Research Article

INTERNATIONAL RESEARCH JOURNAL OF PHARMACY
www.irjponline.com
ISSN 2230-8407 [LINKING]

BIOMEDICAL ACTIVITIES FROM CORAL *JUNCEELLA DELICATA* COLLECTED FROM MADH ISLAND, WEST COAST OF MUMBAI.

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Number of pages: 46
Number of tables: 9
Number of graphs: 5
Number of photographs: 11
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How to cite: Borate M.P. and Zodape G.V, **BIOMEDICAL ACTIVITIES FROM CORAL *JUNCEELLA DELICATA* COLLECTED FROM MADH ISLAND, WEST COAST OF MUMBAI.** International Research Journal of Pharmacy, 2025,16:7:102-144.

Doi: http://doi.org/10.56802/irjp.2025.v16.i07.pp102-144

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ABSTRACT

Background:

The study involved to evaluate the effect of crude extract of coral *Junceella delicata* on different biomedical activities.

Aim of the study: The current study aims to investigate the biomedical activities of the coral *Junceella delicata* collected from Madh island, Mumbai.

Materials and Methods: The corals were collected from the Madh island. The corals were dried and minced in the portal and pestle and were extracted in the mixture of methanol: dichloromethane (1:1). The experiments were conducted by using standard methods to evaluate the biomedical properties of the crude extract of coral *Junceella delicata.*

Results: The crude extract was subjected for its biomedical activities. From the above experiments it was found that the LD_{50} value is greater than 2000mg/kg bodyweight of rats. The histopathological examination was done on different tissues like liver, kidneys, lungs, brain, spleen, ovary, and uterus and it was found that all the tissues showed normal histo-morphological structures. Thus, it confirms that the dose level is greater than 2000 mg/kg bodyweight of rats. In the case of shrimp lethality assay the crude extract showed LC_{50} value 36.307 µg/mL. It is also confirmed that the crude extract contains bioactive compounds which showed strong hemolytic activity by rupturing the red blood cells of chicken and human erythrocytes. In CAM assay, it showed anti-angiogenic activity. The organic crude extract of *Junceella delicata* showed a maximum Na^+-K^+ ATPase activity of 0.096 µPi/mg protein/hour at a dose of 10 µg/µL, with a decrease in activity observed at higher concentrations, indicating a potential neuromodulatory effect. Additionally, the extract exhibited dose-dependent modulation of AChE activity, with the highest activity observed at 500 µg/mL, suggesting its role in enhancing neuronal function. We have also estimated the total protein and it was estimated as 750 µg/mL. The partial purification of the crude protein was done by using SDS-PAGE electrophoresis.

Conclusion: It was concluded that the crude extract and isolated proteins at different kDa levels showed anti-bacterial, anti-inflammatory, hemolytic, haemagglutination, and neuromodulatory and cytotoxic properties. Thus, it confirms that the crude extract of coral *Junceella delicata* have biomedical properties.

Keywords: *Junceella delicata*, crude extract, biomedical properties, hemolytic activity, anti-angiogenic, toxicity

INTRODUCTION

Historically, natural products have been crucial in drug discovery, particularly for cancer and infectious diseases (1). They produce a wide range of molecules such as enzymes, biopolymers, bioactive compounds, and secondary metabolites, which have applications in fields including pharmaceuticals, nutraceuticals, cosmetics, antibiotics, antifouling products, and biomaterials (2). Sponges, cnidarians, molluscs, echinoderms, and ascidians are among the most researched marine invertebrates for bioactive compounds. The existence of drugs developed from natural products isolated from these animals underscores their significance in therapeutic applications (2,3).

Gorgonian corals (soft corals) belong to Phylum Cnidaria and class Anthozoa. The order Gorgonacea is commonly called as a seafans; it is divided into three suborders namely Calcaxonia, Holaxonia and Scleraxonia (4). Soft coral is a rich source of secondary metabolites such as diterpens, sesquiterpenes, furanoditerpenes, terpenoids, capnellenes and steroids from *Lobophytum, Sinularia, Sarcophyton, Capnella, Dendronephthya*. They have HIV inhibitory, cytotoxicity, anti-inflammatory, anticancer and antimicrobial activity (5). The glycosides, cervicosides and prostanoids claviridenones, from the soft corals *Sinularia cervicornis* and *Clavularia viridis* were shown to have antitumour activity against human cancer cells lines. The polyoxygenated steroids from *Alcyonumpata gonicum* and another coral species *Nephteaerecta* were represented the most numerous groups of coral diterpenoids which have mild to strong cytotoxicity to human cancer cell lines. Other cytotoxic and cytostatic compounds from soft corals were eleutherobin and sarcodictyin (6). Several macrolides like bryostatin-1and bryostatin-2 were isolated from Bugulaneritina. Some of these metabolites were showed a high order of antineoplastic activity (7). In *Sinularia sp*., a tetra prenylatedspermine derivative has been isolated, sinulamide, which revealed an ATPase inhibitory activity. Sinulide is a potential antiulcer drug, as it inhibits the production of gastric acid (8). Subergorgic acid, a cardiotoxin, is derived from the pacific gorgonian coral

Subergorgia suberosa (9). It was observed that the toxin inhibited neuromuscular transmission at a concentration of 0.16 µg/mL in an isolated guinea-pig heart assay. Additionally, Pseudopterolide, a unique diterpene with a 12-membered ring obtained from the gorgonian *Pseudopterogorgia acerosa*, exhibited distinctive cytotoxic properties (10). The lobane diterpenes and lobane lacatnes, along with the pacifns from *Sinularia sp.* and *Lobophytum sp*., demonstrated similar cytotoxicity. Several diterpenoids from the xenicane groups found in *Xenia sp*. showed varying levels of cytotoxic activities against human lung carcinoma (H460) and liver carcinoma (HepG2) cell lines. They primarily isolated cembrane substances from *Sinularia triangular* (11).

A recent study by Borate and Zodape highlighted the promising biomedical potential of *Junceella delicata*, demonstrating its antimicrobial, antifungal, and antimycobacterial properties, as well as significant anti-inflammatory effects. The crude extract showed strong inhibition of RBC hemolysis, protein denaturation, and proteinase activity, comparable to the standard drug diclofenac sodium. These findings support its potential as a therapeutic candidate for treating infections and inflammation (12, 13).

MATERIALS AND METHODS

a) Sample Collection:

The coral *Junceella delicata* was collected from Patwadi Village, Madh Island, Malad West Mumbai- 400061, Maharashtra, India (19º 8’ 47652” N, 72º 47’ 17.8116” E) during the low tide. The debris was removed during collection. The sample collected was washed twice with sea water and then rinse three times with distilled water and stored in ice cubes until they were transferred to the deep freezer at -8º C at the Department of Zoology, S.S. & L.S. Patkar College of Arts & Science, and V. P. Varde College of Commerce & Economics, Goregaon West, Mumbai-400104

b) Identification of coral:

Preliminary identification was done by examining the shape and size of the sclerites and by reviewing the literature. The confirmation of identification was done by Dr. Swapnaja Mohite, Professor and Head, Department of Fisheries Biology, College of Fisheries, Shirgaon, Ratnagiri, Maharashtra- 415629

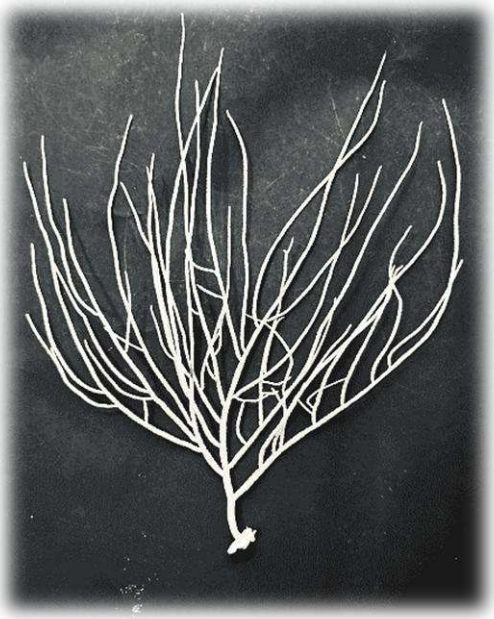
c) Preparation of crude extract:

The coral sample was removed from the deep fridge and bloat with blotting paper and kept in shed dried for 48 hours. After 48 hours the sample was grinded with blender and then macerated by adding an equal volume of MeOH: DCM (1:1) for 24 hours in the water bath at 45º C. The aliquot mixture obtained was filtered through Whatman filter paper No. 1. The homogenate was centrifuged at 10,000 rpm for 15 minutes in cold centrifuge (Remi centrifuge serial No. VCDX- 5983) at -8º C and supernatant was collected. The aliquot was concentrated in a rotary vacuum evaporator at 45º C. The resultant compound was subjected to Millipore filter system and finally dried in vacuum desiccator and stored in the refrigerator at - 20 º C till further use.

d) Ethical approval:

Ethical approval was sought from the Principal Chief Conservator of Forest, Nagpur (Desk-22(8)/Res/CR-25(22-23)/1431/ (22-23) and final approval was taken from the Maharashtra State Biodiversity Board, Nagpur (MSBB/Desk-5/825/2022-23) for collection of *Junceella delicata* samples. The voucher specimen of *Junceella delicata* was submitted to the repository at the Zoological Survey of India, Western Regional Office, Pune (ZSI-WRC Misc/18), India.

e) Classification of coral:



Coral sample: *Junceella delicata* (Grasshoff, 1999)

SYSTEMATIC POSITION

Kingdom: Animalia

Phylum: Cnidaria

Class: Anthozoa

Order: Gorgonacea

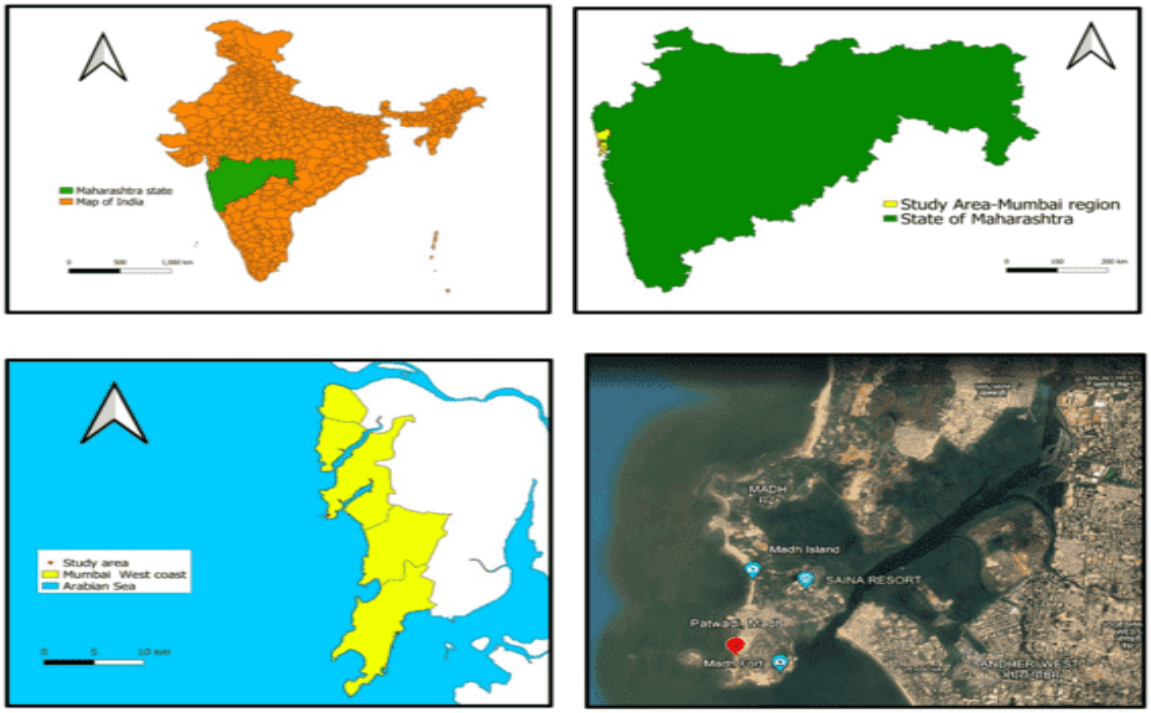
Sub-order: Calcaxonia

Family: Ellisellidae

Genus: *Junceella*

Species: ***Junceella delicata*** (Grasshoff, 1999)

Map of Mumbai showing the site of collection (*Source*- QGIS software) and satellite image (*Source*- Google Earth)



ACUTE ORAL TOXICITY

Twelve female Sprague Dawley rats (150-200 gm) were used for the acute oral toxicity, procured from the National Toxicology Centre, Pune. The experiment was approved by the Ethical Committee at APT Foundation, Pune (CPCSEA: RP. No APTRF/RP-03/2223). The rats were acclimatized for one week in controlled conditions (22 ± 3°C, 50-60% humidity, 12-hour light/dark cycle). They were housed in polypropylene cages with ad libitum access to commercial food, water, and bedding of paddy husk.
Rats were divided into four groups (three rats per group), with two groups receiving the test article at 300 mg/kg body weight and the other two groups at 2000 mg/kg body weight, via a single oral gavage. Observations were made at 30 minutes, 4 hours, 24 hours daily for 14 days, for recording physical changes if any. Rat body weights were recorded before administration of the test article. The body weights of the rats were recorded and intermittently on the day 7 and day 14 and the change in body weight were also recorded.

