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# A Review - Biology, Aquaculture and Medical Use of Seahorse, *Hippocampus* spp

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### Authors' contributions

This work was carried out in collaboration between all authors. Authors YYZ and ZJQ conceived and designed the study. Author YYZ wrote the first draft of the manuscript. Author BMR managed the literature searches. Authors BMR and ZJQ reviewed the first manuscript and wrote the final manuscript. All authors read and approved the final manuscript.

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## ABSTRACT

Seahorse has been used as medicine in Asian countries such as China, Korea, Japan and Vietnam for thousands of years. However, in western countries, the ecology of seahorse has been a focus of attention of many researchers for years. The seahorse aquaculture is popular with aqua farm worldwide for the sake of the increased demand of seahorse. This review described the biology, aquaculture and medical use of seahorse. To present the ecology and highlight the role of seahorse in traditional medicine and biomedical properties, this article based on the information collected from scientific literatures from Elsevier, Wiley, Science direct and CNKI.

**Keywords:** *Hippocampus*; biology; aquaculture; medical use.

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## 1. INTRODUCTION

Seahorse (genus *Hippocampus*), a marine teleost fish, is in the family *Syngnathidae*, which also includes pipefish, pipehorse and seadragon [1]. In the past, people were more interested in their specialized history traits, such as male pregnancy, monogamy, and so on. However, some studies indicated social polygamy still exist in some species [2]. In China, seahorse is one of the rare traditional medicines for its medical use to cure infertility, baldness, asthma and arthritis [3].

According to the CITES Trade Database, there were 70 countries or areas as sources and/or Consumers of seahorses and the annual volume of traded seahorses averaged 7 million individuals. Hong Kong SAR, the top consumer area of seahorses, imported an average of 57% of the total traded volume [4]. A number of fishers profited from catching seahorses for use in ornamental display, curios and traditional medicine [5]. Seahorse life-history characteristics (e.g. low population densities) may tend to make them overexploitation, therefore, all of them are included on CITES Appendix II [6]. In recent years, seahorse aquaculture has been regarded as an important strategy to alleviate the pressure on the wild stock of seahorses and one supplementary solution to substitute wild-caught seahorses to satisfy global demand [7].

This review extracts and integrates available information to provide detailed and comprehensive introduction on ecology and aquaculture of seahorses and medical use of seahorse powder.

## 2. BIOLOGY

### 2.1 Morphology

All seahorses have parallel morphology: a horse-like head is perpendicular to its body; a pair of medium sized-eyes is rolled independently; a long snout has no teeth; trunk is stretched over a series of bony rings. Adult seahorses have no pelvic and caudal fins, and retain one propulsive dorsal fin, two small ear-like pectoral fins and a reduced anal fin [8,9].

### 2.2 Distribution and Species

After the convergence of the African and Eurasian plates, which was to blame for closure

of the Tethyan seaway, the world's tropical biomass can be divided into Atlantic Ocean biome (including the Caribbean and Mediterranean) and Indo-Pacific biome. Seahorses occupy both of the temperate and tropical shallow sea waters distributing from 50 degree north to 50 degree south. So far, there have been 32 species of seahorses recognized and 70% of them are existing in Indo-Pacific [10]. It is difficult to speculate the origin time and place for seahorse accurately due to the lack of reliable seahorse fossils, but some scholars suggest that seahorse would stem from pre-Tethyan [11]. Teske [10] used sequence data derived from four markers (the nuclear RP1 and Aldolase and the mitochondrial 16S rRNA and cytochrome b genes) to determine the phylogenetic relationships among 32 species.

### 2.3 Habits

#### 2.3.1 Inhabit and movement

Seahorses are usually found in coral reefs, mangroves, macroalgae and seaweeds, but certain are accustomed to living in sandy estuary and lagoons [12] and they tend to twist their prehensile tails round the seaweeds when they are inactive. Dorsal fin, as a propeller, plays a pivotal role in movement while their pectoral fins function as rudder, both them facilitate seahorses to move. However, the tail is inoperative actually [9].

#### 2.3.2 Feeding

Seahorses are visual ambush predators, using a technique known as the 'pivot' feeding, which involves rapid movement to overcome prey escape capabilities [13]. They don't have any teeth so they just suck the foods up through the snouts with "cluck cluck". Researchers investigated the diet of *H. guttulatus* and *H. hippocampus* in the Aegean Sea. What they found were that the diets of these seahorses were dominated by amphipoda, anomur, decapoda together with mysidacea, and females possibly had a higher feeding activity than males. Because they are susceptible to macrobenthic fauna, seahorses are prone to forage during daytime [14].

