Effects of nonylphenol on expression levels of GnRH-like polypeptide in *Cipangopaludina chinensis*

Hong-wei WANG[1,2], Fang CAI[3], Xiang LIU [1], Guang WANG[2], Yuan-na Wu[4], Yi-wei LIU[1], Amena HASAN[1]

[1.] College of Life Science, Hebei University, Baoding, P.R. China
[2.] South China Institute of Environmental Sciences, MEP, Guangzhou, P.R .China
[3.] Plant protection station of Beijing City, Beijing, P.R .China
[4.] Chief Engineer Office, Liaoning Academy of Environmental Sciences, Shenyang, P.R. China

Abstract. Nonylphenol plays the role of estrogen in influencing male organisms in water much higher than the female biological. It is typical endocrine disruptors and does harm to biological reproductive system. Gonadotropin-releasing hormone (GnRH) is a sex hormone-containing nerve decape-ptide.The main physiological role is associated with pituitary gonadotropin secretion cells specifically bind to receptors to promote cell gonadotropin secretion. In this experiment, *Cipangopaludina chinensis* was as experimental subjects to detect and observe the trend of nonylphenol on ovary, brain sections, and the visceral ganglion and foot ganglion GnRH-like Peptide expression in *C. chinensis* which lined in bottom sediments. Then, it was discussed on the toxic effects of nonylphenol on *C. chinensis* in bottom sediments and provides some reasonable suggestions to address the environmental impact of hormones on aquatic organisms.

1 Materials and Methods

The experiments based on GnRH-like Peptide ELISA test kit instructions for testing, and then follow the GnRH-like Peptide standard curve calculated the expression level of the sample. (GnRH-like Peptide assay kit was purchased from Shanghai Lu Shang Biological Technology Co., Ltd).

Set nonylphenol blank control group (0 μg /g), ethanol in the control group (0.04 ml / ml), low dose group (1 μg /g), middle dose group (10 μg /g), high dose group (100μg /g), put each group of 10 *C. chinensis*. Then, removed one from each group separately at 12h, 24h, 36h, 48h and measured the expression level of GnRH-like Peptide on ovarian, brain sections, the visceral ganglion and foot ganglion.

Using SPSS19.0 statistical software for data analysis of a dimensional variance (one-way ANOVA) and the resulting data were marked with a bar graph with excels. * P <0.05 indicated treatment group and the control group were significantly different.

2 Results

2.1 GnRH-like Peptide Standard Curve

Table 1 The determination table for Standard concentration

Standard concentration (pg/ml)	OD value
0	0.046
31.2	0.368
62.5	0.810
125	1.117
250	1.621
500	2.318

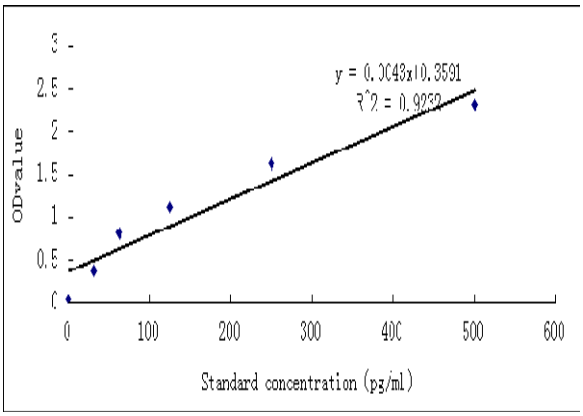

Fig. 1 Standard curve of GnRH-like Peptide

2.2 Expression of GnRH-like Peptide in ovary

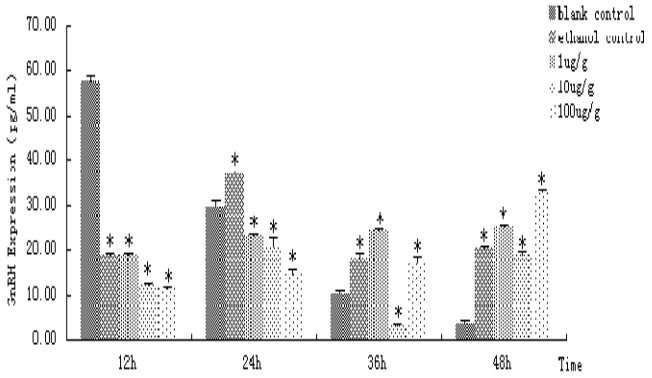

Fig. 2 Expression of GnRH-like Peptide in ovary of C. chinensis (* P<0.05, extremely significantly differences between treatments and the control group)