HISTOLOGICAL EXAMINATION

After 14^{th} day the organs (liver, kidneys, lungs, brain, spleen, ovary and uterus) of the rats were collected after the acute oral toxicity study and processed for histological examination to assess any tissue damage or abnormalities.

SHRIMP LETHALITY ASSAY

Artemia shrimp cysts (Osi Red Ring) were purchased from Keny's Discus Aquarium, Mumbai. The shrimp lethality assay was performed as proposed by Goh *et al.,* 2022 (14).

HEMOLYTIC ASSAY ON HUMAN AND CHICKEN ERYTHROCYTES AND CHICK EMBRYO CHORIO ALLANTOIC MEMBRANE (CAM) ASSAY

The micro-hemolytic assay method was used to assess the hemolytic activity on human and chicken erythrocytes and the CAM assay was tested against the crude extract of coral *Junceella delicata* as proposed by Purushottama *et al.,* 2009 (15).

NEUROMODULATORY ACTIVITY

The neuromodulatory activity of coral *Junceella delicata* on the rat brain was evaluated by conducting ATPase enzyme assays using the method of Green *et al.,* 1957 (16). Additionally, acetylcholinesterase (AChE) activity was assessed using the Ellman *et al.,* 1961 method (17).

ESTIMATION OF PROTEIN AND SDS-PAGE ELECTROPHORESIS

Estimation of protein was done by the Bradford method. The separation of protein was analyzed using SDS-PAGE electrophoresis.

RESULTS AND DISCUSSION

Table No. 1- Showing the effect of crude extract of coral *Junceeella delicata* on acute oral toxicity on female *Sprauge Dawley* rats.

Sample	Step	Dose (mg/kg)	No. of Treated Rats	Terminal Sacrificed	Found Dead
Crude extract of Coral *Junceella delicata*	1	300	03	03	0
Crude extract of Coral *Junceella delicata*	2	300	03	03	0
Crude extract of Coral *Junceella delicata*	3	2000	03	03	0
Crude extract of Coral *Junceella delicata*	4	2000	03	03	0
	TOTAL	-	**12**	**12**	**0**

Table No. 2 - Showing the food consumption, dosing period and body weight (gm) of female Sprague Dawley rats administered with crude extract of coral *Junceella delicata.*

1. Species	Rats
2. Strain	Sprague Dawley Rats
3. Source	APT Testing and Research Pvt Ltd, Pune
4. Age and Sex	Female, 06-08 weeks
5. Body weight range	150 to 200 gm
6. Identification	By unique identification number marked by writing on cage tag and by corresponding colour body markings.
7. No. of animals	Three animals were tested in per group.
8. Acclimatization	The rats were housed in their cages for five days prior to start of dosing in the experimental room after veterinary examination.
9. Environmental Conditions	Room temperature was maintained between 22 ± 3^0C, relative humidity 50-60 % and illumination cycle was set to 12 hours light and 12 hours dark.
10. Accommodation	Three rats per cage housed in polypropylene cages with stainless steel grill top, facilities for food and water bottle, and bedding of clean paddy husk.
11. Diet	Pelleted feed supplied by Supplier.
12. Water	Potable water passed through 'Aquaguard' water filter was provided ad libitum in plastic bottles with stainless steel sipper tubes.

Dose (mg/kg)		**0 Day**	**07 Day**	**14 Day**
G 1- 300 mg/kg	Mean	187.7	217.8	226.3
	SD ±	11.4	12.1	9.0
G 1- 300 mg/kg	Mean	207.7	242.8	257.3
	SD ±	15.0	16.9	17.8
G 2- 2000 mg/kg	Mean	215.0	231.3	230.8
	SD ±	13.9	11.0	6.2

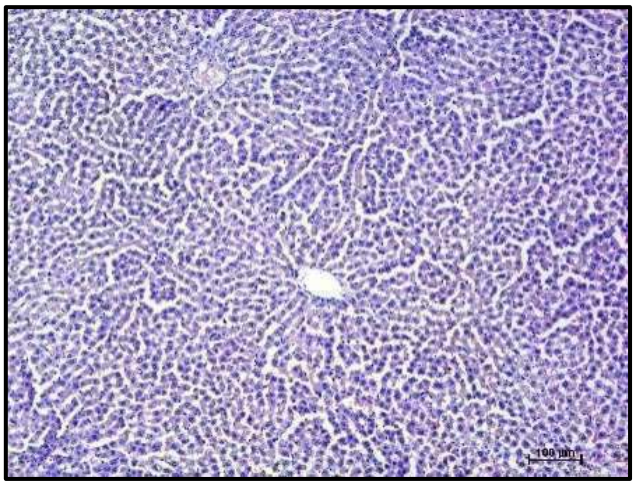
G 2- 2000 mg/kg	Mean	223.0	228.0	245.7
	SD ±	23.3	25.2	17.2

G1- Group 1

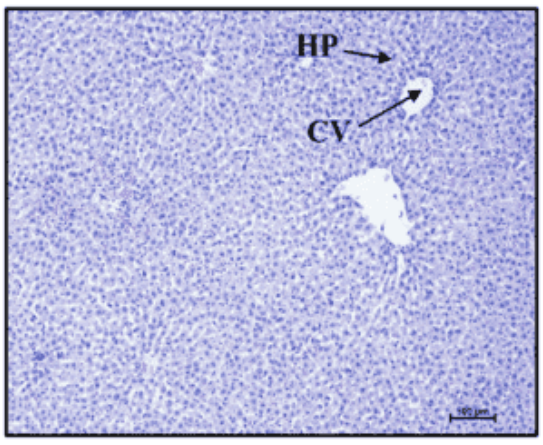
G2- Group 2

SD- Standard deviation

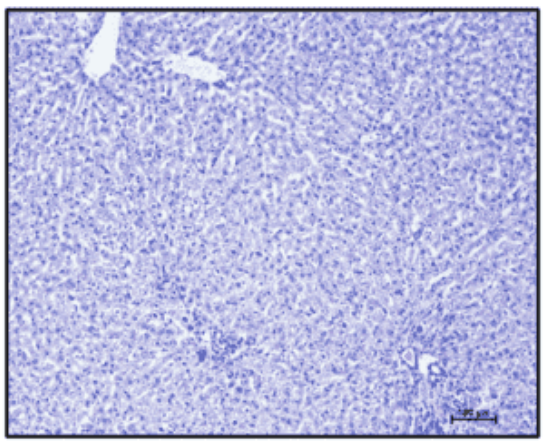
Photograph No. 1 – Showing histopathological observations of normal control, 300 mg/Kg and 2000 mg/Kg of the liver tissue of the female Sprague Dawley rats.



NC-Liver



300 mg/Kg

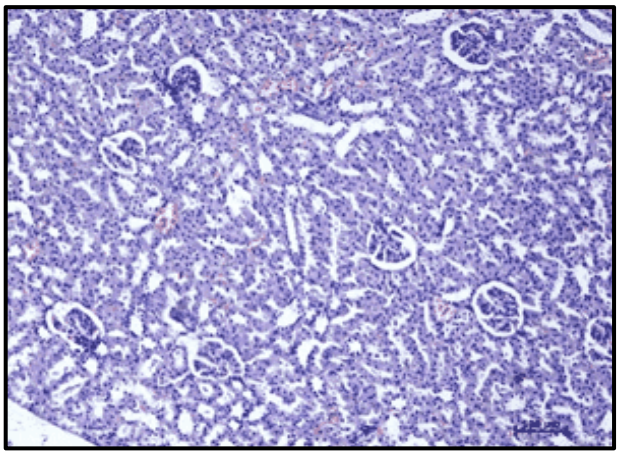


2000 mg/Kg

HP- Hepatocytes

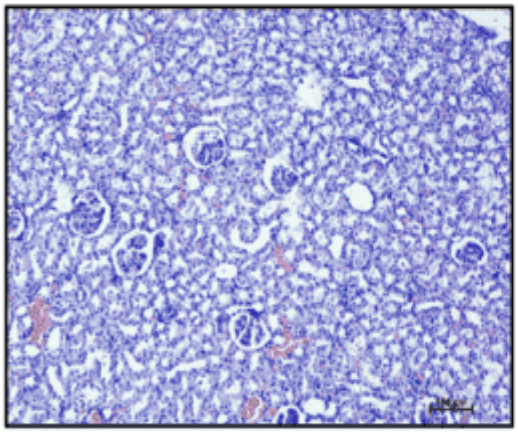
CV- Central vein

Photograph No. 2 – Showing histopathological observations of normal control, 300 mg/Kg and 2000 mg/Kg of the kidney tissue of the female Sprague Dawley rats.

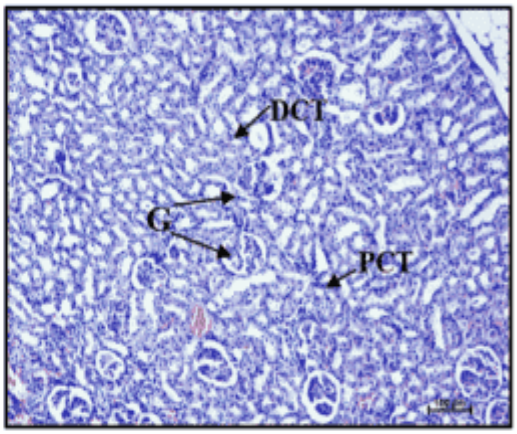


NC- Kidney

300 mg/Kg



2000 mg/Kg

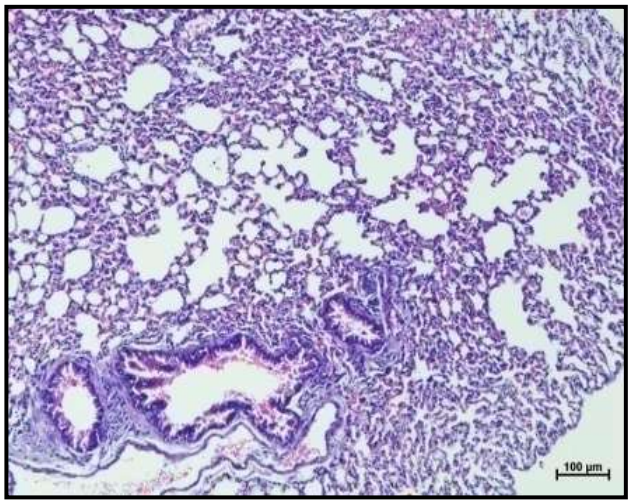


PCT- Proximal convoluted tubule

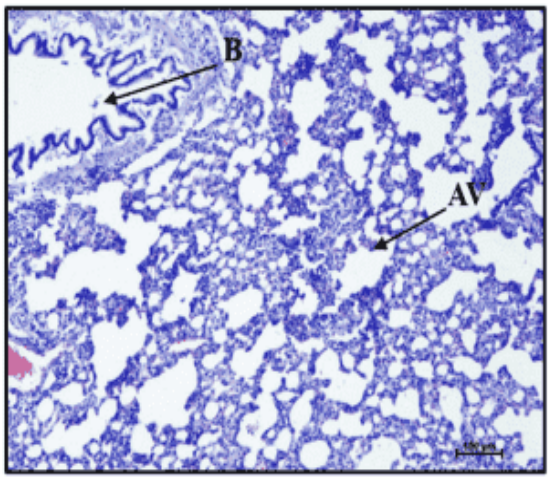
DCT- Distal convoluted tubule

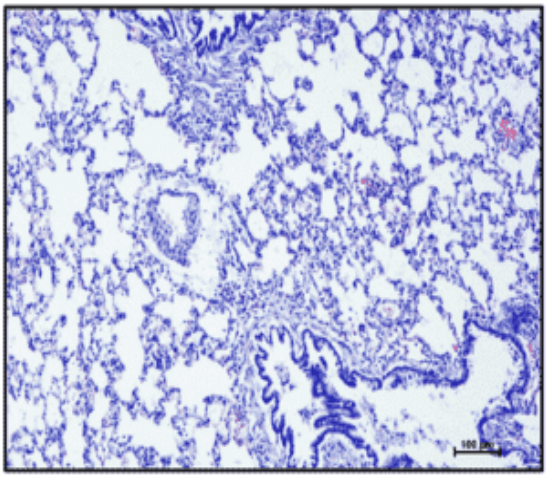
G- Glomerulus

Photograph No. 3– Showing histopathological observations of normal control, 300 mg/Kg and 2000 mg/Kg of the lung tissue of the female Sprague Dawley rats.



