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RESEARCH ARTICLE

FORMULATION AND EVALUATION OF GEL USING INK GLAND EXTRACT FROM INDIAN SQUID *LOLIGO DUVAUCELI*

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ABSTRACT

Marine organisms play a crucial role in almost all biogeochemical processes that sustain the biosphere, and provide a variety of products (goods) and functions (services) which are essential to humankind's well-being. The ink gland of *Loligo duvauceli* consist of melanin granules in a viscous colourless medium. As the ink gland extracts is inspiring new idea for drug development this study focuses on the formulation of gel using ink of Indian squid *Loligo duvauceli*. The antimicrobial activity of ink gland extract was examined using Agar well diffusion method against *Staphylococcus aureus, E.coli, Pseudomonas aeruginosa, Aspergillus niger, Mucor.sp, Rhizopus.sp and Microsporum gypseum*. For topical treatment of skin infections, a wide variety of vehicle ranging from solids, semisolids and liquid preparations is available to clinicians and patients. Within the major group of semisolid preparations, the use of transparent gels has expanded both in cosmetics and in pharmaceutical preparations. The gel was prepared using the dried ink gland extract from the Indian squid *Loligo duvauceli* and Carbapol-940(1%), propylene glycol 400, ethanol, methyl paraben, propyl paraben, EDTA, tri-ethanolamine and distilled water. The experiments was carriedout using ten (Each 5 for gel and cream) adult male Wister rats weighing about 150-200 kg to test for the skin irritation. The area on the back of each rat was shaved prior to the experiment. The shaved areas of skin of each rat was used for the topical application of developed gel.

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INTRODUCTION

In recent years, many bioactive compounds have been extracted, characterized and purified from various marine animals like bacteria, algae, dinoflagellates, tunicates, sponges, soft corals, bryozoans, cephalopods and echinoderms. Inking by the cephalopods has long been recognized as an adaptive response to predation and physical threat, by means of a combination of mechanism that include chemical deterrence, sensory disruption and phago- mimicry. Cephalopod inks are chemical secretions produced by and released from ink sac, which is a hypobranchial gland. This melanin pigment is manufactured in the mature cells of the ink gland, at the end of maturation they are found to secrete the ink into the ink sac. Skin is one of the most accessible organ of human body for topical administration and main route of topical drug delivery system. Number of medicated products is applied to the skin or mucous membrane that either enhances or restores a fundamental function of a skin or pharmacologically alters an action in the underlined tissues. Such products are referred as topical or dermatological products. Hydroxypropyl methylcellulose (HPMC), Carbapol 934p, Sodium alginate has been used as hydrophilic polymers topically in gel drug delivery system. A series of grades based on molecular fractions of these polymers are used at a concentration between 1 to 5% in topical gel formulation. The application of medicinal substance to the skin is a concept doubtless as humanity. In treating skin disease, the primary purpose of applying drug to the skin is to induce local effect at the site of application. In most of the cases, only a small portion of dose finally reaches the site of action, and produce limited local activity. This has been a complicated task due to the highly effective barrier properties of the skin.

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MATERIALS AND METHODS

Sample collection

Cephalopods

The samples were collected from Kasimedu, North Chennai. They were transported to the laboratory at 4˚C. With the help of sterile forceps the ink gland was carefully removed and the

ink was squeezed out gently into sterile brown containers and stored at 4˚C.

Extraction and Antimicrobial activity of squid ink

10 g of the ink was loaded in 6 sterile containers to this 30 ml of solvents such as methanol, ethanol, butanol, acetone, ethyl acetate, acetone were added and incubated at 4˚C for 7 days. After incubation the samples were centrifuged at 15000 rpm for 30minutes and supernatants were collected. This was dried in vacuum at 40˚C in Rota evaporator, the dried sample was suspended in measured volume of the solvents and used for testing. The antimicrobial activity of ink gland extract was examined using Agar well diffusion method against *Staphylococcus aureus, E.coli, Pseudomonas aeruginosa, Aspergillus niger, Mucor sp, Rhizopu sp and Microsporum gypseum*. Muller Hinton Agar plates were seeded with 0.1 ml of bacterial suspension equivalent to 10^7-10^8 CFU/ml. SDA Plates were seeded with fungal strain. The seeded plates were allowed to set and wells were made using well puncher. Each well was filled with 100µl of the extract and incubated for 24hrs at 37^0 C. The diameter of zone of inhibition were measured; ciprofloxacin and Amphotericin B were used as reference compounds for antibacterial and antifungal activities.