We clearly understand from the image above (Fig.2) that expression of GnRH-like Peptide in blank control group presented a obvious decreasing trend and it would

drop rapidly with the extension of exposure time. Within the ethanol controls the expression of GnRH-like Peptide exhibits a maximum at 24h, followed by a rapid decline,and the downward trend is evident. But at 36h and 48h, Expression of GnRH-like Peptide almost unchanged. In low dose group, the expression of GnRH-like Peptide showed a slow upward trend, and it would be slowly increasing with the extension of exposure time. Within the middle dose group the expression of GnRH-like Peptide exhibits a maximum at 24h, followed by a rapid decline, the rapid rise in the 48h, and it had no obvious law.At last, the expression of GnRH-like Peptide in high dose group expressed an upward trend which was obvious than low dose group, and it would be rapidly increasing with the extension of exposure time, especially the increase by a big margin at 48h**.**

2.3 Expression of GnRH-like Peptide in brain sections

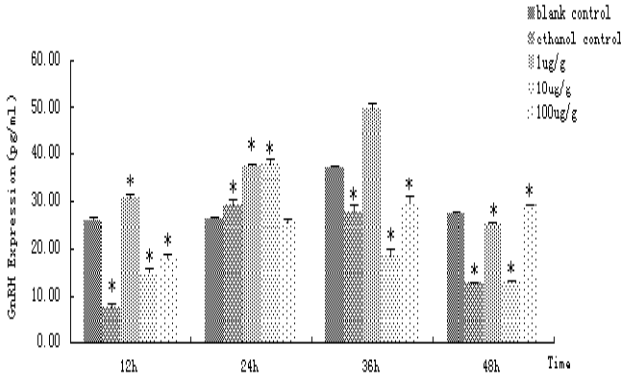

Fig. 3 Expression of GnRH-like Peptide in brain sections of C. chinensis (* *P*<0.05, extremely significantly differences between treatments and the control group)

From the graph (Fig. 3) we can see that blank control group, low dose group and high dose group all showed a maximum value at 36h. Ethanol in the control group and middle dose group showed a maximum at 24h.In blank control group, the expression of GnRH-like Peptide almost had no changes at other perioud except for 36h. Ethanol control group at 24h after GnRH-like Peptide Hormones maximum expression appear, it began to decline, especially at 48h. In the low dose group, the expression of hormones GnRH-like Peptide gradually increased with the extension of exposure time, a maximum value at 36h, but then began a rapid decline, the trend was obvious. GnRH-like Peptide hormone expression in the middle dose group increased rapidly within 24h, and showed a maximum value at 24h. Then, it showed an obvious downward trend with the extension of exposure time. At last, high dose group showed a maximum at 36h, and then began a slow decline, dropping small.

2.4 Expression of GnRH-like Peptide in foot ganglion

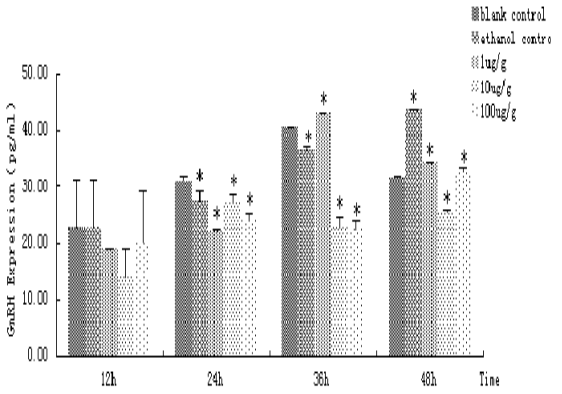

Fig.4 Expression of GnRH-like Peptide in foot ganglion of C. chinensis (* *P*<0.05, extremely significantly differences between treatments and the control group)

