Hepatoprotective and antioxidant effects of dietary Glycyrrhiza polysaccharide against TCDD-induced hepatic injury and RT-PCR quantification of AHR2, ARNT2, CYP1A mRNA in Jian Carp (Cyprinus carpio var. Jian)

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ABSTRACT

To evaluate the protective effects of Glycyrrhiza polysaccharide (GPS) against 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD)-induced hepatotoxicity in Jian carp, the fish were fed diets containing GPS at doses of 0.1, 0.5 and 1.0 g/kg for 60 days before an intraperitoneal injection of 0.6 μg/kg TCDD at a volume of 0.05 mL/10 g body weight. At 72 hr post-injection, blood and liver samples were taken for biochemical analysis and the fish liver samples were used for the preparation of pathological slices. The results showed that increases in alanine aminotransferase (GPT), aspartate aminotransferase (GOT), lactate dehydrogenase (LDH), and alkaline phosphatase (AKP) in serum induced by TCDD were significantly inhibited by pre-treatment with 1.0 g/kg GPS. Following the 1.0 g/kg GPS pre-treatment, total protein (TP), albumin (Alb), catalase (CAT), glutathione peroxidase (GPx), total antioxidant capacity (T-AOC) and superoxide dismutase (SOD) activities in liver tissue increased significantly, malondialdehyde (MDA) formation (P < 0.05 or P < 0.01) was significantly inhibited, and the expression of cytochrome P4501A (CYP1A), aryl hydrocarbon receptor 2 (AHR2) and aryl hydrocarbon receptor nuclear translocator 2 (ARNT2) mRNA (P < 0.05) was significantly enhanced. Histological observations on fish liver were obtained by preparing paraffin tissue sections via HE staining, and the results showed that histological changes were obviously reduced by 0.5 and 1.0 g/kg GPS. GPS significantly reduced liver tissue damage caused by TCDD. Overall, these results proved the hepatoprotective effect of GPS in protecting against fish liver injury induced by TCDD, and supported the use of GPS (1.0 g/kg) as a hepatoprotective and antioxidant agent in fish.

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Introduction

Dioxins are organic pollutants which can exist in soil, air and water, and originate mainly from industrial garbage and
waste incineration. Dioxins pass easily into the food chain and have become a global environmental and public health problem (Chen et al., 2013; Bai et al., 2014). The toxicity mechanism of dioxins has become a hot research topic. There have been reports of dioxin poisoning due to the accumulation of dioxins in the food chain, and fish dioxin levels in the body can be up to ten thousand times higher than those in the environment (Pandelova et al., 2008; Lundstedt-Enkel, 2011). The 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) is one of the most toxic substances, and is an environmental pollutant that can have a negative effect on fish (Liu et al., 2013). Previous experiments have shown that many chemicals, such as carbon tetrachloride, dioxin, and ethanol can cause fish liver damage (Cullen, 2005). As liver is the detoxification organ in animals, it is more vulnerable to damage. Although many laboratories and controlled field investigations have documented that TCDD can cause toxicity in fish, the exact molecular mechanism by which TCDD exerts its effect in fish is still unclear, especially in fish liver (Li et al., 2013; Torre et al., 2015). There are few reports on the effects of TCDD in the literature; however, there were reports indicating that astaxanthin, laurel leaf extract, eicosapentaenoic acid, and glutamine could inhibit the liver damage induced by TCDD (Turkez and Geyikoglu, 2011; Turkez et al., 2012, 2013). In addition, tea polyphenols also had protective effects on liver injury (Liu et al., 2008a, 2008b).