#### 2.3.3 Reproduction

Pregnancy, a privilege for female, is defined as a period during which developing embryos are incubated in the body after egg-sperm union. But

when it comes to seahorse, pregnancy becomes different: the female injects her eggs into the male's pouch, a brooding structure, and then spermatids also enter the pouch. Such fertilization method can reduce sperm movement and shrink the lengthy zygotic migrate to the site of implantation. In fact, a zygote likes a sphere, which is beneficial to exchange of ion and gas around it. At the first few days, the pouch is small, while it will expand with the development of the embryos [15]. The male's pouch releases juveniles after 8-20 day's incubation, and juvenile seahorses are free-living and no further parental care is provided any more [16].

### 3. AQUACULTURE OF THE SEAHORSE

Seahorse once was regarded as a small-scale aquaculture for its low catches and economic value. A large number of seahorses were captured since 1980s as a result of excessive demand for Asian market, increased international trading volume and improvement of trawling technology, which led to the fact that all species of seahorse were listed on Appendix II of CITES or short for Convention for the International Trade in Endangered Species of Wild Fauna and Flora in November 2002 with implementation in May 2004 [17]. The efforts to culture seahorses commercially were not made until 1970s especially in southern China where the seahorses were first produced by captured-born in 1957 at Shantou. However, the farmers did not take a good knowledge of biology and breeding technology of seahorse. So these seahorse farms closed eventually [18]. Recently, an increasing number of experimental works have been conducted to investigate the impact of a series of parameters, including light, salinity, diet and temperature on survival, growth rate and reproduction. These efforts would provide theoretical supports and promote the development of commercial seahorse culturing.

#### 3.1 Light

Light, an essential parameter in rearing seahorse, is exerting an influence on feeding, survival and growth rate, sexual maturity, spawning and endocrine hormone levels [19]. Wong [20] thought there were no significant difference among seahorses (*H. whitei* Bleeker, 1855) in various lights except Gonadosomatic index (GSI). Whereas, a study revealed that light could impact all indexes in seahorse growth markedly. Lin [21] cultured seahorses (*H. erectus* Perry, 1810) with different lights and he found that the juveniles

cultured in 500, 1000 and 1500 lx had a high survival rates and low air-bubble disease rates. The final wet weight and standard length of the juvenile *H. erectus* were different at various lights. In addition, background and substrate color also have effect on seahorse skin coloration. For instance, seahorses (*H. trimaculatus* Leach, 1814) in blue background had the highest coloration rate compared to the others. When cultured with four different substrate colors (yellow, green, red and the mixture, respectively) for 5 days, 77.8% of the seahorses with the yellow substrate changed their skin color to yellow ( $P=0.007$ ), and all seahorses at the red state turned into yellow or yellow-black [22]. Pawar [23] used tanks in green, blue, red, transparent, black and yellow to feed pelagic phase seahorses *H. kuda* (Bleeker 1852). Growth and survival rate were higher in the dark backgrounds than in the light; ingestion of the air-bubble occurred more often in light backgrounds. Martinez [24] studied whether the tank color (transport, white, red, orange, yellow, green, blue and black) can cause difference among tested seahorses (*H. abdominalis*). The results indicated that all seahorses appeared to be able to survive and grow in every tanks. And the researchers needed further study to estimate the interactions of tank color and light intensity, retinal structure of the eye.

#### 3.2 Salinity

Numerous studies reported that salinity has an effect on fish development and growth by regulating osmosensitivity which is related to survival rate, spawning and fertilizing, standard metabolic rate, food intake, food conversion efficiency and hormone stimulation, especially for some sensitive seahorses. Seahorse can live in a wide spectrum of salinity ranging from 8 to 35‰, and the most favorable salinity for juveniles is about 12‰ [25]. Cardenas [26] investigated the salinity tolerance and survival of seahorses (*H. abdominalis*) in different salinities through transferring seahorses from 32‰ to 5, 10, 15, 20, 25, and 32‰. As a consequence, seahorse in 5‰ sank in the tank bottom instead of swimming or feeding and the mortality would up to 100% within 72 h. The survival rates of seahorses cultured in 10 and 15‰ salinities were superior to those cultured at 25 and 32‰ salinities, which can be related to the fact that *H. abdominalis* is considered as pelagic during the first month of life. The optimum salinity for *H. reidi* was 10-25‰. All tested *H. reidi* were died in 0‰ after 10 h while those reared in 10-15‰ possessed the