We clearly understand from the image above (Fig.3-4) that the expression of hormones GnRH-like Peptide showed a maximum at 36h in blank control group, showing an upward trend, followed by a rapid decline. In the ethanol control group, the expression of hormones GnRH-like Peptide showed a gradual upward trend and appeared the maximum at 48h.In the low dose group, the expression of hormones GnRH-like Peptide appeared the maximum at 36h, and showed a rapid upward trend. But it increased slowly within 12h-24h and showed a rapid increase after 24h. In the middle dose group, the expression of hormones GnRH-like Peptide increased rapidly at 24h, but after 24h it had no clear trend. In the high dose group, expression of hormones GnRH-like Peptide had no significant change in the trend, but not much change in the total amount of expression. Then, it showed a maximum at 48h, and increases rapidly.

2.5 Expression of GnRH-like Peptide in the visceral ganglion

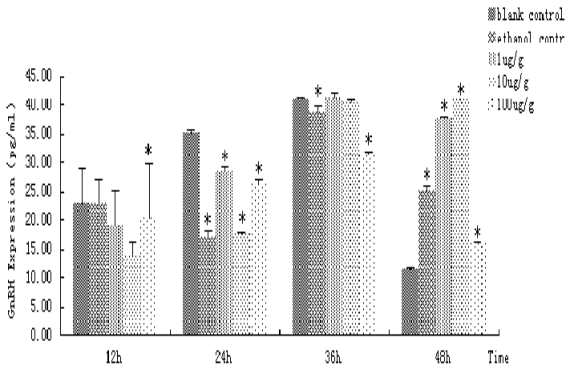

Fig.5 Expression of hormone GnRH-like Peptide in Visceral ganglion of C. chinensis (* *P*<0.05, extremely significantly differences between treatments and the control group)

From the graph (Fig. 5) we can see that the expression of hormones GnRH-like Peptide showed a maximum at 36h in all of the control groups and the experimental groups. In blank control group, the expression of hormones GnRH-like Peptide gradually increased with the extension of exposure time, and showed a maximum at 36h.But at 48h, it had a rapid decline and the trend was obvious. In the ethanol control group, the expression of hormones GnRH-like Peptide showed a downward trend, but then was a rapid rise and the trend is obvious, appeared the maximum at 36h. During the time of 36h-48h it began to decline rapidly. In the low dose group, the expression of hormones GnRH-like Peptide showed a gradual upward trend within 36h with the extension of exposure time, appeared maximum at 36h and then began a slow decline. In the middle dose group, with the extension of exposure time, the expression of hormones GnRH-like Peptide also showed a gradual upward trend and increased rapidly within 24h-36h, then appeared maximum at 36h.But in 36h and 48h, it had no obvious change. In high dose group, the expression of hormones GnRH-like Peptide also increased, but it was slow. At 36h it was highest, and then, it showed a obvious downward trend with the extension of exposure time.

3 Discussion

GnRH-like Peptide is a kind of neurohormonal that containing ten peptide which originally found in some vertebrates. In the Protoxhordata and vertebrate animals people have found 24 kinds of different structures. Studies have shown that many animals may contain many types of GnRH-like Peptide. For example, in some frog fish's body, researchers found sGnRH-like Peptide and cGnRH-like Peptide-II two forms. NP is a kind of environmental hormones which has estrogen effect, and it is hard degradating it is hard. So nonylphenol has a tremendous negative impact on water organisms. Li Zhao took Eriocheir sinensis as experimental materials; found that nonylphenol can have an effect corresponding to estrogen in Eriocheir sinensis. But now, there are not studies about nonylphenol on the expression of GnRH-like Peptide .In this paper, we analyzed the changes of expression of GnRH-like Peptide in different organizations in the role of nonylphenol, thus provide some suggestions for aquaculture.

