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PHARMACOLOGICAL ACTIVITIES OF CEPHALOPOD INK

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ABSTRACT

Cephalopod is any member of molluscan class. It include squid, octopus, and nautilus. Molluscan are small group of highly advanced and organised marine animals. Main characteristics of this organism is their inking behaviour. In between the gills they bear an ink sac. Octopuses produce black ink, squid ink is blue-black colour and cuttlefish ink is a shade of brown. Dark coloured ink have melanin as a major constituent. Cephalopod ink have many medicinal value. The ink is toxic to tumour cells. So it have the anti-tumour activity. The activity is tested against fibrosarcoma. And also it have antihypertensive, anti-inflammatory, antiretroviral, antiulcer genic properties.

INTRODUCTION

Marine animals have a wide variety of action in healing a disease. Cephalopod belongs to the animal kingdom. In the recent years, any bioactive compounds have been extracted, characterised, and purified from various marine animals like bacteria, algae, dinoflagellates, tunicates, sponges, soft corals, bryozoans, echinoderms, and cephalopods. Cephalopods are in the molluscan class. The word Mollusca come from Latin word ‘mollus’ meaning soft. So in this class soft bodied animals are included. But they have an outer hard shell made up of calcium carbonate. Cephalopod evolved reduced, internalized, shells or to entirely losing the shells. Sub classes of cephalopoda are coeleoidea, nautiloidea, orthocertoidea, ammonoidea etc.

Nautiloidea have external shells but in Coeloidea, the shell is either considerably internal or lost. Coeloide represent around 700 species. Among the invertebrates cephalopod possess a well-defined liver, 7-12% of body weight is contributed by this. 6-40% oil content in their body. Squid and Cuttle fish liver is rich source of n3 polyunsaturated fatty acids like eicosapentaenoic acid (EPA) and Docosahexaenoic acid (DHA). Cuttle fish liver oil contained 38.3% saturated fatty acids, 17.4% polyunsaturated fatty acids and 15.6% monounsaturated fatty acids. Among the polyunsaturated fatty acids, omega3 PUFAs were 7.6% and omega6 PUFAs were 1.5%.

Cuttlefish liver oil have the capability to stimulate the immune functions; and also inhibit inflammatory response and platelet aggregation in rats. Antiatherogenic activity decreases the cholesterol level in blood. Antioxidant property of fish oil scavenging the free radicals. The melanin isolated from ink is the great source of standard for natural Eumelanin. The ink contain dopamine and L-Dopa it produce various physiological effects in our body. Presence of special antioxidant in ink prevent rapid degradation of metabolites, melanin granules absorb dopamine in ink and prevent excessive dilution after ejaculation, thus ensuring efficient interaction with target organs in dopamine-mediated inter and intraspecies communication. occurence of large amount of Tyrosinase in for the predator. Various reports suggest the biosynthsesis, localization and fate of catecholamine in the ink gland of cuttlefish.

It contain taurine, aspartic acid, glutamic acid, alanine, and lysine.so it possess phagomimetic properties and used for chemical defence.[1]

Common applications of cephalopod ink

Cytoprotective action of Squid ink extract

Currently, chemotherapy remains the basic therapy for the treatment of tumors. As a general chemotherapeutic method, Cyclophosphamide is an important clinical drug. The drug can kill tumour cells and help patients heal, but on the other hand, it is known to kill healthy cells in many tissues and organs. In this work, we investigate the effects of cyclophosphamide on haematopoietic function, including the spleen organ index and antioxidant capacity, bone marrow nuclear cells and peripheral blood profile.

