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RESEARCH ARTICLE

Developmental Toxicity Assessment of the Extract of *Buddleja officinalis Maxim*

Yuan Li[1,2], Baosheng Huang[2,3]*, Hangping Yu[4], Shijie Sun[4], Zhaoqing Han[5], Hongzeng Wang[5] and Yujie Wang[1]*

[1]School of Safety Science and Emergency Management, Wuhan University of Technology, Wuhan 430070, China; [2]National Engineering Research Center for Agricultural Products Logistics, Shandong Institute of Commerce and Technology, Jinan 250103, China; [3]Shandong GuoNong Logistics Technology Co., Ltd., Jinan 250103, China; [4]Hunter Biotechnology, Inc., Hangzhou 310000, China; [5]College of Agriculture and Forestry, Linyi University, Linyi 276000, China

*Corresponding author: huangbs@stu.hubu.edu.cn (BH), yjwwhut@163.com (YW)

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ABSTRACT

To assess the potential risk of toxicity in fish in the process of applying *Buddleja officinalis Maxim.* extract, zebrafish (Danio rerio) was selected as the model organism for developmental toxicity assessment. The nonlethal concentration (MNLC) of *Buddleja officinalis* extract for zebrafish was 21µg/mL, and the lethal concentration (LC10) was 34µg/mL. The extract showed no significant outcome on the zebra fish hatching rate at tested levels (2.3, 7, 21 and 34µg/mL) ($P>0.05$). The incidence of delayed yolk sac absorption increased in a dose-dependent manner (16.7% in control vs. 30.0%, 36.7%, 66.7% and 66.7% in treatment groups, respectively) ($P<0.05$). No obvious abnormalities were found in other organs or tissues. The *Buddleja officinalis* extract significantly increased the activity of CYP3A4 in zebrafish cytochrome P450 (CYP) at 21µg/mL and 34µg/mL, whereas 34µg/mL significantly increased the activity of CYP2D6. The extract had no significant effect on the activity of Caspase-3/7 in zebrafish at the experimental concentrations. The same situation was found regarding the morphology of zebrafish liver cells. The extract increased the ratio of Bcl-2/Bax at concentrations of 21µg/mL and 34µg/mL and inhibited apoptosis at concentrations of 21µg/mL and 34µg/mL. This study revealed that the extract has no obvious toxicity for the development of zebrafish and does not cause obvious damage to zebrafish liver tissue, which provides a possibility for further research and development of *Buddleja officinalis* extract to alleviate stress in fish.

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INTRODUCTION

Buddleja officinalis Maxim. was first recorded in the Chinese materia medica "Kaibao Bencao"and has been widely documented in traditional Chinese medicine literature. According to the Chinese Pharmacopoeia (2020), it is defined as the dried flowers or buds of *Buddleja officinalis Maxim.* The nature and flavor are sweet and slightly cold, and this plant is associated with the liver meridian (State Pharmacopoeia Committee, 2020). Phytochemical studies have shown that *Buddleja officinalis* contains flavonoids, phenylethanoid glycosides, and triterpenoids (Han *et al*., 2004). Based on team's own Chinese patent technology "Temperature-sensitive Chinese herbal medicine active ingredient extraction and concentration unit" (ZL 201821738845.0), we explored the preparation and process optimization of *Buddleja officinalis Maxim.* extract, including the optimization of extraction temperature, extraction medium, probiotic fermentation, ultrasonic pretreatment and other preparation processes. The extract of *Buddleja officinalis Maxim.* has been shown to alleviate stress in crucian carp and wheat ear fish (Jiang, 2023). At present, as the most important vertebrate model organism in the world, zebrafish have been widely used in the development and function of the nervous system(Mutschler *et al.*, 2024), immune system (Lorimer, 2024), cardiovascular system(Chen *et al.*, 2024), reproductive system(Wu *et al.*, 2023), and other systems, as well as the occurrence and regulation of neurodegenerative diseases (Liu, 2023), skeletal system diseases (Laue *et al.*, 2011), tumors (Sautan *et al.*, 2024), and other diseases, and it has been applied to the large-scale screening of new drugs for small-molecule compounds (Eva *et al.*, 2024). To assess the potential risk of toxicity to fishes in the process of applying *Buddleja officinalis Maxim.* extract, zebrafish

(Danio rerio) was selected as the model organism for developmental toxicity assessment. A systematic study of different drug concentrations may provide support for mechanistic research and product R&D for *Buddleja officinalis* Maxim in the future. Although *Buddleja officinalis* extract has shown promising effects, its developmental toxicity has not been systematically evaluated.

