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Research Article

MITOGENIC EFFECT OF THE SALIVARY GLAND EXTRACTS OF OCTOPUS

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ABSTRACT

Objective: Octopuses from Mumbai waters have been less studied for their bioactive potential. The present study aimed to check the effect of the posterior salivary gland extracts of two species of octopus - *Cistopus indicus* and *Octopus fusiformis* on cell proliferation.

Methods: The posterior salivary glands were extracted in different solvents and partially purified by column chromatography. The samples were tested at different concentrations for their effect on the growing root meristems of *Allium cepa* and on chicken chorio-allantoic membrane.

Results: The extracts increased the mitotic index (MI) with reference to the control system. The mitogenic effect of the extracts decreased with increasing recovery time. The salivary gland extracts of the octopuses, at lower concentrations, did not exhibit pro-angiogenic or anti-angiogenic effect on CAM vasculature. The acetic acid extracts, at a concentration of 0.75 mg/mL, has significantly promoted the proliferation of blood vessels.

Conclusion: The study is indicative of the presence of mitotic promoting factor(s) in low concentrations in the posterior salivary glands. The effect is short-lived.

Keywords: Octopus, *Cistopus indicus, Octopus fusiformis,* Mitotic index, Chorio-allantoic membrane.

INTRODUCTION

The rich and diverse marine environment may inhabit over 80% of the world's flora and fauna, many of which have great bioactive potential [1, 2]. Primary and secondary metabolites of marine life are rich sources of several compounds with high pharmacological potential. Many bioactive compounds have been extracted from various marine animals like tunicates, sponges, corals, nudibranchs, bryozoans, molluscs and other marine organisms [3, 4]. Sessile, soft-bodied marine invertebrates that lack obvious physical defenses are considered prime candidates to possess bioactive metabolites as chemical defenses [5]. Venomous cephalopods have well-developed venom apparatus involving their salivary glands. The posterior salivary glands of octopus have been reported to be a venomous gland and several biogenic amines have been isolated from them [6, 7]. Little attention has been paid to the studies on cephalopods along the Mumbai Coast. The objective of the present study was to check the presence of the effect of posterior salivary gland extracts of the octopus *Cistopus indicus* and *Octopus fusiformis* on cell proliferation using onion root meristem assay system and CAM vasculature.

MATERIALS AND METHODS

Specimen collection and Extraction

Cistopus indicus and *Octopus fusiformis* were collected from the fish landing centre at Marve Beach, Mumbai and were brought live to the laboratory and immediately dissected to isolate the posterior salivary glands. The posterior salivary glands of the two species of octopus were separately extracted in acetic acid [8], methanol [9] and methanol: chloroform 1:2 [10] and will be hereafter referred to as Crude A, Crude M and Crude MC respectively. The extracts were subjected to partial purification and the fractions collected [11, 12]. One unadsorbed fraction (FAU, FMU, FMCU) and ten adsorbed fractions (FA1-10 for Crude A, FM1-10 for Crude M and FMC1-10 for Crude MC) were eluted and subjected to the following assays.

Table 1: Mitotic Index of extracts of *Cistopus indicus* & *Octopus fusiformis* @ 0.25 mg/mL

Octopus species	Recovery time (in hours)	Mitotic Index (MI)		
		Crude A	Crude M	Crude MC
Cistopus indicus	6	8.43±0.63*	8.51±0.26*	8.39±0.54*
	12	8.27±0.24*	8.23±0.31*	8.27±0.48*
	18	8.14±0.17*	7.68±0.09	8.14±0.12*
	24	7.57±0.33	7.16±0.41	7.63±0.37
	30	6.42±0.25	7.02±0.28	7.25±0.16
	36	6.37±0.41	6.58±0.32	6.87±0.28
	42	6.31±0.52*	6.41±0.16*	6.43±0.43
	48	6.24±0.28*	6.23±0.51*	6.16±0.19*
Octopus fusiformis	6	8.32±0.47*	8.42±0.14*	8.82±0.32*
	12	8.17±0.22*	8.37±0.26*	8.61±0.28*
	18	7.54±0.12	8.21±0.09*	7.92±0.09
	24	7.23±0.09	7.64±0.42	7.53±0.17
	30	7.21±0.23	7.32±0.36	6.84±0.13
	36	6.42±0.52	7.08±0.29	6.62±0.61
	42	6.32±0.08	6.51±0.16	6.52±0.24
	48	6.16±0.19*	6.42±0.24*	6.16±0.18*

At a concentration of 0.5 mg/mL, all the samples, except Crude A of *Cistopus indicus*, showed decreasing MI with increasing recovery time (Table 2).

