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Protective Efficacy of Aqueous Extract of *Naringi Crenulata* Leaves against Shampoo (Endocrine Disruptor) Induced Toxicity in *Labeo Rohita*

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Abstract

The protective effect of different concentrations of leaf extracts of *N. crenulata* was tested against shampoo induced toxicity. 250mg/L/day of leaf extract brought the protein (25.7 ± 0.11), carbohydrate (30.7 ± 0.8) and lipid (5.13 ± 0.4) contents in its normal level in shampoo intoxicated fish. However, in shampoo intoxicated fish lipid content was found highest and decreasing with the increasing concentration of leaf extracts. Length and weight increased with the increase of leaf extract concentration and was observed equal to normal at 250 mg/L (length 6.63 ± 0.2, weight 3.7 ± 0.7). The results also showed that there is positive correlation between length and weight gain, leaf extract concentration and length gain. These results indicate that aqueous leaf extract of *N. crenulata* acts as an antidote against shampoo toxicity in *L. rohita*.

Keywords: Shampoo intoxicated fish; Length gain; Weight gain; Protein; Carbohydrate; Lipid; Antidote

Introduction

There is rising interest in the possible health threat posed by endocrine-disrupting chemicals (EDCs), which are substances in our environment, food and consumer products that hinder with hormone biosynthesis, metabolism or action resulting in a divergence from normal homeostatic control or reproduction [1,2]. EDCs are a threat to both humans and wildlife species because they can imitate, block or alter the actions of natural endogenous hormones. They pose a novel challenge to species that have evolved in the absence of these exogenous compounds, because they disturb important developmental processes such as sexual differentiation [3], and adult processes such as ovarian and estrous cycles [4]. The majority of consumer soaps and shampoos claiming to be "antibacterial" or "antimicrobial" contain the chemicals triclosan or triclocarban. FDA first proposed a rule that would have removed these chemicals from soaps in 1978. Until this rule is finalized, these chemicals can be widely used with no regulatory oversight — despite evidence that they are not effective and numerous studies associate them with serious health risks. The growing use of these chemicals in products has led to widespread residues in the environment and in people. Laboratory studies have shown that these chemicals are endocrine-disruptors capable of interfering with hormones critical for normal development and reproduction. Such hormonal interference has the potential to cause long-term health problems including poor sperm quality and infertility, metabolic disorders and damage to the developing brain leading to poor learning and memory.

Antidote is given in order to prevent an acute intoxication and its efficacy will be demonstrated in animal models. The development and evaluation of substances to counteract the disruption of EDCs is principally a task for the scientific community. Thus, in view of this, an attempt has been made to find out whether leaf extract of *N. crenulata* rich in bioactive compounds, has any protective efficacy against shampoo intoxication or not, using fish model (*L. rohita*).

Materials and Methods

Fish and experimental condition

Apparently healthy *L. rohita* fingerlings with an average body weight of 2.26 ± 1.04 gram were obtained from private farm at Kanater, Kalubia Governorate. Fish were kept in trough measuring (100 x 50 x 30 cm) and maintained in aerated de-chlorinated fresh water at 22°C ± 2 for 5 days prior to use in experiments. The health status was examined throughout the acclimatization period. Water pH was measured by using electric digital pH meter and water temperature was recorded daily using a glass thermometer.

Experimental design

The duration of the experiment was 45 days. The healthy fingerlings were distributed into six groups, ten in each trough and were acclimatized for the experimental conditions for 5 days prior to the start. During that period fish were adapted on feeding of control diet (without any additives). Water was changed every week to maintain good water quality. Water temperature and pH were adjusted at 20-25°C and 7.4 respectively during the experimental period. After acclimation period, five groups of fish were exposed to sublethel concentration of 424 mg/L/day [5] to induce toxicity.

Growth measurements

Weights and length of fingerlings in each experimental group were measured and recorded weekly throughout the experimental period.

Determination of total protein, carbohydrate and lipid contents

The protein, carbohydrate and lipid contents were estimated by adopting the standard methods of the Biuret method of [6-8], respectively.

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Statistical analysis

Analysis of variance (ANOVA; SPSS, 10.0) was used to determine whether significant variation between the treatments existed. Difference between means were determined and compared by ANOVA. All tests used a significance level of $P < 0.05$. Data are reported as means ± standard errors.

Results and Discussion

Protein and carbohydrate content of normal fish and those treated with 250mg/L/day (leaf extract) was significantly higher than that of other groups of the same experiment. The result showed that 250mg/L/day of leaf extract brought the protein (25.7 ± 0.11), carbohydrate (30.7 ± 0.8) and lipid (5.13 ± 0.4 contents in its normal level in shampoo intoxicated fish. In shampoo intoxicated fish lipid content was found highest and decreasing with the increasing concentration of leaf extracts (Figures 1-3). One way analysis of variance done showed that protein, carbohydrate and lipid contents among these six groups (Table 1) differed significantly ($P < 0.05$).