As part of the haemopoietic system, the spleen participates in the mechanism of regulation of bone marrow hemopoiesis and in the elimination of red blood cells and platelets. Body index and antioxidant capacity reflect your health. In this study, we found that cyclophosphamide

significantly reduced the organ index and antioxidant parameters of the spleen, that is, cyclophosphamide caused damage to this organ. A decrease in the number of nuclear cells in the bone marrow as a haemopoietic organ also indicates that chemotherapy may affect the haematopoietic function of the bone marrow. Other evidence of cyclophosphamide-induced haemopoietic bone marrow damage is a change in the profile of peripheral blood. This study showed that cyclophosphamide strictly regulated the levels of erythrocytes, leukocytes and haemoglobin, but had no effect on platelets. From the previous data, it can be concluded that the hemopoietic function of mice is effectively affected by cyclophosphamide, so that a balanced haematopoietic system in the clinical treatment of a tumour can adversely affect chemotherapy and, consequently, the development of cellular protective drugs is necessary and urgent.[2]

Anti-Parkinson potential of *holathuria scabra*

Marine invertebrates have wide-range of medicinal values. Sea cucumber is seafood an organism used as a traditional food, and a medicine that promotes longevity and mental acuity. *Holothuria scabra* is a sea cucumber that is mostly a Indian Pacific region, including Thailand. Sea cucumber have antioxidant, anti-tumor, antiviral, anti-inflammatory, antibacterial and anti-bacterial antifungal activities. Therefore, in this study *H. scabra* extracts had Parkinson's activity. PD is a complicated neurodegenerative disease of which The mechanism of pathogenesis could not be easily antioxidant in the ink can prevent the rapid oxidation of L-dopa.[3]

Antioxidant and antimicrobial activities of squid ink powder

Squid ink powder are collected and analyse the protein content from it, high amount of protein found in that where the majority of it composed with myofibril protein (80%), followed by myoplastic protein (12-20%) and the least was myostroma protein (2-3%) which composed of collagen . The ability of the protein to function as an ingredient in food products depends on their functional properties such as amino acid composition and environmental factors including pH, temperature and ions present in it. The value of fat content in squid ink powder is slightly higher compared with raw squid which was about 1.0 to 2.0%, where the value considered lowest among all types of seafood. Seafood contains various amounts of minerals due to minerals concentration in seawater contribute to it via food chain generally. The mineral content of squid and other seafood varies according to their species, environment, habitat, and maturity. Squid ink did possess antioxidant activity. separation of

melanin from squid ink did not abolish the antioxidant properties of squid ink where it tested with several assays including DPPH assay. The radical scavenging activity of melanin free squid ink was 179.6 ± 2.1%. Squid ink polysaccharide also possessed antioxidant activity from the scavenging ability of the sample with DPPH radicals.

Water extraction of squid ink powder have the highest value in total antioxidant activity DPPH assay when compared with ethanol extraction and hexane extraction. Polarity changes of solvent have a significant role in affecting the antioxidant activity. The solvents used in experimental procedures as well as combination and concentration in extraction process influenced the type, yield, and potency of the antioxidant compounds in the cephalopod ink.

Majority of antioxidant content has been found in more polar solvents. This analysis, showed that there is a significant difference at $P < 0.05$ between the three solvent extracts of squid ink powder. These solvents with different polarity were used to extract the antioxidant compound from squid ink powder. From the results, water extraction has the highest value compared to ethanol and hexane extracts. Thus, it showed that the polar solvent has a high affinity in extracting out the antioxidant compound from the sample.

Gram positive and Gram negative bacteria showed susceptibility towards different extracts of squid ink powder, where the inhibition zone ranging from 7 mm to 8.5 mm for distilled water extract and from 8.5 mm to 15 mm for ethanol extract. The inhibition zone for positive control, gentamicin was ranged from 17.5 mm to 28 mm. Antimicrobial activity had been reported from various parts of cephalopod. Squid (*Loligo duvauceli*) showed antimicrobial activity on *E. coli* and *S. aureus* from its accessory nidamental gland with inhibition range of 7 mm. Fresh squid ink extract of *Loligo duvauceli* did present antimicrobial properties on *E. coli* with inhibition range was 11 mm . The result recorded from this study showed that water extract of squid ink powder, as well as ethanol extract, presented inhibition zone on all bacteria used in this analysis. Ethanol extract of squid ink also showed antimicrobial activity from selected bacteria while hexane extract also reported exhibiting the same properties.[4]

Therapeutic significance of *loligo vulgaris* ink extract

Exact composition of the crude squid ink was studied and found to have protein as the major component over lipid and carbohydrates. Bioactive fractions of squid ink were extracted with ethanol, and therapeutic applications such as haemolytic, antioxidant, antimicrobial, and *in vitro* anti-inflammatory properties were analysed using standard methods.

