asthma. In animals depending on the drug duration the studies may be classified as acute, sub-acute or chronic [14, 15].

In acute inflammatory studies, the animal is given a single dose of drug to determine the immediate effect; toxicological data helps to make decision whether a new drug should be adopted for clinical use or not [16]. The main objective of this research was to validate the anti-inflammatory potential of ethyl acetate extract of *Streptomyces carpaticus* (designated as SC-EAextract) by carrageenan-induced rat paw edema and by cotton induced granuloma methods. Toxicity of SC-EA extract was evaluated by analyzing the liver function tests using an acute oral toxicity test in rats.

MATERIAL AND METHODS:

Acute Toxicity: Adult healthy Wister albino rats weighing 120-150g were kept in large spacious cages and maintained in controlled environment at 25 ± 2 °C temperature with the dark and light cycle of 14/10 h. Actue oral toxicity of SC-EA extract (2000mg/kg) of *Streptomyces carpaticus* was determined by monitoring the Changes in autonomic and behavioural responses and also for symptoms of toxicity and death within 24 h and at regular time intervals for next 14 days [17] according to OECD guide lines 423(1677/PO/Re/S/2012/ CPCSEA/30).

Assessment of Liver Function Test: The serum from the control, SC-EA extract treated groups was separated from the blood by centrifugation at 2500 rpm for 10 min. The levels of Alanine aminotransferase (ALT), Aspartate amino-transferase (AST), Alkaline phosphatase (ALP), Bilirubin, Albumin and Total proteins in the serum were analyzed using commercially available enzyme kit (AGAPPE, India).

Effect of SC-EA Extract on Acute Inflammation: Carrageenan-induced rat paw edema is the most widely used *in vivo* model to determine acute anti-inflammatory activity of lead compounds. The animals were grouped with six animals each. The control rats were given saline orally, the remaining animals were treated with Diclofenac (30mg/kg) and different concentrations of SC-EA extracts (50mg/kg and 100mg/kg) respectively. After 1 h of oral administration, inflammation was induced by an injection of by 0.1 ml of 1% carrageenan into subplantar region of the right hind paw of the rats [18] in all the groups maintained in four different groups. The Paw edema was monitored as the percentage increase in the thickness of the paw at four different time points (0, 1, 2, 3 and 4h) using a Plethysmometer. The percentage inhibition of paw edema was measured using the formula [19].

Cotton Pellet Granuloma Method: The effect of SC-EA extracts on chronic inflammation was analyzed by cotton pellet granuloma model as per the method adopted by [20]. Briefly the cotton pellets weighing 5mg were implanted through subcutaneous skin incision at the back of the animals. After implantation the animals were orally administrated with standard drug, vehicle control (saline 10ml/kg), standard drug (Diclofenac 30mg/kg) and SC-EA extract 50mg/kg and 100mg/kg for seven consecutive days. After treatment for 7 days the animals were subjected to anesthesia and the size of the granuloma was measured as per the standard protocol [21].

Estimation of Hydroxyproline: Hydroxyproline is as a quantitative measure of collagen deposition and fibrosis. The dried granuloma tissues were acid-hydrolyzed at 130 °C for 4 hours in 6N HCl, The hydrolysate was neutralized to pH 7.0 and the mixture was oxidized with chloramine-T for 20

min. After 20 minutes of brief incubation the oxidation reaction is terminated by adding 0.4M perchloric acid further Ehrlich reagent was added to the oxidized reaction mixture and incubated at 60 °C and the developed color was measured at 557nm using UV-Visible spectrophotometer [22].

RESULTS:

Non-Toxic Properties of SC-EA Extract in Wistar Rats: The acute toxicity analysis of SC-EA extract (2000mg/kg) was found to be non-toxic and symptoms with reference to the mortality or morbidity were not observed during the 14 day period of administration in all the animals. As per the results, no impact was found with SC-EA extract on colour of the skin, physical movements of the animal as well as eye balls.

