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Comparison Effect of Alcoholic and Non-Polar Extract of Persian Gulf Sea Cucumber (*Holothuria leucospilota*) on Steroid Hormones Levels in Molly Fish (*Poecilia sphenops*)

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Abstract

Background: sea cucumber is important aquatics with nutritional and medical properties.

Aim: So, the aim of this study was to determine comparison effect of alcoholic and non-polar extract of Persian Gulf sea cucumber on steroid hormones levels in Molly fish.

Methods: A total 210 Molly fish were randomly divided into 7 experimental groups (n=30). In Group 1, fish kept as control (without injection). In Group 2, fish injected with ethanol (100 mg/kg, i.m.) for 20 days. In Group 3, fish injected with hexane (100 mg/kg, i.m.) for 20 days. In Groups 4-5, fish injected with alcoholic extract of Persian Gulf sea cucumber (AEPGSC, 100 and 200 mg/kg, i.m.) for 20 days, respectively. In Groups 6-7, fish injected with non-polar extract of Persian Gulf sea cucumber (NPEPGSC, 100 and 200 mg/kg, i.m.) for 20 days, respectively. In all groups, the injection was given on alternate days. Then 24 h after the last injection, fish euthanized using PI_{222} (Pars Imen Daru, Tehran, Iran). The biometric indexes body length (cm) and weight (g) were determined. Then the gonads carried out and the body mass homogenized, body testosterone (ng/ml), β-estradiol (ng/ml), cholesterol (mg/dl) and germ cell index (mm) were determined.

Results: According to the results, injection of AEPGSC and NPEPGSC significantly diminished body β-estradiol levels (ng/ml) in the Moly fish compared to control group (P=0.012). Injection of AEPGSC (200 mg/kg) and NPEPGSC (100 and 200 mg/kg) significantly diminished body testosterone (P=0.000) and cholesterol (P=0.003) levels in the Molly fish.

Conclusion: these results suggest Persian Gulf sea cucumber extract has an effect on the production of steroid hormones in Molly fish.

Keywords: Persian Gulf sea cucumber extract; Germ cells division; Testosterone; 17-β estradiol; Molly fish

Introduction

Sea cucumbers are abundant worm-like and soft-bodied echinoderms found in nearly every marine environment [1]. To date approximately 1400 living species of sea cucumbers have been identified worldwide including Persian Gulf [2]. Based on the analysis it is reported sea cucumber is cholesterol-free and contains approximately 55% protein and 15% mucopolysaccharides, saponins and collagen peptides [3]. Sea cucumbers are aquatic creatures and have nutritional and therapeutic properties on human health [4]. It is reported polysaccharides isolated from sea cucumbers have anticoagulant, antitumor and immune modulating activity [5]. Recently it is reported polysaccharide derived from sea cucumbers have anti-hyperlipidemic effects [6]. It is reported injection of sea cucumber extract decreased serum total cholesterol and improved lipid metabolism in rats fed high-cholesterol diet [7]. On the other hand, it is known, saponins has effect on lipid metabolism [7]. For instance, aqueous extract of Malaysian sea cucumber (*Stichopus chloronotus)* minimize lipid biosynthesis [8].

Recently interests increased for curative effects of the biological components on reproduction where saponin significantly affects reproduction in animals [9]. The negative effect of saponins on animal reproduction is well documented [9] however controversial reports exist. Recently, Delghandi Moghadam et al. [10] studied effect of Persian Gulf sea cucumber (*Holothuria leucospilota*) on maturation of mice oocyte and granulosa cells. Based on their report, sea cucumber saponin enhanced follicle growth in mice, but precise mechanisms still unclear [10]. The saponin of sea cucumber suppressed pancreatic lipase and inhibiting triglyceride and cholesterol absorption in rat [7]. It is known that cholesterol is the main structure of sex steroid hormones which decrease cholesterol levels impair gonad estradiol, estrogen (E_2) and testosterone production [11].

Holothuria leucospilota are commercially important aquatics in the Persian Gulf and Oman Sea. These aquatics have important composition and medical properties [2] but there is no information about the biological activity of *Holothuria leucospilota* on sex hormone levels. Based on the literature, the sea cucumber has properties to diminish body lipid and cholesterol profile [6] as well because of correlation between cholesterol level and sex hormone biosynthesis [10], the hypothesis of the current study was to determine possible curative or adverse effect in the *Holothuria leucospilota* extract on

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steroid hormones levels in Molly fish. So, the aim of the current study was to determine comparison effect of alcoholic extract Persian Gulf sea cucumber extract (AEPGSC) and non-polar Persian Gulf sea cucumber extract (NPEPGSC) on steroid hormones levels in Molly fish (*Poecilia sphenops*).