The application of traditional Chinese medicine has a long history in our country, and contains many types of chemical components, such as polysaccharides, flavonoids, etc. (Deng and Zhou, 2014). Chinese herbal medicine is widely used in the treatment of diseases. Licorice is one of the most commonly used medicinal herbaceous plants and is a perennial leguminous plant. Licorice is known as the king of Chinese herbal medicine, and is also known as Ural licorice. Glycyrrhiza polysaccharide (GPS) is a type of a-D-pyran polysaccharide extracted from licorice. Previous experiments had shown that GPS has many functions including antioxidant properties, and can remove a variety of free radicals (Yang et al., 2007). It was reported that GPS inhibited the growth of liver tumors in rats (Zhang et al., 2013). It was also reported that GPS might have therapeutic implications in the clinical management of hepatocellular carcinoma patients (Chen et al., 2013). However, the effects of GPS against TCDD-induced liver injury in fish and its associated mechanisms have been rarely studied. This is the first time the effects of GPS against TCDD-induced liver injury have been studied in Jian carp.

In this study, the protective effect of GPS on acute liver injury induced by TCDD in Jian carp was evaluated by measuring changes in pathological sections and biochemical indices in liver tissues, and the hepatic expression of AHR2, ARNT2, and CYP1A mRNA. The findings of this study will provide a theoretical basis for the development of new drugs against TCDD-induced liver injury.

### 1. Materials and methods

#### 1.1. Fish

Jian carp were obtained from the Freshwater Fish Research Center of the Chinese Academy of Fishery Sciences, Wuxi, China. They were about 2 months old at the start of the experiment, with an average weight of (50.0 ± 1.0) g, and an average length of (12.0 ± 1.5) cm. The fish were reared at 26°C in a recirculation system and fed ad libitum twice a day with commercial diets containing approximately 32% crude protein, 4% crude lipid, and 15% ash. Fish were acclimated for 7 days prior to the experiment.

#### 1.2. Chemicals and Instruments

Dimethyl sulfoxide (DMSO) was purchased from Sigma-Aldrich, USA; Glycyrrhiza polysaccharide (GPS) was purchased from Nantong Universal Plant Extract Co., Ltd., China; alanine aminotransferase (GPT), aspartate aminotransferase (GOT), and other measurement kits were purchased from Nanjing Jiancheng Biology Engineering Research Institute, China; TCDD was purchased from Toronto Research Chemicals, Canada (CAS1746-01-6, purity 98%).

A 723 visible spectrophotometer was purchased from Shanghai Xinmao Instrument Co., Ltd., China; an MK3 microplate reader was purchased from Thermo Company, USA.

#### 1.3. Establishment of model for liver injury induced by TCDD

The 180 Jian carp were randomly and equally divided into 6 groups. Five test groups were intraperitoneally injected with TCDD at 0.1, 0.3, 0.6, 1.2, and 2.4 μg/kg at a volume of 0.05 mL/10 g body weight, respectively, in addition to one control group. At 24, 48, 72, 96, and 120 hr post-injection, blood and liver samples were obtained for biochemical analysis.

#### 1.4. Experimental design

The 120 healthy Jian carp without injury were selected for study and were randomly divided into six groups, 20 fish in each of three treatment groups, the TCDD control group, the GPS control group and the normal control group. The GPS control group was fed the basal diet supplemented with GPS at 1.0 g/kg. The TCDD control group and the normal control group were fed with the basal diet. Three treatment groups were fed the basal diet supplemented with GPS at 0.1, 0.5 and 1.0 g/kg. After 60 days of feeding, the normal control group was intraperitoneally injected with 0.1% DMSO at a volume of 0.05 mL/10 g body weight, while the TCDD control group and the GPS treatment groups were intraperitoneally injected with TCDD at a volume of 0.05 mL/10 g body weight. At 72 hr post-injection, blood and liver samples were obtained for analysis.

#### 1.5. DPPH assay

To determine the radical scavenging activity of GPS, a 2,2-diphenyl-1-picrylhydrazyl (DPPH) assay was conducted according to the methods described previously (Prevc et al., 2013). Different methanolic dilutions of extract were prepared (0.05–0.35 mg/mL). Briefly, 2.0 mL GPS extract was added to 2 mL DPPH solution (90 μmol/L in methanol) as the free-radical source. The mixtures were shaken vigorously and allowed to stand at room temperature for 30 min. The decrease in solution absorbance due to proton donating activity of the components of each extract was determined at 519 nm. Lower absorbance of the
reaction mixture indicated higher free-radical-scavenging activity. DPH with corresponding solvents (without plant material) served as controls. The DPH radical-scavenging activity (A, %) was calculated by Eq. (1):

\[
A = \left( \frac{A_0 - A_1}{A_0} \right) \times 100\%
\]

where, \(A_0\) is the absorbance of the control and \(A_1\) is the absorbance of the extract or standard sample.