NC- Lung





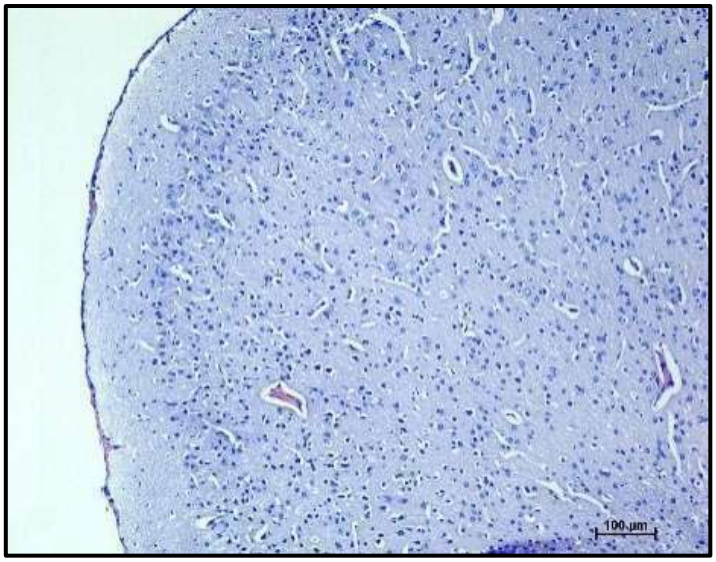
300 mg/Kg

2000 mg/Kg

B- Bronchiole

AV- Alveoli

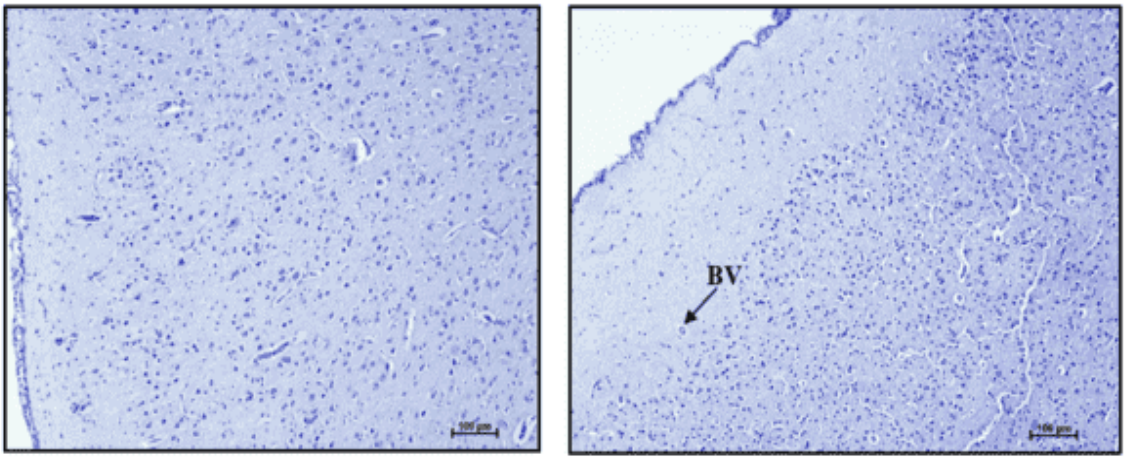
Photograph No. 4 – Showing histopathological observations of normal control, 300 mg/Kg and 2000 mg/Kg of the brain tissue of the female Sprague Dawley rats.



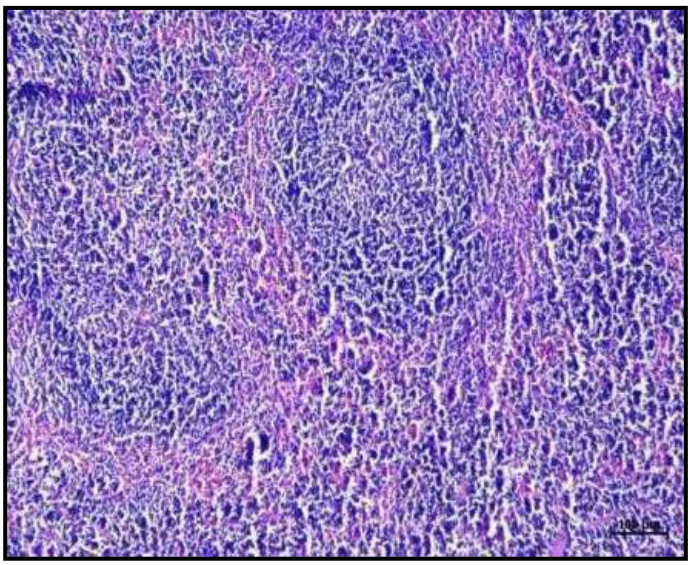
NC- Brain

300 mg/Kg 2000 mg/Kg

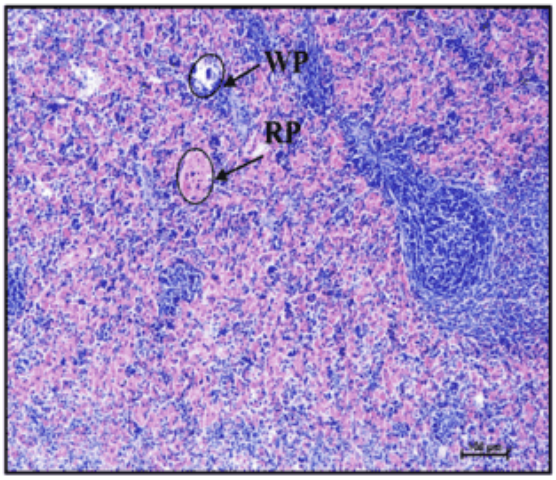
BV- Blood vessel



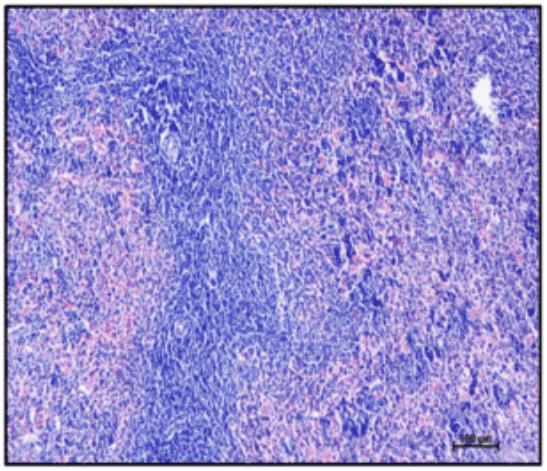
Photograph No. 5 – Showing histopathological observations of normal control, 300 mg/Kg and 2000 mg/Kg of the spleen tissue of the female Sprague Dawley rats.



NC-Spleen



300 mg/Kg

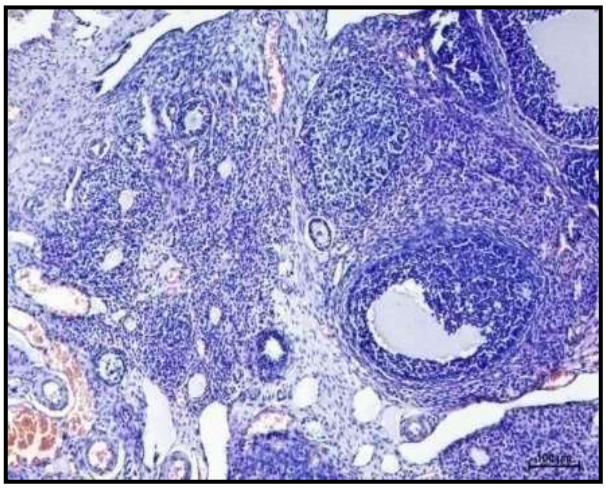


2000 mg/Kg

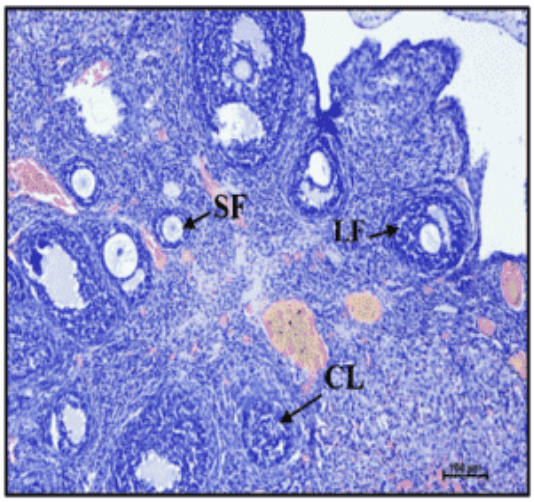
WP- White pulp

RP- Red pulp

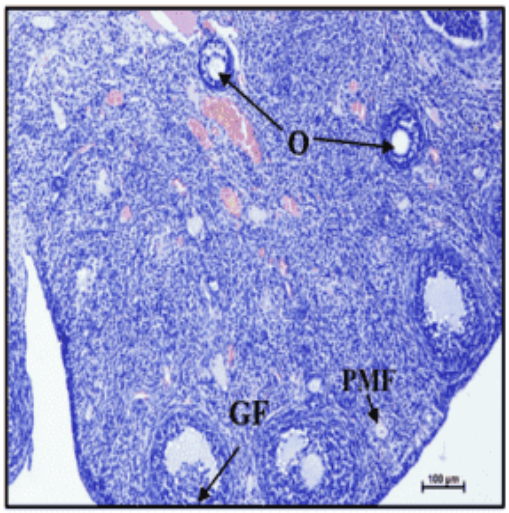
Photograph No. 6 – Showing histopathological observations of normal control, 300 mg/Kg and 2000 mg/Kg of the ovary tissue of the female Sprague Dawley rats.



