

## **The Influence of Licorice Root (*Glycyrrhiza glabra*) on Sex Reversal in Guppy *Poecilia reticulata***

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**Abstract:** The roots and rhizomes of licorice (*Glycyrrhiza*) species have long been used worldwide as a herbal medicine and natural sweetener. Licorice is commonly present in menopausal botanical supplements in the United States. The estrogenic activities of different licorice species are variable and likely depend on the type and amounts of bioactive compounds. This study examined the effects of Licorice root extract on sex reversal in guppy, *Poecilia reticulata*. Newly born guppy fry ( $0.014 \pm 0.001$  mean weight), were randomly distributed into 30 L aquaria at a density of 80 fish per aquarium and subjected to a sex-reversal treatment by immersion application of licorise root extract for 30 days. The licorise root extract was incorporated into the aquarium water as follows: 0 (control), 0.25 and 0.5 g/L licorise root extract. Each of the treatment group was randomly assigned to triplicate groups of fish. The treatment was repeated twice (in the beginning and half way through the experiment) during 30 days. At the end of experiment, the highest feminization (88%) was observed at 0.5 g/L licorise root extract group. Morphological and histological examinations of the gonads in all groups revealed no intersex fish. Histological examination of fish treated with licorise root revealed no damage to the testes or ovaries. This study demonstrated successful sex reversal with treatment of licorise root on new-born progenies of *P. reticulata*.

**Keywords:** Guppy, *Poecilia reticulata*, Licorice Root, *Glycyrrhiza glabra*, Sex reversal

### **1. INTRODUCTION**

In last decade, the total world fishery production decreased slightly and the human consumption for aquatic product increased. The reduction in capture fisheries was partly compensated for the fast growth of aquaculture industry and this sector is the world's fastest growing sector of agricultural [1]. With the increasing intensification and commercialization of aquaculture production, fish culture is globally expanding into new directions, intensifying and diversifying for enhanced disease resistance, feed efficiency, and growth performance of cultured organisms. Therefore, several growth promoters and hormones have been tested for enhancing feed conversion efficiency and for increasing fish culture productivity [2]. However, the recent consumer demand for farmed fish has increasingly stressed quality and safety, and the absence of pollutants, antibiotics and carcinogens. Thus, along with growth performance, the fish rearing strategy needs to focus on food hygiene. This global demand for safe food has prompted the search for natural alternative growth promoters to be used in aquatic feeds. There has been heightened research in developing new dietary supplementation strategies in which

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various health and growth promoting compounds as probiotics, prebiotics, synbiotics, phytobiotics and other functional dietary supplements have been evaluated [3].

Phytoestrogens are estrogenic compounds found in >300 species of plants that can mimic oestrogen activity [4]. Phytoestrogens of current medical interest are the isoflavones, which are widely used as oestrogen replacement in human post-menstrual treatment and as a breast cancer preventive treatment [5]. A conspicuous feature of the chemical structure of phytoestrogen is the presence of a phenolic ring that, with few exceptions, is prerequisite for binding to the oestrogen receptor (ER). It was initially believed that phytoestrogens action would be predominantly hormonal, but as this field of study has expanded over the past decade, it has become apparent from the diversity of properties of isoflavones that no single action can explain many of the in vivo and in vitro effects in the same organism [5, 6]. The oestrogenic potency of these compounds on fish has been documented [7-11].

The roots and rhizomes of licorice (*Glycyrrhiza*) species have long been used worldwide as a herbal medicine. The active component of licorice, glycyrrhizic acid, is hydrolyzed in vivo to glycyrrhetic acid, which is responsible for most of its pharmacological properties. In ancient Chinese medicine and during Roman times, licorice was also recommended to cure sterility of women [12, 13]. Theophrastus and Pliny also reported an efficacy of licorice in reducing hunger and thirst [13, 14]. In addition, licorice extract has some estrogen-like, anti-viral, and antioxidant properties [12]. The estrogenic activities of different licorice species are variable and likely depend on the type and amounts of bioactive compounds.