Chicken Chorio-Allantoic Membrane Assay

To study the angiogenic nature of the salivary gland extracts, the chicken chorioallantoic membrane (CAM) assay was carried out [14] in embryonated chick eggs procured from Central Poultry Development Organization, (CPDO). Three concentrations (0.75 mg/mL, 0.5 mg/mL & 0.25 mg/mL) of the extracts and fractions were used. Discs soaked in Phosphate buffer were used as control.

The number of macroscopic blood vessels that converge towards the disc were counted from day 8 to day 12 [15]. Angiogenesis was

quantified by analyzing the number and thickness of branching blood vessels within the area of each disc.

Data Analysis

Experiments were carried out in five sets, each with a sample size of ten. Data are expressed as mean ± SEM and evaluated by one way ANOVA. * indicates statistical significance at 0.05 level.

RESULTS

All the crude extracts, at a concentration of 0.25 mg/mL, showed decreasing MI with increasing recovery time (Table 1).

The mitogenic effect of FA1-10 and FM1-10 of *Cistopus indicus* decreased with increasing duration of recovery (Table 3). The MI of the control group treated with distilled water is 7.42±0.31

Table 2: Mitotic Index of extracts of *Cistopus indicus* & *Octopus fusiformis* @ 0.5 mg/mL

Octopus species	Recovery time (in hours)	Mitotic Index (MI) Crude A	Crude M	Crude MC
	6	8.57±0.41*	7.92±0.31*	8.14±0.43*
Cistopus indicus	12	8.64±0.52*	7.91±0.40*	8.02±0.21*
	18	7.24±0.61	7.23±0.12	7.65±0.19
	24	8.16±0.18	6.74±0.09	7.42±0.08
	30	9.47±0.25*	6.38±0.24	7.16±0.32
	36	7.63±0.32	5.92±0.16*	7.07±0.36
	42	8.29±0.11*	5.64±0.32*	6.52±0.17
	48	8.51±0.24	5.02±0.45*	6.31±0.29*
	6	8.47±0.37*	8.57±0.25*	8.43±0.31*
Octopus fusiformis	12	8.42±0.21*	8.52±0.61*	8.21±0.29*
	18	8.31±0.42*	7.47±0.52	8.19±0.27*
	24	7.84±0.16	7.14±0.12	7.87±0.42
	30	7.32±0.51	7.08±0.43	7.63±0.53
	36	6.76±0.32	6.72±0.24	6.89±0.14
	42	6.52±0.61	6.72±0.16	6.72±0.62
	48	6.31±0.47*	6.65±0.28*	6.43±0.43*

Table 3: Mitotic Index of the fractions (0.5 mg/mL) of extracts of *Cistopus indicus*