NC-Ovary



300 mg/Kg



2000 mg/Kg

GF- Graafian follicle

O- Ova

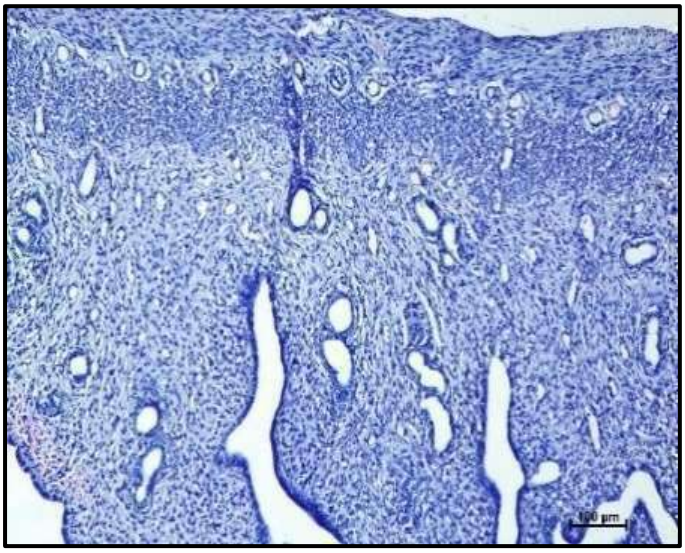
CL- Corpus luteum

PMF- Primordial follicle

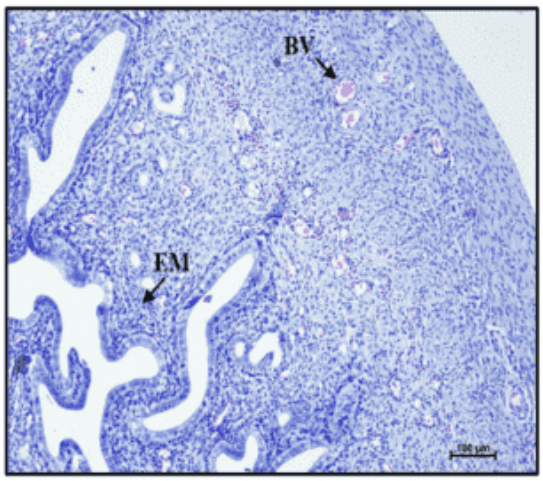
SF- Secondary follicle

LF- Large follicle

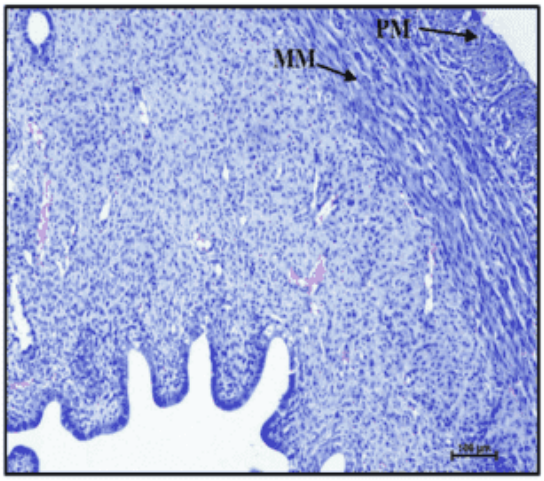
Photograph No. 7 – Showing histopathological observations of normal control, 300 mg/Kg and 2000 mg/Kg of the uterus tissue of the female Sprague Dawley rats.



NC- Uterus



300 mg/Kg



2000 mg/Kg

EM- Endometrium
MM- Myometrium

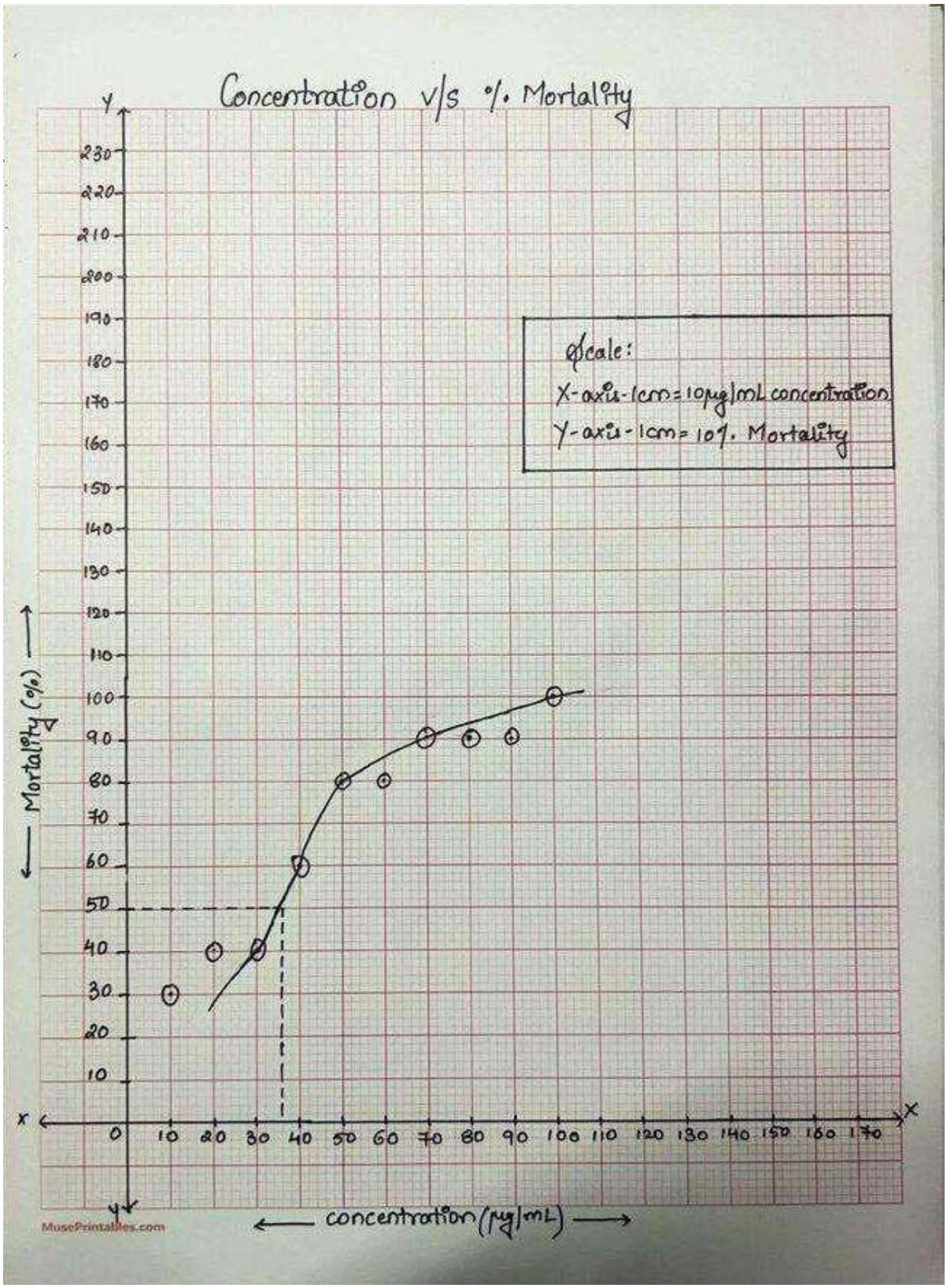
PM- Perimetrium
BV- Blood vessel

SHRIMP LETHALITY ASSAY

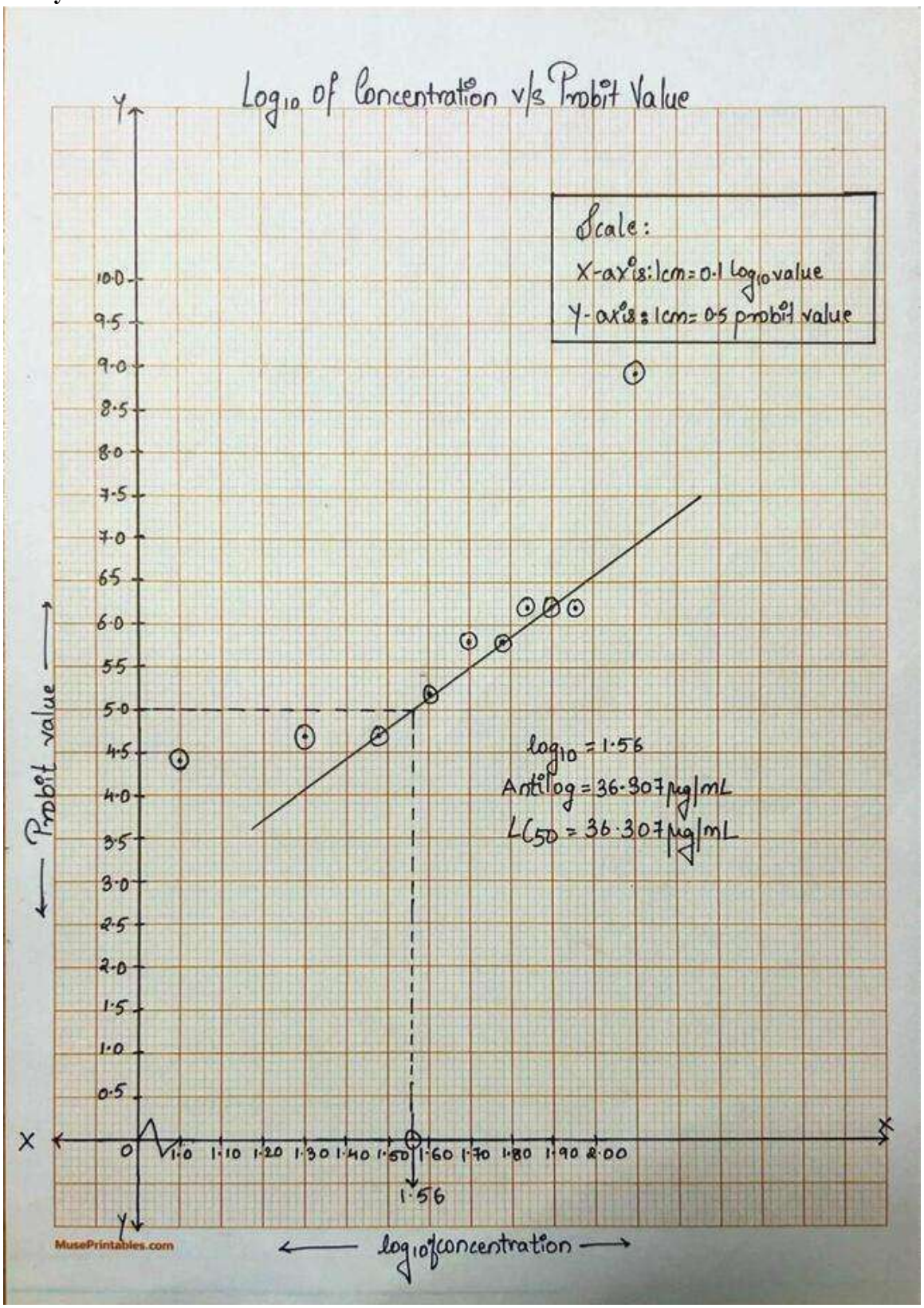
Table No. 3 - Showing the effect of crude extract of Coral *Junceella delicata* on *Artemia* Shrimp

Sr. No.	Concentration of sample (µg/mL)	Log_{10} of concentration	No. of live shrimp in each tube	No. of live shrimp	No. of dead shrimp	Mortality (%)	Probit value
1.	10	1.0000	10	07	03	30%	4.48
2.	20	1.3010	10	06	04	40%	4.75
3.	30	1.4771	10	06	04	40%	4.75
4.	40	1.6021	10	04	06	60%	5.25
5.	50	1.6990	10	02	08	80%	5.84
6.	60	1.7782	10	02	08	80%	5.84
7.	70	1.8451	10	01	09	90%	6.28
8.	80	1.9031	10	01	09	90%	6.28
9.	90	1.9542	10	01	09	90%	6.28
10.	100	2.0000	10	00	10	100%	8.96

Graph No. 1 - Showing the Concentration of crude extract of coral *Junceella delicata* v/s % Mortality in Shrimp Lethality Assay



Graph No. 2 - Showing Log_{10} of Concentration v/s Probit Value in Shrimp Lethality Assay