Licorice root (*Glycyrrhiza glabra*) is also able to affect androgen metabolism in human [13]. Various studies have demonstrated that licorice blocks the activity of 3- $\beta$ -hydroxysteroid dehydrogenase (3HSD), 17- hydroxysteroid dehydrogenase (17HSD) and 17-20 lyase [13, 15] and stimulates the activity of aromatase [16-18]. All these enzymes are involved in the synthesis and/or metabolism of androgens and estrogens. A possible compensatory mechanism is the binding of glycyrrhetic acid to sex binding globulin, thus increasing free testosterone and estradiol values [19].

Considering these aspects, the present study was focused to evaluate the efficacy of licorise root as potent alternatives for induction of sex reversal in fish. The live-bearing guppy, *Poecilia reticulata* is a popular freshwater species among aquarium hobbyists. In this study, guppies have used as a model fish for investigating the influence of Licorise root (*Glycyrrhiza glabra*) on sex reversal in fish.

## 2. MATERIAL and METHODS

Newly born guppy fry ( $0.014 \pm 0.001$  mean weight), were randomly distributed into 30 L aquaria at a density of 80 fish per aquarium and subjected to a sex-reversal treatment by immersion application of licorise root extract for 30 days.

Licorise root was provided from Hatay province (Turkey). The aqueous extracts of Licorise root was prepared by boiling 7.5 and 15 g Licorise root in 250 ml distilled water for 30 min and then filtering it with a whatman filter paper twice [20,21]. The licorise root extract was incorporated into the aquarium water as follows: 0 (control), 0.25 and 0.5 g/ L licorise root extract. The prepared solutions were poured in the aquariums. The aquariums were aired strongly to spread the solutions in water. Each of the treatment group was randomly assigned to triplicate groups of fish. The treatment was repeated twice (in the beginning and half way through the experiment) during 30 days [10]. The water of the aquaria was changed entirely every 15 days. The aquaria were controlled temperature ( $26 \pm 1$  °C) and equipped with aeration. The photoperiod was maintained on a 12-h light: 12-h dark schedule. A control group was also included in this experiment. Hatchlings were fed ad-libitum three times a day until satiation.

The basic food was ornamental fish diet (Aquamaks, Turkey: 28% protein, 12% lipid (on wet basis)). After completion of the treatment, they were transferred to larger aquaria. When individuals were 2 months old, the sex of each was determined by external examination, with gonopodium and other morphological characters [22]. In addition, for histological examination of gonads, guppy were fixed in 10% neutral formalin. After fixing the samples, specimens, excluding head and caudal regions were processed for histology using routine de-hydration and parafin-embedding procedures. Cross-sections of thickness 4-6  $\mu$ m, were stained with Mayer's haematoxylin and eosin phloxine B solution examined, and microphotography [23]. Growth and survival rate were monitored to determine the growth in each treatment groups during the experiment. Each fish was individually anesthetized with 300 mg L<sup>-1</sup> lidocaine-HCL\1000 mg L<sup>-1</sup> NaHCO<sub>3</sub> [24], weighed and measured (total length) to the nearest 0.0001 g and 0.01 cm, respectively. A Chi-square ( $\chi^2$ ) test was used to determine whether observed sex ratios are different from an expected 1:1. Also, differences in growth were assessed by one-way ANOVA test and Duncan test was used to analyze which dosage groups cause the difference [25].

### 3. RESULTS and DISCUSSIONS

In the present investigation, both 0.25 and 0.5 g/L groups of licorise root extract– treated fish exhibited more female number than male number comparing to the control group. The sex ratio in the *P. reticulata* in these groups significantly changed ( $p < 0.001$ ; Table 1) and the highest feminization (88.35%) was observed at 0.5 g/L licorise root extract group. Also, the sex ratio observed in 0.25 g/L groups of licorise root extract– treated fish was 137:70 (female:male); this difference was statistically significant. The sex ratio observed for the *P. reticulata* was nearly the expected ratio of 1:1 (female:male) in control group. (Table, 1). Morphological and histological examination of the gonads in all treatment groups revealed that no intersex fish were identified in guppy. Histological examination of fish treated with licorise root revealed no damage to the testes or ovaries.