Type of Fraction	Mitotic Index (MI) taken after various recovery time (in hours)							
	6	12	18	24	30	36	42	48
FA1	8.38±0.24	8.27±0.12	8.21±0.31	8.09±0.32	7.76±0.26	7.54±0.32	7.53±0.14	6.89±0.08
FA2	8.42±0.32	8.28±0.17	8.26±0.41	7.72±0.26	7.65±0.09	7.52±0.28	7.50±0.19	6.92±0.41
FA3	8.51±0.42	8.42±0.16	8.42±0.23	8.14±0.51	7.92±0.34	7.87±0.08	6.91±0.21	6.63±0.44
FA4	8.42±0.32	8.36±0.16	8.21±0.41	7.67±0.28	7.52±0.43	7.43±0.09	6.82±0.24	6.70±0.18
FA5	8.37±0.24	8.23±0.31	8.21±0.08	8.18±0.22	7.49±0.05	7.32±0.28	6.66±0.16	6.28±0.35
FA6	8.43±0.32	8.27±0.17	8.18±0.08	7.74±0.21	7.72±0.24	6.53±0.32	6.51±0.16	6.48±0.19
FA7	8.36±0.42	8.26±0.31	8.26±0.19	7.87±0.06	7.82±0.24	7.52±0.23	6.94±0.17	6.81±0.08
FA8	8.42±0.21	8.36±0.16	8.32±0.32	7.82±0.11	7.47±0.28	7.18±0.09	6.32±0.24	6.16±0.42
FA9	8.39±0.32	8.27±0.16	8.27±0.24	7.85±0.28	7.28±0.15	6.56±0.31	6.71±0.22	6.24±0.30
FA10	8.37±0.21	8.37±0.36	7.81±0.42	7.52±0.17	7.32±0.08	7.21±0.29	6.88±0.11	6.25±0.36
FAU	8.42±0.32	8.32±0.08	8.01±0.12	7.88±0.43	7.62±0.21	6.73±0.06	6.28±0.06	6.01±0.17
FM1	8.43±0.13	8.24±0.38	8.21±0.27	7.69±0.41	7.52±0.26	6.84±0.18	6.81±0.35	6.72±0.23
FM2	8.47±0.21	8.41±0.37	8.32±0.42	8.01±0.09	7.84±0.18	7.63±0.38	7.27±0.26	6.92±0.50
FM3	8.38±0.13	8.21±0.46	7.88±0.09	7.76±0.42	7.24±0.25	6.58±0.32	6.51±0.19	6.23±0.28
FM4	8.41±0.21	8.38±0.32	7.62±0.19	7.59±0.42	7.42±0.28	6.82±0.35	6.72±0.26	6.65±0.08
FM5	7.32±0.19	7.26±0.22	7.21±0.31	7.18±0.29	7.07±0.30	6.84±0.17	6.63±0.28	6.41±0.42
FM6	8.42±0.42	8.38±0.18	8.25±0.09	7.57±0.61	7.41±0.53	7.33±0.32	6.84±0.18	6.59±0.25
FM7	8.27±0.24	8.21±0.18	7.63±0.31	7.52±0.44	6.49±0.29	6.28±0.32	6.16±0.25	6.24±0.42
FM8	8.32±0.32	8.31±0.32	8.29±0.28	7.95±0.42	7.82±0.33	7.64±0.19	7.56±0.26	6.82±0.28
FM9	8.52±0.32	8.48±0.16	8.05±0.41	7.62±0.08	7.64±0.29	7.52±0.33	6.69±0.28	6.31±0.16
FM10	8.28±0.31	8.14±0.09	7.82±0.24	7.61±0.18	7.48±0.22	6.52±0.31	6.43±0.28	6.21±0.08
FMU	8.44±0.27	8.13±0.36	8.09±0.38	7.88±0.25	7.72±0.28	6.86±0.42	6.58±0.27	6.17±0.32

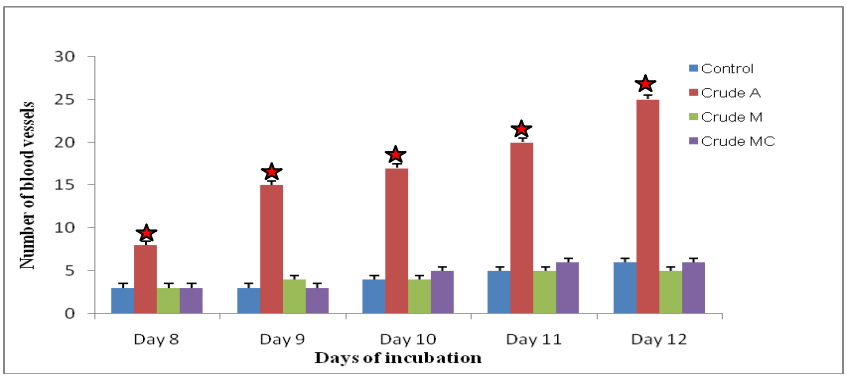

Fig. 1: Angiogenic response of the crude extracts of *Cistopus indicus* @ 0.75 mg/mL

The salivary gland extracts of the octopuses did not show pro-angiogenic or anti-angiogenic effect at the lower concentrations. Crude A of *Cistopus indicus*, at a concentration of 0.75 mg/mL, showed a very significant angiogenic activity, seen increasing from day 8 of incubation when compared with the control (Figure 1).