HEMOLYTIC ASSAY ON HUMAN AND CHICKEN ERYTHROCYTES

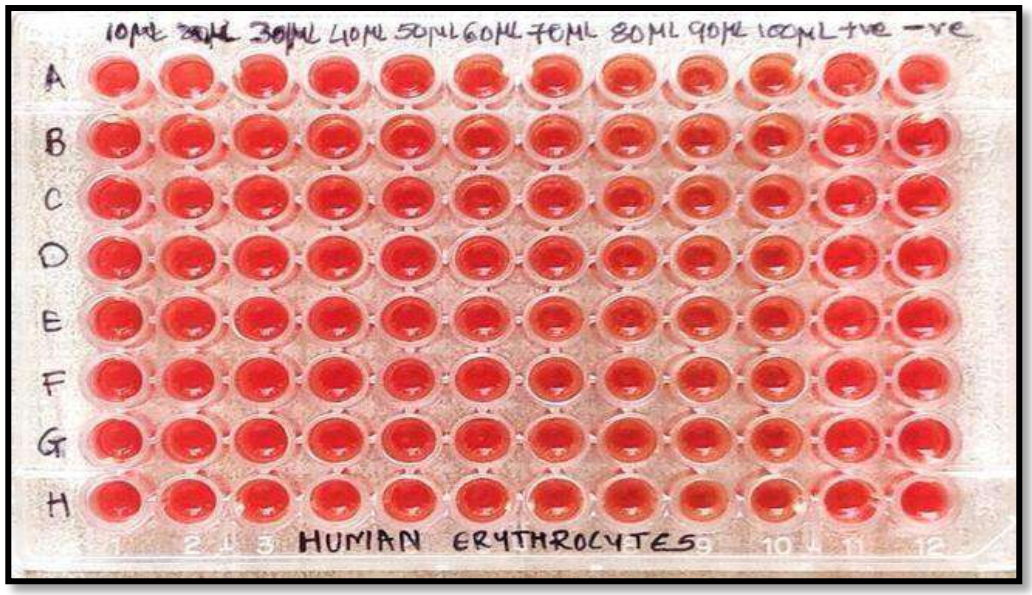
Table No. 4 – Showing the effect of crude organic extract of coral *Junceella delicata* on the hemolytic activity on human erythrocytes

Name of sample	Protein (mg)	Total hemolysis (up to dilution)	Hemolytic titre	Specific hemolytic activity (HT/mg)
Junceella delicata	0.75	50	32	42.67 HT/mg

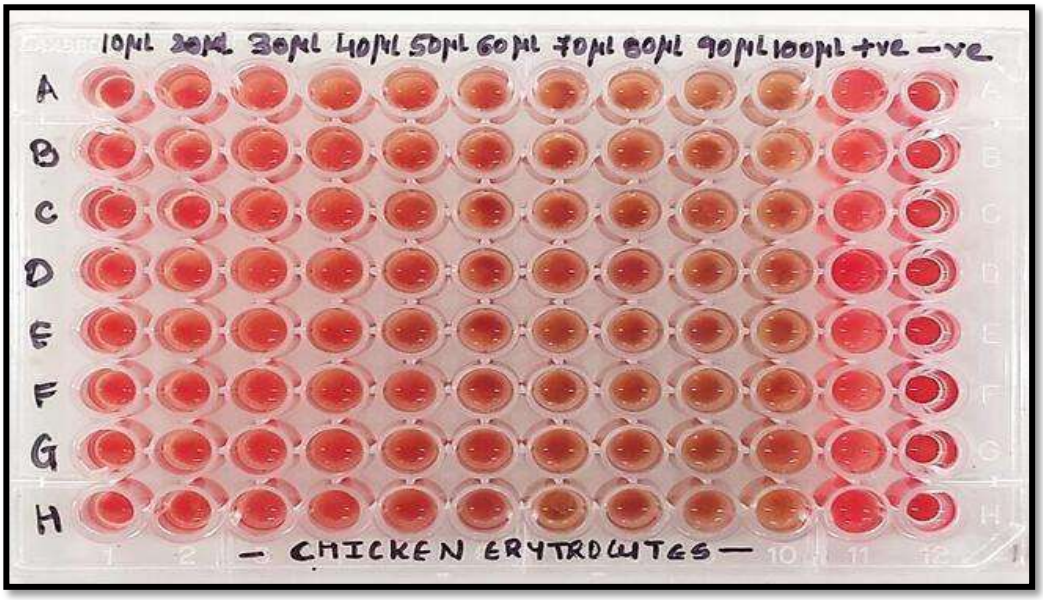
Table No. 5 - Showing the effect of crude organic extract of coral *Junceella delicata* on the hemolytic activity on chicken erythrocytes

Name of sample	Protein (mg)	Total hemolysis (up to dilution)	Hemolytic titre	Specific hemolytic activity (HT/mg)
Junceella delicata	0.75	60	64	85.33 HT/mg

Photograph No. 8 – Showing the effect of crude organic extract of coral *Junceella delicata* on the hemolytic activity on human erythrocytes



Photograph No. 9 – Showing the effect of crude organic extract of coral *Junceella delicata* on the hemolytic activity on chicken erythrocytes



+ve: Positive hemolysis -ve: Negative hemolysis

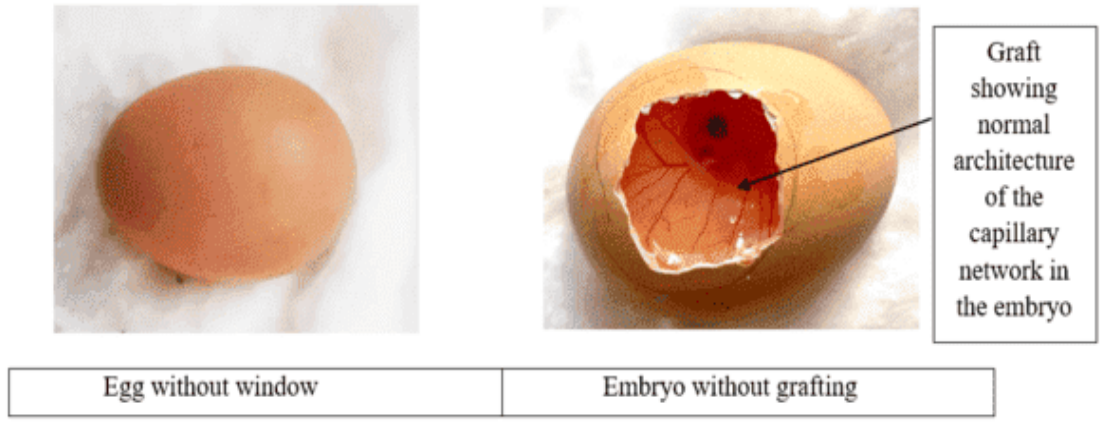
CHICK EMBRYO CHORIO ALLANTOIC MEMBRANE (CAM) ASSAY

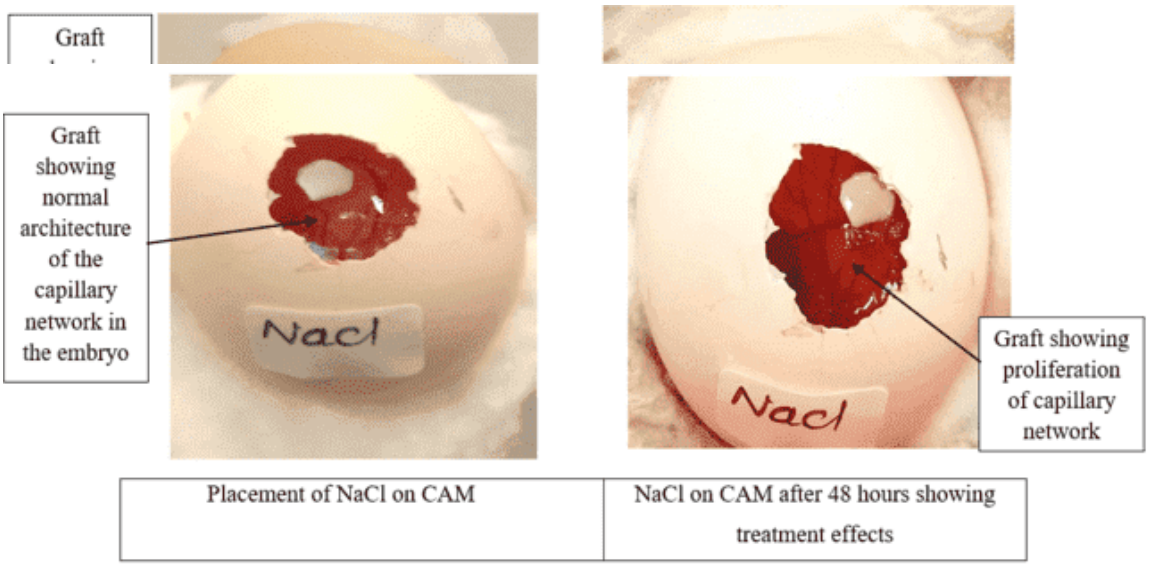
Table No. 6 - Showing the effects of various concentrations of crude extract of coral *Junceella delicata* on a 5-day-old egg in a CAM assay

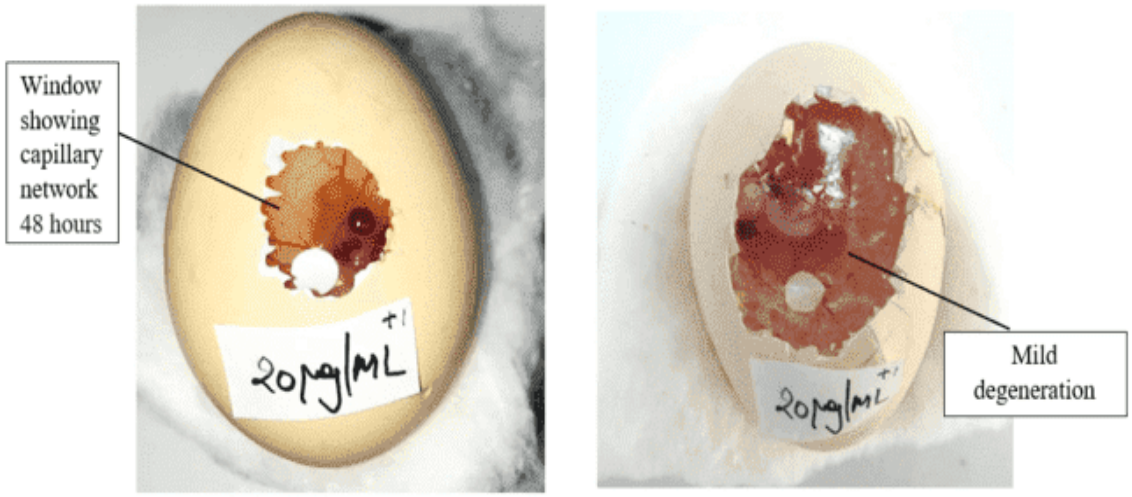
Concentration (µg/mL)	Levels
20 µg/mL	+
40 µg/mL	++
60 µg/mL	+++
80 µg/mL	+++

+: mild; ++: moderate; +++: severe.