GnRH-like Peptide exists not only in ovarian tissue of *C. chinensis*, but also in each ganglion. In *C. chinensis* there are four ganglia, brain sections, side ganglion, and the visceral ganglion and foot ganglion. Since side ganglion in ordinary dissection microscope can not discriminate clearly, so we did not choose it as experimental subjects.

Ovarian as the most important reproductive organs, contains higher levels of estrogen. GnRH-like peptide is the role of sex hormones in the regulation of expression. From the graph (Fig.3-2) we can see that the expression of GnRH-like Peptide Hormones in the control group also decreased with the extension of feeding time, consumption of nutrients. In low-dose and high-dose groups, with prolonged exposure time, the expression of GnRH-like Peptide hormones gradually increased. Compared with the control group, nonylphenol on the expression of GnRH-like Peptide hormones exhibit inhibition with toxic side effects, then it exhibited inducing action. Maybe it is related to the estrogen effect of nonylphenol. In the middle dose group, the trend is not obvious. However, compared with the control group, before 36h are expressed as toxicity, effects of estrogen appear at 48h. This is consistent with the trend of low-dose and high-dose groups. Therefore, GnRH-like peptide hormone in ovarian tissue can be used as an important indicator to detect nonylphenol pollution.

Brain sections as the nerve center of *C. chinensis*, plays a regulatory role on the body's life activities. Thus, GnRH-like Peptide is little overall change in expression. Expression levels peaked at 36h, because the body may be in a state of chronic hunger and needs to adjust its living activities. This resulted in a rapid increase in the expression of GnRH-like Peptide and later returned to the basic level. At three different concentrations of nonylphenol, we can clearly see that the expressions of GnRH-like Peptide are all first increased, then began declining, the peak time are different. In three doses, peak maximum is in low dose group, and with the NP concentration increased, the peak showed a gradual downward trend. This indicates that small doses of nonylphenol can promote the expression of GnRH-like Peptide, and the greater the concentration of nonylphenol is, the smaller the induced effect is. But with the extension of exposure time, nonylphenol enriched in the organism, showing side effects, and then resulted in the expression of GnRH-like Peptide began to decrease. Therefore, the role of environmental hormones on aquatic organisms can not be ignored.

In foot ganglion, the expressions of GnRH-like Peptide are much lower relative to the ovarian tissue and brain sections. In low dose group, nonylphenol had great influence on the expression of GnRH-like Peptide , first exhibited inhibitory at 24h, then increased rapidly even reached a peak at 36h. This trend is the same as the control group. Probably because the low dose and short time inhibit the expression of GnRH-like Peptide gene, While with the addition of reaction time, the body is hungry, coupled with the effects of estrogen, resulting in the increase of expression. In middle dose group and high dose group, nonylphenol showed toxicity, resulting in that the expression of GnRH-like Peptide is not always high, but the general trend is not obvious. Thus, a low concentration of nonylphenol has serious influence on the expression of GnRH-like Peptide in foot ganglion of *C. chinensis*.

In the visceral ganglion, the overall trend of GnRH-like Peptide expression in blank contral group is relatively similar with it in brain section and foot ganglion. It appeared the maximum at 36h and then began to gradually reduce the amount of expression, which may be related to the body's nerve regulation. In the low dose group, middle dose group and high dose group all had a maximum value at 36h, this trend was similar with the control group. But at 12h and 24h the values were lower compared with the control, indicating that nonylphenol inhibits the expression of GnRH-like Peptide. At 36h, the expression

of GnRH-like Peptide had no significant difference with the control group in middle dose group, which may be related to detoxification effect of liver. At 48h, the low dose group, middle dose group and high dose group all exhibited induction, and the effect is obvious, because of nonylphenol may be enriched in the body, showing the role of estrogen.

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