maximum wet weight and survival rate [27]. Hilomen-Garcia [28] evaluated survival, growth, and total body water content after 4 and 18 days of 9-week-old *H. kuda* cultured in 0-85‰ transferred from salinity of 32-33‰. *H. kuda* could survive neither in 0‰ nor 55‰. The upper limit of *H. kuda* salinity tolerance was 50‰. Although 10‰ cultured seahorses had higher growth and survival compared with the control (30‰ seawater), total body water had a significant advance (10‰). Therefore, like majority fish, *H. kuda* are accustomed to seawater salinities of 15 and 20‰. The growth of *H. erectus* is bound up to salinity. When the salinity was 31-33‰, *H. erectus* had the greatest wet weight and survival rate compared with others [21]. Salinity changes may have a significant effect on numbers of juveniles, which peaked at 10‰ while decreased at higher salinities, rather than mating behavior of *H. kuda*. In addition, the diverse salinities also induced changes of biochemical components in seahorses. For instance, the levels of SOD, CAT, MDA, AKP and ACP in *H. kuda* varied with salinity [29].

### 3.3 Diet

Diet difference may influence the juvenile seahorses. Therefore, it is a necessary that the diet should be changed with seahorse's growth. The new-born seahorses, having a small-scale snout with an average of 0.56 mm ranging from 0.45-0.68 mm, only can seize some microplanktons such as rotifer, microalgae and etc, while senior seahorses would be fond of Cladocera and Copepods or something larger. Segade [30] observed the same trend in fish survival, growth, protein, ash or humidity content, except for color changes when they bred the seahorses (*H. hippocampus*) with Artemia and frozen Mysis. According to Segade [30], Woods [31] thought there was no significant difference among tested seahorses cultured with *Artemia* sp. enriched with Algamac-3050 and frozen mysids *Amblyops kempfi*. Enriched with chlorella could be conducive to seahorse's feeding and could expand the biodiversity of breeding grounds, mediate the water quality and optimize the survival circumstances [32]. Several studies showed that diet also had a pivotal effect on seahorse's reproduction. For instances, seahorses cultured with Artemia combined with tilapia or frozen mysids had a higher frequency of delivery and had more broods. Were they cultured with fresh-mysid rather than frozen-mysid, seahorses (*H. erectus*) had a superior

nutritional status, which would improve their survival and growth [33].

Artemia density is a key factor to breed seahorses. A high density could cause improvement of corruption and organic matters, and aggravate the deterioration of water quality-low pH and high ammonia nitrogen and nitrite nitrogen content, which were favorable for *Vibrio* growth instead of seahorses. However, low density could not supply sufficient nutrition for seahorses [34]. Therefore, a proper Artemia density could both maintain water quality and cut down the cost without impacting survival and growth. Yin [35] investigated the effect of various Artemia densities on juvenile seahorse (*H. erectus*). What he found was that 1-10 day seahorses should be cultured with Artemia with 10 individual/mL. After a month, the density of 10 individual/mL was appropriate.

### 3.4 Temperature

Temperature, a primary parameter during fish life, seems to be related to feeding, metabolism, immune function, the incubation rate and the gender of fishes [19], so do seahorses. Temperature mutations influenced the growth, biochemical composition and enzyme activity of seahorse (*H. kuda*). Sun [36] put the seahorses survived in 23°C to 15, 28 and 33°C respectively and he thought a proper mutation could facilitate the growth, biochemical composition and enzyme activity of seahorses. When the mutation was excessive, it led to poor health and death. Gonadosomatic index (GSI), fecundity and spawning, fertilization and hatching rate were all in the charge of temperature. Lin [20,37] demonstrated that the higher the temperature, the higher fecundity and spawning and fertilization rate, and the GSI was peaked at 28°C—the optimal temperature for reproduction. The sum of effective temperature was 14066.9°C h<sup>-1</sup>. Furthermore, hatching behaviors of juvenile seahorses (5-10 day) were subject to temperature. At 18°C, seahorses sank into bottom without hatching or swimming, and they began to hatch with a low-rate at 22°C. But the maximum hatching rate reached at 26°C. A 107 day experiment conducted by Wong [20] showed that the growth rate of *H. whitei* were 0.14 mm/d and 0.26 mm/d at 17°C and 26°C, respectively. *H. trimaculatus* accommodated themselves to the water at 26°C [38]. Planas [39] described the survival and wet weight of *H. guttulatus* cultured in 15, 18 and 21°C. Those in 15°C died earlier than the other with a short swimming time. The