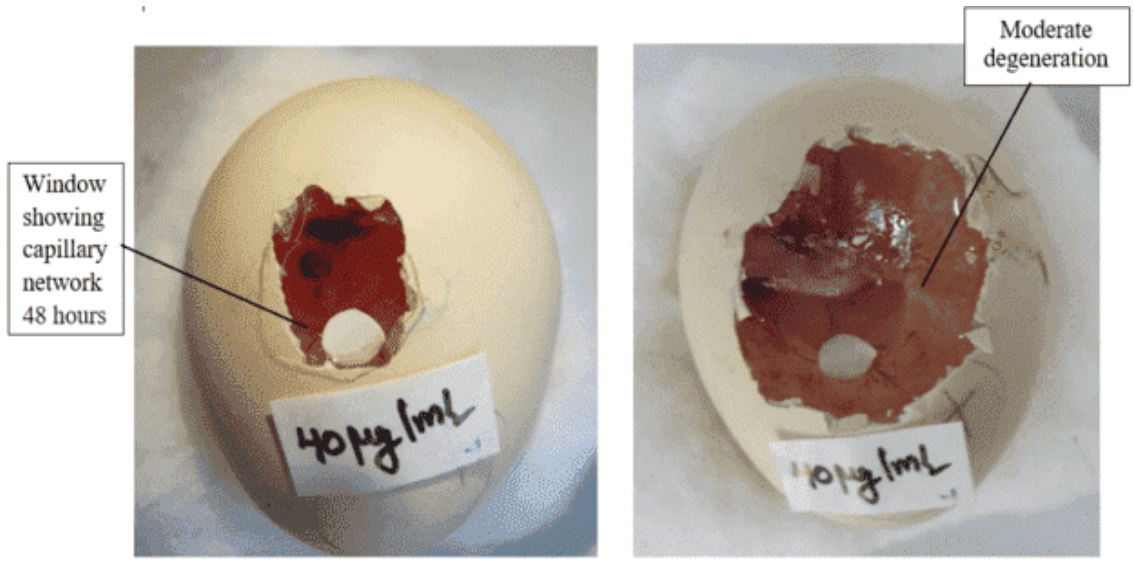
Photograph No. 10 – Showing the effect of crude extract of *Junceella delicata* on CAM



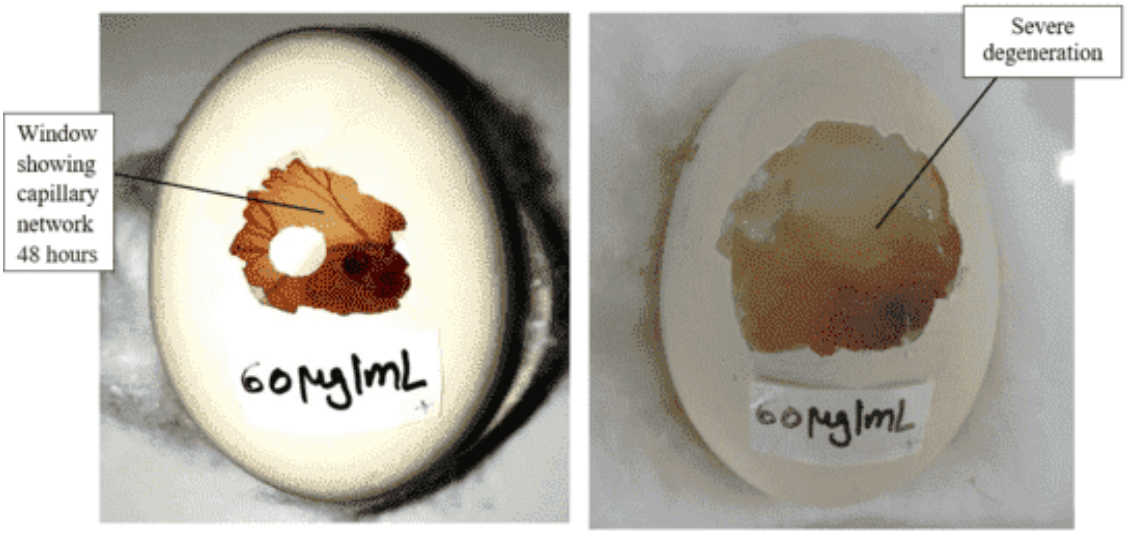




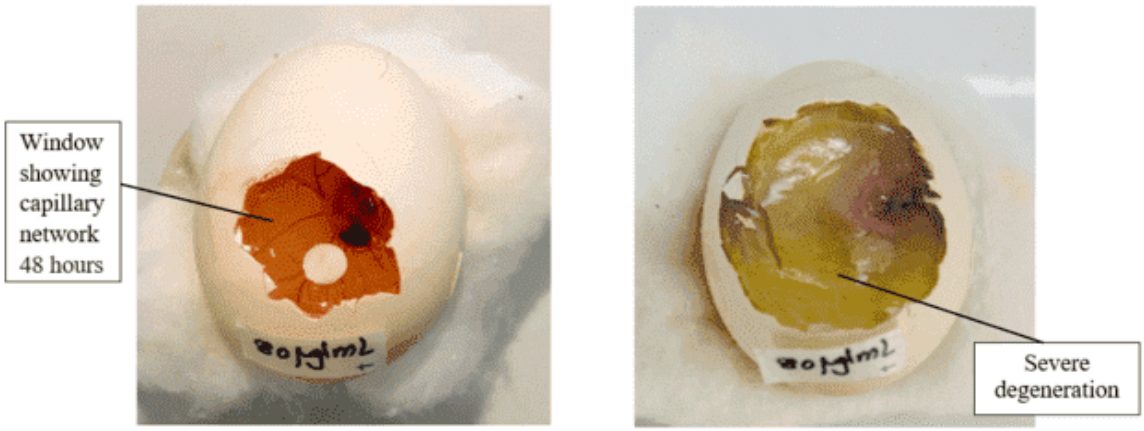
Placement of 20 µg/ml sample on CAM	20 µg/ml sample on CAM after 48 hours showing treatment effects



Placement of 40 µg/ml sample on CAM	40 µg/ml sample on CAM after 48 hours showing treatment effects



Placement of 60 µg/ml sample on CAM	60 µg/ml sample on CAM after 48 hours showing treatment effects



Placement of 80 µg/ml sample on CAM	80 µg/ml sample on CAM after 48 hours showing treatment effects

Table No. 7 - In-vitro effect of methanolic establish of coral *Junceella delicata* on rat (20±2g) brain Na+ -K+ ATPase activity.

Sr. No.	Concentration of Toxins (µg/mL)	Na^+-K^+ATPase activity (µPi/mg protein/hour) of Coral *Junceella delicata*
1.	10 µg/mL	0.096
2.	20 µg/mL	0.090
3.	30 µg/mL	0.085
4.	40 µg/mL	0.080
5.	50 µg/mL	0.075
6.	60 µg/mL	0.070
7.	70 µg/mL	0.068
8.	80 µg/mL	0.067
9.	90 µg/mL	0.066
10.	100 µg/mL	0.065

(All results are average of triplicate sets)

Table No. 8 - In-vitro effect of methanolic establish of coral *Junceella delicata* on the rat brain AchE activity.

Sr. No.	Concentration of Toxins (µg/mL)	Level of Modulation (%)
1.	50 µg/mL	9
2.	100 µg/mL	12
3.	150 µg/mL	18
4.	200 µg/mL	26
5.	500 µg/mL	30

(All results are average of triplicate sets)

Graph No. 3 - In-vitro effect of methanolic establish of coral *Junceella delicata* on rat (20±2g) brain Na^+-K^+ ATPase activity.

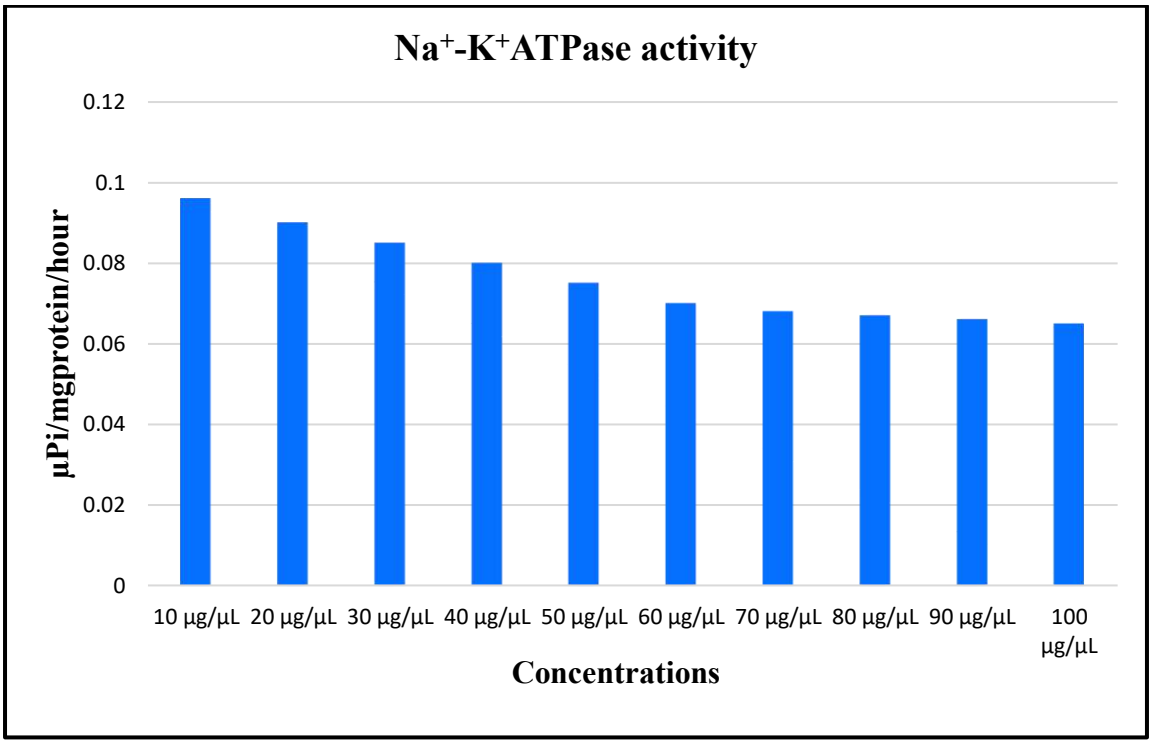
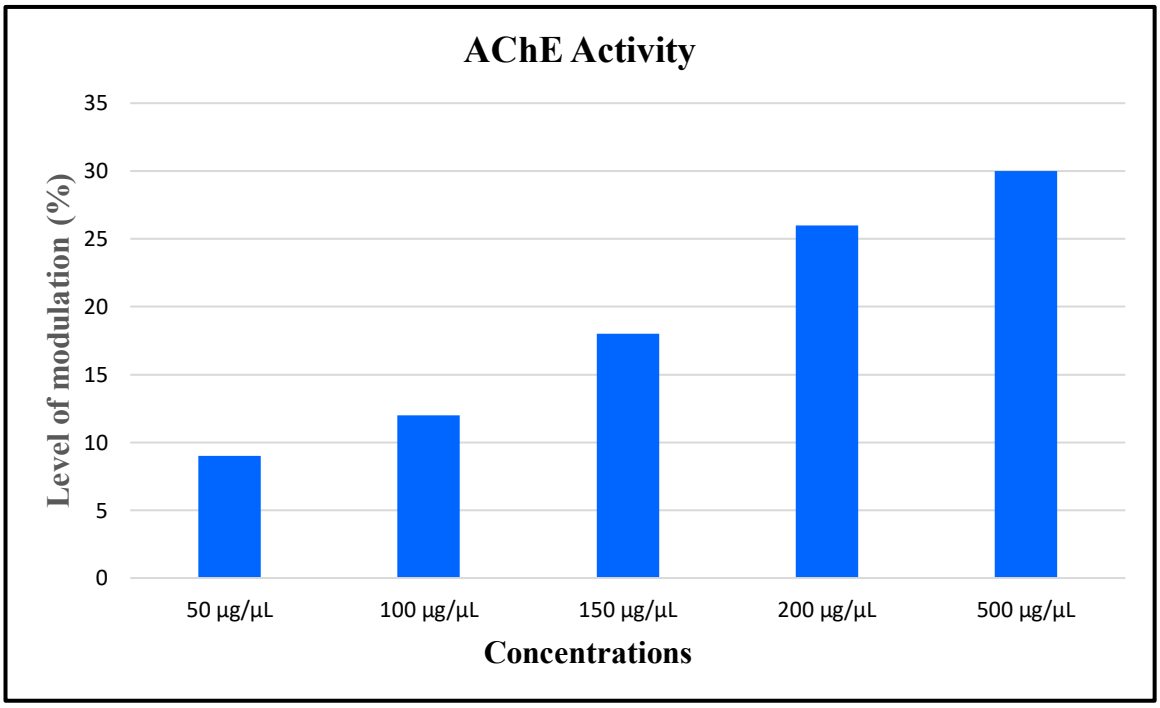
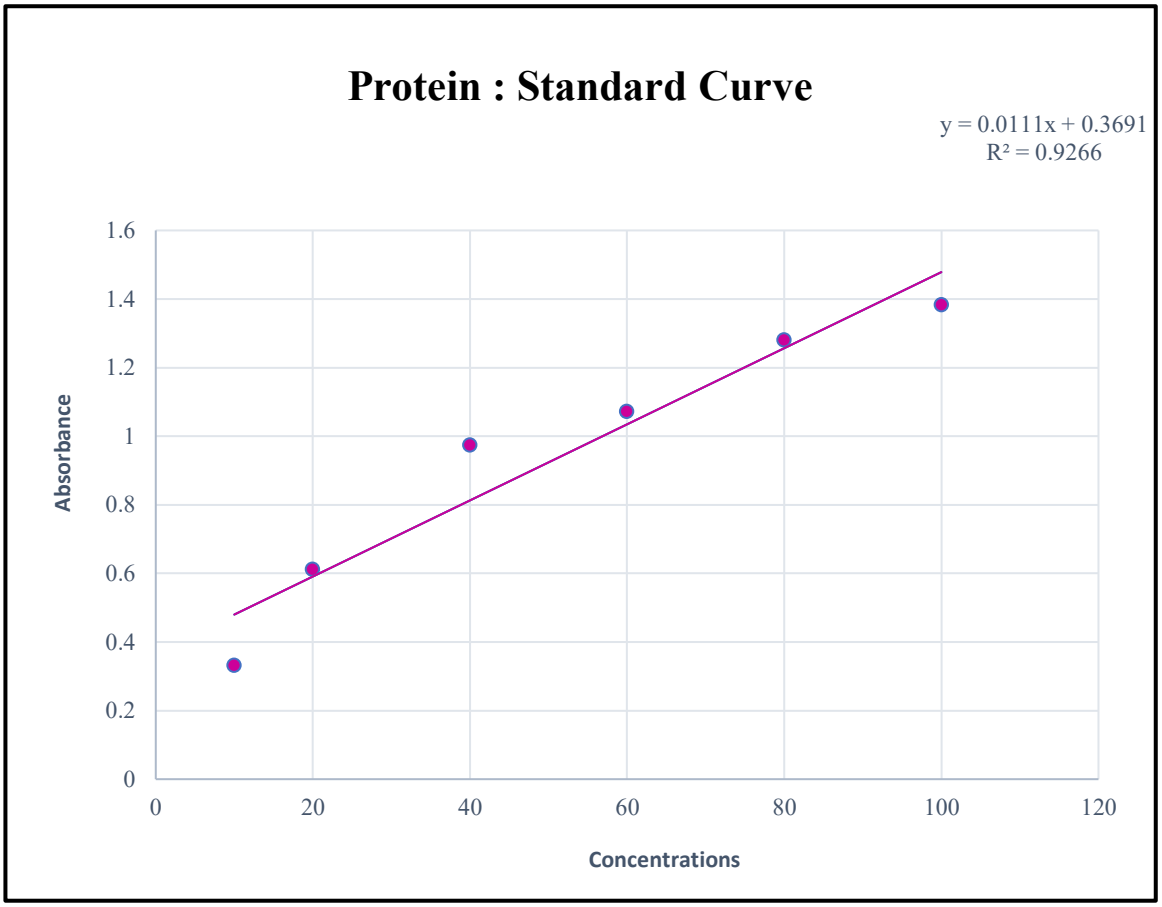