Liver Function Tests (LFTs): The nontoxic nature of SC-EA extract was assessed through the analysis of liver function markers such as aspartate amino transferase (AST), alanine transaminase (ALT) [23], alkaline phosphatase (ALP), protein, albumin and globulin [24], bilirubin [25]. The serum analysis of these biomarkers provides information about the non-toxicity of the SC-EA extract and its dose after acute toxicity test (**Table 1**).

TABLE 1: EFFECT OF SC-EA EXTRACT ON BIOCHEMICAL PARAMETERS OF WISTAR RATS

Male Rats	Aspartate aminotransferase (AST), U/L	Alanine aminotransferase (ALT), U/L	Serum alkaline phosphatase (ALP), U/L	Bilirubin, mg/dL	Albumin, g/dL	Total Protein (g/dL)
Control	214.00 ± 1.23	53.40±1.54	164.87 ± 1.3	0.46 ± 0.3	3.2 ± 1.0	6.4±0.3
SC-EA extract (2000mg/kg)	214.01 ± 0.12	54.18 ±0.13	167± 0.48	0.46 ±0.4	3.1 ± 0.65	6.6±0.1

Mean ± SEM of three independent experiments.

Carrageenan Induced Paw Edema: In the course of the examination for anti-inflammatory efficacy of SC-EA extract on Carrageenan induced rat paw edema method, it was evident that a gradual decrease in paw volume was observed in SC-EA extract compared with the standard drug Diclofenac at a dose level of 30mg/kg body weight as shown in the **Fig. 1**. The paw volume was reduced to 46.78% inhibition by diclofenac after carrageenan induction whereas SC-EA extract at 50mg/kg and 100mg/kg shows 42.18% and 47.08% inhibition (**Table 2**). Based on this it is inferred that SC-EA extract is strong and more potential to control the paw volume on comparison with standard NSAID.

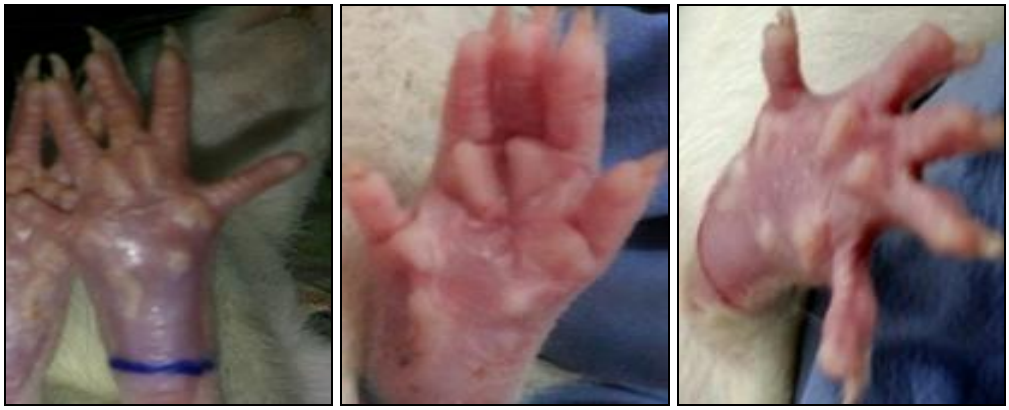

FIG. 1: VENTRAL VIEW OF CARRAGEENAN INDUCED PAW EDEMA. (A) CONTROL (1% CARRAGEENAN) AND (B) SC-EA EXTRACT (50mg/kg) (C) SC-EA EXTRACT (100mg/kg)

TABLE 2: INHIBITION OF INFLAMMATION BY SC-EAEXTRACT AND DICLOFENAC ON RAT PAW EDEMA INDUCED BY CARRAGEENAN

	1hr	2hr	3hr	4hr
Normal Control	-	-	-	-
Disease control (carrageenan 1%)	0.00	0.00	0.00	0.00
Diclofenac30mg/kg	38.46**	58.56***	57.89***	46.78***
SC-EA extract (50mg/kg)	6.67	3.77	41.05***	42.18***
SC-EA extract (100mg/kg)	21.54	41.44***	43.68***	47.08***

Results were expressed as the mean ± SEM of six individual observations with Statistical significance *$p < 0.05$, ** $p < 0.001$ and ***$p < 0.0001$when compared to disease control.

