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PHARMACOLOGICAL ACTIVITIES OF METABOLITE FROM *STREPTOMYCES FRADIAE* STRAIN GOS 1

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ABSTRACT

Streptomyces species being renowned for their secondary metabolites and their diversified activities. The present study focused on assessing the anti-inflammatory, analgesic and antipyretic activity of an antibiotic metabolite from *Streptomyces fradiae* strain GOS1 isolated from Western Ghats of Agumbe, Karnataka. The results reveal moderate anti-inflammatory, analgesic and mild antipyretic activity, thus suggesting the pharmacological significance of an antibiotic metabolite.

Key words: *Streptomyces fradiae strain* GOS1, CNS activity, Western Ghats

INTRODUCTION

Actinomycetes are the most widely distributed groups of microbes in nature. Soil actinomycetes are prokaryotes with extremely various metabolic possibilities[1]. These are gram positive bacteria frequently filamentous and sporulating organisms with DNA rich in G + C from 57-75%. Actinomycetes of about 100 genera exist in soil[2]. The genus *Streptomyces* represents the largest group of actinomycetes and is the largest antibiotic-producing genus in the microbial world discovered so far. The number of bioactive compounds reported from the species of this genus per year has increased almost exponentially for about two decades[3].

Western Ghats of Karnataka are one of the 34 biodiversity hotspots and few studies have been carried out on the biological activity of *Streptomyces* isolated from these regions[4-9]. In context of the rich diversity of Western Ghats and the bioactive metabolites from *Streptomyces*, the present study focused on the pharmacological activities of antibiotic

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metabolite produced from *Streptomyces fradiae* strain GOS1 (NCBI accession number JF 682780) (GOS1) isolated from Western Ghats of Agumbe regions of Karnataka, India[10].

EXPERIMENTAL

Material and methods

Extraction of metabolite

The isolate GOS1 was inoculated into Starch Casein Nitrate (SCN) broth medium and was incubated at 32ºC for 12 days. After incubation the broth was filtered through Whatmann grade 01 filter paper and the obtained crude metabolite was subjected to drying at 40^{o}C[4,10]. The dried metabolite was reconstituted in distilled water and was orally dosed to animals at respective dosages.

Animals

The pharmacological study of the potent metabolite of GOS1 was carried out on Sprague Dawley rats and Swiss Albino mouse models. The animals were procured from Sri Venkateshwara Enterprises, Banglore and were maintained at National College of Pharmacy, Shimoga. The study was conducted as per study protocol, relevant Standard Operating Procedures (SOP) of the testing facility, Committee for the Purpose of Control and Supervision on Experiments on Animals (CPCSEA) guidelines and Institutional Animal Ethics Committee (IAEC) guidelines.

Ethical committee clearance certificate No. NCP/IAEC/CLEAR/06/12/2010-11.

Anti-inflammatory activity

The anti-inflammatory effect of the metabolite was studied by carrageenan-induced paw edema in rats[11]. The paw edema was measured using plethysmograph. Eighteen rats (143-165 g) of both sex were randomly divided into three groups of six rats each. Group I served as control and were administered distilled water (2 mL/Kg b.w.), group II rats were treated with standard drug diclofenac sodium (25 mg/Kg b.w.) and group III was treated with GOS 1 metabolite at 200 mg/Kg b.w. respectively. The hind paw's of all animals were marked just beyond the tibio-tarsal junction to ensure uniform dipping of paw and constant paw volume. The initial paw volume was measured by mercury displacement method. Thirty minutes post administration of the metabolite and diclofenac sodium, 0.5 mL of carrageenan (1% w/v) in normal saline was injected into the sub plantar area of the hind paw. The change in paw volume due to carrageenan-induced paw swelling was measured at 0.5 h, 1 h, 2 h, 3 h, 4 h and 5 h after injecting carrageenan.

Analgesic activity

The analgesic activity of the metabolite was studied in mice by tail flick method[11]. Eighteen mice (25-30 g) of both sex were randomly divided into three groups of six mice each. Group I served as control and were administered distilled water (2 mL/Kg b.w.), group II rats were treated with standard drug diclofenac sodium (25 mg/Kg b.w.) and group III was treated with GOS1 metabolite at 200 mg/Kg b.w. respectively. 1-2 cm of mice tail was immersed in warm water kept constant at 55^{o}C, the reaction time was measured as the time taken by the mice for tail deflection. A latency period of 20 seconds was defined as complete analgesia and the measurement was then stopped to avoid injury to mice. The tail-flick response was determined at 0.0 h, 0.5 h, 1 h, 2 h, 3 h, 4 h and 5 h after administration of drugs.