MATERIALS AND METHODS

Experimental animals: Wild-type AB strain zebrafish were bred via paired mating. Each experimental group consisted of 30 larvae aged 6 hours after fertilization (6 hpf). The fish were reared in water (28°C). The quality of water was maintained by dissolving 200mg of instant sea salt in 1L of reverse osmosis water. The prepared water showed a conductivity ranging from 480 to 510 µS/cm, a pH between 6.9 and 7.2, and a hardness value of 53.7–71.6mg/L as CaCO3. All experimental procedures were approved by the Institutional Animal Care and Use Committee (IACUC) (Approval No.: IACUC-2019-2100-01). Feeding management adhered to the requirements of international AAALAC certification.

***Buddleja officinalis Maxim.* Extract:** The extraction involved aqueous extraction, concentration, and freeze-drying. *Buddleja officinalis Maxim.* extract was prepared via extraction of the active ingredients of a temperature-sensitive Chinese herbal medicine and concentrated in the laboratory. A brown solid was obtained after freeze-drying and stored in a refrigerator at a low temperature of 4°C. When used for research, the original liquid with a concentration of 100mg/mL was prepared with ultrapure water and diluted for the experimental design.

Instruments and reagents: Instruments and reagents used in this study included an anatomical microscope (Olympus, SZX7) and a microscope-mounted camera system (Germany, VertA1); a precision electronic balance (Ohaus, USA, CP214); a multifunctional microplate reader (Berthold Technologies, Germany, LB940); 96-well microtiter plates (Corning Incorporated, USA); 6-well plates (Fisher Scientific, USA); methylcellulose (Sigma, USA); dimethyl sulfoxide (Sigma, USA); a caspase-3 kit (Promega, USA); a caspase-9 kit (Promega, USA); a CYP3A4 kit (Promega, USA); 4% paraformaldehyde (Dingguo Biological, China); xylene (Aladdin, China); ammonia water (Sinopharm Group, China); PBS buffer (Boster Bioengineering Co., Ltd., China); Mayer's hematoxylin staining solution (Yihe Biological, China); eosin staining solution (Ech Bio, China); a neutral resin sealing agent (Yihe Biological, China); soft wax (melting point 50–52°C, Shanghai Huayong Paraffin Co., Ltd., China); and hard wax (melting point 60–62°C, Shanghai Huayong Paraffin Co., Ltd., China).

Assessment of the maximum nonlethal concentration (MNLC) and lethal concentration (LC10) of the *Buddleja officinalis* Maxim extract: A total of 180 normal 6 hpf wild-type AB strain zebrafish were randomly selected, placed in six-well plates and treated with diluted extracts of *Buddleja officinalis Maxim.* at 15.6, 31.3, 62.5, 125 and 250µg/mL. Fish culture water treatment was used in the normal control group. At the same time, each experimental group was treated with 30 zebrafish; during the experiment, the deaths of the zebrafish were recorded every day, and the dead fish were removed immediately. After 120 h of treatment, the number of zebrafish that died and the degree of toxicity in each experimental group were determined. Using Origin 8.0 statistical software, the optimal concentration-effect curve was plotted, and the minimum no-lethal concentration (MNLC) of the extract from Flos Buddlejae on zebrafish was calculated to be 21µg/mL, with a lethal concentration 10 (LC10) of 34µg/mL.

Developmental toxicity and teratogenicity of the *Buddleja officinalis Maxim.* Extract: A total of 150 normal 6 hpf wild-type AB strain zebrafish were randomly selected, placed in six-well plates and treated with diluted extracts of *Buddleja officinalis Maxim.* at drug concentrations of MNLC/9, MNLC/3, and MNLC/LC10. Fish culture water treatment) was used in the normal control group. The zebrafish heart, brain, jaw, eye, liver, intestine, trunk/tail/notochord, muscle/somite/movement, body color, circulatory system, body edema, hemorrhage, fin and other developmental toxic reactions were recorded under a microscope from 114 h to 5 dpf. The incidence of developmental toxicity in each experimental group was determined according to the developmental toxicity of each organ, and the typical organs associated with developmental toxicity were photographed.

Effects of *Buddleja officinalis* extract on cytochrome P450 (CYP) and caspase activity: A total of 300 normal 6 hpf wild-type AB strain zebrafish were randomly selected, placed in six-well plates and treated with diluted extracts of *Buddleja officinalis Maxim.* at drug concentrations of MNLC/9, MNLC/3, and MNLC/LC10. Fish culture water treatment) was used in the normal control group.