Material and Methods

Sample preparation

Samples of Persian Gulf sea cucumber *(Holothuria leucospilota)* were collected from Persian Gulf, at the depth of 25-30 meters in 2016. Then samples transported and stored at -20°C in laboratory of Fisheries, Department of Pharmacology, Islamic Azad University, Tehran, Iran.

Persian Gulf sea cucumber extract

In this study two extracts type of Persian Gulf sea cucumber were done. The AEPGSC obtained using ethanol and NPEPGSC by hexane. The sea cucumber sample (2000 g wet weight) was cut into small pieces (1 cm), they were put in freeze dried until the tissue was dry (150 g dry weight). Extracts of powder sea cucumber were obtained by using three different solvents including: n-hexane, diethyl ether and methanol. After 24 hours of exposure in n-hexane, the extract was concentrated under low pressure at 30°C by rotary evaporation. The diethyl ether extract was ready after 48 h, and then the solvent was removed by rotary evaporation at 35°C. The methanol extract was ready after 72 h then the solvent was removed by rotary evaporation at 40°C. Ether-methanol was formed by adding Ether in order to separate the methanol-aqueous extract, then the upper phase was separated by separating funnel. The upper phase was combined by n-butanol and aqueous extract was separated by separating funnel. Each extract was shaken by mechanical shaking at room temperature (25°C). All processes were carried out on dark condition. Finally, both crude extracts were kept in freezer.

Study protocol

A total 210 Molly fish (*Poecilia sphenops*) (3 ± 0.2) were randomly divided into 7 experimental groups (n=30). The fish were kept in 2 m^3 tanks with a flow-through circuit, suitable aeration and filtration system and natural photoperiod. The water temperature ranged from 25.1 to 27.8°C. The environmental parameters, mortality and food intake were recorded daily.

In Group 1, fish kept as control (without injection).

In Group 2, fish injected with ethanol (100 mg/kg, i.m.) for 20 days (given on alternate days).

In Group 3, fish injected with hexane (100 mg/kg, i.m.) for 20 days (given on alternate days).

In Group 4, fish injected with AEPGSC (100 mg/kg, i.m.) for 20 days (given on alternate days).

In Group 5, fish injected with AEPGSC (200 mg/kg, i.m.) for 20 days (given on alternate days).

In Group 6, fish injected with NPEPGSC (100 mg/kg, i.m.) for 20 days (given on alternate days).

In Group 7, fish injected with NPEPGSC (200 mg/kg, i.m.) for 20 days (given on alternate days).

The doses for the extracts were used based on the previous reports [12-14] and our pilot studies (un-published).

Tissue extract and hormone assay

Then 24 h after the last injection, fish euthanized using PI_{222} (Pars Imen Daru, Tehran, Iran). The biometric indexes body length (cm), weight (g) and germ cell index (mm) were determined. Then the gonads carried out and the body mass homogenized. Then, body testosterone (ng/ml), β-estradiol (ng/ml) and cholesterol (mg/dl) were determined using commercial ELISA or colorimetric detecting kits [15].

Statistical analyses

Effect of AEPGSC and NPEPGSC was analyzed by repeated measure two-way analysis of variance (ANOVA) using SPSS 16.0 for Windows (SPSS, Inc., Chicago, IL, USA). Data is presented as mean ± SEM. For treatment showing a main effect by ANOVA, means compared by Tukey-Kramer test. $P<0.05$ was considered as significant differences between treatments.

Results

The comparison effect of AEPGSC and NPEPGSC on steroid hormones (testosterone, β-estradiol and cholesterol) levels in Molly fish (*Poecilia sphenops*) is presented in Figures 1-4. As seen in Figure 1, i.m. injection of AEPGSC and NPEPGSC significantly diminished body β estradiol levels (ng/ml) in the Molly fish compared to control group ($P=0.012$; $F=4.126$). However, there was no significant difference on body β estradiol levels (ng/ml) using different levels of the AEPGSC (100 and 200 mg/kg) and NPEPGSC (100 and 200 mg/kg) compared to ethanol (100 mg/kg) and non-polar base (100 mg/kg) ($P>0.05$).