### 1.6. Biochemical assays

Serum was separated by 3500 r/min centrifugation for 10 min at 4°C, and then stored at –20°C for analysis. A 0.9% saline solution was used for cleaning the fish liver and the surface was blotted dry with a clean filter paper. 0.1 g of liver tissue was accurately weighed and a 9-fold volume of 0.9% saline solution was added, and a 10% liver homogenate was prepared in an ice bath using a glass homogenizer. The liver homogenate was also stored at –20°C for analysis.

Changes in the following biochemical indices were determined: the serum levels of GOT, GPT, LDH, AKP were measured with an MK3 microplate reader using kits obtained from Nanjing Jiancheng Bioengineering Institute according to the manufacturer’s instructions; the serum levels of Alb and TP were measured with a 723 spectrophotometer using commercially available kits obtained from Nanjing Jiancheng Bioengineering Institute according to the manufacturer’s instructions; TP and Alb were expressed as g/L, GOT and GPT were expressed as IU/L, and LDH and AKP were expressed as U/L.

SOD, CAT, GPx, T-AOC and MDA in the liver tissue homogenate were measured with a 723 spectrophotometer using commercially available kits obtained from Nanjing Jiancheng Bioengineering Institute according to the manufacturer’s instructions; SOD, CAT, GPx, and CAT were expressed as U/mg protein, and MDA was expressed as nmol/mg protein.

### 1.7. Histopathological observation

The collected liver was placed in Bouin’s liquid and fixed for 48 hr, and histological observations of the fish liver were obtained by preparing paraffin tissue sections via HE staining.

### 1.8. Quantitative real-time PCR analysis of CYP1A, AHR2, and ARNT2 mRNA levels

Total RNA was extracted from fish liver using a fast pure RNA kit (Dalian Takara, China), according to the manufacturer’s instructions. The RNA quality was evaluated by gel electrophoresis, and the RNA concentration was determined by a GeneQuant 1300 (GE Healthcare Biosciences, USA), and normalized to a common concentration with DEPC-treated water (Invitrogen, China) before proceeding to cDNA synthesis. The procedure for reverse transcription was carried out according to the manufacturer’s instructions (Invitrogen, China), and the products (cDNA) were then stored at –20°C for qRT-PCR. The sequence of AHR and ARNT were determined by Shanghai BioSune Biotechnology Co. Ltd., China using an ABI 377 automated sequencer (Perkin Elmer, USA). Sequences were determined for each strand and each strand was sequenced at least 3 times. The results showed that the AHR and ARNT types were AHR2 and ARNT2.

Real-time quantitative PCR (qRT-PCR) was performed to detect the gene expression of AHR2, ARNT2, and CYP1A in fish liver using SYBR Premix Ex Taq (Dalian Takara, China), and the reaction was performed on an ABI PRISM 7500 Detection System (Applied Biosystems, USA). The program was set to run for one cycle at 95°C for 30 sec, 33 cycles at 95°C for 5 sec and at 60°C for 34 sec. The primers used in this study are listed in Table 1. The specificity of PCR amplification was confirmed by agarose gel electrophoresis and melting curve analysis. The gene expression results were analyzed using the 2^(-ΔΔCt) method (Livak and Schmittgen, 2001).