**Table 1.** Effects of treatment with Licorise root extract on sex reversal of the *Poecilia reticulata*.

	Dose (g/L)	Treatment Duration (day)	Sex Distribution	Sex Ratio	$\chi^2$
			(n) ♀ : ♂	(%) ♀ : ♂	
Licorise root extract	0	30	102 : 107	48.80 : 51.20	-
	0.25	30	137 : 70	66.18 : 33.82	12.851***
	0.5	30	182 : 24	88.35 : 11.65	75.105***

$\chi^2$  values are for comparisons of sex ratios with the control (\*,  $P < 0.05$ ; \*\*,  $P < 0.01$ ; \*\*\*,  $P < 0.001$ ).

Table 2 shows the survival rate and growth rates in total length and body weight of the *P. reticulata*. Total survival rates and growth rate in all treatments and controls were uniformly high ranging from 85.83% to 87.08% and 0.197 to 0.189 g respectively ( $p > 0.05$ ). This indicates that Licorise root extract has no negative effect on survival and growth rate of *P. reticulata* at the tested concentrations (Table 2).

**Table 2.** Effects of treatment with Licorise root extract on survival and growth of the *Poecilia reticulata*.

	Dose (g /L)	Survival Rate (%)	Total length (cm)	Body weight (g)
Licorise root extract	0	87.08 ± 1.10 <sup>a</sup>	1.85 ± 0.06 <sup>a</sup>	0.197 ± 0.01 <sup>a</sup>
	12.5	86.25 ± 0.72 <sup>a</sup>	1.79 ± 0.08 <sup>a</sup>	0.192 ± 0.01 <sup>a</sup>
	25	85.83 ± 0.42 <sup>a</sup>	1.80 ± 0.11 <sup>a</sup>	0.189 ± 0.01 <sup>a</sup>

Values (mean±S.E. of triplicate) with same superscripts in each column indicate insignificant differences ( $P>0.05$ ) (Water temperature 26±1°C).

To our knowledge, this is the first report regarding the potential of Licorise root extract as a feminization agent in ornamental fish. The 0.5 g/L licorise root extract group was the most potent dose in this study, having duration of 30 days for feminization. Although the treatment of the newly-born progenies using Licorise root extract significantly increased the percentage of females to 88.35%, we cannot conclude that this potency was caused by an increase of estrogen since we did not measure plasma estrogen levels. The Licorise root extract did not produce 100% females; a higher dose may have generated better results. Most literature on Licorise treatments in humans [13,26,27] and rats [28,29] endocrine system because it contains isoflavones (phytoestrogens), which are chemicals found in plants that may mimic the effects of estrogen. Licorice may also reduce testosterone levels, which can contribute to hirsutism in women. Therefore, usage of a higher dose may lead to the production of all-female *P. reticulata* population. Most authors have reported similar observation for other fish species, treated with various phytoestrogens [8,9,10,11,30]. However, the use of Licorice plant in fish studies was rather limited so far [7]. Also, survival rate of the Licorise -treated *P. reticulata* was similar to that of control group of the experiment. Similarly, survival was high in studies on guppy *P. reticulata* and zebra cichlids *C. nigrofasciatum* [10].

In summary, this study demonstrated that sex differentiation of guppies was successfully diverted toward the female gender with treatment of licorise root extract. The findings of this study indicate the probability of using herbal compounds as an alternative method for production of all-female guppy population. However, further studies will be required to determine the optimum treatment regime for induction of 100% sex reversal with these chemicals in guppy.

#### Conflict of Interests

Authors declare that there is no conflict of interests.

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