Crude A of *Octopus fusiformis* showed angiogenic activity from day 10 (Figure 2). The Crude M and MC extracts of both the species failed to elicit any significant pro-angiogenic or anti-angiogenic response on the CAM vasculature in all the tested concentrations.

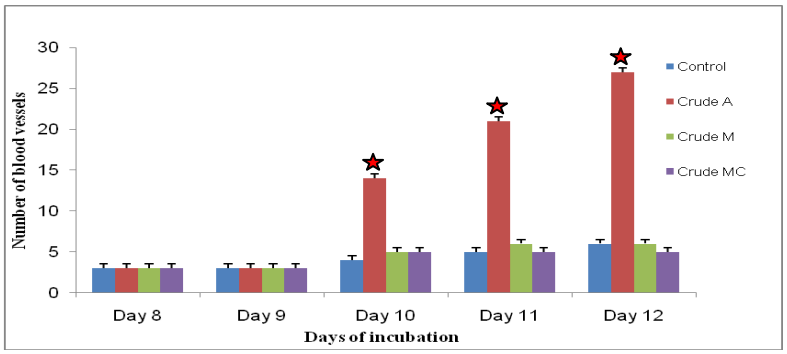

Fig. 2: Angiogenic response of the crude extracts of *Octopus fusiformis* @ 0.75 mg/mL

Among the fractions, significant angiogenic activity was seen in the fractions FA4 and FA5 (Figure 3), at a concentration of 0.75 mg/mL. Significant angiostatic activity (inhibition of new capillary growth) was not seen in any of the samples. FMC1-10 of *Cistopus indicus* and all the fractions of *Octopus fusiformis* and did not show any significant effect in both the assays.

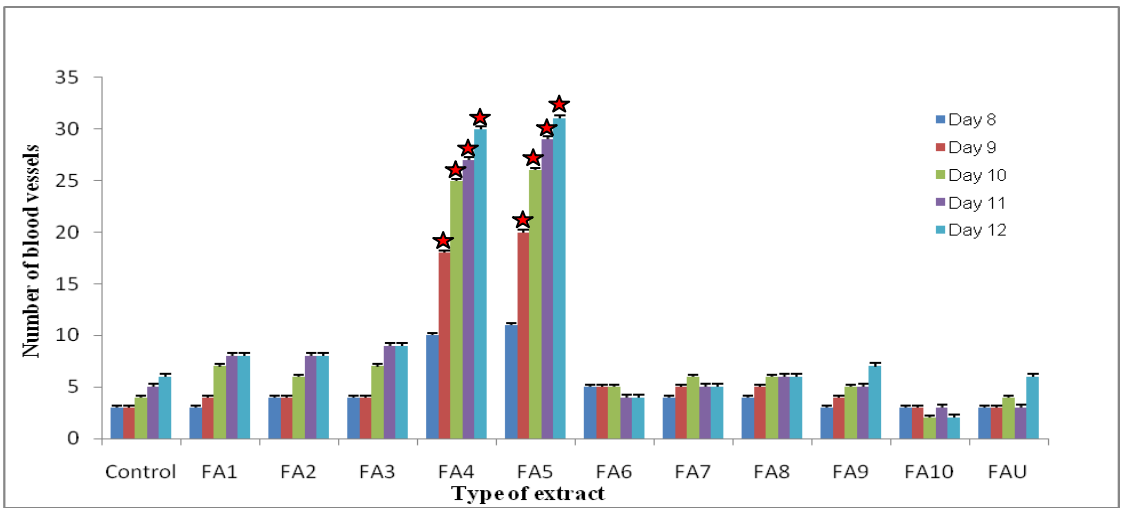

Fig. 3: Angiogenic response of the Crude A fractions of *Cistopus indicus* @ 0.75 mg/mL