Cotton Pellet-Induced Granuloma Inflammation: The cotton pellet-induced granuloma is widely used to assess the transudative and proliferative components of chronic inflammation. The weight of cotton pellet granuloma was measured and compared with the disease control and standard drug diclofenac at 30 mg/kg body weight. It was observed that the dry weight of granuloma was found to be 92.0 ± 1mg and the weight was reduced to 41.0 ± 1.2 and 42.79 ± 1.9 with SC-EA extract and diclofenac respectively. The data infers the anti-inflammatory activity of SC-EA extract (**Fig. 2**).

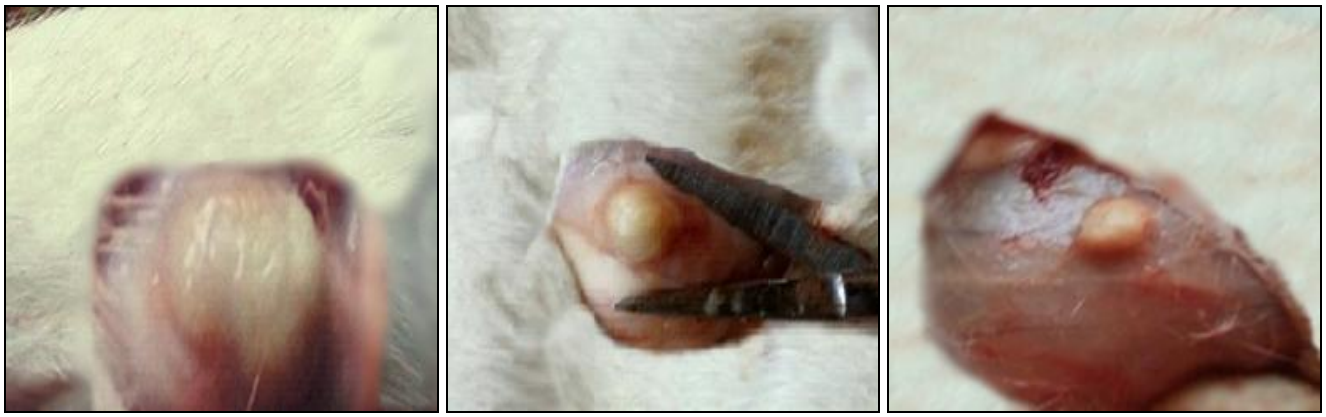

FIG. 2: CHANGES IN THE SIZE OF GRANULOMA IN RATS (A) CONTROL; (B) SC-EA EXTRACT (50mg/kg); (C) SC-EA EXTRACT (100mg/kg)

TABLE 3: PERCENT INHIBITION AND MEAN WEIGHT OF COTTON PELLET IN TREATED GROUPS

Groups	Dry weight of cotton pellet (mg)	% inhibition of granuloma
Disease control	92.0 ± 1	0.00
Diclofenac (30mg/kg)	42.79 ± 1.9***	56.5
SC-EA extract (50mg/kg)	35.79 ± 1.7***	47.2
SC-EA extract (100mg/kg)	41.0 ± 1.2***	58.3

Values are expressed as mean ± SEM (n = 6), *p < 0.05, ** p < 0.001 and ***p < 0.0001vs disease control group.