### 1.9. Determination of liver and spleen indexes

Liver index (LI, %) and spleen index (SI, %) were calculated by Eqs. (2) and (3):

\[
LI = \frac{W_l}{W_s} \times 100\%
\]

\[
SI = \frac{W_s}{W_l} \times 100\%
\]

where \(W_l\) (g) is the liver weight of each fish at the end of the experiment, \(W_s\) (g) is the spleen weight of each fish at the end of the experiment and \(W_l\) (g) is the weight of each fish at the end of the experiment.

### 1.10. Statistical analysis

Statistical analyses were carried out using SPSS version 17.0 software. Data were expressed as mean ± standard deviation. The differences between different groups were analyzed using one-way analysis of variance (ANOVA). * \(P<0.05\) and ** \(P<0.01\) were considered statistically significant.

### 2. Results

#### 2.1. Effects of different concentrations of TCDD on GOT, GPT, LDH, GST, and T-AOC activities in serum and liver tissue of Jian carp

Leakage of the marker enzymes (GOT, GPT, LDH, GST and T-AOC) from liver tissue and serum following exposure to TCDD are presented in Fig. 1. There were significant increases (\(P < 0.01\), \(P < 0.05\)) in GOT, GPT and LDH leakage from serum (Fig. 1a-c) at TCDD concentrations ≥0.3 μg/kg 72 hr post-injection. There

<table>
<thead>
<tr>
<th>Gene</th>
<th>PCR primer</th>
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<tbody>
<tr>
<td>Ahr2</td>
<td>F: ATTCCTTTGTCTTTAAGAACCGT</td>
</tr>
<tr>
<td></td>
<td>R: AGTCCAGATGTCGACGGGT</td>
</tr>
<tr>
<td>Arnt2</td>
<td>F: TGATCCAGCAGGTGACTCCTGTG</td>
</tr>
<tr>
<td></td>
<td>R: CGCTTGTTGAGCATCTCGCC</td>
</tr>
<tr>
<td>Cyp1A</td>
<td>F: TGACAGGAGAACGAATCCCGAGAG</td>
</tr>
<tr>
<td></td>
<td>R: TAGAGCAGCGCCAGAAGAGAGAG</td>
</tr>
<tr>
<td>β-Actin</td>
<td>F: GTCAATTCCTTTTAGGATGTGCCA</td>
</tr>
<tr>
<td></td>
<td>R: GGATGATGACCTTGAGCATGAGC</td>
</tr>
</tbody>
</table>
were significant decreases ($P < 0.01$, $P < 0.05$) in the antioxidant enzyme GST activity and total antioxidant capacity (T-AOC) leakage level from liver tissue (Fig. 1d, e) at TCDD concentrations $\geq 0.3 \mu g/kg$ 48 hr and 72 hr post-injection.

2.2. Effects of GPS on liver and spleen index in Jian Carp

As shown in Table 2, after the fish were treated with TCDD, the liver and spleen indexes were significantly decreased compared with the normal group ($P < 0.01$ or $P < 0.05$). When the carp were fed with GPS at the concentrations of 0.1, 0.5, and 1.0 g/kg, the liver and spleen indexes were significantly increased to different degrees ($P < 0.01$ or $P < 0.05$).

2.3. Radical scavenging activity of GPS

To verify the antioxidant power of GPS and its free-radical scavenging potency, the DPPH assay was performed. The results showed that GPS even at low concentrations exerted a potent radical scavenging activity (Fig. 2).

2.4. Effects of GPS on biochemical indices

The activities of the marker enzymes (GOT, GPT, LDH, AKP) from serum of fish treated with GPS are presented in Fig. 3; after the fish were treated with TCDD, the activities of GOT, GPT, LDH, and AKP in serum significantly increased ($P < 0.01$)

Fig. 1 – Effects of TCDD on alanine aminotransferase (GPT) (a), aspartate aminotransferase (GOT) (b), lactate dehydrogenase (LDH) (c), glutathione S-transferase (GST) (d), and total antioxidant capacity (T-AOC) (e) activities in serum and liver tissue of Jian carp. Values are expressed as mean ± SD ($n = 10$). *$P < 0.05$; **$P < 0.01$, compared with control group alone. TCDD: 2,3,7,8-Tetrachlorodibenzo-p-dioxin.
compared with the control group. When the carp were fed with GPS at the concentrations of 0.1, 0.5, and 1.0 g/kg, the activities of GOT, GPT, LDH, and AKP in serum significantly decreased to different degrees ($P < 0.01$ or $P < 0.05$).