In haemolytic assay, the squid ink extract exhibited a maximum haemolytic activity of 128 haemolytic unit against tested erythrocytes. In DPPH assay, the ethanolic extract of squid ink has exhibited an antioxidant activity of 83.5%. The squid ink was found to be potent antibacterial agent against the pathogens tested. 200 µL of *L. vulgaris* ink extract showed remarkable antibacterial activity as zone of inhibition against *Escherichia coli* (28 mm), *Klebsiella pneumoniae* (22mm), *Pseudomonas aeruginosa* (21mm), and *Staphylococcus aureus* (24 mm). The 68.9% inhibition of protein denaturation by the squid ink extract indicated that it has very good *in vitro* anti-inflammatory properties. The Fourier transform infrared spectroscopy analysis of the ethanolic extracts of the squid ink indicated the presence of functional groups such as 1° and 2° amines, amides, alkynes (terminal), alkenes, aldehydes, nitriles, alkanes, aliphatic amines, carboxylic acids, and alkyl halides, which complements the biochemical background of therapeutic application.

Hence, results of this study point out that the ethanolic extract of *L. vulgaris* has many therapeutic applications such as antimicrobial, antioxidant, and anti-inflammatory activities.[5]

Drugs

Cephalopod ink used to modulate human health and treat various ailments. Medicinal effects is not only in ink, but also other tissues of cephalopods. Many health benefits of cephalopod ink used as a traditional medicine, both in Western culture (ancient Greece and Rome) and Eastern culture (China). More recently, cephalopod ink has been used in an attempt to develop new drugs, through the search for new natural compounds with beneficial health effects. This is an especially active field in Asia, where cephalopods are a major fishery catch, for which ink sacs are a bi-product and where homeopathic medicine has deep roots. It should be noted that the ink used in drug discovery is not always fresh, often being taken from dead animals, sometimes from preserved animals. Homogenized chemically digested ink sacs are often used as the source material. Therefore, caution must be used in assuming that compounds identified through this process of drug discovery might also be present in naturally released ink and used by cephalopods in their natural environment. Furthermore, since such drug discovery involves intellectual property with commercial applications, identified bioactive compounds may not be reported. Consequently, published work in this field tends toward phenomenology in which effects are identified, but underlying molecules or mechanisms are not.

Antimicrobial properties

Cephalopod ink possess antimicrobial properties against a diversity of organisms, including human pathogens. Antimicrobial activity is found in different extracts of ink, including aqueous and organic solvents, and in the melanin fraction.[6,12]

Potential anticancer properties

Based on *in vitro* studies of various cell types and cell lines determined the potential anticancer property of cephalopod ink. The effect is often through the induction of apoptosis and is often associated with different chemicals in ink.

Apoptotic effect is due to the production of dopaquinone, which is known to interact with nucleophiles to produce protein-bound DOPA through a 5-*S*-cysteinyldopa residue, which, in turn, can oxidatively damage cellular molecules.

Peptidoglycans from squid and cuttlefish ink can have anti-tumour effect of ink is due to the presence of Peptidoglycans. Mechanisms underlying the effects of cephalopod peptidoglycans may include fragmentation of DNA and apoptosis, perhaps resulting in the inhibition of embryonic development.