DISCUSSION

Plants are very useful for preliminary toxicity assays and due to the high degree of concordance between results obtained from mammalian assays and plant assays [16, 17], hence the onion root tip system was used in the present study. Glycoproteins having mitogenic activity and those with wound healing properties have been reported in plants [18-20]. Some species of marine sponges have been shown to produce metabolites with endocrine-altering and cell growth regulatory properties [21, 22]. The present study of the cytotoxic, phytotoxic and mitogenic effect of the salivary gland of octopus in *Allium cepa* root tip meristems model revealed mitogenic effect. The increase in the recovery time period after exposure to the extracts brought down the MI. This indicates the effect of the mitotic promoting factor(s) in the extracts is significant for only a short period of time.

In the present study, the effect of low concentration of crude A is comparable in both the species. After 48 hours of recovery in tap water, the effect of 0.5 mg/mL of crude A of *Cistopus indicus* is considerably higher than that of *Octopus fusiformis* whereas at high concentration, the effect of the former is more than the latter. This indicates the presence of a longer-lasting mitogenic component in crude A of *Cistopus indicus.* Also, the present observations show decline in MI with increasing concentration and agrees with earlier findings [23]. The fractions enhanced mitosis of the onion root tip meristem without indicating cytotoxicity. The lack of significant variability in the effect of the crude and fractions indicates presence of various mitogenic components in different fractions and the effect of the crude extracts could be cumulative of these. This may be the first report on the quantitative and qualitative data of the salivary gland extracts of *Cistopus indicus* and *Octopus fusiformis* on the mitotic index of onion root tip.

CAM assay study is one of the important parameters to assess the angiogenic effects of various compounds. It is widely used as a model to examine angiogenesis and anti-angiogenesis [24]. Under normal conditions, this tightly regulated process occurs only during embryonic development, in the female reproductive cycle, and during wound repair [25].

However, in pathological conditions such as malignant growth, atherosclerosis and diabetic retinopathy, angiogenesis becomes persistent. It has been demonstrated that this prevalence is mainly due to an imbalance in the interplay between pro-angiogenic and anti-angiogenic signals that control this process [26]. In the present study of CAM assay, the lower concentrations of the crude extracts of the octopuses did not exhibit any pro-angiogenic or anti-angiogenic effect on the CAM vasculature, whereas at higher

concentration, Crude A has significantly promoted the proliferation of blood vessels. This indicates that the angiogenic promoters are present in low concentrations in Crude A.

The Crude A of *Cistopus indicus* showed significant increase in the number of blood vessels by 24 hours of its administration, i.e., from day 8 onwards. This effect was detected progressively till day 12. The present observation points towards the possible existence of fast-acting angiogenic stimulator in this extract which is effective *in vivo*. The Crude A of *Octopus fusiformis* showed enhancement in the number of blood vessels from day 10 onwards. By day 11, the augmentation in the number of blood vessels due to the crude A of both the species was comparable. The present observations correlate with the earlier study of dose-dependant effect of methanol extract of crustaceans on CAM [27].

Present results suggest the presence of compounds of either different chemical nature or with varying effect or affecting different mechanism in promoting angiogenesis in the Crude A extracts of these two species of octopus. The proliferative phase in wound healing is characterized by angiogenesis, followed by collagen deposition, granulation tissue formation, epithelialization and wound contraction [28, 29]. Any of these steps, which are potential targets for pharmacological intervention, could have been effected by the crude A extracts. The present observations are strengthened by the earlier reports of skin extract of an Arabian Gulf catfish, *Arius thalassinus* by Al-Hassan *et al*. [30, 31]. Angiogenic stimulators can be therapeutically administered to diabetic wounds to accelerate neovascularization and promote healing [32-34]. The promising stimulation of angiogenesis can be used for wound healing therapeutics and also to promote cell growth in culture.

The salivary gland extracts from the two species of octopus namely *Cistopus indicus* and *Octopus fusiformis* show significant acceleration of cell proliferation which could be used in effective wound healing. Further purification of extracts and analysis are planned to be executed.

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