As shown in Fig. 4, the levels of TP and Alb in serum decreased significantly ($P < 0.01$) compared with the control group. This trend was inhibited by the addition of GPS, and from the results, GPS at the concentration of 1.0 g/kg enhanced the levels of TP and Alb more than the other concentrations.

The activities of antioxidant enzymes (SOD, GPx, CAT, T-AOC) in liver treated with GPS are presented in Fig. 5; following injection of TCDD, the activities of SOD, GPx, CAT and T-AOC in the liver homogenate decreased significantly ($P < 0.01$ or $P < 0.05$) compared with the control group. GPS at the concentration of 1.0 g/kg had the best effect, and enhanced the activities of SOD, GPx, CAT and T-AOC ($P < 0.05$).

The localization of radical formation resulting in lipid peroxidation, measured as MDA in the liver, is shown in Fig. 6; following injection of TCDD, the content of MDA in liver homogenate increased significantly ($P < 0.01$) compared with the control group. GPS at the concentrations of 0.5 and 1.0 g/kg effectively inhibited the generation of MDA, and prevented TCDD-induced injury in fish liver.

### 2.5. Effects of GPS on hepatic tissue structure

The histopathological changes are shown in Fig. 7; observations using an optical microscope showed that the liver nuclei and cytoplasm had no abnormal changes in the control group. The hepatic tissue structure in the TCDD group was destroyed, parts of the nuclei of hepatocytes disintegrated, and vacuole formation was observed. When the fish were treated with 3 different concentrations of

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**Table 2 - Effects of GPS on liver and spleen indexes of TCDD treated carp.**

<table>
<thead>
<tr>
<th>Group</th>
<th>Liver index (%)</th>
<th>Spleen index (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal control</td>
<td>1.1854 ± 0.0903</td>
<td>0.2024 ± 0.0123</td>
</tr>
<tr>
<td>TCDD control</td>
<td>0.6076 ± 0.0352</td>
<td>0.1267 ± 0.0148</td>
</tr>
<tr>
<td>GPS control</td>
<td>1.1505 ± 0.0539</td>
<td>0.1958 ± 0.0155</td>
</tr>
<tr>
<td>0.1 g/kg GPS</td>
<td>0.5703 ± 0.0275</td>
<td>0.1324 ± 0.0153</td>
</tr>
<tr>
<td>0.5 g/kg GPS</td>
<td>0.8299 ± 0.0658</td>
<td>0.1651 ± 0.0160</td>
</tr>
<tr>
<td>1.0 g/kg GPS</td>
<td>0.7411 ± 0.0531</td>
<td>0.1253 ± 0.0119</td>
</tr>
</tbody>
</table>

Values are expressed as mean ± SD ($n = 10$). *$P < 0.05$; **$P < 0.01$, compared with the TCDD control group only.

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**Fig. 2** - Radical scavenging activity of GPS: the inhibition of DPPH changed as the concentration of GPS varied. GPS: Glycyrrhiza polysaccharide.

**Fig. 3** - Effects of GPS on serum alanine aminotransferase (GPT) (a), aspartate aminotransferase (GOT) (b), lactate dehydrogenase (LDH) (c) and alkaline phosphatase (AKP) (d) in TCDD-treated fish. Ctrl: fish were fed the basal diet without GPS; GPS Ctrl: fish were fed the basal diet supplemented with GPS at 1.0 g/kg; TCDD: fish were intraperitoneally TCDD at the concentration of 0.6 μg/kg. Values are expressed as mean ± SD ($n = 10$). *$P < 0.05$; **$P < 0.01$, compared with the group treated with TCDD alone.
GPS, different changes in hepatic tissue structure were observed. In the 0.1 g/kg GPS treatment group, the hepatocytes of Jian carp showed no obvious changes; in the 0.5 g/kg GPS treatment group, hepatocyte injury was ameliorated and hepatocyte contour gradually improved; in the 1.0 g/kg GPS treatment group, hepatocyte injury was markedly decreased.

