

Epigallocatechin-3-gallate attenuates the povidone iodine-induced immunotoxicity in *Procambarus clarkii*

Qingri Jin

Hangzhou Medical College

Zixuan Guo

Hangzhou Medical College

Ziyang Li

Hangzhou Medical College

Yong Wang

Zhejiang A & F University

Fei Zhu

zhufei@zafu.edu.cn

Zhejiang A & F University

Research Article

Keywords: EGCG, povidone iodine, crayfish, immunotoxicity

Posted Date: October 19th, 2026

DOI: <https://doi.org/10.21203/rs.3.rs-10767863/v1>

License:   This work is licensed under a Creative Commons Attribution 4.0 International License.

[Read Full License](#)

Additional Declarations: No competing interests reported.

Abstract

Objective

Povidone iodine (PVP-I) is a broad-spectrum disinfectant known for its effectiveness in preventing and treating bacterial and viral diseases in aquatic animals. EGCG, a component of tea polyphenols, possesses antioxidant, antibacterial, and immune-enhancing properties. This study aimed to investigate the effects of EGCG on the povidone iodine (PVP-I)-induced immunotoxicity in crayfish.

Methods

This study conducted enzyme activity assay, apoptosis analysis of crayfish hemocytes, histopathological analysis of the hepatopancreas, survival analysis after *V. alginolyticus* challenge, and the real time quantitative polymerase chain reaction (RT-qPCR) assay for the expression of multiple immune-related genes in crayfish

Results

The findings indicated that PVP-I suppressed the expression of immune-related genes, but the expression levels of these genes were increased by EGCG. The activities of enzymes such as phenoloxidase (PO), catalase (CAT), peroxidase (POD), lysozyme (LZM), acid phosphatase (ACP), glutathione S-transferase (GST) and glutathione (GSH) in the PVP-I group were reduced, but the activities of these enzymes were increased by EGCG. Additionally, PVP-I caused damage to the hepatopancreas tissue, leading to a decrease in total hemocyte counts and an increased rate of apoptosis. EGCG increased total hemocyte counts, and decreased the apoptosis. The PVP-I-induced immunotoxicity contributed to an elevated mortality rate among crayfish infected with *V. alginolyticus* but EGCG reduced the mortality of crayfish after challenge.

Conclusion

This study firstly confirmed that EGCG can attenuate the PVP-I-induced immunotoxicity and make immune parameters tend to be normal in aquatic animals. This study contributes to fully understand the mechanisms of EGCG in reducing the immunotoxicity of antibiotic residues on aquatic animals.

1. Introduction

Crayfish is increasingly recognized as a valuable dietary option due to its high protein content, excellent meat quality, and comprehensive nutritional profile. In addition to providing a palatable culinary experience, crayfish farming significantly enhances the income of farmers. The diversity of crayfish species is considerable, with over 640 species identified to date^[1–2]. Among these, *Procambarus clarkii*

has gained popularity for aquaculture^[3–4]. This species is distributed globally, with significant populations in Europe, Asia, and Africa. However, the excessive use of antibiotics and aquaculture drugs in recent years has complicated the prevention, control, treatment, and health management of crayfish diseases. The prolonged application of these substances can have detrimental effects on aquatic organisms and ecosystems, even at concentrations deemed safe, leading to environmental pollution. Antibiotic residue pollution severely impacts the aquatic environment and poses significant risks to the health of aquaculture animals and consumers.

Povidone iodine, a versatile fungicide, demonstrates efficacy against bacteria, fungi, and certain viruses^[5]. It is also extensively utilized in aquaculture as a crucial chemical disinfectant to sanitize aquatic environments and equipment^[6]. The mechanism underlying its antimicrobial action is primarily attributed to polyvinylpyrrolidone (PVP), a non-ionic surfactant that enhances the affinity of povidone iodine for bacterial cell membranes. This affinity facilitates the interaction of available iodine with bacterial cell membranes and cytoplasm. Concurrently, free iodine reacts with bacterial proteins, enzymes, and lipids, leading to the disruption of protein biological activity and bacterial membrane integrity, ultimately resulting in microbial death^[7]. Povidone-iodine (PVP-I) is widely employed in aquaculture, including crayfish culture, to prevent and treat bacterial infections. However, the continuous use of such agents has been associated with the development of physical resistance and adverse environmental impacts on surrounding fauna. Previous research has confirmed the toxicity of sulfamethoxazole (SMZ) on *Scylla paramamosain* when exposed to contaminated water^[8]. The adverse effects of povidone iodine have been documented for an extended period. In a study conducted by Ma et al., the impact of various common aquaculture drugs on the immune system of crayfish was investigated, revealing that povidone iodine (PVP-I) can diminish the innate immunity and disease resistance of crayfish^[7]. Antibiotics are predominantly employed to treat and prevent diseases; however, their use is associated with several challenges, including low bioavailability, drug residues, and overuse, which contribute to the development of drug resistance and environmental pollution. Epigallocatechin-3-gallate (EGCG), a polyphenolic compound primarily derived from green tea, constitutes approximately 60% of its total polyphenol content^[9]. As the most abundant and biochemically active catechin^[10], EGCG plays a crucial role in alleviating the detrimental effects caused by bacteria, viruses, and oxidative stress^[11–15]. Several aquaculture studies have demonstrated the efficacy of EGCG in treating infections such as Singapore grouper iridovirus and white spot syndrome virus^[16–19]. In our prior investigations and research, we identified that epigallocatechin gallate (EGCG) can inhibit the replication of white spot syndrome virus (WSSV) by regulating the expression of WSSV genes^[18], and it can also enhance the organism's immune resistance. The various functions and safety attributes of EGCG have been widely recognized. Drawing upon existing literature, we hypothesized that EGCG might mitigate sulfamethazine (SMZ)-induced toxicity by modulating the innate immune response and metabolic pathways in crayfish, a hypothesis grounded in our previous studies^[18].

In the present study, we examined the potential of EGCG to attenuate povidone-iodine (PVP-I)-induced immunotoxicity in crayfish, with a particular emphasis on its effects on the innate immune response in

crayfish exposed to PVP-I.

2. Materials and methods

2.1 Chemicals and experimental animals

The solution of povidone iodine was purchased from Henan Nanhua Qianmu Biotechnology Co., Ltd. And the Epigallocatechin-3-gallate (EGCG, purity $\geq 98\%$) was purchased from Shanghai Ica Biotechnology Co., Ltd (China). In this study, the EGCG was completely dissolved in purified water and make a stock solution of 1 mg/mL, which was stored at 4°C. The povidone iodine (PVP-I, 10% content) purchased from South Ranch company (China) was strictly used according to the packaging instructions to ensure effective use and safety. Healthy crayfish (each crayfish weighs about 26g) were bought from an aquaculture farm in Anhui province, China. Crayfish were kept in aquaculture tanks (60 cm $\times$ 40 cm $\times$ 30 cm) containing 15 L aerated tap water. Each tank was fitted with PVC pipes to create a suitable habitat for the crayfish and reduce the potential for physical confrontation and harmful behaviors. The water in each group was changed every 24 hours, while the crayfish were fed pellets without any antibiotics.

2.2 Treatment of different groups

The trials were divided into three groups, namely the control group (soak in aerated water for six days), PVP-I immersion for three days followed by water treatment for three days, and PVP-I immersion for three days followed by EGCG treatment for three days. The number of crayfish used for the trial was 12 (in a tank) per group. Dissolving PVP-I in 5 L of aerated water is enough to completely cover the crayfish. The concentration of EGCG was 1 mg/mL, the injection volume based on the weight of the crayfish (the average weight of each group). Dilute the EGCG stock solution with autoclaved PBS and intramuscular injected 100 μ L each crayfish (PBS, pH = 7.4). This experiment was repeated as three independent replicate per treatment.

2.3 Histopathology examination

After 6 days of treatment according to the above test method, the hepatopancreas of crayfish were randomly taken, cut into appropriate sizes (5 mm $\times$ 5 mm), soaked in formalin with a concentration of 10%, fixed for 48 hours and sent to a company in Tianjin for histopathological analysis.

2.4 Enzyme activity assay

In order to analyze the effects of PVP-I on various biochemical indicators and oxidative stress in crayfish, several parameters such as Phenoloxidase (PO), acid phosphatase (ACP), superoxide dismutase (SOD), peroxidase (POD), lysozyme (LZM), catalase (CAT), glutathione S-transferase (GST) and glutathione (GSH) activities, and total hemocyte count (THC) were detected after six days with the treatment. The above enzyme activity detection kits are all from Nanjing Jiancheng Bioengineering Institute. This experiment was repeated as three independent replicate per treatment.