Antipyretic activity

The antipyretic activity of metabolites was evaluated by yeast induced pyrexia in rats[12]. Fever was induced by administering 20 mL/Kg b.w. of 20% aqueous suspension of Brewer's yeast in normal saline subcutaneously in the paw 18 h before the metabolite administration. The rectal temperature was recorded before the injection of yeast and 18 h after yeast administration the animals with a minimum of 0.6°C increase in temperature were considered for dosing. Eighteen rats (143-165 g) of both sex were randomly divided into three groups of six rats each. Group I served as control and were administered distilled water (2 mL/Kg b.w.), group II rats were treated with standard drug paracetamol sodium (150 mg/Kg b.w.) and group III was treated with GOS1 metabolite at 200 mg/Kg b.w. respectively, the dose was administered orally. Rectal temperature was determined by thermal probe Eliab thermistor thermometer at 1 h, 2 h, 3 h, 4 h, 5 h and 6 h after dose administration.

Statistical analysis

The results were statistically analysed by one way ANOVA using Statistical Analysis Software (SAS) version 9.1.3, CARY, USA.

RESULTS AND DISCUSSION

Anti-inflammatory activity

Carrageenan induced hind paw edema is the standard experimental model of acute inflammation. Carrageenan is the phlogistic agent of choice for testing anti-inflammatory drugs as it is known to be antigenic and is devoid of apparent systemic effects. Carrageenan

induced edema is a biphasic response; the first phase is mediated through the release of histamine, serotonin, and kinins where as the second phase is related to the release of prostaglandin and slow reacting substances which peak at 3 hour[13]. The carrageenan induced hind paw edema experimental model exhibits a high degree of reproducibility[14] and thus has been adapted in the present investigation. The decrease in edema was time dependent and was prominent in the drug treated group and moderate results were observed in the metabolite treated group. A gradual reduction in inflammation was observed from 0.5 h to 5 h post dose time point (Table 1). Similar results on anti-inflammatory activity of *Streptomyces* metabolites have been observed by Lee *et al.*[15] and Kuriyama *et al.*[16]

Table 1: Anti-inflammatory activity of GOS1 metabolite

Time (h)	Paw volume in mL (mean ± SD)			% Edema inhibition	
	Control	Diclofenac	GOS1	Diclofenac	GOS1
00 h	0.57 ± 0.04	0.57 ± 0.03	0.58 ± 0.03	-	-
0.5 h	0.57 ± 0.03	0.47 ± 0.02*	0.57 ± 0.03	18.44%	2.83%
01 h	0.57 ± 0.03	0.38 ± 0.04*	0.52 ± 0.01*	34.29%	10.48%
02 h	0.56 ± 0.02	0.25 ± 0.03*	0.47 ± 0.01*	56.19%	18.69%
03 h	0.55 ± 0.02	0.23 ± 0.02*	0.44 ± 0.01*	59.65%	23.79%
04 h	0.54 ± 0.02	0.22 ± 0.01*	0.43 ± 0.01*	61.67%	26.06%
05 h	0.53 ± 0.01	0.21 ± 0.01*	0.42 ± 0.01*	63.40%	28.04%

*Indicates $p < 0.05$ level of significance

The exact mechanism of action of the metabolite on the reduction of paw volume is yet to be deduced; the reduction can be the result of anti-inflammatory activity similar to nonsteroidal drugs. Diclofenac sodium being a non steroidal anti-inflammatory drug showed significant decrease in edema.

Analgesic activity

Analgesia is defined as a state of reduced awareness to pain and analgesics are substances which decrease pain sensation by increasing the threshold to painful stimuli[11]. The analgesic property of a drug can be due to the blockade of the effects or the synthesis and / or release of prostaglandins and / or other endogenous substances that excite pain nerve endings[17]. The tail flick method is carried out to differentiate between central and peripheral

analgesics. The thermal model of tail flick is considered to be central reflex involving spinal cord, but could also involve higher neural structures[18].

In the present study, the increase in mean basal latency to sensation in both the standard drug and the GOS1 metabolite treated group was observed, the control group showed no variation. The tolerance towards heat exposure was prominent in the drug treated group, but was moderate in the metabolite treated group. The analgesic activity was prominent at 2nd and 3rd hour, and a decline in activity was observed (Table 2).