2.6. Effects of GPS on CYP1A, AHR2, and ARNT2 mRNA expression

As shown in Fig. 8, AHR2, ARNT2, and CYP1A mRNA expression decreased significantly in the TCDD treatment group compared to the normal control. When the diets contained 3 different concentrations of GPS, all three concentrations of GPS significantly increased the expression of AHR2, ARNT2, and CYP1A mRNA to different degrees. GPS at 0.1 g/kg and 0.5 g/kg in the diet had no significant effect on AHR2, ARNT2, and CYP1A mRNA expression, while GPS at 1.0 g/kg significantly enhanced AHR2, ARNT2, and CYP1A mRNA expression, compared to the TCDD control.

3. Discussion

TCDD can induce liver injury, including hepatomegaly, fatty liver, and hepatic atrophy (Li et al., 2011). It has not only occurred in mammals but also in fish. Previous reports had peroxidation in zebrafish (Liu et al., 2008a, 2008b), and altered EROD activity in hepatocytes of rare minnow (Gobiocypris perryi) and Japanese medaka (Oryzias latipes) (Ma et al., 2010). In the present study, Jian carp injected with TCDD at the concentration of 0.6 μg/kg exhibited characteristics of liver injury (intracellular enzyme leakage and reduced oxidation resistance),
demonstrating that TCDD succeeded in inducing liver injury. At present, two views are often expressed regarding the toxicological mechanism of TCDD: one theory involves the aryl hydrocarbon receptor/aryl hydrocarbon receptor nuclear transporter pathway, and the other theory involves the aromatic hydrocarbon receptor/protein phosphorylation signal transduction pathway (Hassoun et al., 1995). The present study mainly focused on the first theory, in which TCDD is thought to cause a series of oxidative stress reactions and changes in enzyme activity (Jin et al., 2010).

In vivo experiments were performed to determine the hepatoprotective effect of the traditional Chinese medicine, GPS, against TCDD-induced liver injury. Similar results were also reported in rats and fish (Ciftci et al., 2013; Jia et al., 2014). After adding different concentrations of GPS to the diets, the increase in these 4 cytosolic enzymes was inhibited, which showed that GPS had a protective effect in TCDD-induced liver injury.

The liver plays an important role in the process of protein metabolism, and almost all the main proteins in plasma are produced by the liver. When the liver is damaged by external physical means, the protein synthesis is disrupted (Liu et al., 2012). In the present study, TCDD caused liver tissue damage leading to a loss of TP and Alb content in serum; however, the addition of GPS to the diets increased the content of these parameters to varying degrees, which showed that GPS alleviated TCDD-induced damage and that protein synthesis in liver tissue was resumed.

The indices SOD, GPx, CAT and T-AOC are important antioxidant enzymes in the body. The function of SOD is to prevent oxygen free-radicals, disproportionation of oxygen free-radicals, and to prevent the generation of hydroxyl radicals by inhibiting hydrogen peroxide (H$_2$O$_2$) combined with O$_2$ (Liu et al., 2010; Wang et al., 2011). Catalase (CAT) is a terminal oxidase in living organisms, and the main function of CAT is to catalyze H$_2$O$_2$, to prevent high levels of H$_2$O$_2$ in the

![Graph](image1)

**Fig. 6** – Effects of GPS on malondialdehyde (MDA) level in TCDD-treated fish. Ctrl: fish were fed the basal diet without GPS; GPS Ctrl: fish were fed the basal diet supplemented with GPS at 1.0 g/kg; TCDD: fish were intraperitoneally TCDD at the concentration of 0.6 μg/kg. Values are expressed as mean ± SD (n = 10). *P < 0.05; **P < 0.01, compared with the group treated with TCDD alone.