2.5 Real time quantitative polymerase chain reaction (RT-qPCR)

RNA was extracted based on the report we previously published using Trizol reagent (Invitrogen, CA, USA) after low-temperature centrifugation^[19], followed by RNA concentration determination and reverse transcription. cDNA obtained after transcription was subsequently used for SYBR Green real-time fluorescent quantitative PCR (qPCR) analysis and the relative gene expression determined with the $2^{-\Delta\Delta C_t}$ method^[20]. This experiment was repeated as three independent replicate per treatment.

2.6 Apoptosis analysis of crayfish hemocytes

After six days with the treatment, the rate of apoptosis was carried out depending on the method previously published in a study^[21]. The data obtained from the three independent experiments were expressed as the mean $\pm$ SD. This experiment was repeated as three independent replicate per treatment.

2.7 Kaplan-Meier survival analysis

The crayfish were randomly divided into four groups of 20 individuals each (in a tank). One group was injected with PBS alone as a negative control, another group was injected with *V. alginolyticus* alone as a positive control. The remaining groups are PVP-I + *V. alginolyticus* and PVP-I+EGCG + *V. alginolyticus*, respectively. The challenged crayfish were injected with 100 μ L of *V. alginolyticus* (ATCC17749) suspension (10^6 CFU/mL; the LD50 dose, determined in preliminary 6 experiments as the concentration achieving 50% mortality within 72h), while the remaining four groups received corresponding treatments without bacterial challenge. To ensure reproducibility, *V. alginolyticus* was freshly cultured to OD = 0.6 (corresponding to 10^6 CFU/mL) before each injection. All groups were observed for seven days, during this period, the water was changed every 24 hours and the number of dead crayfish was recorded every 12 hours. This experiment was repeated as three independent replicate per treatment. The data was evaluated using Microsoft GraphPad 8.0 for statistical analysis.

2.8 Statistical analysis

Statistical analysis was performed using SPSS software (IBM, NY, USA). All measurements were performed in triplicate, and the data are expressed as the mean $\pm$ standard deviation (SD) of the mean. A multivariate t-test was used to analyze differences. * $P < 0.05$, ** $P < 0.01$ and *** $P < 0.001$ indicate significant and highly significant differences, respectively. All software is consistent with a previous report^[21].

3. Results

3.1 The expression of immune-related genes

RT-qPCR was used to evaluate the impact of PVP-I on the expression of multiple immune-related genes in crayfish (Fig. 1), including *β-actin* (internal reference gene), *NF-κb*, *lectin*, *ferritin*, *Hemocyanin*, *STAT*, *HsP70*, *TLR*, *ALF* and *Crustin*. Following exposure to PVP-I, the expression of *NF-κb*, *lectin*, *ferritin*, *Hemocyanin*, *STAT*, *HsP70*, and *TLR* were observed to decrease, while the expression of *ALF* and *Crustin* increased. After EGCG treatment, the genes downregulated by PVP-I were remarkably enhanced. The expression of *Crustin*, *NF-κb*, *lectin*, *ferritin*, *Hemocyanin*, and *HsP70* was significantly ($P < 0.001$) higher in PVP-I+EGCG compared to the PVP-I, but there was no significant difference in *ALF* gene between the two groups. These changes in gene expression suggested that exposure to PVP-I may affect several crucial biological functions, such as oxidative stress, apoptosis, antibacterial and antioxidant capabilities, as well as gene-regulated signaling pathways. And EGCG can reverse these changes caused by PVP-I in crayfish.

3.2 The immunological parameters

The activities of key enzymes associated with crayfish immunity were changed after exposure to PVP-I (Fig. 2). In comparison to the control group, the activity of PO (Fig. 2A) exhibited a notable reduction after treatment with PVP-I, while it increased significantly ($P < 0.001$) in PVP-I+EGCG group compared to the PVP-I group. The activity of SOD in the PVP-I group was increased, but the EGCG reduced the SOD activity after PVP-I exposure (Fig. 2B). Exposure to PVP-I resulted in a significant ($P < 0.001$) reduction in the activities of POD (Fig. 2D) and CAT (Fig. 2F) in comparison to the PBS group. However, EGCG significantly ($***P < 0.001$) enhance the activities of the two enzymes after PVP-I exposure, which is similar to that of PBS group. Similarly, the effect of the combination of PVP-I and EGCG on the activity of GSH (Fig. 2C) and GST (Fig. 2E) was higher than the PBS group, even though exposure to PVP-I still led to a decrease in the activity of both enzymes. As POD, CAT, GSH and GST were all associated with the antioxidant activity of crayfish, EGCG seems to enhance the antioxidant activity which is reduced by PVP-I. After treatment with PVP-I, the activity of ACP (Fig. 2G) also declined when compared to the control group, yet no significant difference was observed in LZM (Fig. 2H). Compared to PVP-I+ EGCG and PVP-I, the activity of both ACP and LZM was found to be increased. As ACP and LZM were associated with the antibacterial activity of crayfish, EGCG seems to enhance the antibacterial activity which is reduced by PVP-I.

3.3 The histopathological analysis of the hepatopancreas

The hepatopancreas, which is an essential immune organ of crayfish, was evaluated for its response to PVP-I exposure through a pathological tissue analysis (Fig. 3). The objective is to investigate the impact of PVP-I on the immune function of the crayfish. Following PVP-I exposure, the sample exhibited a large number of mononuclear cell infiltration (yellow arrow, Fig. 3D) as well as eosinophilic clumps (red arrow, Fig. 3C) compared to the control group (Fig. 3A and B). In addition, hepatic tubular epithelial cells exhibited an abnormal structure as well as visible swelling, steatosis (blue arrow). Extensive accumulation of dense round vacuoles of varying sizes was observed in the cytoplasm, indicating cellular dysfunction. A substantial increase in hepatic tubular epithelial pigmentation (green arrow) was

also identified, potentially reflecting the damage of tissue. In contrast to PVP-I group, PVP-I+EGCG group showed lower numbers of eosinophilic clumps (red arrow, Fig. 3E), fewer mononuclear cells (yellow arrow), and less pigmentation of hepatic tubular epithelium (green arrow, Fig. 3F). The organizational morphology of hepatopancreas in PVP-I + EGCG group is similar to that in PBS group.

3.4 The apoptosis and total count of hemocytes

Hemocyte, an integral component of crustacean immune defense, were observed to be significantly ($P < 0.05$) lower following PVP-I exposure compared to the control group. Conversely, EGCG treatment resulted in a significant ($P < 0.05$) increase in the total hemocyte count, although still lower than the control group (Fig. 4E). In the analysis of apoptosis (Fig. 4D), the apoptosis rates of the control group (Fig. 4A), PVP-I group (Fig. 4B), and PVP-I + EGCG group (Fig. 4C) were found to be 43.8%, 46%, and 30.9%, respectively. It was showed that the rate of apoptosis was significantly ($P < 0.01$) higher following PVP-I exposure compared to the control group. However, the apoptosis rate in the PVP-I + EGCG group significantly ($***P < 0.001$) decreased. The results of apoptosis rate are consistent with the total number of hemocytes, indicating that PVP-I has significant toxicity to hemocytes.

3.5 Impacts of EGCG on the survival of PVP-I-exposed crayfish after *V. alginolyticus* challenge

In order to determine the effect of EGCG on the antibacterial immunity of crayfish exposed to PVP-I, *Vibrio alginolyticus* challenge experiments were carried out (Fig. 5). The results showed that all crayfish in the control group (injected with PBS) survived, Injected with *V. alginolyticus* began to die within 24 hours, and the final survival rate was 50%. However, exposure to PVP-I reduced the survival rate of crayfish infected with *V. alginolyticus* (35%). Moreover, exposure to PVP-I in combination with EGCG treatment significantly improved the survival rate of crayfish (75%). These findings suggested that PVP-I exposure reduce the survival rate of crayfish after *V. alginolyticus* challenge, while EGCG effectively attenuates the harmful effects on the survival of *V. alginolyticus*-challenged crayfish caused by PVP-I exposure and enhances the survival rate of crayfish.

3.6 Impacts of EGCG on the survival of PVP-I-exposed crayfish after *V. alginolyticus* challenge

In order to determine the effect of EGCG on the antibacterial immunity of crayfish exposed to PVP-I, *Vibrio alginolyticus* challenge experiments were carried out (Fig. 6). The results showed that all crayfish in the control group (injected with PBS) survived, Injected with *V. alginolyticus* began to die within 24 hours, and the final survival rate was 50%. However, exposure to PVP-I reduced the survival rate of crayfish infected with *V. alginolyticus* (35%). Moreover, exposure to PVP-I in combination with EGCG treatment significantly improved the survival rate of crayfish (75%). These findings suggested that PVP-I exposure reduce the survival rate of crayfish after *V. alginolyticus* challenge, while EGCG effectively attenuates the

harmful effects on the survival of *V. alginolyticus*-challenged crayfish caused by PVP-I exposure and enhances the survival rate of crayfish.