The activities of CYP3A4 and CYP2D6 were detected via a cytochrome P450 kit after the zebrafish were treated with *Buddleja officinalis Maxim.* extract for 5 days. The signal intensity of the relative luminescence units (RLU) was detected with a microplate reader. The effects of the *Buddleja officinalis Maxim.* extract on the activities of CYP3A4 and CYP2D6 in zebrafish were evaluated via RLU statistical analysis. The formula for calculating the inhibitory effect of *Buddleja officinalis Maxim.* extract on CYP3A4/CYP2D6 activity is as follows:

Activity induction

$$(\%)=\left[\frac{S(\text{experiment al group})}{S(\text{control group})}-1\right]\times 100\%$$

The activity of caspase-3/7 of the cysteinyl aspartate-specific proteinase (caspase) family related to apoptosis was detected via a caspase kit after the zebrafish were treated with *Buddleja officinalis Maxim.* extract for 5 days. The RLU signal intensity was detected with a microplate reader. The effects of different concentrations of *Buddleja officinalis Maxim.* extract on the activity of Caspase-3/7 were evaluated by using statistical technique of RLU. The statistical analysis results are expressed as the means±SEs. The effects of *Buddleja officinalis* extract on caspase-3/7 activity were calculated as follows:

$$\text{Caspase inducement (\%)} = \left(\frac{\text{S (experimental group)}}{\text{S (control group)}} - 1\right) \times 100\%$$

Effects of *Buddleja officinalis* extract on liver tissue: A total of 150 normal 6 hpf wild-type AB strain zebrafish were randomly selected, placed in six-well plates and treated with diluted extracts of *Buddleja officinalis Maxim.* at drug concentrations of MNLC/9, MNLC/3, and MNLC/LC10. Fish culture water treatment was used in the normal control group. After 5 days of exposure, zebrafish were collected and fixed in 4% paraformaldehyde, followed by standard hematoxylin and eosin (H&E) staining. extract. After staining, histopathological sections of zebrafish livers from each experimental group were observed.

Effects of *Buddleja officinalis* maxim extract on the expression of related genes (Bax and Bcl-2): A total of 150 normal 6 hpf wild-type AB strain zebrafish were randomly selected, placed in six-well plates and treated with diluted extracts of *Buddleja officinalis Maxim.* at drug concentrations of MNLC/9, MNLC/3, and MNLC/LC10. Fish culture water treatment was used in the normal control group.

The total RNA of the zebrafish in each experimental group was extracted via the classical TRIzol method after 5 days of treatment with *Buddleja officinalis Maxim.* extract. The concentration and purity of total RNA were determined with a Thermo Ultra-Micro spectrophotometer. β-actin was used as the housekeeping gene; PCR conditions were added. The transcription levels of the Bax and Bcl-2 genes related to the apoptosis pathway were detected via q-PCR, and the relative RNA expression levels of the target genes were calculated (Table 1). The calculation formula is as follows:

$$\text{RNArelative expression} = 2\string^\Delta\Delta C(t)$$

$$\Delta\Delta C(t) = \overline{\Delta C(t)}_{\text{control group}} - \Delta C(t)_{\text{experimental group}}$$

$$\Delta C(t) = C(t)_{\text{targeted gene}} - C(t)_{\beta\text{-actin}}$$

Table 1: Details of Primer sequence used

Gene	Primer sequences 5'→3'	
β-actin	Forward	TCGAGCAGGAGATGGGAACC
	Reverse	CTCGTGGATACCGCAAGATTC
Bax	Forward	GACTTGGGAGCTGCACTTCT
	Reverse	TCCGATCTGCTGCAAACACT
Bcl-2	Forward	CACTGGATGACTGACTACCTGAA
	Reverse	CCTGCGAGTCCTCATTCTGTAT

Data analysis: SPSS 27.0 statistical software was used for data processing and analysis, and the results are expressed as the means±standard deviations (±SDs). Origin 8.0 was used to generate the corresponding curve; statistical analysis was carried out through analysis of variance (ANOVA) followed by Dunnett's T test (P<0.05).

RESULTS

Assessment of the MNLC and LC10 of the *Buddleja officinalis Maxim.* Extract: After 114 h of treatment, two zebrafish in the control group died, corresponding to a mortality rate of 6.7% (Table 2). When the concentration of *Buddleja officinalis Maxim.* extract was set to 15.6, 31.3, 62.5, 125 and 250µg/mL, 2-, 26-, and 30-tailed zebrafish death was induced, and the mortality rates were 6.7%, 86.7%, 100% and 100%, respectively. The best concentration-effect curve was generated via Origin 8.0 statistical software. Through further calculation, the MNLC and LC10 of *Buddleja officinalis* extract for zebrafish were determined to be 21µg/mL and 34µg/mL, respectively (Fig. 1).