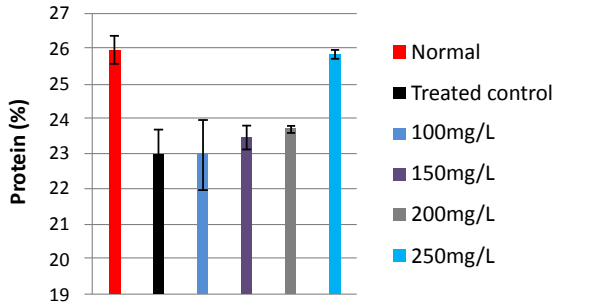

Figure 1: Showing the protein content among normal, treated control and leaf extract treated groups of *L. rohita*.

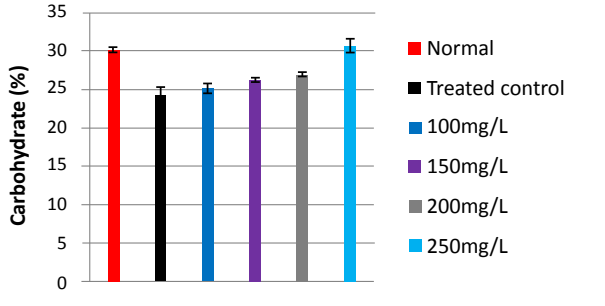

Figure 2: Showing the carbohydrate content among normal, treated control and leaf extract treated groups of *L. rohita*.

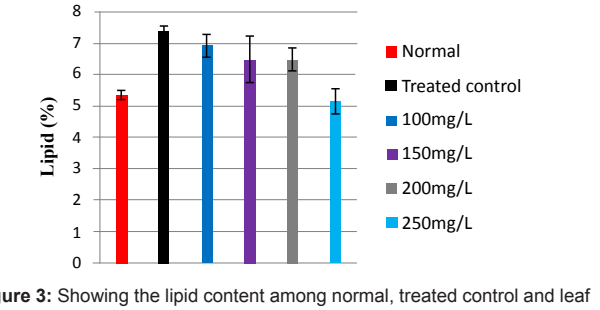

Figure 3: Showing the lipid content among normal, treated control and leaf extract treated groups of *L. rohita*.

Parameters	Sum of squares between groups	Sum of squares within groups	Degree of freedom Between groups	Degree of freedom within groups	F	P
Protein	28.81167	3.553333	5	12	19.4	2.21E-05
Carbohydrate	107.7016	5.084067	5	12	50.8	1.16E-07
Lipid	11.53778	2.013333	5	12	13.7	0.000129

Table 1: Analysis of variance for protein, carbohydrate and lipid contents among normal, treated control and extract treated groups of *L. rohita*.

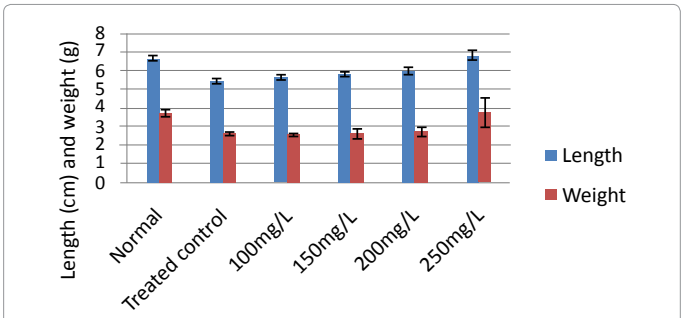

Figure 4: Showing the length and weight gain among normal, treated control and leaf extract treated groups of *L. rohita*.

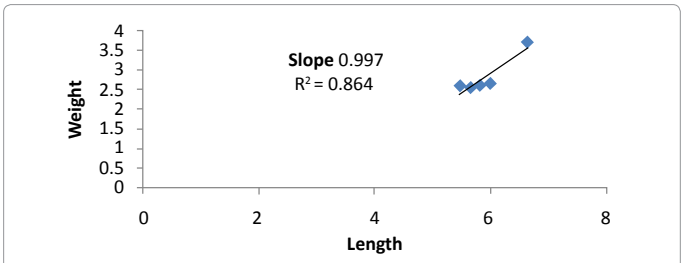

Figure 5: Correlation of length and weight gain of normal, treated control and leaf extract treated groups of *L. rohita* (r2 = 0.86, slope 0.997).

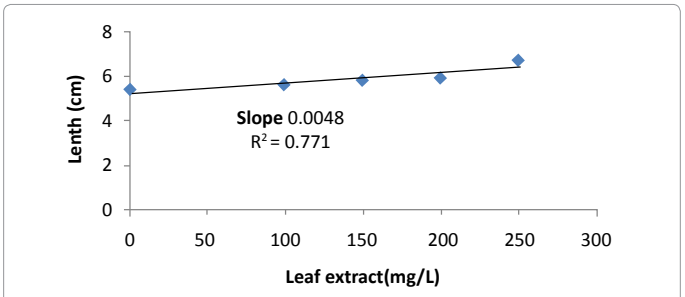

Figure 6: Correlation of length gain at different concentration of leaf extracts of *L. rohita* (r2 = 0.771, slope 0.004).