survival and wet weight were 21.1%, 85.9% and 81.1%, and 14.7 mg, 51.9 mg and 106.43 mg, eventually.

### 3.5 Disease

Seahorse aquaculture has encountered many bottlenecks including diseases. A study conducted by researchers from four countries showed that inflammatory processes caused by bacterial, parasitic and virus dominated the pathological progress. Neoplasms or neoplastic-like lesions, environment and congenital factors were the secondary cause [40]. Some researchers [41,42] compared the intestinal bacterial flora in health and intestinal-diseased seahorse and the intestinal microbiota differences between wild and cultured seahorses. What they found were that *Vibrionaceae* commonly represented the dominant population in the intestinal tract of all seahorses. In addition, *Enterobacteriaceae*, *Rhodobacteraceae*, *Sphingomonadaceae*, *Flavobacteriaceae*, and *Alcaligenaceae* were also identified in seahorse intestinal tract. In another experiment, a new pathogenic bacteria or *V. fortis* which could cause enteritis in cultured seahorses [43]. *V. parahaemolyticus* isolated from infected seahorses was sensitive to *Streptomyces Rifampicin*, *kanamycin*, ciprofloxacin, norfloxacin, tetracycline, compound Daxin, bacteria must rule, Ned acid [44]. Declercq [45] thought that the white necrotic tail not only associated with *Vibrio* sp. and *Mycobacterium* sp., but also influenced by Uronema-like parasite.

## 4. MEDICAL USE

Seahorses have been used widely as a traditional medicine and invigorant from time immemorial, whose edible and medicinal effects have been seen in Compendium of Materia Medica (《本草纲目》) and Shennong Materia Medica(《神农本草》). Traditional medicinal uses and local names of some prescription authorized by CFDA are listed in Table 1. During the past decades, considerable research efforts have been directed to reveal their therapeutic potentials, such as anti-fatigue, anti-inflammatory, anti-tumoral activities [3].

### 4.1 Hormone-like Effect (Table 2)

A group of researchers [46,47] worked with Sun Yat-sen University investigated the Invigorating Yang and tonifying kidney function of Haima

capsule (Qinyangchun, Wutongtang) by intragastric administration to castrate mice. After 20 days, levels of load swimming time, serum testosterone and weight of the reproductive organs had improved. Another study feeding the kidney deficiency mouse with Haima capsule for 12 days found that capsule might possess androgenic hormone-like physiological function which enhanced the sexual response and mating ability and shortened the ejaculation latency time [48]. Meng [49] verified the Haima capsule could also treat benign prostatic hyperplasia (BPH). Similarly, Lu [50] employed Kidney Yang-deficiency mouse to illuminate the effect of Haima tonify Yang capsule, and they confirmed that this kind of capsule could alleviate impotence and enhance the mating ability.

Researchers contended that the acetic ether extract of *H. trimaculatus* had therapeutical effect on castration and testosterone-induced benign prostatic hyperplasia and oligospermism explicated by decreasing serum ACP activity and reducing the expression of PCNA and bFGF in the prostate. Compared to Finasteride, a drug used to reduce symptoms of prostate enlargement, the extract did not induce finasteride-related adverse event [51].

Zhang [52] mixed the powder of *H. japonicas* with CMC-Na (0.5%) to prepare the suspension (0.59 g/ml). After the intragastric administration of the suspension to Hydrocortisone-treated mice, levels of peripheral serum hemolysin and cAMP were increased and the weight of prostate and testes were rising.