Table No. 4 - In-vitro effect of methanolic establish of coral *Junceella delicata* on the rat brain AchE activity.



ESTIMATION OF PROTEIN

Graph No. 5 - Standard Curve for Protein Estimation Using the Bradford Method



PARTIAL PURIFICATION OF CRUDE EXTRACT BY SDS-PAGE ELECTROPHORESIS

Photograph No. 11 - Showing the separation of proteins by SDS-PAGE gel electrophoresis.

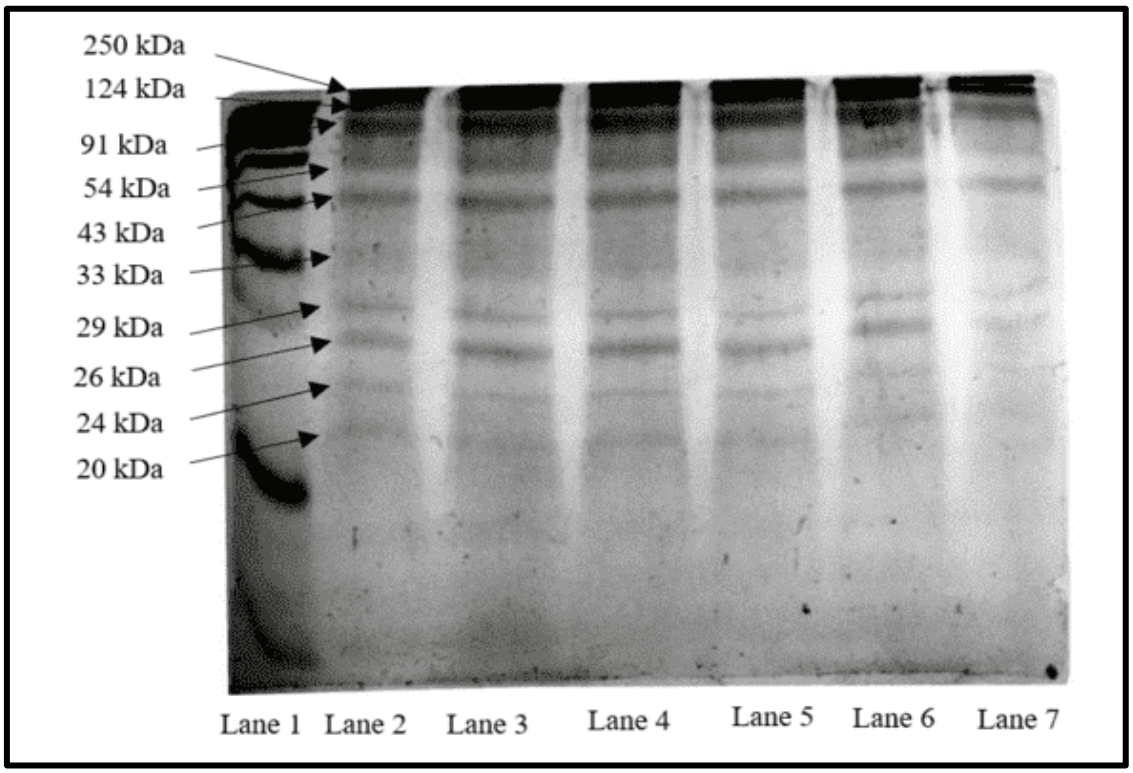


Table No. 9- Showing the different lanes of separation of proteins by SDS-PAGE

Lane No.	Volume loaded µL	Sample Id
Lane 1	6	Standard protein markers
Lane 2	30	*Junceella delicata*
Lane 3	30	*Junceella delicata*
Lane 4	40	*Junceella delicata*
Lane 5	40	*Junceella delicata*
Lane 6	60	*Junceella delicata*
Lane 7	60	*Junceella delicata*

DISCUSSION:

ACUTE ORAL TOXICITY

Bhadekar and Zodape *et al.* (2021) evaluated the LD_{50} value of marine sponges *Sigmadocia fibulata* and *Suberites carnosus* and found that the LD_{50} value of both the marine sponges is greater than 2000 mg/Kg (18,19). Hanafi *et al.* (2019) investigated the ethanolic extract of sea cucumber *Holothuria atra* for acute oral toxicity in mice. The acute toxicity results demonstrated that oral single administration of the crude extract at doses of 300 and 2000 mg/kg did not result in mortality or sudden death. Consequently, the LD_{50} of the ethanolic extract of the sea cucumber *Holothuria atra* is greater than 2000 mg/kg (20).

The acute oral toxicity of the crude extract of *Junceella delicata* was tested in female Sprague Dawley rats at doses of 300 mg/kg and 2000 mg/kg. No mortality or signs of intoxication were observed throughout the 14-day study period. There were no significant changes in skin, fur, eyes, mucous membranes, or behaviour. Additionally, no pathological alterations were found in any of the organs, confirming that the crude extract does not exhibit toxic effects at the given doses. Thus, the acute oral toxicity of the extract is higher than 2000 mg/kg body weight.

HISTOLOGICAL EXAMINATION

Garcia-Arredondo *et al.* (2015) carried out a study on the effect of aqueous extract of coral *Millepora complanata* against male Wistar rats for histopathological examination. The histopathological examination involves the microscopic examination of brain, liver, kidneys, and lungs tissues of mice which died within 24 hours after being injected with the *M. complanata* extract. The examination revealed severe damage to kidney and lung tissues, characterized by congestion, edema, and the presence of erythrocytes and proteinaceous material. However, no abnormalities were observed in the brain or liver. The histopathological changes mentioned were not observed when the heat-denatured extract was administered. The aqueous extract of the fire coral *Millepora complanata*, which contains cytolysins, primarily phospholipases A, induced histopathological damage to the kidneys and lungs. This damage

ultimately led to the gradual demise of the mice. Additionally, the extract comprises thermostable neurotoxins that trigger quick convulsions and fatality in mice (21). Sudharsan *et al.* (2013) carried out an effect of the methanolic crude extract and three fractions of sea anemone *Stichodactyla mertensii* against male albino mice for histopathological examination of brain, heart liver, kidney and lungs tissue. The crude extract of sea anemone *Stichodactyla mertensii* affected all the examined organs, including the brain, heart, liver, kidney, and lungs (22).

In this study, we investigated the histopathological effects of the organic crude extract of *Junceella delicata* on various organs (liver, kidneys, lungs, brain, spleen, ovary and uterus) in female Sprague Dawley rats, administered at doses of 300 mg/Kg (G1) and 2000 mg/Kg (G2). Table No. 2 presents food consumption, dosing period, and body weight data. The histopathological analysis (Photographs No. 1-7) revealed normal tissue structures in all organs at both doses. The liver showed intact hepatocytes, kidneys exhibited normal glomeruli and tubules, and the lungs displayed healthy bronchi and alveolar tissues. The brain showed no pathological changes, and the spleen maintained normal red and white pulp architecture. Ovarian tissues displayed developing follicles and corpus luteum, and uterine tissues showed normal mucosa and myometrium. These results confirm that the crude extract of *Junceella delicata* does not cause any adverse histological effects at doses of 300 mg/Kg and 2000 mg/Kg.

SHRIMP LETHALITY ASSAY

Widagdo and Susilaningsih (2008) carried out effect of LD_{50} on sponge *Haliclona sp* and gorgonian *Isis hippuris* against nauplius *Artemia salina*. The calculated LD_{50} value was found to be 17 ppm (23). Goh and Chou (1998) studied eight gorgonians (*Subergorgiidae, Subergorgia suberosa, S. mollis, Acabaria robusta, Ctenocella.Umbracella cf.umbraculum, C.Umbracella sp.A, Echinogorgia sp.C, Echinogorgia sp.E, Euplexaura cf. pinnata*) for their

acute and chronic toxicities against the *Artemia salina*. From the above study, they found that *S. mollis*, *Ctenocella* (*Umbracella*) *sp*., *Euplexaura cf. pinnata* and *Echinogorgia sp*. showed higher toxicity. The study found that higher concentrations led to higher death rates in *Artemia salina* (14).

The LC_{50} value of the organic crude extract of *Junceella delicata* was evaluated using the brine shrimp lethality assay (*Artemia salina*). The LC_{50} value was determined to be 36.307 µg/mL, indicating that as the concentration of the crude extract increased, the mortality rate of *Artemia salina* also increased. This result supports the presence of potent bioactive compounds in the crude extract, which could have pharmaceutical applications, particularly in drug development.

HEMOLYTIC ASSAY ON HUMAN AND CHICKEN ERYTHROCYTES

Bragadeeswaran and their colleagues studied the effect of organic extract epidermal mucus of fish *C. arel* and *A. caelatus* showed significant hemolytic activity on chicken and goat erythrocytes. They found that the 11 fractions of the crude extract of mucus of fish showed hemolytic activity in goat erythrocytes whereas, they noted that the chicken blood was more susceptible to lysis in fish *C. arel* muscle extract. The crude extract of *C. arel* showed maximum hemolysis in chicken blood whereas the maximum hemolysis was noted in goat blood against *A. caelatus.* Thus, it confirms that both the fish's epidermal extract showed hemolysis against goat and chicken erythrocytes (24). Similarly, Karthikayalu *et al.* (2010) explored the effects of the soft coral *Sarcophyton trocheliophorum* and observed its inhibitory activity against human erythrocytes on a blood agar plate (25).