The effect of different concentration of leaf extract on total length and weight gain of *L. rohita* is shown in Figure 4. Length and weight increased with the increase of leaf extract concentration and was observed equal to normal at 250 mg/L/day (length 6.63 ± 0.2, weight 3.7 ± 0.7). The results also showed that there is positive correlation between length and weight gain, leaf extract concentration and length gain (The slope is significantly non-zero) (Figure 5 and 6), however, no such correlation was found between weight gain and leaf extract concentrations (slope 0.003) (Figure 7). ANOVA done showed that length and weight gain (Table 2) at different leaf extract concentration among normal, treated control and extract treated differed insignificantly ($P > 0.05$).

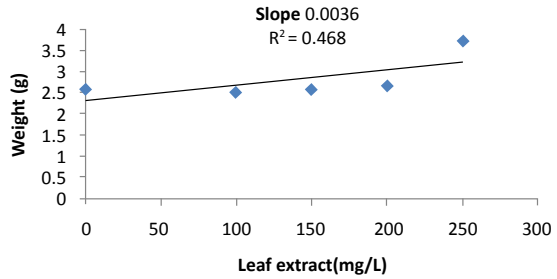

Figure 7: Correlation of weight gain with different concentration of leaf extracts of *L. rohita* (r2 = 0.468, slope 0.0036).

	SS	DF	MS	F	P
Total	96.1	35	2.7		
Length	9.3	5	1.9	23.3	1.69675E-08
Weight	84.6	1	84.6	1058.0	2.31E-21
Interaction	0.2	5	0.0	0.5	0.7792
Res. error	1.9	24	0.1		

Table 2: Analysis of variance for length and weight among normal, treated control and extract treated groups of *L. rohita.*

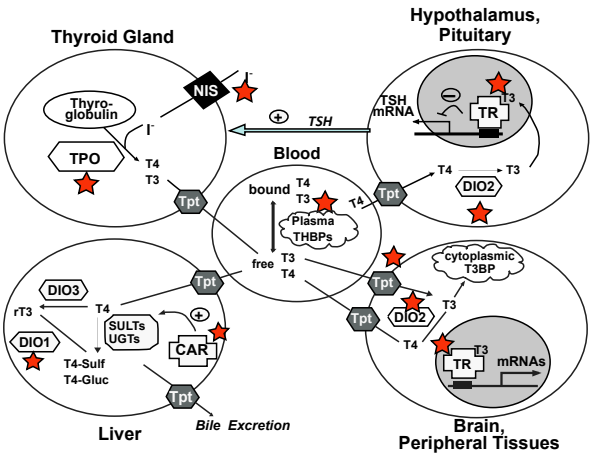

Figure 8: A schematic view of the thyroid hormone regulatory network and thyroid disruption endpoints. Cytoplasmic T3BP: Cytoplasmic T3-binding protein; DIO1,2,3: deiodinases type1, 2, 3; NIS: sodiumiodidesymporter; Plasma THBPs: plasmathyroid hormone-binding proteins; rT3: reverse-T3 (inactive); SULT: sulfotransferase; T4- Gluc: T4 glucuronide (inactive); T4-Sulf: T4-Sulfate (inactive); TPO: thyroperoxidase; Tpt: membrane transporter; TR: thyroid hormone receptor; TSH: thyrotropin; UGT, glucuronosyltransferase; ( ) thyroid disruption endpoints.(Adopted from Line Jugan et al., 2010).

Biochemical constituents in animals are known to vary with season, size of the animal, stage of maturity, temperature and availability of food and exposure of neuro endocrine disruptors etc. The results reported in the present study indicated that the extraction of *N. crenulata* leaves plays a major role in bringing carbohydrate, protein, lipid contents and length and weight gain back to it normal level in the intoxicated *L. rohita*. Kanlayavattanakul *et al.* [9] studied pharmacognostic specification of *Naringi crenulata* stem wood, on twelve wood samples from different sources. Pongpunyayuen *et al.* [10] studied the skin whitening agent in Naringi crenulata and Sampathkumar and Ramakrishnan [11] has isolated 13 bioactive compounds from methanolic extract of *Naringi crenulata* leaves using GC-MS. The majority of consumer soaps and shampoos claiming to be "antibacterial" or "antimicrobial" contain the chemicals triclosan or triclocarban [12] which manly effects the thyroid glands and subsequent effects on metabolism (Figure 8). The results of these investigations are in agreement with earlier studies of Colborn *et al.* [1]; Matthiessen [2]; Woo Park *et al.* [13]; Sartaj *et al.* [5]. The results of the present study on leaf extract of *N. crenulata* showing that it contains bioactive compounds that have Protective efficacy against triclosan (constituent of majority of consumer soaps and shampoos) intoxication.

This is an exciting time for research on antidote search. There is increased interest in the topic due to medicinal and aquaculture applications (fish) and the focus on fish as keystone aquatic animals which are mostly exposed to neuro endocrine disruptors present in aquatic environment.

Acknowledgement

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