### 4.2 Antioxidant Activity (Table 3)

Wang [53] investigated the antioxidant activity of the water, methanol, n-butanol and ethyl acetate and chloroform extracts of seahorse (*H. japonicus* Kaup). The results showed that water extract possessed the highest antioxidant activity measured by DPPH radical scavenging activity with an equation of linear regression:  $Y=99.998-0.698X$ ,  $r^2=0.960$ . The dry-seahorse was ground into powder and extracted with methanol within 30 min, then filtered it with ultrasonic. The authors studied antioxidant activity of seahorse by measuring the oxidation time required for the reduction of potassium permanganate, and the clearance rate of superoxide anion and the total and the clearance rate of superoxide anion and the total reducing power compared with ascorbic acid, which were 21.28 s, 31.98% and 0.48 of seahorse extract and 15.8 s, 42.42% and 0.686

of ascorbic acid. Therefore, the researcher believed that the seahorse filtrate exhibited excellent antioxidant activity [54]. Using Sephadex G-10 gel to separate the water extract of *H. trimaculatus* could obtain 6 kinds of fractions containing various amounts of phenolics and amino acids among which FrIV had the highest antioxidant activity with IC<sub>50</sub> value of 0.25 mg/mL. Chen [55] thought the antioxidant activity was related to types and contents of the amino acids, small molecular peptide and phenolic compounds derived from the extract. In another study, the water extract of *H. trimaculatus* could reduce the peroxide value of oils, and it had the highest anti-oxidative activity when the extract gained in the optimum condition (pH=3.3, 40°C, 40 min) [56]. Qian [57] extracted *H. kuda* with water, methanol and ethanol and studied the free-radical and ROS scavenging activities of

these different extracts which were measured with various antioxidant assay in vitro (e.g. reducing power, total antioxidant, DPPH radical scavenging, hydroxyl radical scavenging). Moreover, the inhibitory effects on intracellular ROS on RAW264.7 and myeloperoxidase (MPO) activity in HL60 cells were also evaluated. In 2012, two new phthalate derivatives from the methanol extract of *H. kuda* were isolated. Similar to the former, both of them possessed antioxidant activities and had no cytotoxicity on RAW264.7 and MRC-5 cells [58].

Chen [59] employed the alkali protease to hydrolyze *H. trimaculatus* and studied the antioxidant activity of this product. As a result, the antioxidant activity of this protein hydrolysate was 4.73 times higher than that of ascorbic acid on the same condition. Papain enzyme,

**Table 1. Traditional medicine containing *Hippocampus* in China**

<b>Drug Name</b>	<b>Main Composition</b>	<b>Function</b>
Haimasanshen Pellet	<i>Hippocampus, Epimedium, Morinda officinalis How, Velvet antler, donkey kidney, etc.</i>	Invigorating Yang, Tonifying kidney
Haimawanying Paste	<i>Hippocampus, Saussurea costus, notopterygium, Angelica dahurica, Angelica sinensis, etc.</i>	Accelerating circulation of blood, Acesodyne, Bruise
Haimiaduobian Pellet	<i>Hippocampus, gecko, cynomorium songaricum, Semen Cuscutae, etc.</i>	Invigorating Yang, Beneficial to the sperm
Haimabaji Capsule	<i>Hippocampus, Morinda officinalis How, pilose antler, Cnidium monnieri, etc.</i>	Invigorating Yang, Tonifying kidney, Anti-fatigue, Impotence
Jingzhihaimazhuifen Paste	<i>Hippocampus, Unprocessed, Achyranthes, parsnip, eucommia, cinnamon, saffron, etc.</i>	Accelerating circulation of blood, Acesodyne
Shexianghiamazhui feng Paste	<i>Hippocampus, Unprocessed, Achyranthes, parsnip, eucommia, cinnamon, saffron, etc.</i>	Accelerating circulation of blood, Acesodyne
Shenronghaima Pellet	<i>Hippocampus, dog kidney, pilose antler, ginseng, Epimedium, etc.</i>	Invigorating Yang, Tonifying kidney, Anti-fatigue
Haimaqliangshen Pellet	<i>Hippocampus, Radix Rehmanniae Preparata, Fructus Corni, pilose antler, deer kidney, etc.</i>	Spermatorrhea, Asynodia, Tonifying kidney
Haimashuhuo Pellet	<i>Hippocampus, panax notoginseng, daemonorops draco, red flower, Ligusticum wallichii, etc.</i>	Bruise, Reducing swelling, Alleviating pain
Haimabushen Pellet	<i>Hippocampus, rehmannia, donkey kidney, dog kidney, etc.</i>	Nourishing yin, Tonifying kidney, Strong brain
Haimaxiangcao Wine	<i>Hippocampus, Ginseng, Cistanche, Epimedium, etc.</i>	Invigorating Yang, Tonifying kidney, Fatigue, Impotence