4. Discussion

Disinfectants are commonly employed in the treatment and management of diseases following outbreaks^[22]. While short-term exposure to high concentrations of certain chemicals can be effective, prolonged exposure may result in adverse environmental impacts and harm to surrounding organisms. Even at low concentrations, pesticides have been documented to exert immunotoxic effects on aquatic species such as fish, crabs, and shrimp, thereby posing a significant threat to their health^[23]. Similarly, although polyvinylpyrrolidone-iodine (PVP-I) is widely used as an aquatic disinfectant against bacterial and viral diseases, recent research has indicated its potential toxicity to aquatic organisms. Several studies have demonstrated the toxic effects of PVP-I on carp, including histological damage^[24–25]. Furthermore, research has shown that various substances, such as copper sulfate, norfloxacin, and polyethylene iodine, can suppress the innate immune response in crayfish^[26]. However, there remains a significant gap in the literature concerning the effects of PVP-I exposure on the innate immunity of crayfish.

Due to the absence of adaptive immunity, crustaceans predominantly rely on innate immune mechanisms for protection against infections^[27]. Innate immunity in these organisms is characterized by the activation of pattern recognition receptors or proteins (PRRs or PRPs)^[28]. Invertebrate immune responses are generally divided into humoral defenses, which involve the production of soluble effector molecules such as antimicrobial peptides (AMPs), and cellular defenses, which are mediated by hemocytes^[29–31]. Upon external stimulation, crustaceans produce reactive oxygen species (ROS), which activate a complex oxidative stress response system to maintain ROS levels within a physiological range^[32]. This regulatory mechanism is essential for mitigating oxidative damage associated with increased ROS concentrations resulting from aerobic metabolism^[33]. ROS not only play a role in metabolic processes but also act as regulators in immune responses. Furthermore, oxidative stress is identified as a key mechanism in pollutant-induced toxicity in aquatic organisms^[34–35]. Superoxide dismutase (SOD) and catalase (CAT) are critical components of the antioxidant defense system in crustaceans. Superoxide dismutase (SOD) plays a crucial role in the elimination of excess superoxide ions^[36], while catalase (CAT) is adept at decomposing hydrogen peroxide, a highly reactive oxygen species. Peroxidase (POD) is a specialized oxidoreductase that neutralizes harmful hydrogen peroxide and phenolic compounds within the organism. Glutathione (GSH), another vital antioxidant, serves as a reliable marker for assessing overall antioxidant capacity^[8]. Research had demonstrated that GSH levels tend to diminish under specific external stimuli^[37]. Exposure to polyvinylpyrrolidone-iodine (PVP-I) has been shown to reduce the activity of SOD, CAT, POD, GSH, and other enzymes associated with antioxidant defense, suggesting a potential impact on the antioxidant capacity of crayfish. The hydrolases acid phosphatase (ACP) and lysozyme (LZM) constitute the primary defense mechanism

within humoral immunity, significantly influencing antibacterial and inflammatory responses^[38]. Furthermore, prophenoloxidase (proPO), a precursor to phenoloxidase (PO), can be activated by endogenous and exogenous stimuli^[39]. Upon activation, PO is rapidly released into the hemolymph, facilitating a swift and robust response against invading pathogens. Subsequent research has indicated that proPO mRNA is predominantly expressed in hemocytes and the hepatopancreas^[39–41]. One study has demonstrated that reductions in PO activity are associated with exposure to environmental stressors and a decrease in disease resistance^[42]. Glutathione S-transferase (GST) is a multi-component enzyme that not only facilitates detoxification but also plays a critical role in safeguarding tissues against the deleterious effects of oxidative stress^[43]. It is well-established that oxidative modifications of enzymes and structural proteins can significantly contribute to disease development^[44]. Therefore, a reduction in the activity of these essential enzymes suggests that PVP-I may potentially compromise crayfish immunity, rendering them more susceptible to pathogenic invasion. Considering that POD, CAT, GSH, and GST are all involved in the antioxidant activity of crayfish, epigallocatechin gallate (EGCG) appears to enhance antioxidant activity, which is otherwise diminished by PVP-I.

Hemocytes are integral to the immune system of crustaceans^[45]. Their immune functions, including phagocytosis, apoptosis, and antioxidant capabilities, are significantly influenced by their ability to proliferate and regenerate^[46]. Our findings demonstrated a marked reduction in the total hemocyte count following exposure to PVP-I, suggesting potential cytotoxic effects. According to a previous study, both humoral and cellular immunity are reliant on the functionality of hemolymph filtration and detoxification organs^[47]. The hepatopancreas, recognized as the primary immune and metabolic organ in decapod crustaceans^[48], is integral to numerous physiological and metabolic functions. Previous studies have indicated that exposure to polychlorinated biphenyls (PCBs) in aquatic environments can induce apoptosis and damage to the hepatopancreas^[49]. In the current study, PVP-I exposure was also associated with an increased rate of apoptosis (though the increase was not statistically significant) and resulted in hepatopancreas cell reduction, disorganized hepatic tubules, cellular swelling, and the presence of cytoplasmic vacuoles of varying sizes. Glutathione S-transferase (GST), an enzyme involved in detoxification predominantly located in the liver, showed a significant decline in activity in response to damage^[50]. Consequently, we propose that PVP-I-induced hepatopancreas damage may be linked to a reduction in antioxidant enzyme activity, potentially leading to the excessive accumulation of reactive oxygen species (ROS) and the upregulation of apoptosis.

The alterations in the expression levels of immune-related genes and signaling pathways can reflect the modifications in the crayfish's innate immunity and disease resistance. The exposure of PVP-I significantly decreased the expression levels of immune-related genes in crayfish, which included *NF-κB*, *lectin*, *ferritin*, *Hemocyanin*, *STAT*, *HsP70*, and *TLR*. NF-κB has been demonstrated to play a crucial role in humoral immune responses, thereby being essential for the host defense system^[51]. Additionally, it has been implicated in the regulation of apoptosis-related genes, which can either promote or inhibit apoptosis^[52]. Lectins exhibit a variety of biological activities in invertebrate hemocytes and are integral

to immune response processes. The presence of several pattern recognition receptors (PRRs), including lectins, initiates a cascade of immune responses in target cells upon recognizing pathogen-associated molecular patterns (PAMPs)^[53]. The novel ferritin gene identified in the red swamp crayfish, *Procambarus clarkii*, is designated as PcFer^[54]. The role of ferritin in iron storage and antioxidant stress has been extensively studied^[55]. Furthermore, ferritin may confer resistance to bacterial proliferation^[56,57]. Hemocyanin, a multifunctional protein, rapidly responds to a broad spectrum of pathogens, including bacteria, fungi, and viruses, rendering it a crucial component of innate immune defense mechanism^[58, 59]. Members of the signal transducer and activator of transcription (STAT) protein family are pivotal in mediating cell proliferation, apoptosis, and differentiation^[60–61]. Heat shock proteins (HSPs) are highly conserved across all organisms and play a crucial role in the body's defense mechanisms against various threats^[62], including pathogenic infections, oxidative stress, heavy metals, and exogenous stressors^[63,64]. HSP70 is a member of the heat shock protein family. Toll-like receptors (TLRs) are essential for mediating inflammatory responses in aquatic organisms^[65]. Specifically, TLRs are involved in regulating the expression of crustins (Cru1, Cru2), anti-lipopolysaccharide factor 2 (ALF2), and lysozyme 1 (Lys1)^[66]. Exposure to PVP-I has been shown to reduce the expression levels of these genes, indicating a detrimental effect on the innate immunity of crayfish. EGCG has demonstrated therapeutic efficacy by mitigating this adverse effect. Crustin, an antimicrobial peptide found in crustaceans, plays a vital role in defending against bacterial and viral pathogens^[67]. The anti-lipopolysaccharide factor (ALF) is a type of antimicrobial peptide (AMP) that exhibits broad-spectrum antimicrobial activity^[68,69]. Functional studies on ALF suggest that it not only inhibits bacterial growth but also suppresses viral replication. The expression of *ALF* and *Crustin* genes was up-regulated after exposure to PVP-I, which may be related to the induction of NF- κ B, STAT, TLR and other signaling pathways.