As seen in Figure 2, only i.m. injection of AEPGSC (200 mg/kg) and NPEPGSC (100 and 200 mg/kg) significantly diminished body testosterone levels (ng/ml) in the Molly fish ($P=0.000$; $F=16.868$). However, administration in the ethanol (100 mg/kg), non-polar base (100 mg/kg), AEPGSC (100 mg/kg had no significant effect on body testosterone levels (ng/ml) compared to control group ($P>0.05$).

The effect of i.m. injection of AEPGSC and NPEPGSC on body cholesterol levels (mg/dl) is presented in Figure 3. According to the results, injection of the AEPGSC (100 and 200 mg/kg) significantly decreased body cholesterol levels in the Moly fish compared to control group ($P=0.003$; $F=6.041$). As observed in Figure 4, injection of AEPGSC and NPEPGSC had no effect on GIS in Molly fish compared to control group ($P>0.05$).

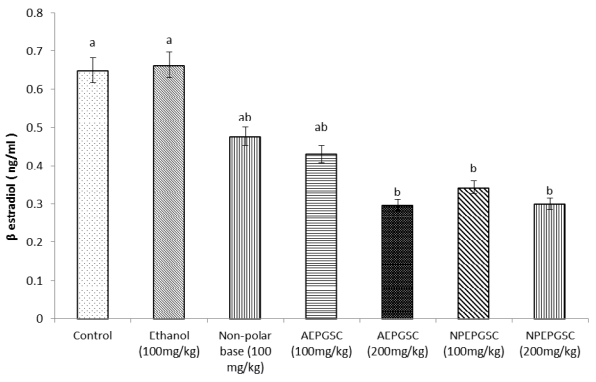

Figure 1: The effect of i.m. injection of AEPGSC and NPEPGSC on body β estradiol levels (ng/ml) compared to control group in Molly fish. i.m.: intramuscular, alcoholic extract Persian Gulf sea cucumber extract: AEPGSC, non-polar Persian Gulf sea cucumber extract: NPEPGSC. There are significant differences between groups with different superscripts in a column (a and b; $P<0.05$).

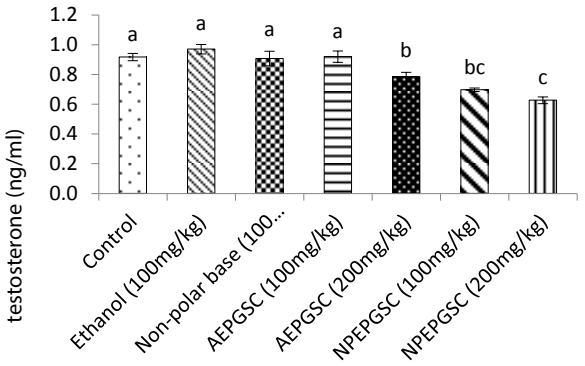

Figure 2: The effect of i.m. injection of AEPGSC and NPEPGSC on body testosterone levels (ng/ml) compared to control group in Molly fish. i.m.: intramuscular, alcoholic extract Persian Gulf sea cucumber extract: AEPGSC, non-polar Persian Gulf sea cucumber extract: NPEPGSC. There are significant differences between groups with different superscripts in a column (a, b and c; P<0.05).

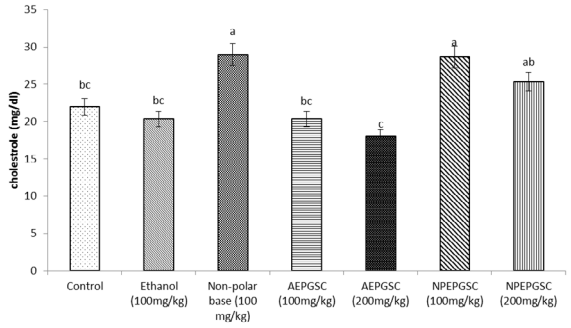

Figure 3: The effect of i.m. injection of AEPGSC and NPEPGSC on body cholesterol levels (mg/dl) compared to control group in Molly fish. i.m.: intramuscular, alcoholic extract Persian Gulf sea cucumber extract: AEPGSC, non-polar Persian Gulf sea cucumber extract: NPEPGSC. There are significant differences between groups with different superscripts in a column (a, b and c; P<0.05).