Table 2: Statistical data of zebrafish mortality under different *Buddleja officinalis Maxim.* extract concentrations

Group	Experimental concentration of *Buddleja officinalis Maxim.* extract (µg/mL)	Number of deaths (tail)	Mortality (%)
Control group	-	2	6.7
Experimental group	15.6	0	0
	31.3	2	6.7
	62.5	26	86.7
	125	30	100
	250	30	100

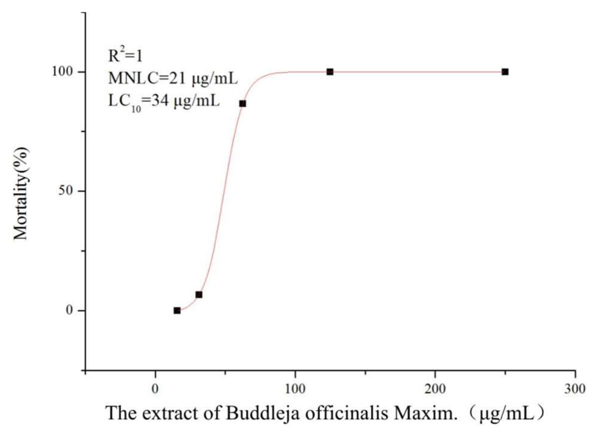

Fig. 1: Concentration–lethal effect curve for the extract of *Buddleja officinalis Maxim.*

Developmental toxicity and teratogenicity of *Buddleja officinalis Maxim.*

The concentrations of MNLC/9 and MNLC/3, as well as the MNLC and LC10, for zebrafish were calculated to be 2.3, 7, 21 and 34µg/mL, respectively, according to the MNLC of 21µg/mL and the LC10 of 34µg/mL. The experiments involving the above four treatment groups and the control group revealed that after 114 h of treatment with *Buddleja officinalis* extract until 5 dpf, no deaths occurred in any of the concentration groups. When the *Buddleja officinalis* extract was treated at 48 hpf, no egg condensation, abnormal somite formation or tail extension were observed in any of the experimental groups. At 72 hpf, the hatching rates of zebrafish in the 2.3, 7, 21 and 34µg/mL concentration groups were 100%, 100%, 100% and (P>0.05), 96.7%, respectively, in comparison to those in normal control group (100%) suggesting that the extract of *Buddleja officinalis* had no significant effect on the hatching rate of zebrafish under the experimental concentration conditions. The incidence of delayed absorption of zebrafish yolk sacs in the normal control group was 16.7%; the absorption of zebrafish yolk sacs was delayed by 30.0%, 36.7%, 66.7% and 66.7% in the 2.3, 7, 21 and 34μg/mL groups, respectively. Although the extract still had a slight effect on development, no severe morphological abnormalities were observed. The incidences of developmental toxicity and teratogenicity are shown in Figure 2, Figure 3 and Table 3.

Effects of *Buddleja officinalis Maxim.* extract on cytochrome P450 (CYP) and caspase activity

Effects of *Buddleja officinalis* extract on cytochrome P450 (CYP) activity: In this study, the RLU values of zebrafish CYP3A4 activity in the 2.3, 7.0, 21 and 34μg/mL *Buddleja officinalis* extract groups were 504, 539, 558 and 594, respectively. In Comparison to those in the normal control group (489) (P>0.05, P<0.05 and P<0.001), the induction of CYP3A4 activity in zebrafish was 3%, 10%, 14% and 22%, respectively. These findings suggest that the *Buddleja officinalis* extract significantly increased the activity of CYP3A4 in zebrafish cytochrome P450 (CYP) at 21 and 34μg/mL.

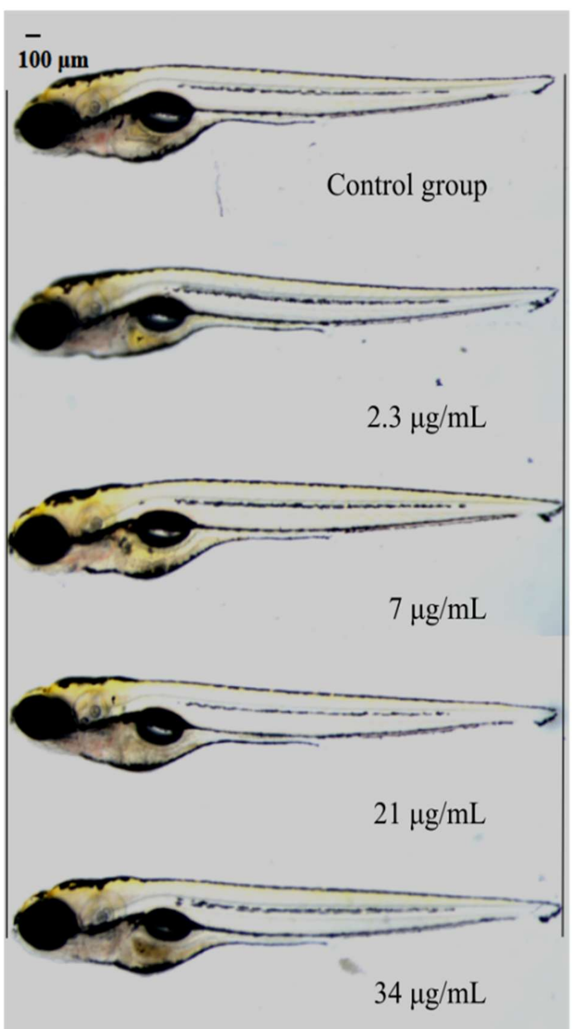

Fig. 2: The body length phenotype map of zebrafish.