**Table 2. The hormone-like effect of seahorse**

Subject	Processing method	Experimental model	Reference
Haima capsule		Castration mouse	[46, 47]
Haima capsule		Kidney deficiency mouse	[48]
Haima capsule		Castration rats	[49]
Haima tonify Yang capsule		Kidney Yang-deficiency mouse	[50]
<i>H. trimaculatus</i>	Acetic ether extract	Castration rats	[51]
<i>H. japonicus</i>	Powder	Hydrocortisone-treated mice	[52]

**Table 3. The antioxidant effect of seahorse**

Subject	Processing method	Experimental model	Reference
<i>H. japonicus</i>	Ethanol extract	DPPH radical scavenging activity	[53]
Seahorse	Ethanol extract	Scavenging activity on superoxide anion	[54]
<i>H. trimaculatus</i>	Water extract	Total antioxidant activity	[55]
		DPPH free radical scavenging	
		Hydroxyl radical scavenging	
		Total reducing power	
		Ferrous ion chelating ability	
<i>H. trimaculatus</i>	Water extract	Antioxidative activities on fat and oil	[56]
<i>H. kuda</i>	Ethanol extract	Free radical scavenging activities	[57]
	Water extract	Reactive oxygen species	
	Methanol extract		
<i>H. kuda</i>	Methanol extract		[58]
<i>H. trimaculatus</i>	Enzymatic hydrolysis	DPPH radical scavenging activity	[59]
<i>H. kuda</i>	Enzymatic hydrolysis	DPPH free radical scavenging	[60]
		Hydroxyl radical scavenging	
		Ferrous ion chelating ability	

selected out from 5 kinds of proteases, was used to hydrolyze seahorse protein. After the filtration and freeze drying, researchers [60] found 5 kinds of fractions of which component 3, 4 and 5 had excellent activity with IC<sub>50</sub> of 0.896, 0.982, and 0.814 mg/mL respectively. All of the fractions could both significantly eliminate DPPH radical and hydroxyl radical and had iron-chelating ability. ACE inhibitory peptides obtained from the hydrolysate exhibits antioxidant as well as hypotensive function. Similarly, Liu [61] thought that the hydrolysate prepared by neutral protease could scavenge DPPH radical and superoxide anion radical.

#### 4.3 Anti-Fatigue Activity (Table 4)

To explore the anti-fatigue activity of seahorse, neutral protease was availed to hydrate seahorse ponder. The researchers reported that the enzymatic extract of *H. kelloggi* had anti-fatigue activity evaluated by the activity of SOD and MAD, as well as the levels of blood lactic acid and serum urea, besides, the extracts could

inhibit *Escherichia coli*, *Bacillus gasoformans* and *Proteusbacillus vulgaris* [61]. Suspending liquid of eight kinds of marine creatures-*Syngnathus* sp., *H. trimaculatus*, oyster, tortoise plastron, *Oplopanax elatus* Nakai, *Asteria*, *Spirulina* and seatangle, had different anti-fatigue activity on tested mice after 14 days [62].

Zhu [63] reported that ethanolic extract of *H. trimaculatus* Leach played a significant part in anti-fatigue and enhancing hypoxia tolerance. In another experiment, the result showed that the enzymatic hydrolysis of *H. kuda* prolonged the swimming time, cold resistance and hypoxia tolerance time, as well as decreased serum lactic acid and serum urea nitrogen content after swimming [64].