Hydroxyproline Estimation: Wound healing is a complex process of the skin or the body tissues in order to repair itself after injury. During this process, an increase in production of collagen occurs. The major component of the protein collagen is hydroxyproline which is a non-proteinogenic amino acid produced by the amino acid proline by hydroxylation.

In the present study, the hydroxyproline content was estimated in the standard drug treated groups and the content of hydroxyl proline was increased to 15.6µg/mg in presence of Diclofenac sodium and similar accelerator trend of hydroxyproline was noticed as 10.3µg/mg and 17.3µg/mg with SC-EA extract at 50mg and 100mg respectively. These results indicate that treatment with in SC-EA extract 100mg/kg effectively helped in healing the wound induced by cotton pellet on comparison with diclofenac (**Fig. 3**).

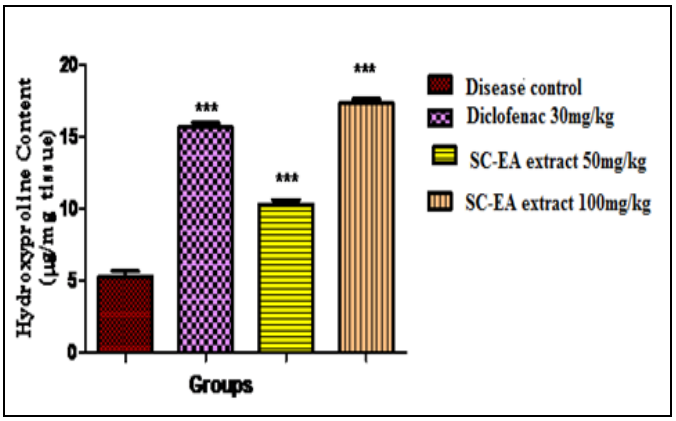

FIG. 3: CHANGES IN HYDROXYPROLINE LEVELS OF GRANULOMA TISSUE

Statistical Analysis: All the results are performed in triplicates, and values represented are mean ± SEM. Statistical significance were calculated by Dunnet's test using Graph pad prism software version 5.0 and is denoted by an asterisk (*) when p values are *p < 0.05, ** p < 0.001 and ***p < 0.0001.

DISCUSSION: In the present study, systematic approach was made to find out the efficacy of SC-EA extracts of marine *Streptomyces carpaticus* against inflammation. Acute inflammation may be

regarded as the first line of defense against injury. Carrageenan induced models are renowned to determine the inhibition of acute inflammation. Carrageenan injection into the sub plantar surface of rat paw is characterized by biphasic response with the involvement of different inflammatory mediators. The early phase observed (1-2 h) is mediated by histamine and serotonin and delayed phase (3-4 h) is mediated with the release of prostaglandins [26]. Our results indicate that the administration of SC-EA extracts inhibited the edema during acute phase of inflammation probably by inhibiting the chemical mediators of inflammation.

The cotton pellet granuloma method is being widely used to assess the transudative and proliferative components of chronic inflammation. Chronic inflammation occurs by means of the development of proliferate Chronic inflammation occurs by means of the development of proliferate smooth muscle cells. Cytokines, such as IL-1 and TNFα, as well as growth factors influence this proliferation.

The reduction in the size of granuloma with different doses of SC-EA extracts shows the significant anti-inflammatory activity in chronic inflammatory conditions. Hydroxyproline is the repetitive unit of collagen which denotes the intact collagen and further wound healing. Our present work was carried out to assess the effect of SC-EA extracts on hydroxyproline content and it shows increase in tissue formation and it is increased compared to Dicolfenac .

CONCLUTION: From the above study it was observed that the SC-EA extracts of marine *Streptomyces carpaticus* has no toxicity up to 2000mg/kg by observing the liver function tests with the control and it has a pronounced anti-inflammatory activity by both acute and chronic inflammatory studies compared to Diclofenac. The hydroxylproline content is also high in SC-EA extract treated groups than the Diclofenac.

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CONFLICT OF INTEREST: There is no conflict of interest.

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