Table 3: The incidence of developmental toxicity and teratogenicity data

Malformation type		Control group	Experimental concentration of *Buddleja officinalis Maxim.* Extract (μg/mL)			
			2.3	7	21	34
Heart	Pericardial edema	-	-	-	-	-
	Abnormal blood rate	-	-	-	-	-
	No ventricular atrium	-	-	-	-	-
Blood system	Blood flow too fast	-	-	-	-	-
	Blood flow too slow	-	-	-	-	-
	Cycle direction defect	-	-	-	-	-
Kidney	Body edema	-	-	-	-	-
Hemorrhage and thrombus		-	-	-	-	-
Brain	Brain degeneration	-	-	-	-	-
	Became smaller	-	-	-	-	-
	Irregular shape	-	-	-	-	-
Eye	Became smaller	-	-	-	-	-
	Irregular shape	-	-	-	-	-
Mandible	Malformation	-	-	-	-	-
Liver	Deletion	-	-	-	-	-
	Tumefaction	-	-	-	-	-
	Became smaller	-	-	-	-	-
	Liver degeneration	-	-	-	-	-
Intestinum	Lack of internal cavity	-	-	-	-	-
	Lack of intestinal folds	-	-	-	-	-
Trunk/Tail/Notochord	Bending	-	-	-	-	-
Muscle	Denaturation	-	-	-	-	-
Body coloring	Abnormality	-	-	-	-	-
Trunk Length	Abnormality	-	-	-	-	-
Tail fin	Deletion	-	-	-	-	-
	Became smaller	-	-	-	-	-
	Finfold deformity	-	-	-	-	-
Delayed absorption of yolk sac		16.7	30.0	36.7	66.7	66.7
Mortality		-	-	-	-	-

CYP2D6 is highly important for the metabolism of clinically used drugs, and approximately 20–25% of these compounds are metabolized by this enzyme (Ingelman-Sundberg, 2005). The RLU values of CYP2D6 activity in zebrafish at 2.3, 7.0, 21 and 34μg/mL were 52599, 53493, 56383 and 66295, respectively. Compared with the normal control group (44451) (P>0.05, P>0.05, P>0.05 and P<0.001), the induction of CYP2D6 activity in zebrafish was 10%, 12%, 18% and 39%, respectively. These findings suggest that the extract of *Buddleja officinalis* significantly affects the activity of CYP2D6 in zebrafish cytochrome P450 (CYP) at a concentration of 34μg/mL (Table 4).

Table 4: Quantitative results of CYP3A4 and CYP2D6 activity in zebrafish after treatment with *Buddleja officinalis* extract (n=5, mean±SE)

Group	Experimental concentration of *Buddleja officinalis Maxim.* Extract (μg/mL)	CYP3A4		CYP2D6	
		RLU	Inducement effect (%)	RLU	Inducement effect (%)
Control group	-	489±13	-	44451±3823	-
Experimental group	2.3(MNLC/9)	504±24	3	52599±1034	10
	7.0(MNLC/3)	539±9	10	53493±3157	12
	21(MNLC)	558±10*	14*	56383±2148	18
	34(LC10)	594±17***	22***	66295±3615***	39***

Note: The values were compared with those of normal control group; *P<0.05 and ***P<0.001 were statistically significant.

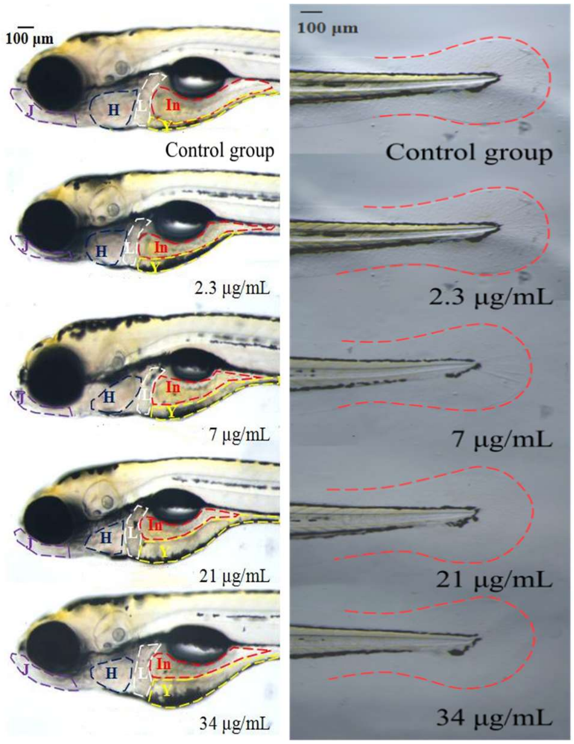

Fig. 3: Left: Organ phenotypes (heart, brain, jaw, eye, liver, intestine) of zebrafish. Right: tail fin phenotype map of zebrafish; Note: J = mandible; h = heart; l = liver; y = yolk sac; in = intestinal tract; e = eyes.