In the present study, the organic extract of *Junceella delicata* exhibited hemolytic activity on both human and chicken erythrocytes (Table No. 4, Photograph No. 8, Table No. 5, and Photograph No. 9). Hemolysis was observed at 50 µL dilution for human erythrocytes, with a hemolytic titre of 32 at 0.75 mg protein concentration and a specific hemolytic activity

of 42.67 HT/mg. For chicken erythrocytes, total hemolysis occurred at 60 µL dilution, with a hemolytic titre of 64 and specific hemolytic activity of 85.33 HT/mg. These results indicate that the crude extract of *Junceella delicata* contains potent toxins that induce hemagglutination, lysis, and hemolysis in both human and chicken erythrocytes. Notably, chicken erythrocytes were more susceptible to lysis. Our findings are comparable to studies on *C. arel* and *A. caletus* (24). This is one of the few studies on hemolytic activity in Indian soft corals, supporting previous findings on different animal groups.

CHICK EMBRYO CHORIO ALLANTOIC MEMBRANE (CAM) ASSAY

Corals have been identified as a potential source of anti-angiogenic compounds, which may contribute to cancer treatment (26). According to Senthilkumar *et al.* (2016) and Quesada *et al.* (2014), marine organisms, including corals, contain a diverse array of bioactive compounds with unique chemical structures and mechanisms of action, making them promising candidates for anti-angiogenic therapies (27, 28). The anti-angiogenic compounds derived from corals showed inhibition in neovessel development, which inhibits tumour growth thus, it shows a unique approach to cancer treatment (29). The study carried out by Shaaban *et al.* (2020) in Egypt revealed two new hydroazulenes in the organic extract of marine soft coral *Sarcophyton glaucum*, along with eight recognized compounds in vitro showed promising activity against anti-cancer and anti-angiogenic properties (30).

In our study, the chorio-allantoic membrane (CAM) assay was used to assess the angiogenic properties of the crude extract of *Junceella delicata*. Fertilized eggs were treated with varying concentrations of the extract and incubated for 48 hours. Heparin (100 µg/mL) served as the negative control, and 0.9% NaCl as the positive control. The CAM assay results (Table No. 6, Photograph No. 10) showed that NaCl (positive control) maintained normal vasculature, while heparin inhibited new blood vessel formation. The crude extract caused hemolysis at 20 µg/mL, worsening at higher concentrations. At 40 µg/mL, hemolysis

increased, and at 60 µg/mL and 80 µg/mL, complete disintegration of blood vessels, hemorrhage, and coagulation were observed. These findings confirm the strong anti-angiogenic activity of the extract, with increasing hemolysis and inhibition of blood vessel formation at higher concentrations. The crude extract may be useful for treating conditions like tumor progression, atherosclerosis, psoriasis, arthritis, and diabetic retinopathy. Further structural analysis and pre-clinical studies are recommended to develop the extract into a therapeutic agent for cancer and other diseases.

NEUROMODULATORY ACTIVITY

The ATPase enzyme system is well-known for utilizing energy from ATP hydrolysis to vigorously carry Na+-K+ ions. The bile extracts from freshwater carps had the same neuroinhibitory effect on the Na^+ -K^+ ATP-ase enzyme system in mammalian models (31). Marine organisms have a potential source of AChE has not been thoroughly investigated. In the study on bioactive compounds from marine organisms, a total of eighty-nine organic extracts from various soft corals and sponge species were examined using an AChE inhibitory bioassay and bioautography technique. They found that, there were thirty soft coral extracts and three sponge extracts demonstrated inhibition of AChE (32, 33). The examination of AChE inhibitors in the marine organisms analyzed indicated that the extracts from soft corals have potential as a source of AChE. In particular, the extracts from *Eunicea* and *Pseudoplexaura* genera showed the highest level of activity. The most potent compound in the soft coral *P. porosa* was analyzed chemically, leading to the identification of 14-acetoxycrassine as an inhibitor of AChE (34).

In our present investigations, we observed the neuromodulatory activity on the Sprague Dawley rat brain, as shown in Table No. 7 and Graph No. 3, which shows the in vitro effect of the crude extract of the soft coral *Junceella delicata* on the Na+-K+ ATPase activity of the Sprague Dawley rat brain (20±2g). The average Na^+- K^+ ATPase activity of the organic crude

extract of soft coral *Junceella delicata* was 0.076 µPi/mg protein/hour in Sprague Dawley rat brain extract. It was found that the activity decreases as the concentration of the dose level increases. The maximum Na^+-K^+ ATPase activity was noted at 0.096 µPi/mg protein/hour at a dose of 10 µg/µL (100%). The organic crude extract of soft coral *Junceella delicata* showed the lowest Na^+-K^+ ATPase activity at 0.065 µPi/mg protein/hour at a dose of 100 µg/µL (67.71%). The decline suggests that while lower concentrations promote neuromodulatory activity, higher concentrations may impair these functions. Nevertheless, the initial increase in modulation at lower concentrations emphasizes the importance of compounds derived from corals like *Junceella delicata* in influencing neuronal signalling pathways, highlighting their relevance in understanding neuromodulation across species. The results indicate that the toxins derived from *Junceella delicata* show a significant inhibitory effect on Na^+-K^+ ATPase activity. This inhibition suggests that these compounds possess neuromodulatory properties, which could be beneficial for developing therapeutic strategies in conditions related to neuronal dysfunction.

Table No. 8 and Graph No. 4 shows in vitro effect of organic extract of soft coral *Junceella delicata* on the Sprague Dawley rat brain AChE activity. The maximum AChE activity was observed at a concentration of 500 µg/mL, corresponding to 30% modulation. The lowest AChE activity was found at 50 µg/mL, at a dose of 9% in the Sprague Dawley rat brain extract. This trend indicates that higher concentrations of the organic extract enhance AChE activity, suggesting its potential role in modulating neuronal function.

ESTIMATION OF PROTEIN AND SDS-PAGE ELECTROPHORESIS

The study on the crude extract of the marine sponge *Halichondria panicea* was done to determine its protein content. The crude protein was extracted using methanol, chloroform:methanol (2:1), and aqueous extracts. The protein concentrations in each crude extract were found to be 0.096 mg/mL, 0.192 mg/mL, and 0.124 mg/mL, respectively (15). A

study on the protein content in the crude extract of the soft coral *Sarcophyton trocheliophorum*. Their findings showed that the protein concentration in the extracted fraction was 0.46 mg/mL, while the fraction precipitated by ammonium sulfate had a protein concentration of 30 mg/mL (25).

Bhadekar and Zodape conducted a study on two marine sponges, *Suberites carnosus* and *Sigmadocia fibulata* using SDS-PAGE and they found approximately nine bands in both sponges. The protein bands identified in *Suberites carnosus* were 12 kDa, 16 kDa, 20 kDa, 25 kDa, 27 kDa, 42 kDa, 51 kDa, 57 kDa, and 59 kDa, whereas in *Sigmadocia fibulata* were 12 kDa, 16 kDa, 20 kDa, 22 kDa, 24 kDa, 35 kDa, 52 kDa, 57 kDa, and 62 kDa. The presence of proteins at 20 kDa and 22 kDa exhibited hemolytic activity. Additionally, it was confirmed that both sponges contain proteins at 24 kDa, confirming the presence of lectins in both species (18,19). Rahman *et al.* (2005) used SDS- PAGE and studied alcyonarian coral, *Synularia polydactyla* and their study showed the presence of proteins with molecular masses of 109, 83, 70, 63, 41, 30 and 22 kDa. Among these, the 83 and 63kDa proteins were found to be glycosylated, while the 109 and 63kDa proteins showed radioactivity as calcium-binding proteins (35).

The estimated protein concentration in *Junceella delicata* was found to be 750 µg/mL. In our study, SDS-PAGE analysis (Photograph No. 11 and Table No. 9) of *Junceella delicata* revealed ten protein bands with molecular weights ranging from 16 kDa to 250 kDa. These bands included 16 kDa, 20 kDa, 24 kDa, 26 kDa, 29 kDa, 33 kDa, 54 kDa, 91 kDa, 124 kDa, and 250 kDa. Our findings are consistent with literature cited above. The 20 kDa protein exhibited hemolytic activity, while the 24 kDa band confirmed the presence of lectins with haemagglutination properties. Proteins in the 30 kDa to 45 kDa range were identified as α-hemolysin (HlyA), a cytolysin produced by *E. coli*. The 54 kDa protein binds to actin, regulating its function, and the 91 kDa band showed TTX binding protein, which inhibits

sodium channels. The 124 kDa band corresponded to phytochrome, which binds liposomes without losing its photochromic properties. The 250 kDa protein exhibited hemolytic toxin activity. These findings confirm that *Junceella delicata* contains binding proteins with cytotoxic, hemolytic, haemagglutination, and neuromodulatory activities.

CONCLUSION

From the above results, we found that the LD_{50} value in the female Sprague Dawley rats is greater than 2000 mg/kg. However, the brine shrimp, *Artemia salina*, lethality assays showed LC_{50} calculated at 36.307 µg/mL. In histological examination of liver, kidney, lungs, brain, spleen, ovary and uterus of the female Sprague Dawley rats showed normal histo-architecture thus it confirms that the screened crude extract of coral *Junceella delicata* showed LD_{50} is greater than 2000 mg/kg in Sprague Dawley rats. We have tested the crude extract by performing experiments such as hemolytic activity, CAM assay, Neuromodulatory activity, and protein content to find the biomedical properties. Partial purification of the crude extract was carried out to screen the binding proteins by SDS-PAGE and the protein bands at different KDa were checked for its biological properties. From the above results it confirms that crude extract of coral *Junceella delicata* showed biomedical applications. It also confirms that the binding proteins showed wide applications in biomedical studies. These marine compounds demonstrate various strong biological effects related to pharmaceutical activities such as hemolytic, cytotoxic, anti-angiogenic and neuromodulatory activity. H.owever, further research is necessary to fully understand its mechanisms and potential of the drug in clinical studies.

ACKNOWLEDGEMENTS

The authors thank Dr. Swapnaja Mohite, Professor and Head, Department of Fisheries Biology, College of Fisheries, Shirgaon, Ratnagiri, for final species identification and confirmation. The thanks are also due to The Director, Principal Chief Conservator of Forest,

Nagpur and The Director, Maharashtra State Biodiversity Board, Nagpur, for permitting the collection of species. Authors would like to thank to, Director, APT Testing & Research Pvt. Ltd. (ATR) Pune, for providing the female Sprague dawley rats used for the experiment and approved by Ethical committee at APT Research Foundation, experimentation. Pune prior to the experimentation.

FUNDING SOURCE

Authors are thankful to Mahatma Jyotiba Phule Research & Training Institute (MJPRF), Nagpur, Govt. of Maharashtra, for their financial support and cooperation for the research.

CONFLICT OF INTEREST

Authors have no conflict of interest.

DATA AVAILABILITY STATEMENT

This statement does not apply to this article

ETHICAL STATEMENT

Principal Chief Conservator of Forest, Nagpur (Desk-22(8)/Res/CR-25(22-23)/1431/(22-23) and final approval was taken from the Maharashtra State Biodiversity Board, Nagpur (MSBB/Desk-5/825/2022-23) for collection of *J. delicata* samples.

VOUCHER SPECIMEN NUMBER

The voucher specimen *J. delicata* was submitted to the repository at the Zoological Survey of India, Western Regional Office, Pune India. (ZSI-WRC Misc/18).

INFORMED CONSENT STATEMENT

This study did not involve human participants, and therefore informed consent was not required.

CLINICAL TRIAL REGISTRATION

This research does not involve any clinical trials.

PERMISSION TO REPRODUCE MATERIAL FROM OTHER SOURCES

Not applicable.

AUTHORS CONTRIBUTIONS

Meenakshi Prakash Borate: data collection, analysis and writing. Prof. Dr. G.V. Zodape: Conceptualization and supervision.

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