Sepiella maindroni ink polysaccharide (SIP), derived by enzymatic digestion of the peptidoglycans, when treated with chlorosulfonic acid, yields a sulphated SIP, called SIP-SII, having a sulphate content of ~35%. SIP-SII has anti-cancer activity, which may result from several of its properties: (1) suppression of the invasion and migration of carcinoma cells via inhibition of matrix metalloproteinase-2. (2) Suppression of melanoma metastasis via inhibition of tumour adhesion mediated by intercellular adhesion molecule 1; and (3) inhibition of angiogenesis mediated by basic fibroblast growth factor.

Sepia ink oligopeptide (SIO), extracted from enzymatically digested ink sacs, also has anti-cancer properties. Its mode of action in prostate cancer cells is by induction of apoptosis via activation of caspase-3 and elevation of the ratio of Bax/Bcl-2 protein.[7,11]

Hematopoietic effects

Cuttlefish ink modulate immune responses by affecting haematopoiesis. It promotes the proliferation and differentiation of granulocyte-monocyte progenitor cells.

Anti-Hypertensive actions

An angiotensin-converting enzyme purified from squid ink causes dilation of blood vessels, resulting in lower blood pressure. This represents a potential treatment of hypertension. The bioactivity can be traced to peptide derivative of ~294 Da.

Anti-Retroviral activity

Ink isolated from *Loligo duvauceli* and *Sepiella inermis* has been determined an anti-retroviral activity.

Potential Anti-Ulcerogenic actions

Ink from squid and octopus inhibits gastric secretion of rats and, thus, has potential in the development of anti-Ulcerogenic drugs. The active fraction contains an unidentified low molecular weight melanoprotein that might be responsible for the activity, by enhancing the glycoprotein activity in the gastric mucosa.

Anti-Inflammatory activity

Anti-inflammatory activity of squid ink determined these fraction also inhibit gastric secretions.

Anti-Oxidant activity

Melanin and melanin-free fractions of squid ink possess anti-oxidant activity. This anti-oxidant activity may be related to some of the other effects such as anti-cancer effects, as well as its photo protective effects.[8]

Anti-bacterial activity

Cephalod ink have anti-bacterial property against various pathogens. *K. pneumoniae* (17mm) in *Sepia brevimana* extract, extracts of *Octopus cyaneus* exhibited medium zone of inhibition against *E. coli* and *V. cholera* (15mm) respectively and *Sepia brevimana* extract showed activity against E. coli, *V. cholerae* and *S. aureus* (13 mm). The least inhibition zone of 12mm, 11mm was observed against *E.coli* and *S. aureus* respectively with extracts of *Sepioteuthis lessoniana*. 100µl concentration showed the highest zone of inhibition against *K. pneumoniae* (19mm) in *S. brevimana* extract. *O. cyaneus* extract showed significant activity against *E. coli* and *V. cholera* and moderate activity was observed against *K. pneumoniae* and S. aureus (15mm). *S. brevimana* extract exhibited moderate activity against *E. coli* and *V. cholerae* (15mm). Gentamicin was used as positive control and methanol was used as

negative control in this evaluation. The antibacterial potential evaluation showed that methanolic extract from different cephalopods possessed antibacterial activity towards all five pathogenic strains with two concentrations 50µl and 100 µl.[9]

Presence of amine oxidase in cephalopod ink

Amine oxidase occurs in all species of vertebrates, echinoderms and molluscs also contain the presence of the enzyme. Sepia oficinalis has determined the highest proportion of this enzyme. Liver of species is the major site of production. The enzyme acts upon many sympathomimetic amines, The part which the enzyme takes in the in vivo inactivation of adrenaline and noradrenaline, the two substances of this group which are most active in vertebrates, and which are known to be substrates of the enzyme *in-vitro*. The occurrence of tyramine in the posterior salivary glands of Octopus macropus is determined earlier. Tyramine is one of the amines most rapidly oxidized by amine oxidase. The amine has since been found in the posterior salivary glands of 0. Vulgaris where, in addition to tyramine, another monophenolic sympathomimetic amine has been described; this compound is called octopamine.

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