In the context of crayfish, the antibacterial activity is associated with the presence of PO, ACP, and LZM, while EGCG appears to enhance this activity, which is otherwise diminished by PVP-I. Survival analysis indicated that exposure to PVP-I compromises the immune system of crayfish, thereby increasing their susceptibility to *V. alginolyticus* and leading to a significant increase in mortality rates. EGCG has demonstrated potential health benefits, including antioxidant properties, cancer chemoprevention, cardiovascular health improvement, facilitation of weight loss, protection against ionizing radiation-induced skin damage, and mitigation of respiratory disorders^[70–73]. The aquaculture industry is experiencing rapid growth globally, with China being the largest producer and exporter of aquatic products. Antibiotics are extensively used in aquaculture to prevent and treat diseases. A total of 32 different antibiotics have been detected in Chinese aquatic products, raising concerns that humans may experience adverse drug reactions or encounter antibiotic-resistant bacteria through the consumption of antibiotic-contaminated aquatic products^[74]. Considering the aquaculture cycle, EGCG can be used as feed additive for enhancing immunity and antioxidant properties in the middle stage of aquaculture to reduce the usage of antibiotics. EGCG can also be used for removing drug residues in aquatic products, especially after the use of fishery drugs, in the later stages of aquaculture to improve the quality of aquatic products and pass quality inspection. Although EGCG cannot completely replace fishery drugs,

its functions of enhancing disease resistance, reducing drug toxicity, and reducing drug residues have unique effects in reducing the usage and residues of fishery drugs in aquatic environments.

5. Conclusion

The findings of our research demonstrate that PVP-I possesses the capacity to diminish the activity of antioxidant enzymes, trigger apoptosis, and modulate the expression of various signaling pathways, ultimately resulting in pathological changes in hepatopancreatic tissue. This PVP-I-induced immunotoxicity was associated with increased mortality rates in crayfish infected with *V. alginolyticus*. This study is the first to confirm that EGCG can mitigate PVP-I-induced immunotoxicity, thereby normalizing immune parameters in aquatic animals. Our research enhances the understanding of EGCG's mechanisms in reducing antibiotic content in aquatic products and addressing the immunotoxic effects of antibiotic residues on aquatic organisms. Beyond its known roles in regulating innate immunity and enhancing disease resistance in crustaceans^[18, 75], EGCG demonstrates novel biological functions and holds promise as a multifunctional, environmentally friendly immune protective agent.

Declarations

FUNDING

This study was financially supported by the Open Research Fund of Key Laboratory of Healthy Freshwater Aquaculture, Ministry of Agriculture and Rural Affairs (ZJK202103).

CONFLICTS OF INTEREST

The authors declare that they have no conflicts of interest.

ACKNOWLEDGMENTS

Author Contribution

We have made substantial contributions to the conception or design of the work; or the acquisition, analysis, or interpretation of data for the work. FZ conceived and designed research. QJ and ZG conducted experiments. ZG and YW analyzed data. QJ wrote the manuscript. All authors read and approved the manuscript. The corresponding author is responsible for ensuring that the descriptions are accurate and agreed by all authors. We have drafted the work or revised it critically for important intellectual content. We have approved the final version to be published. We agree to be accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved. Qingri Jin: Investigation, Writing original manuscript Zixuan Guo: Investigation, Data curation Ziyang Li: Validation Yong Wang: Data curation, Software Fei Zhu: Conceptualization, Methodology, Investigation, Writing-Reviewing and Editing

DATA AVAILABILITY

The DNA sequence data have been deposited in GenBank, and the generated data of the current study are stored on the personal computers of the authors and are open to everyone who needs them upon request.

ETHICS STATEMENT

The experimental animals are invertebrates, it is not applicable.

References

1. L.S. Dai, X.M. Yu, M.N. Abbas, Essential role of the peroxiredoxin 4 in *Procambarus clarkii* antioxidant defense and immune responses, *Fish Shellfish Immunol.* 75 (2018) 216–222.
2. K.A. Crandall, J.E. Buhay, Global diversity of crayfish (Astacidae, Cambaridae, and Parastacidae- Decapoda) in freshwater, *Hydrobiologia.* 595 (2008) 295–301.
3. S.H. Chu, L. Liu, M.N. Abbaas, Peroxiredoxin 6 modulates Toll signaling pathway and protects DNA damage against oxidative stress in red swamp crayfish (*Procambarus clarkii*), *Fish Shellfish Immunol.* 89 (2019) 170–178.
4. S. Fisher, E.A. Grice, R.M. Vinton, Evaluating the biological relevance of putative enhancers using Tol2 transposon-mediated transgenesis in zebrafish, *Nat. Protoc.* 1 (2006) 1297–1305.
5. P. Wutzler, A. Sauerbrei, R. Klocking, Virucidal and chlamydicidal activities of eye drops with povidone-iodine liposome complex, *Ophthalmic Research* 32 (2000) 118–125.
6. A.D. Scarfe, C.S. Lee, J.O. Patricia, *Aquaculture biosecurity: prevention, control, and eradication of aquatic animal disease.* Blackwell Publishing 2006.
7. X.C. Ma, F. Zhu, Q.R. Jin, Antibiotics and chemical disease-control agents reduce innate disease resistance in crayfish, *Fish Shellfish Immunology* 86 (2019) 169–178.
8. Q. Wang, X.J. Zhou, Q.R. Jin, Effects of the aquatic pollutant sulfamethoxazole on the innate immunity and antioxidant capacity of the mud crab *Scylla paramamosain*, *Chemosphere.* 349 (2024) 140775.
9. J.V. Higdon, B. Frei, Tea catechins and polyphenols: health effects, metabolism, and antioxidant functions, *Crit. Rev. Food Sci. Nutr.* 43 (1) (2003) 89–143.
10. N. Khan, H. Mukhtar, Tea polyphenols for health promotion, *Life Sci.* 81 (7) (2007) 519–533.
11. Q.Y. Eng, P.V. Thanikachalam, S. Ramamurthy, Molecular understanding of Epigallocatechin gallate (EGCG) in cardiovascular and metabolic diseases, *J. Ethnopharmacol.* 210 (2018) 296–310.
12. W.C. Reygaert, The antimicrobial possibilities of green tea, *Front. Microbiol.* 5 (2014) 434.
13. L.G. Xiong, Y.J. Chen, J.W. Tong, J.A. Huang, J. Li, Y.S. Gong, Z.H. Liu, Teapolyphenol epigallocatechin gallate inhibits *Escherichia coli* by increasing endogenous oxidative stress, *Food Chem.* 217 (2017) 196–204.

14. 14. Y. Qiao, J. Cao, L. Xie, X. Shi, Cell growth inhibition and gene expression regulation by (-)-epigallocatechin-3-gallate in human cervical cancer cells, *Arch. Pharm. Res.* 32 (2009) 1309–1315.
15. 15. M. Nakayama, K. Shimatani, T. Ozawa, N. Shigemune, D. Tomiyama, K. Yui, M. Katsuki, K. Ikeda, A. Nonaka, T. Miyamoto, Mechanism for the antibacterial action of epigallocatechin gallate (EGCG) on *Bacillus subtilis*, *Biosci. Biotechnol. Biochem.* 79 (2015) 845–854.
16. 16. P.F. Li, S.S. Huang, S.Y. Xiao, Y.H. Xu, X.X. Wei, J. Xiao, Z.B. Guo, Q. Yu, M.Z. Liu, Antiviral activities of green tea components against grouper iridovirus infection in vitro and in vivo, *Viruses*. 14 (2022) 1227.
17. 17. Y. Cheng, M. Liu, Q. Yu, S. Huang, S. Han, J. Shi, H. Wei, J. Zou, P. Li, Effect of EGCG extracted from green tea against Largemouth Bass Virus infection, *Viruses*. 15 (2023) 151.
18. 18. Z. Wang, B. Sun, F. Zhu, Epigallocatechin-3-gallate inhibit replication of white spot syndrome virus in *Scylla paramamosain*, *Fish Shellfish Immunology* 67 (2017) 612–619.
19. 19. J. Gong, X. Pan, X. Zhou, F. Zhu, Dietary glycerol monolaurate protects *Cherax quadricarinatus* against white spot syndrome virus infection. *Fish Shellfish Immunology* 131 (2022) 1085–1091.
20. 20. K.J. Livak, T. Schmittgen, Analysis of relative gene expression data using real-time quantitative PCR and the 2-DDCt method, *Methods*. 25 (2001) 402–408.
21. 21. W.X. Jin, Y.Y. Lai, F. Zhu, Effect of dietary fucoidan on innate immune response of *Procambarus clarkii* and disease resistance against white spot syndrome virus, *Aquaculture* 534 (2021) 736233.
22. 22. X. Chen, C. Lai, Y. Wang, Disinfection effect of povidone-iodine in aquaculture water of swamp eel (*Monopterus albus*). *Peer Journal* 6 (2018) e5523.
23. 23. K. Vijayavel, S. Gopalakrishnan, R. Thiagarajan, H. Thilagam, Immunotoxic effects of nickel in the mud crab *Scylla serrata*, *Fish Shellfish Immunology* 26 (2009) 133–139.
24. 24. A. Hedayati, F. Darabtabar, T. Bagheri, Histopathological impairment of common carp (*Cyprinus carpio*) induced through povidone-iodine exposure, *Microscopy Research Technique* 81 (2018) 1257–1260.
25. 25. M. Forouhar Vajargah, A. Mohamadi Yalsuyi, A. Hedayati, Acute toxicity of povidone-iodine (Betadine) in common carp (*Cyprinus carpio* L. 1758), *Pollution*. 3 (2017) 589–593
26. 26. L.X. He, L.Y. He, F.Z. Gao, M. Zhang, J. Chen, W.L. Jia, G.G. Ying, Mariculture affects antibiotic resistome and microbiome in the coastal environment. *Journal of Hazardous Materials*. 452 (2023) 131208.
27. 27. X.Z. Shi, X.F. Zhao, J.X. Wang. A new type antimicrobial peptide astacidin functions in antibacterial immune response in red swamp crayfish *Procambarus clarkii*. *Dev Comp Immunol* 43 (2014) 121–128.
28. 28. C.Z. Li, S. Wang, J. He, The Two NF- κ B Pathways Regulating Bacterial and WSSV Infection of Shrimp, *Frontiers in Immunology*. 10 (2019) 1785.
29. 29. S. Akira, S. Uematsu, O. Takeuchi, Pathogen recognition and innate immunity, *Cell*. 124 (2006) 783–801.