Effects of *Buddleja officinalis Maxim.* extract on the activity of caspase-3/7: The RLU values of caspase-3/7 activity in zebrafish in the 2.3, 7.0, 21 and 34µg/mL groups were 804950, 792167, 785481 and 730748, respectively. Compared with those in the normal control group (808470), the RLU values of caspase-3/7 activity in zebrafish in each concentration group were 0%, -2%, -3% and -10%, respectively. Negative values indicate a decrease relative to control.These findings suggested that the *Buddleja officinalis* extract had no significant effect on the activity of Caspase-3/7 in zebrafish at the experimental concentrations (Table 5).

Table 5: The activity of Caspase-3/7 in zebrafish treated with *Buddleja officinalis* extract (n=5, mean±SE).

Group	Experimental concentration of *Buddleja officinalis Maxim.* extract (µg/mL)	Caspase-3/7	
		RLU	Inducement effect (%)
Control group	-	808470±40724	-
Experimental group	2.3 (MNLC/9)	804950±20636	0
	7.0 (MNLC/3)	792167±30205	-2
	21 (MNLC)	785481±34762	-3
	34 (LC10)	730748±29824	-10

Effects of *Buddleja officinalis Maxim.* extract on liver histopathology: In this study, the normal control group zebrafish liver cell outline was clear, there was no visible swelling or vacuoles, the cytoplasm was evenly distributed, there was no visib006Ce foreign matter, and the nucleus size and shape were regular and interlaced. The cytoplasm of liver cells in the 2.3, 7, 21 and 34µg/mL *Buddleja officinalis* extract groups was clear, without visible swelling or vacuoles, and the cytoplasm was evenly distributed without visible foreign bodies (Figure 4). The size and shape of the nucleus were regular and intertwined, similar to those of the normal control group. These findings suggest that under the concentration conditions used in this study, the extract of *Buddleja officinalis* had no significant effect on the morphology of zebrafish liver cells.

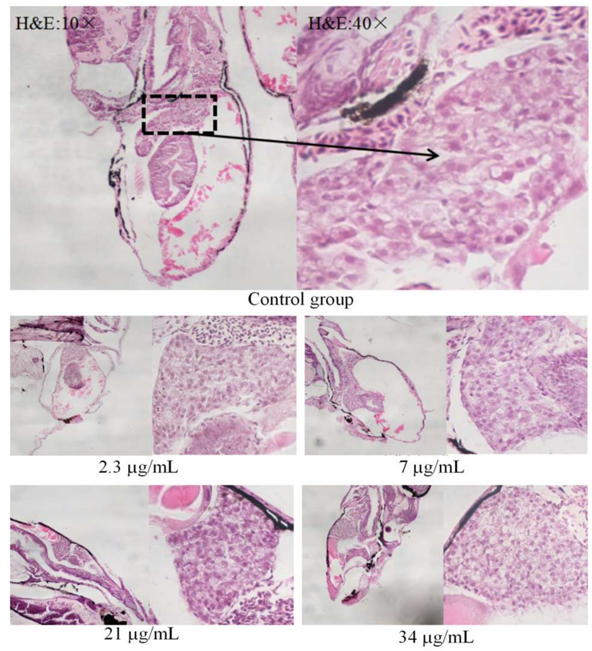

Fig. 4: Typical diagram of the effects of *Buddleja officinalis* extract on the morphology of zebrafish liver tissue cells.

Effects of *Buddleja officinalis Maxim.* extract on the expression of zebrafish-related genes

RNA extraction results: After treatment with Buddleja extract, total RNA was extracted from the zebrafish, and the concentration and purity of the RNA (A260/A280 ratio) were determined via an ultramicro spectrophotometer (Table 6). The A260/A280 ratio was between 2.00 and 2.04, indicating that the quality of total RNA extracted from zebrafish was good and that it could be used for subsequent q-PCR experiments (Table 6).

Table 6: Concentration and purity of total RNA

Group	Experimental concentration of *Buddleja officinalis Maxim.* extract (µg/mL)	Total RNA concentrations (ng/µL)	A260/A280
Control group	-	1065.3	2.00
Experimental group	2.3 (MNLC/9)	1471.6	2.03
	7.0 (MNLC/3)	1603.3	2.01
	21 (MNLC)	1158.9	2.01
	34 (LC10)	1281.4	2.03

Effects of *Buddleja officinalis* maxim extract on the transcription levels of the Bax and Bcl-2 genes related to the apoptosis pathway: The relative expression level of the Bcl-2 gene was 1.00 for normal control group, and the values of 0.84, 0.94, 1.90 and 2.11 were found for 2.3, 7.0, 21 and 34µg/mL treatment groups respectively. These findings suggest that the *Buddleja officinalis* extract can upregulate the relative expression of Bcl-2 at concentrations of 21 and 34µg/mL.