Formulation of topical gel

The gel was prepared using the dried ink gland extract from the Indian squid *Loligo duvauceli.* The gel was prepared using Carbapol-940(1%), propylene glycol 400, ethanol, methyl paraben, propyl paraben, EDTA, tri-ethanolamine and distilledwater in a quantity sufficient to prepare 100 g of gel incase of blank gel. Water required for theseformulations was divided in to two parts. In one part theexact amount of extract was dissolved and to thiscalculated quantity of propylene glycol 400 and ethanolwas added and in other part, carbapol-940 was dissolved and to this solution methylparaben, propylparaben and EDTAwas added. Both of these solutions were mixed in a beaker and tri-ethanolamine was addedto the mixture dropwise to obtain the gelconsistency. The same procedure was used for preparationof Diclofenac sodium gel as standard. Compositionof gel formulation is given in Table 1.

Experimental Animals

The experiments was carriedout using ten (Each 5 for gel and cream) adult male Wister rats weighing about 150-200 kg to test for the skin irritation. They were kept carefully following an acclimation period of 7 days to ensure their suitability for the study. Test animals were kept within a limited access rodent facility with environmental conditions set to a temperature of 25 ± 2º C, a humidity of 60-90% RH and a 12-h light / 12-h dark cycle. Animals were provided ad-labium access to a commercial rat and drinking water. The experimental protocol was approved by the Institutional Animal Ethics Committee (IAEC) and the care of laboratory animals was taken according to the guidelines of CPCSEA, Ministry of Forests and Environment, Government of India.

Preparation of Animals prior to testing

The area onthe back of each rat was shaved prior to the experiment. The shaved areas of skin of each rat were divided into two marked area. The two Markedarea of respective animals were used for the topical application of developed gel and gel of different concentration (1 and 1.5%) respectively while remaining marked area was considered as blank sample for testing the skin irritation as per the method of Driaze (Draize *et al*., 1944).

Application of gels

Half a gram of thegel and gel of ink gland extract respectively (1 and 1.5%), as the test substances, were applied to shaved area of approximately 6 cm^2 of skin of respective animals. Both the treated sites for gel and cream on respective rabbits were covered by gauze and the back of the rat was wrapped with a non-occlusive bandage. The animals were then returned to their cages. After 24 hours, the bandage and the test materials were removed and 1 hour later the sites were examined for skin irritation. Observation of the sites was done at 24 hours after application, and repeated at 48 and 72 hours thereafter. The reactions, defined as erythema and edema, were evaluated according to the scoring system for skin reactions (Table 2).

Evaluation methods

Evaluation is being performed to assess various parameters like homogeneity, loss of drying, PH, Viscosity, Spreadability. Results were recorded and tabulated (Table-3).

Appearance and Homogeneity

All developedgels were tested for physical appearance andhomogeneity by visualobservation.They were tested for their appearance with no lumps.

Test of loss on drying

Loss of drying was determined by placing gel in Petri dishes on water bath and dried for 1050 C.

$$\text{Percentage loss of drying} = \frac{W-w}{W} X100$$

Test of Ph

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The pH value of gelformulation was determined by using a pH meter.The measurement was performed at 1, 30 days after preparation to detect any pH changes withtime.

Test of Viscosity

The measurement of viscosity ofthe prepared gel was done withBrookfield viscometer (Model RVTDV II). The reading was taken at 100 rpm using spindle no.6.