30. 30. C. Yang, J. Zhang, F. Li, H. Ma, Q. Zhang, A Toll receptor from Chinese shrimp *Fenneropenaeus chinensis* is responsive to *Vibrio anguillarum* infection, *Fish Shellfish Immunol.* 24 (2008) 564–574.
31. 31. L.S. Yang, Z.X. Yin, J.X. Liao, X.D. Huang, C.J. Guo, S.P. Weng, et al. A Toll receptor in shrimp. *Mol Immunol.* 44 (2007) 1999–2008.
32. 32. Y. Huang, Y. Hong, Z. Huang, H. He, Cytotoxicity induced by abamectin exposure in haemocytes of Chinese mitten crab, *Eriocheir sinensis*, *Environ. Toxicol. Pharmacol.* 77 (2022) 103384.
33. 33. Y. Yang, A.V. Bazhin, J. Werner, S. Karakhanova, Reactive oxygen species in the immune system, *Int. Rev. Immunol.* 32 (2013) 249–270.
34. 34. V.I. Lushchak, Environmentally induced oxidative stress in aquatic animals, *Aquat. Toxicol.* 101 (2011) 13–30.
35. 35. A. Valavanidis, T. Vlahogianni, M. Dassenakis, M. Scoullas, Molecular biomarkers of oxidative stress in aquatic organisms in relation to toxic environmental pollutants, *Ecotoxicol Environ Saf.* 64 (2006) 178 – 89.
36. 36. A.I. Campa-Cordova, N.Y. Hernandez-Saavedra, P.R. De. Philippis, F. Ascencio, Generation of superoxide anion and SOD activity in hemocytes and muscle of American white shrimp (*Litopenaeus vannamei*) as a response to beta-glucan and sulphated polysaccharide, *Fish Shellfish Immunology.* 12 (2002) 353–366.
37. 37. A.V. Ivanina, A.S. Cherkasov, I.M. Sokolova, Effects of cadmium on cellular protein and glutathione synthesis and expression of stress proteins in eastern oysters, *Crassostrea virginica Gmelin*, *J. Exp. Biol.* 211 (2008) 577–586.
38. 38. S. Rajalakshmi, A. Mohandas, Copper-induced changes in tissue enzyme activity in a freshwater mussel, *Ecotoxicol Environ Saf.* 62 (2005) 140–143.
39. 39. C.H. Liu, D.Y. Tseng, C.Y. Lai, W. Cheng, C.M. Kuo, Molecular cloning and characterisation of prophenoloxidase cDNA from haemocytes of the giant freshwater prawn, *Macrobrachium rosenbergii*, and its transcription in relation with the moult stage, *Fish Shellfish Immunology.* 21 (2006) 60–69.
40. 40. S. Gopalakrishnan, H. Thilagam, W.B. Huang, K.J. Wang, Immunomodulation in the marine gastropod *Haliotis diversicolor* exposed to benzo(a)pyrene, *Chemosphere.* 75 (2009) 389–397.
41. 41. Y.C. Wang, P.S. Chang, H.Y. Chen, Tissue expressions of nine genes important to immune defence of the Pacific white shrimp *Litopenaeus vannamei*, *Fish Shellfish Immunology.* 23 (2007) 1161–1177.
42. 42. C.C. Chang, P.P. Lee, C.H. Liu, W. Cheng, Trichlorfon, an organophosphorus insecticide, depresses the immune responses and resistance to *Lactococcus garvieae* of the giant freshwater prawn *Macrobrachium rosenbergii*, *Fish Shellfish Immunology.* 20 (2006) 574–585.
43. 43. M. Teles, M. Pacheco, M.A. Santos, *Anguilla anguilla* L. liver ethoxyresorufin Odeethylation, glutathione S-transferase, erythrocytic nuclear abnormalities, and endocrine responses to naphthalene and β -naphthoflavone, *Ecotoxicol. Environ. Saf.* 55 (2003) 98–107.

44. 44. I. Dalle-Donne, R. Rossi, D. Giustarini, A. Milzani, R. Colombo, Protein carbonyl groups as biomarkers of oxidative stress, *Clin Chim Acta*. 329 (2003) 23–38.
45. 45. X. Lin, I. Soderhall, Crustacean hematopoiesis and the astakine cytokines, *Blood* 117 (2011) 6417–6424.
46. 46. S. Jeyachandran, K. Park, I.S. Kwak, V. Baskaralingam, Morphological and functional characterization of circulating hemocytes using microscopy techniques, *Microsc. Res. Tech.* 83 (2020) 736–743.
47. 47. P.S. Gross, T.C. Bartlett, C.L. Browdy, R.W. Chapman, G.W. Warr, Immune gene discovery by expressed sequence tag analysis of hemocytes and hepatopancreas in the Pacific White Shrimp, *Litopenaeus vannamei*, and the Atlantic White Shrimp, *L. setiferus*, *Dev Comp Immunol.* 25 (2001) 565–577.
48. 48. G. Vogt, Cytopathology and immune response in the hepatopancreas of decapod crustaceans, *Dis. Aquat. Org.* 138 (2020) 41–88.
49. 49. C.Y. Xiao, Y.F. Zhang, F. Zhu, Immunotoxicity of polychlorinated biphenyls (PCBs) to the marine crustacean species, *Scylla paramamosain*, *Environmental Pollution*. 291 (2021) 118229.
50. 50. V. Roncalli, M.C. Cieslak, Y. Passamaneck, A.E. Christie, P.H. Lenz, Glutathione S Transferase (GST) gene diversity in the crustacean *Calanus finmarchicus*—contributors to cellular detoxification, *PLoS One*. 10 (2015) e0123322.
51. 51. S. Mitchell, J. Vargas, A. Hoffmann, Signaling via the NFκB system, *Wiley Interdiscip Rev Syst Biol Med.* 8 (2016) 227–241
52. 52. J. Dutta, Y. Fan, N. Gupta, G. Fan, C. Gelinas, Current insights into the regulation of programmed cell death by NF-kappaB, *Oncogene*. 25 (2006) 6800–6816.
53. 53. T. Mekata, S. Okugawa, M. Inada, M. Yoshimine, J. Nishi, T. Kono, M. Sakai, T. Itami, R. Sudhakaran, Class B scavenger receptor, Croquemort from kuruma shrimp *Marsupenaeus japonicus*: Molecular cloning and characterization. *Mol Cell Probes*. 25 (2011) 94–100.
54. 54. H. Yang, Z. Liu, Q. Jiang, J. Xu, Z. An, Y. Zhang, D. Xiong, L. Wang, A novel ferritin gene from *Procambarus clarkii* involved in the immune defense against *Aeromonas hydrophila* infection and inhibits WSSV replication, *Fish Shellfish Immunology*. 86 (2019) 882–891.
55. 55. K. Orino, L. Lehman, Y. Tsuji, H. Ayaki, S.V. Torti, F.M. Torti, Ferritin and the response to oxidative stress, *Biochem. J.* 357 (2001) 241–247.
56. 56. P. Kong, L. Wang, H. Zhang, Z. Zhou, L. Qiu, Y. Gai, Two novel secreted ferritins involved in immune defense of Chinese mitten crab *Eriocheir sinensis*, *Fish Shellfish Immunology*. 28 (2010) 604–612.
57. 57. L. Zheng, Z. Liu, B. Wu, Y. Dong, L. Zhou, J. Tian, Ferritin has an important immune function in the ark shell *Scapharca broughtonii*, *Dev. Comp. Immunol.* 59 (2016) 15–24.
58. 58. S.G. Kim, B.W. Jung, H. Kim, Hemocyanin-derived phenoloxidase activity with broad temperature stability extending into the cold environment in hemocytes of the hair crab *Erimacrus isenbeckii*,