The relative expression of the Bax gene in the normal control group was 1.00, and the relative expression of the Bax gene in the 2.3, 7.0, 21 and 34µg/mL groups was 0.88, 0.92, 1.12 and 1.15, respectively. Compared with that in the normal control group, the $P>0.05$ for each concentration group suggested that the extract of *Buddleja officinalis* did not show any significant effect in the relative expression of Bax at specified concentration being used in the study.

The value of Bcl-2/Bax in the normal control group was 1.00, and the values of Bcl-2/Bax in the 2.3, 7.0, 21 and 34µg/mL groups were 0.95, 1.02, 1.70 and 1.84, respectively ($P>0.05$, $P>0.05$, $P<0.001$ and $P<0.001$, respectively), suggesting that the extract of *Buddleja officinalis* can increase the ratio of Bcl-2/Bax at concentrations of 21 and 34µg/mL and inhibit apoptosis at concentrations of 21 and 34µg/mL (Table 7).

Table 7: Effects of *Buddleja officinalis* extract on the transcription levels of genes related to the apoptosis pathway (n=3, mean±SE).

Group	Experimental concentration of *Buddleja officinalis Maxim.* Extract (µg/mL)	Relative expression of Bax	Relative expression of Bcl-2	Bcl-2/Bax
Control group	-	1.00±0.04	1.00±0.05	1.00±0.05
Experimental group	2.3 (MNLC/9)	0.88±0.05	0.84±0.09	0.95±0.05
	7.0 (MNLC/3)	0.92±0.06	0.94±0.09	1.02±0.04
	21 (MNLC)	1.12±0.04	1.90±0.04***	1.70±0.08***
	34 (LC10)	1.15±0.08	2.11±0.05***	1.84±0.08***

DISCUSSION

The word 'stress' was first proposed by Canadian pathophysiological scientist Selye H in 1936; it is an important concept in the field of medical physiology (Selye, 1956). When the living environment of aquatic products changes, factors such as high or low temperature, oxygen content, water quality deterioration, transportation vibration, environmental noise, external light intensity changes and other external factors can lead to a stress response in aquatic products. Aquatic products undergo a series of compensatory reactions to adapt or respond to these changes. Mazeaud (*et al*., 1977) suggested that primary, secondary and tertiary reactions are a series of physiological reactions that may help aquatic products maintain balance in the body under cold stress. The primary reactions are related to the nervous and endocrine systems; the secondary reactions are related to immunity, hemodynamics and metabolism; and the tertiary reactions are related to the growth, development and behavioral characteristics of fish (Chen *et al*., 2021). When the body cannot adapt to these changes, there can be stress injury and even death.

Cytochrome P450 enzymes are a large family of isozymes (Meunier *et al*., 2004) that are primarily located in liver microsomes and participate in the biotransformation of many endogenous and exogenous substances in organisms. Interactions between many clinically relevant drugs are associated with the inhibition and/or induction of CYP enzymes (Mei-Wei *et al*., 2023). CYP3A4 is a member of the cytochrome P450 oxidase family, accounting for approximately 50%.

Caspases are an evolutionarily conserved family of cysteine-dependent endonucleases whose substrates are hydrolyzed after specific aspartic acid residues. They are composed of a variable-sized N-terminal domain followed by large and small catalytic subunits, with 20 kDa and 10 kDa combined to form a protease domain. Recent studies have shown that caspase-mediated cell lysis (necroptosis, a lysis-regulated cell death pattern driven by receptor-interacting protein (Rip) kinases) is involved. In addition, the mechanism by which inflammatory caspases promote pyroptosis has been discovered in recent years; this is another major lytic cell death pattern that is associated with the secretion of the inflammatory cytokines IL-β and IL-18 (Xu *et al*., 2025). Increasing evidence shows that the abnormal activation of caspases plays an important role in the occurrence and development of tumors and autoimmune and infectious diseases. Apoptotic caspases are functionally subdivided into induced caspases (caspases 8, 9, and 10) and effector caspases (caspases 3, 6, and 7) (Opdenbosch *et al*., 2019).