Table 2: Analgesic activity of GOS1 metabolite

Time (h)	Tail flick latency in seconds (Mean ± SD)			% Analgesia	
	Control	Diclofenac	GOS-01	Diclofenac	GOS-01
00 h	2.36 ± 0.19	2.64 ± 0.99	2.91 ± 0.74	13.21%	14.55%
0.5 h	2.46 ± 0.22	5.37 ± 1.93*	3.07 ± 0.73	26.86%	15.35%
01 h	2.54 ± 0.29	8.44 ± 4.14*	3.77 ± 0.59*	42.21%	18.88%
02 h	2.53 ± 0.32	14.69 ± 2.36*	5.13 ± 0.84*	73.45%	25.68%
03 h	2.55 ± 0.28	15.04 ± 1.55*	5.20 ± 0.88*	75.2%	26.01%
04 h	2.48 ± 0.24	14.35 ± 1.52*	4.87 ± 0.84*	71.78%	24.35%
05 h	2.39 ± 0.18	14.21 ± 1.60*	4.53 ± 0.77*	71.08%	22.69%

*Indicates $p < 0.05$ level of significance

The analgesic effects of the metabolite represented it may act via centrally mediated analgesic mechanism, exact mechanism of action is yet to be deduced. The analgesics are also classified as opoid and nonopoid based on their mode of action. The nonopioid analgesics act to decrease the generation of the mediators of pain at the site of tissue damage, although several drugs also have some effects within the central nervous system (CNS). The opioid analgesics are unique in that they not only block the incoming nociceptive signals to the brain but also act at higher brain centers, controlling the affective components of the pain. Diclofenac sodium represents non opioid analgesics[19].

Antipyretic activity

Tortora and Grabowski[20] stated fever is an elevation of core temperature that results from the resetting of the hypothalamic thermostat. Yeast induced pyrexia is called

pathogenic fever; its etiology includes production of prostaglandins which set the thermoregulatory centre at a lower temperature. Antipyretics are agents which reduce the elevated body temperature; regulation of body temperature requires a delicate balance between production and loss of heat and the hypothalamus regulates the set-point at which body temperature is maintained[21]. In the present study, the metabolite and standard paracetomol were assayed for their antipyretic potential. The significant increase in rectal temperature was observed in all groups after yeast administration. Reduction in temperature was observed after administration of drug and metabolite. The effect of paracetomol was statistically significant ($p < 0.05$) in comparison to the metabolite. A mild decrease in temperature after 2 hrs of metabolite administration was observed (Fig. 1).

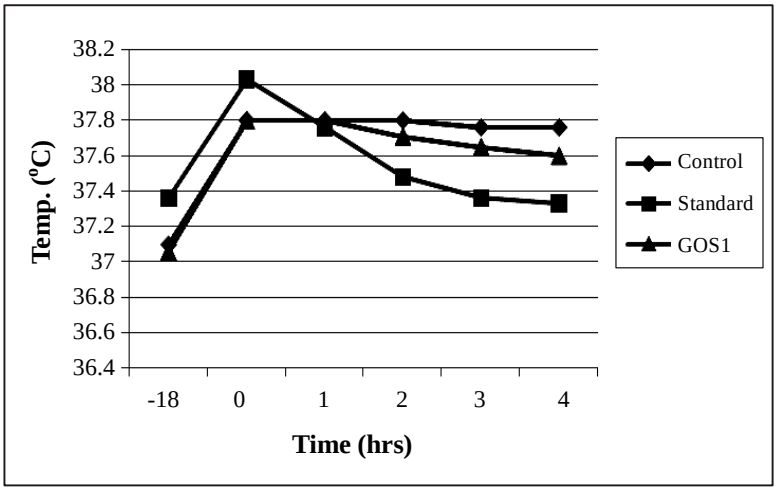

Fig. 1: Antipyretic activity of GOS1 metabolite

Usually most anti-inflammatory and analgesic drug possesses antipyretic activity. In general non steroidal drugs produce their antipyretic action through the inhibition of prostaglandin synthetase with in the hypothalamus[22]. Therefore from the above results, it was concluded that the anti-inflammatory, analgesic and antipyretic property of the metabolite can be attributed to the inhibition of prostaglandin synthesis within the hypothalamus.

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