- Comparative Biochemistry and Physiology Part B Biochemistry and Molecular Biology 159 (2011) 103–108.
59. 59. F. Yan, Y. Zhang, R. Jiang, M. Zhong, Z. Hu, H. Du, J. Lun, J. Chen, Y. Li, Identification and agglutination properties of hemocyanin from the mud crab (*Scylla serrata*), Fish Shellfish Immunology. 30 (2011) 354–360.
60. 60. E.K. Deenick, S.J. Pelham, A. Kane, C.S. Ma, Signal transducer and activator of transcription 3 control of human T and B cell responses, Frontiers in Immunology. 9 (2018) 168.
61. 61. A. Subramaniam, M.K. Shanmugam, E. Perumal, Potential role of signal transducer and activator of transcription (STAT)3 signaling pathway in inflammation, survival, proliferation and invasion of hepatocellular carcinoma, Biochim Biophys Acta. 1835 (2013) 46–60.
62. 62. K.C. Kregel, Heat shock proteins: modifying factors in physiological stress responses and acquired thermotolerance, J. Appl. Physiol. 92 (2002) 2177–2186.
63. 63. M.E. Feder, G.E. Hofmann, Heat-shock proteins, molecular chaperones, and the stress response: evolutionary and ecological physiology, Annu. Rev. Physiol. 61 (1999) 243–282.
64. 64. P. Moseley, Stress proteins and the immune response, Immunopharmacology 48 (2000) 299–302.
65. 65. W.Q. Zhu, X.Q. Yuan, H.J. Luo, J.C. Shao, X.H. Chen, High percentage of dietary soybean meal inhibited growth, impaired intestine healthy and induced inflammation by TLR-MAPK/NF- κ B signaling pathway in large yellow croaker (*Larimichthys crocea*), Aquacult. Rep. 20 (2021) 100735.
66. 66. Z. Wang, Y.H. Chen, Y.J. Dai, J.M. Tan, Y. Huang, J. F. Lan, Q. Ren, A novel vertebrates Toll-like receptor counterpart regulating the anti-microbial peptides expression in the freshwater crayfish, *Procambarus clarkii*, Fish Shellfish Immunology. 43 (2015) 219–229.
67. 67. Z.Q. Du, Y. Wang, H.Y. Ma, X.L. Shen, K. Wang, J. Du, X.D. Yu, W.H. Fang, X.C. Li, A new crustin homologue (SpCrus6) involved in the antimicrobial and antiviral innate immunity in mud crab, *Scylla paramamosain*, Fish Shellfish Immunology. 84 (2019) 733–743.
68. 68. L. Wang, Y. Zhang, L. Wang, J. Yang, Z. Zhou, Y. Gai, L. Qiu, L. Song, A new anti-lipopolysaccharide factor (EsALF-3) from *Eriocheir sinensis* with antimicrobial activity, Afr. J. Biotechnol. 10 (2011) 17678–17689.
69. 69. J.K. Zhang, F.H. Li, H. Jiang, Y. Yu, C.Z. Liu, S.H. Li, B. Wang, J.H. Xiang, Proteomic analysis of differentially expressed proteins in lymphoid organ of *Fenneropenaeus chinensis* response to *Vibrio anguillarum* stimulation, Fish Shellfish Immunology. 29 (2010) 186–194.
70. 70. F. Wei, D. Li, X. Chen, Y. Li, Y. Zeng, Y. Cai, Y. Zeng, Y. Chen, X. Ma, J. Zeng, Therapeutic effects of epigallocatechin-3-gallate for inflammatory bowel disease: A preclinical meta-analysis. Phytomedicine. 128 (2024) 155408.
71. 71. B.N. Singh, S. Shankar, R.K. Srivastava, Green tea catechin, epigallocatechin-3-gallate (EGCG): mechanisms, perspectives and clinical applications. Biochem Pharmacol. 82 (2011) 1807–1821.
72. 72. M. Alam, S. Ali, G.M. Ashraf, A.L. Bilgrami, D.K. Yadav, M.I. Hassan, Epigallocatechin 3-gallate: From green tea to cancer therapeutics. Food Chem. 379 (2022) 132135.

73. 73. D. Mokra, J. Adamcakova, J. Mokry, Green Tea Polyphenol (-)-Epigallocatechin-3-Gallate (EGCG): A Time for a New Player in the Treatment of Respiratory Diseases? Antioxidants (Basel). 11 (2022) 1566.

74. 74. X. Liu, J.C. Steele, X.Z. Meng, Usage, residue, and human health risk of antibiotics in Chinese aquaculture: A review. Environ Pollut. 223 (2017) 161–169.

75. 75. Z. Wang, B. Sun, F. Zhu, Epigallocatechin-3-gallate protects Kuruma shrimp *Marsupeneaus japonicus* from white spot syndrome virus and *Vibrio alginolyticus*. Fish Shellfish Immunology. 78 (2018) 1–9.