As an intermediary related to various organs and tissues, the liver is an important metabolic organ that regulates energy metabolism. The liver is one of the largest internal organs of fish and plays a variety of roles in fish activities, such as nutrient processing and storage, protein synthesis, detoxification of autometabolites, bile production and secretion, and drug metabolism. As a key link between material metabolism and immune protection in fish, the liver (or hepatopancreas) plays an indispensable role in resisting or alleviating heat stress damage when facing temperature stress (Feng *et al*., 2022). Studies have shown that temperature stress can cause changes in the structure of fish liver tissue, resulting in hepatocyte vacuolization, nuclear translocation and cell disintegration, thereby damaging the basic functions of the liver and ultimately causing metabolic imbalances and endangering health (Guan *et al*., 2014). A study of P. argenteus juveniles by Peng *et al*. (2011) revealed that, in a stressful environment, the energy of juveniles mainly depends on the rapid transformation of liver glycogen. The liver cells of the CK and NC treatment groups exhibited vacuolization; this may be due to the sharp increase in oxygen consumption in crucian carp when they experience a sudden decrease in temperature (Hochachka and Somero, 2014) , which in turn promotes the decomposition of liver glycogen or fat and ultimately causes vacuolization of liver cells. The optimal dose of coriander essential oil for anesthetizing rainbow trout is 200mg/L, which does not cause damage to liver tissue. The results of this study were similar to those of Yigit and Kocaayan (2023).

Bax is a death gene, and an increase in Bax expression promotes the occurrence of apoptosis. Bcl-2 (B-cell lymphoma-2 gene, B-cell lymphoma-2) is a survival gene. An increase in Bcl-2 expression inhibits the occurrence of apoptosis. The ratio of Bcl-2/Bax determines the degree of apoptosis (Del *et al*., 2003; Gao *et al*., 2025) The ratio of Bcl-2/Bax was increased, and apoptosis was inhibited. The ratio of Bcl-2/Bax was decreased, which promoted apoptosis.

In nature, many plant active ingredients have a wide range of functions. Compared with antibiotics, plant active ingredients have outstanding characteristics, such as high efficiency and low toxicity. In the past decade, our team has been committed to providing technical solutions for the transportation of live aquatic products to ensure their survival. We are convinced that stress alleviation is the key to ensuring a high survival rate of transportation. The extract of *Buddleja officinalis Maxim.* has been shown to alleviate stress in crucian carp and wheat ear fish. Liu *et al*. (2010) studied the effects of anthrone extract on Macrobrachium rosenbergii under high-temperature stress. The results showed that after 10 weeks of feeding with 0.1-0.2% anthrone extract, the resistance of Macrobrachium rosenbergii to high-temperature stress improved, and the growth of Macrobrachium rosenbergii increased. It is clear that plant extracts play an active role in promoting the growth, antioxidation and immune regulation of aquatic animals. The most widely studied and commonly used plant anesthetic is eugenol, which effectively affects zebrafish (Gressler *et al*., 2021), spotted sea bass (He RP and Lei B, 2020), and hybrid Amazon catfish (De Oliveira *et al*., 2019). To assess the potential risk of toxicity to fishes in the process of applying *Buddleja officinalis Maxim.* extract, zebrafish (Danio rerio) was selected as the model organism for developmental toxicity assessment. Overall, the present findings suggest that *Buddleja officinalis* extract induces limited physiological modulation in zebrafish under the tested conditions, without causing obvious developmental or hepatic structural toxicity. However, these conclusions are restricted to early developmental stages and acute exposure, and further chronic toxicity and dose–response studies are required to fully evaluate its biological safety and aquaculture potential.

Conclusions: At the experimental concentration used in this study, the *Buddleja officinalis* extract may have no obvious teratogenic effects, which mainly involves a certain degree of absorption delay in the zebrafish yolk sac. More than 70% of the components in the yolk sac are neutral fat, which provides nutrition for the early growth and development of zebrafish. The incidence of delayed absorption of the yolk sac in zebrafish in the normal control group was 16.7%. With increasing concentrations of *Buddleja officinalis* extract, the absorption of the yolk sac may be slowed by affecting its metabolic rate. At 48 hpf, no egg condensation, abnormal somite formation or tail extension was observed in any of the experimental groups. There was no significant difference in the hatching rate between the experimental and normal control groups at 72 hpf, suggesting that the *Buddleja officinalis* extract had no significant effect on the hatching rate of zebrafish under the concentration conditions of this experiment. Further studies revealed that the *Buddleja officinalis* extract significantly promoted the activity of CYP3A4 and CYP2D6. There was no significant effect on the activity of Caspase-3/7 or the morphology of liver cells; it can increase the ratio of Bcl-2/Bax and inhibit apoptosis. The results of this study suggest that the extract of *Buddleja officinalis* has no obvious toxicity to the development of zebrafish and does not cause obvious damage to liver tissue, which provides a possibility for further research and development of *Buddleja officinalis* extract to alleviate stress in fish. Overall, these biochemical changes should be interpreted as preliminary associations rather than confirmed mechanistic pathways.

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