Spread ability Test

The spread ability of thegelformulations was determined by measuring thespreading diameter of 1 g of gel between two horizontalplates (20cm X20cm) after one minute. The standard weight applied on the upper plate was 125g. Control marked area on animals were prepared in the same manner and 2% of the gel base, (without extract) and gel base (with extract) were applied to the control animals and observations were made similar to the test animals.

RESULTS AND DISCUSSION

The formulated gel was found to be homogenous with even distribution of all the ingredients. The formulation suits with the pH of the skin without causing any discomfort.

Table 1. Composition of ink gels

Formulation	Carbapol-940	Extract	Propyleneglycol	Ethanol	Methylparaben	Propylparaben	EDTA	Water
	(%)	(%)	(%)	(%)	(%)	(%)	(%)	(%)
Ink 2%	1	2	4	3	0.2	0.02	0.03	Up to100

Table 2. Classification system for skin reaction

Reactions	Score
Erythema	
No erythema	0
Very slight erythema	1
Well defined erythema	2
Moderate to severe erythema	3
Severe erythema to scar formation	4
Edema	
No edema	0
Very slight edema	1
Well defined edema	2
Moderate edema	3
Severe edema	4
Total possible score for primary irritation	8

Table 3. Evaluation parameters of prepared ink gel

Formulation	Ph	Appearance	Homogenecity	Spreading diameter after 1 min (mm)	Viscosity (cp)
gel	6.15	White	Good	55	4500
Ink gel	6.45	Black	Good	42	4700

Table 4. Dermal Observations

Animals	Reaction	24 Hours		72 hours	
		Intact	Abraded	Intact	Abraded
Rabbit No.1	Erythema	0	0	0	0
Only gel	Edema	0	0	0	0
Rabbit No.2	Erythema	0	0	0	0
Only gel	Edema	0	0	0	0
Rabbit No.3	Erythema	0	0	0	0
Only gel	Edema	0	0	0	0
Rabbit No.4	Erythema	0	0	0	0
Gel+ extract	Edema	0	0	0	0
Rabbit No.5	Erythema	0	0	0	0
Gel+ extract	Edema	0	0	0	0
Rabbit No.6	Erythema	0	0	0	0
Gel+ extract	Edema	0	0	0	0

Primary Irritation Test:0/6 = 0.00

Fig. 1. *Loligo duvauceli*

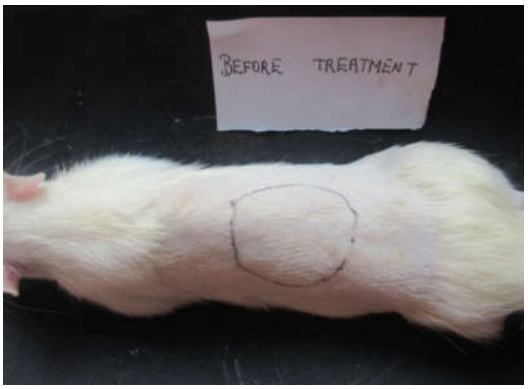

Fig.2. Before Treatment (48 hrs)

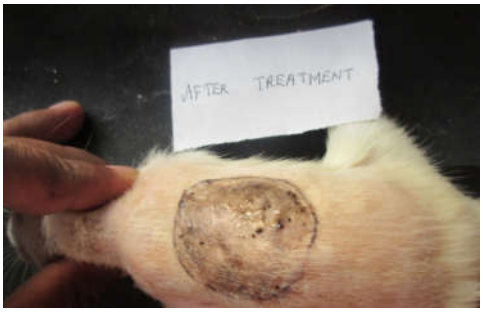

Fig. 3. After 48 hrs Treatment with Ink gel

Antimicrobial activity was demonstrated effectively by the gel. There have not been any changes in the pH, viscosity with the 3 months of the study. The Score of Primary Irritation (SPI) was calculated for each rat as the following. Scores for erythema and edema at 24, 48 and 72 hours were summed and divided by the number of the observations for the treated sites. The Primary irritation index of the test article was calculate to be 0.00; No irritation was observed on the skin of the rabbits. Individual results of dermal scoring appear in Table (4).

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