Figures

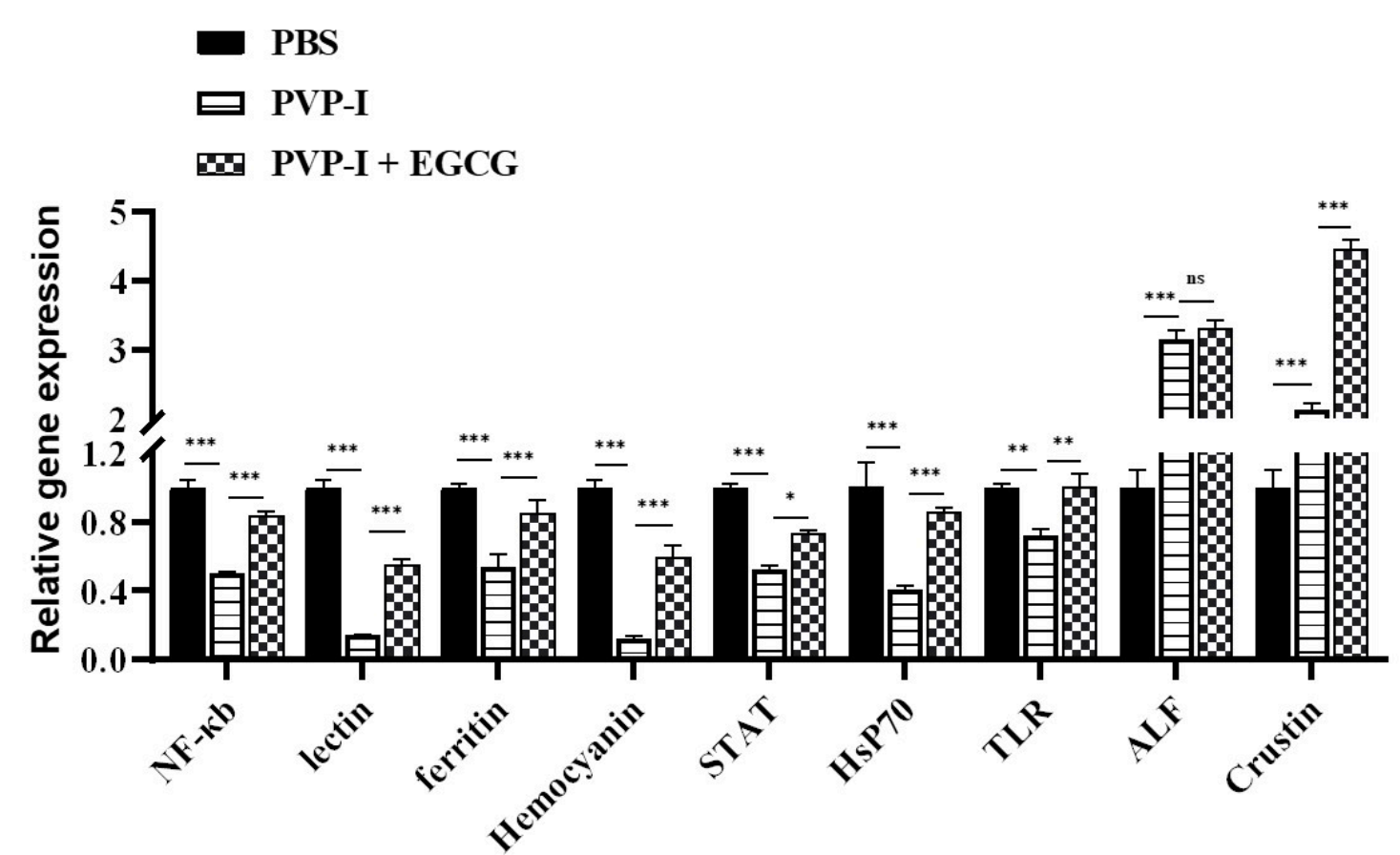


Figure 1

Expression levels of 9 immune-related genes (*Hsp70*, *TLR*, *STAT*, *Lectin*, *Hemocyanin*, *ferritin*, *NF-κB*, *ALF*, *Crustin*) in the hemocytes of crayfish treated with EGCG. Data are depicted as the mean value with its corresponding standard error, "ns" indicates no significant difference ($P\geq0.05$), while significance is indicated with an asterisk. (** $P<0.01$, * $P<0.05$)

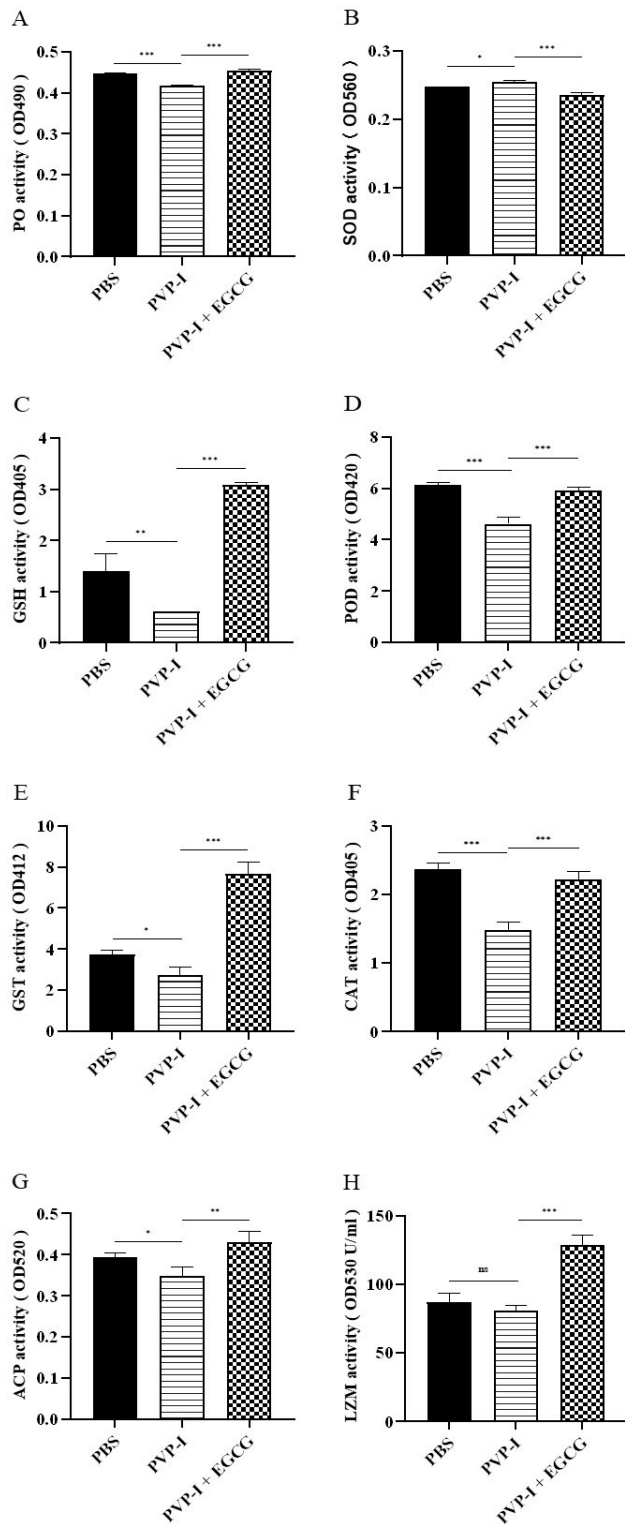


Figure 2

The impact of PVP-I exposure on the immune parameters of crayfish. PO (A), SOD (B), GSH (C), POD (D), GST (E), CAT (F), ACP (G), LZM (H). P values are expressed as ns $P \geq 0.05$, * $P < 0.05$, ** indicates $P < 0.01$, *** indicates $P < 0.001$.

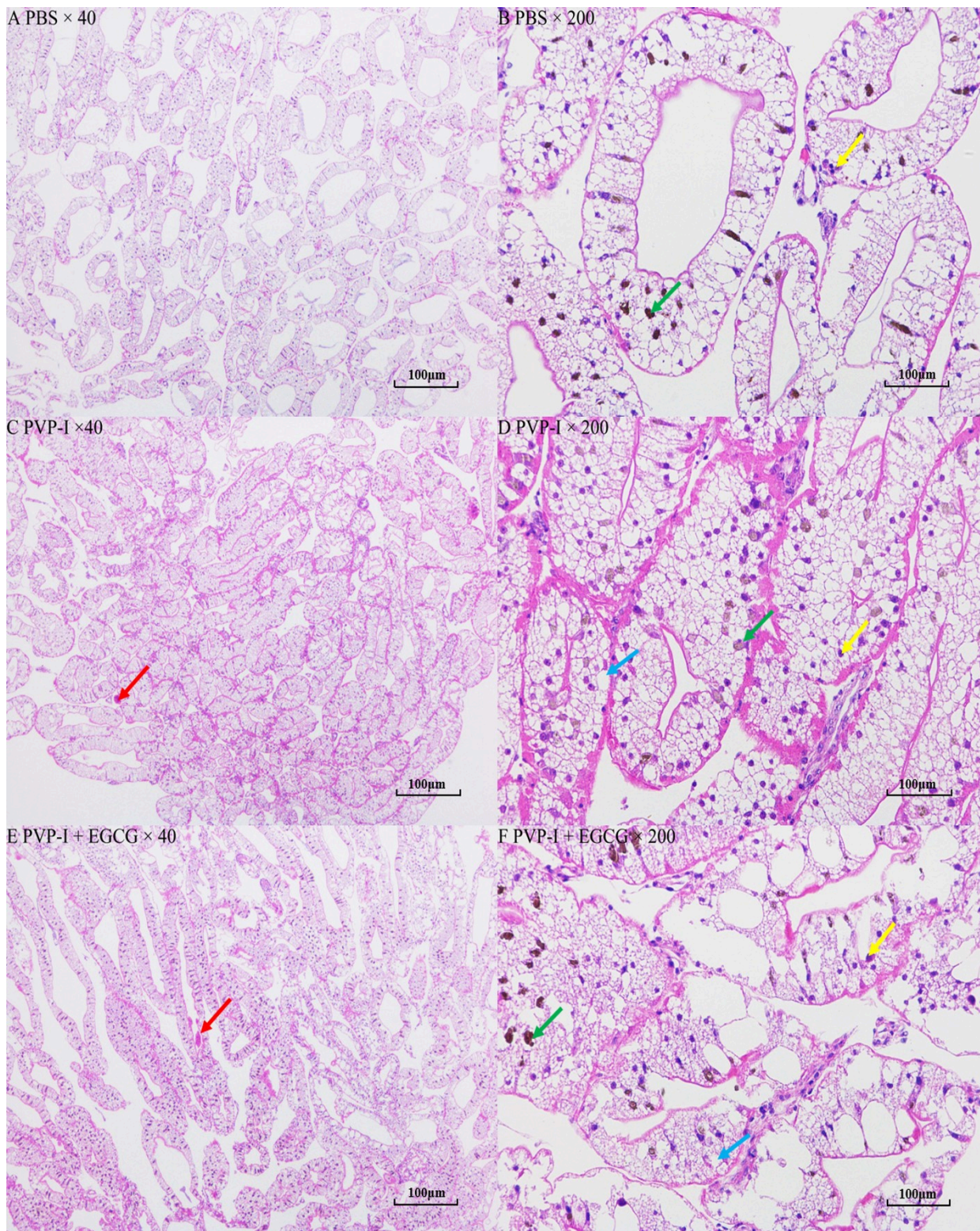


Figure 3

Pathological changes in hepatopancreatic tissue after exposure to PVP-I, with different colored arrows indicating different changes. Mononuclear cell infiltration (yellow arrow), Eosinophilic mass (red arrow), Steatosis (blue arrow), Hepatic tubular epithelial pigmentation (green arrow).