#### 4.4 Anti-inflammatory, Anti-tumoral and Immune-Modulating Activities (Table 5)

Chen [65] proved that the petroleum ether extract of seahorse was composed of phospholipids, unsaturated fatty acid and phenolic compounds,

**Table 4. Anti-fatigue activity of seahorse**

Subject	Processing method	Experimental model	Reference
<i>H. kelloggi</i>	Enzymatic hydrolysis	Mice forced swimming test	[61]
<i>H. trimaculatus</i>	Water extract	Mice forced swimming test	[62]
<i>H. kuda</i>	Ethanol extract	Mice forced swimming test	[63]
<i>H. kelloggi</i>	Enzymatic hydrolysis	Mice forced swimming test	[64]

**Table 5. Anti-inflammatory, anti-tumoral and immune-modulating activities of seahorse**

Subject	Processing method	Experimental model	Reference
<i>H. kuda</i>	Petroleum ether extract	RAW264.7 cell	[65]
<i>H. kuda</i>	Enzymatic hydrolysis	MG-63 and SW-1353 cell	[67]
<i>H. erectus</i>	Powder	<sup>60</sup> Co-γ treated mice	[68]
<i>H. kuda</i>	Ethanol extract	Intragastric administration	[63]

which suppressed the production of NO in LPS-induced RAW264.7 cell. To manifest the anti-neuro inflammatory function of paeonol or 1-(2-hydroxy-4-methoxyphenyl) ethanone, isolated from *H. kuda* Bleeler, Himaya [66] studied the inflammatory response in LPS-induced BV-2 microglial and RAW264.7 macrophage cells, and they found that the paeonol could inhibit the pro-inflammatory cytokines in both activated BV-2 and RAW264.7 cells in a dose-dependent manner. Moreover, MAPK and NF-κB signal pathway were inhibited as well. Ryu [67] optimized the enzymatic hydrolysis of *H. kuda* protein and investigated the anti-inflammatory activity of the peptide DPFKDDWDNWK (1821 Da) purified from the hydrolysis. As expected, this peptide promoted the formation of collagen and mineralization by reducing the expression of MMP-13, iNOS and COX-2 in TPA-induced MG-63 and SW-1353 cells.

The effect of suspend of *H. erectus* powder was studied by intragastric administration to <sup>60</sup>Co-γ treated mice. Compared with control group (<sup>60</sup>Co-γ treated only), the levels of peripheral white blood cells and platelets were higher for 3 days and were significantly higher for 7 days. When it lasted for 30 days, the survival rates of experimental groups (2 groups) and control group were 33.3% (20 mg/kg), 53.3% (40 mg/kg) and 6.7% respectively [68]. Zhu [63] concluded that ethanolic extract of seahorse possessed immune-modulating and anti-allergy properties by enhancing the phagocytosis of macrophage and reducing the swelling degree caused by 2, 4-Dinitrochlorobenzene.

#### 4.5 Other Activities

Xu [69] used methanol to extract the supernatant of the raw powder (*H. kuda*) twice and then obtained the jasmine crystal followed by

lyophilization of the extract. They measured the inhibitory rate of the extract on carotid artery thrombosis and cerebral thrombosis with Aspirin as a positive control group, and the results showed that rate were 24.2%, 40.5%, and 46.8% at doses of 50 mg/kg, 100 mg/kg and 200 mg/kg while the Aspirin was 44.7% at 10 mg/kg. It was unsaturated fatty acids that formed the main constituents of the extract, which could reduce blood viscosity and raise blood flow velocity.

In traditional Chinese medicine, seahorse is used for tranquilizer and sedative. To explore this medicinal effect, Zhang [70] determined them by the calcium mobilization assay. The result showed that the water and ethanol extract of the *H. kelloggi*, *H. trimaculatus*, *H. japonicas*, and *H. kuda* could significantly inhibit the calcium influx in rat neuron, which underlied this medical mechanism.

The middle-cerebral-artery-occlusion-induced focal ischemia/reperfusion (I/R) rat brain damage model similar to human ischemic stroke was used to study the effects of seahorse extraction on neurological deficit score (NDS), cerebral infarction volume and cerebral water content after I/R. In briefly, the seahorse powder was suspended into 0.3% CMC-Na solution. The suspension was given by intra gastric administration to mice at a dose of 10 ml/kg an hour before I/R. The results showed that the extract lowered the NDS significantly and reduced cerebral infarct volume and brain edema reaction at the interval of 1 h, 24 h and 72 h after I/R [71].

#### 5. CONCLUSION

As described above, seahorses have been shown to played diverse roles in tradition medicine and pharmacologic properties. The majority of the trade was for TCM and the

demand continued to rise. Although the seahorse aquaculture has been developed for several years combined with updated technical, there are still many difficulties in this industry. Therefore, it is imperative for us to encourage technical advance to alleviate the pressure on wild population.

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## COMPETING INTERESTS

Authors have declared that no competing interests exist.

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