![Image](image2)

**Fig. 7** – Effects of GPS on Jian carp liver injury induced by TCDD (HE, ×200). (a) Control group; (b) GPS control group; (c) TCDD control group; (d) 0.1 g/kg GPS treatment group; (e) 0.5 g/kg GPS treatment group; (f) 1.0 g/kg GPS treatment group.
significantly increased, suggesting that GPS enhanced Jian carp were treated with GPS, the activities of SOD, GPx, CAT and T-AOC were significantly inhibited by GPS. This inhibitory effect may be related to the radical scavenging ability of GPS.

CYP1A is an important subgroup of CYP450, and its activity level can directly influence the therapeutic effect of drugs and their toxic effects (Rowlands et al., 2011; Kim et al., 2013). It can predict the effectiveness and potential toxicity of drugs in vitro and in vivo. The aryl hydrocarbon receptor is a member of the family of basic helix-loop-helix transcription factors. AHR can bind several exogenous ligands such as natural plant flavonoids, polyphenolics and indoles, as well as synthetic polycyclic aromatic hydrocarbons and dioxin-like compounds. In previous studies, AHR was found to play an important role in the toxic effects of dioxins. It is a ligand for transcription factors in a variety of aromatic compounds, and regulates the biological effects of dioxin (Iwata et al., 2010). ARNT has also been proposed as a factor in regulating biological responses, and it was proposed that recruitment of ARNT to complex with AHR2 may affect the formation of dimers with other partners (Holmes and Pollenz, 1997). In the present study, the preliminary findings showed the opposite: following injection of TCDD for 72 hr, as the expression of CYP1A, AHR2, and ARNT2 mRNA decreased. Similar results have been observed in mouse hepatoma cells and rats liver treated with TCDD or dioxin (Prokipcak and Okey, 1991; Giannone et al., 1998; Peng and Gao, 2009); the authors concluded that the AHR or ARNT is down-regulated by TCDD. CYP1A induction is one of many biochemical responses to TCDD and, although well characterized, is probably not involved directly in acute TCDD toxicity (Poland and Knutson, 1982). It was reported that AHR expression correlated with ARNT and CYP1A mRNA expression. The environmental contaminant TCDD could induce CYP1A gene transcription, but this process required two basic helix-loop-helix regulatory proteins, the aromatic hydrocarbon receptor (AHR) and the aromatic hydrocarbon receptor nuclear translocator (ARNT) (Abbott et al., 1999). The inhibition of CYP1A gene expression may be related to the decrease of AHR2 and ARNT2 activities in liver of Jian Carp. When 3 different concentrations of GPS were added to the diets, GPS at 1.0 g/kg showed the best effects, and upregulated CYP1A, AHR2, and ARNT2 expression, which showed that GPS had a good effect on relieving TCDD damage.

**Fig. 8 – Effects of GPS on cytochrome P4501A (CYP1A) (a), aryl hydrocarbon receptor 2 (AHR2) (b), and aryl hydrocarbon receptor nuclear translocator 2 (ARNT2) (c) mRNA expression levels in liver injury induced by TCDD.** Ctrl: fish were fed the basal diet without GPS; GPS Ctrl: fish were fed the basal diet supplemented with GPS at 1.0 g/kg; TCDD: fish were intraperitoneally TCDD at the concentration of 0.6 μg/kg. Values are expressed as mean ± SD (n = 10). *P < 0.05; **P < 0.01, compared with the group treated with TCDD alone.

**4. Conclusions**

It can be concluded that GPS at the concentration of 1.0 g/kg had the best effect in protecting against liver injury in Jian carp. The protective mechanism of GPS may be related to its antioxidant and free-radical scavenging ability, and its regulation of AHR2, ARNT2, and CYP1A mRNA expression. Overall, these results show that GPS could be used as a protective drug against TCDD-induced liver damage in fish. These findings may also lay the foundation for the development of new drugs against TCDD-induced liver injury.

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