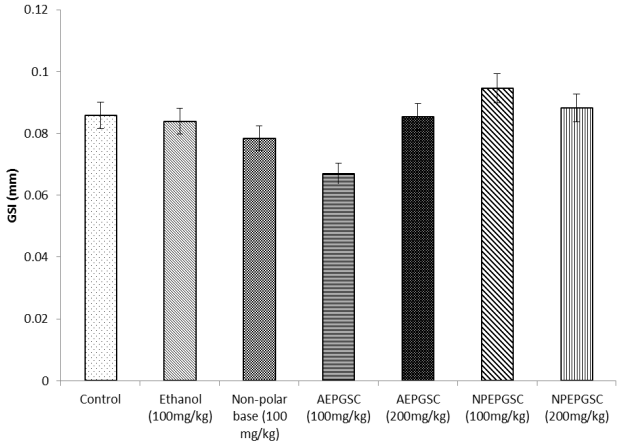

Figure 4: The effect of i.m. injection of AEPGSC and NPEPGSC on GIS (mm) compared to control group in Molly fish. i.m.: intramuscular, alcoholic extract Persian Gulf sea cucumber extract: AEPGSC, non-polar Persian Gulf sea cucumber extract: NPEPGSC, GSI: germ cell index. P<0.05 was considered as significant differences between treatments.

Discussion

During the past decade, growing attention has been centered towards discovery of novel drugs from marine organisms due to containing pharmacologically bioactive compounds. To our knowledge this is the first report on effect of AEPGSC and NPEPGSC. According to the results, injection of AEPGSC and NPEPGSC had no effects on body length and weight in the Molly fish while injection of the AEPGSC decreased body cholesterol levels in the Moly fish. Dry sea cucumber contains approximately 20 mg/g glucosylceramide. It is reported dietary sea cucumber contacting glucosylceramide decreased liver cholesterol and triglyceride compared to glucosylceramide free sea cucumber in mice but not affect body weight [16]. As observed in our study AEPGSC and NPEPGSC had no effect on body weight in Moly fish which was in agreement with previous reports. Fish have also been shown to exhibit stress reactions to the presence of saponins in water [9]. Bureau et al. [17] observed that saponins damaged the intestinal mucosa in rainbow trout and Chinook salmon at dietary levels above 1.5 g/kg. Polysaccharides can act as stimulators of bile acid synthesis and circulation and increasing the fecal excretion of bile acids so that fewer bile acids return to the liver [16]. On the other hand, antioxidant activity of polysaccharides might play major role in modify LDL formation but there is also increasing evidence to support the idea [18].

Herein, Injection of AEPGSC and NPEPGSC diminished body β-estradiol and testosterone levels in the Moly fish. To the best of our knowledge, there are no reports on the effects of Persian Gulf sea cucumber on reproductive hormones in Moly fish. Most of surveys have been reported about terrestrial saponin compounds. The negative effects of terrestrial saponins on animal reproduction have been introduced these bioactive compounds as abortifacient metabolites [9]. Injection of the saponin-rich extracts from *Combretodendron africanum* into female rats blocked the oestrous cycle [18]. Also, saponins directly inhibits the steroidogenesis genes and suppresses the proliferation of follicle-stimulating hormone-modulated granulosa cells in the mice ovarian follicle via similar mechanism as saponin-induced proliferation of tumor cells [9]. The same reports exist for male animals which lower Gonad-somatic index observed in male tilapia receiving a continuous supply of dietary saponin [19]. Despite the direct mechanism for reported results is not elicited but presumably interactions exist between saponins and steroid receptors given the similarities between the basic chemical structures of saponins and steroid hormones [20]. Presumably saponins block the expression of the genes coding for androgen receptors and 5-areductases that converts testosterone into the dihydrotestosterone [21].

As reported the saponins and polysaccharides are the main bioactive compounds in the sea cucumber [5] which decreased serum cholesterol in rats [7]. These results suggest Persian Gulf sea cucumber extract has impairs steroid hormone synthesis in Molly fish. So, the observed results might because of the bioactive components in the *Holothuria leucospilota*. However, there was no previous report on effect of sea cucumber on testosterone, β-estradiol and cholesterol in moly fish. So, further researches needed to determine accuracy of the results and the possible molecular and cellular mechanisms for effect of AEPGSC and NPEPGSC on steroid hormones levels in Molly fish. Also, because of differences in sex hormone generation between marine and human, further researches needed to determine administration of sea cucumber for human clinical trials.

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