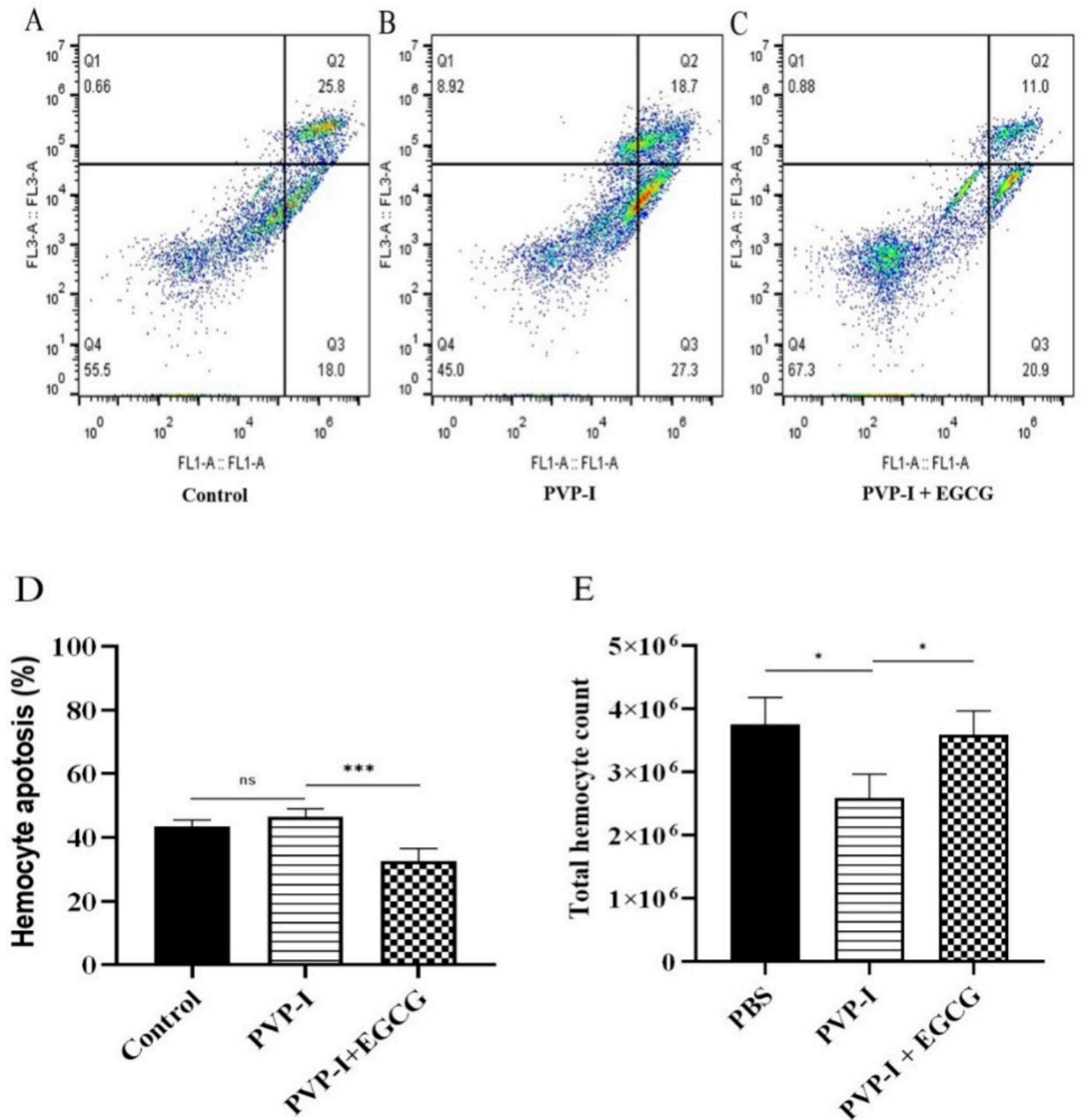


Figure 4

Crayfish apoptosis rate was measured by flow cytometry, and the total hemocyte count was determined by direct microscopic examination and cell count plate enumeration. Control (A), PVP-I (B), PVP-I+EGCG (C), Column chart of apoptosis rate (D) and THC (E). All data are presented as the means $\pm$ SEM, and the asterisks indicate significant differences (ns $P \geq 0.05$, * $P < 0.05$, *** $P < 0.001$).

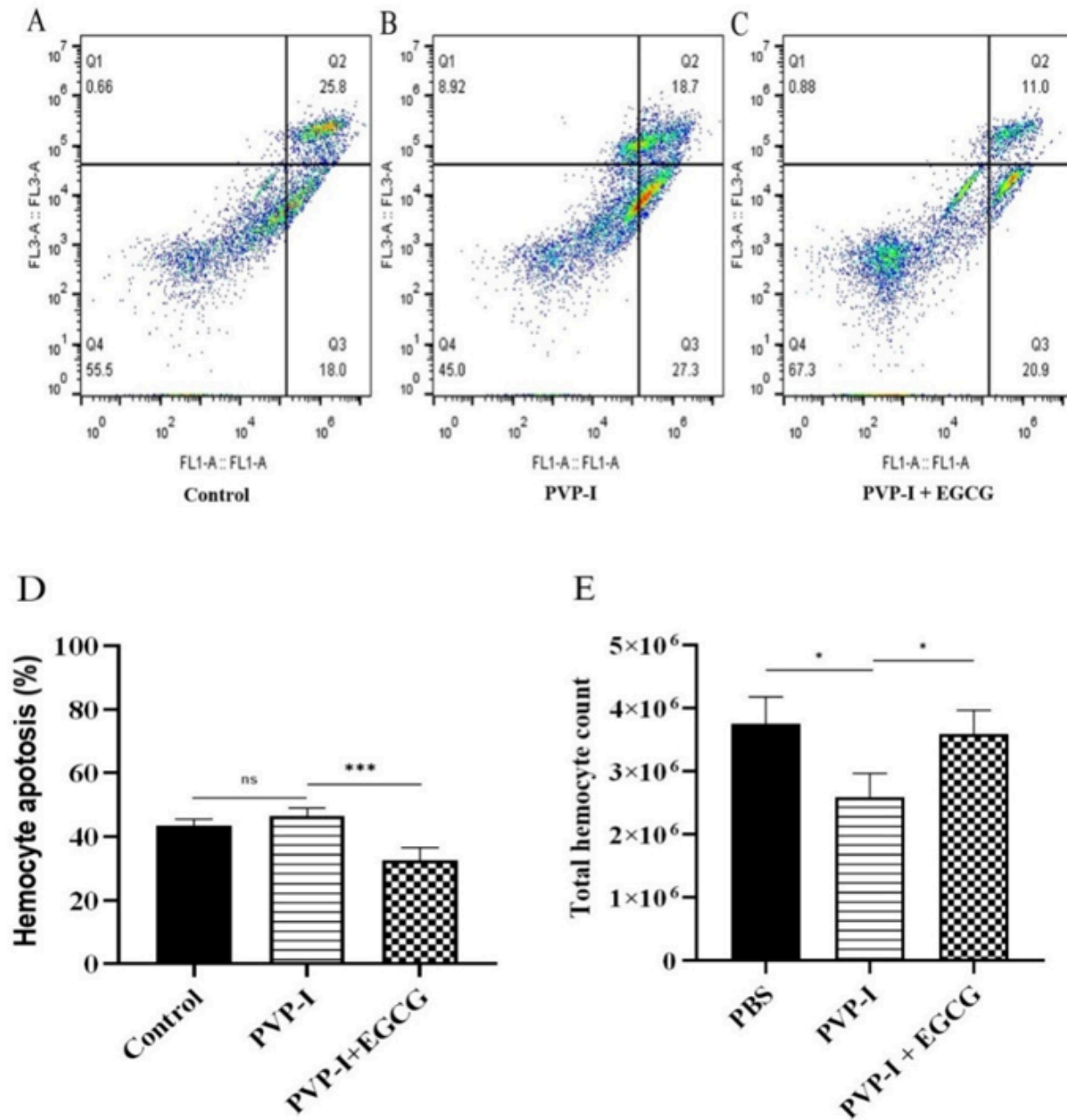


Figure 5

Crayfish apoptosis rate was measured by flow cytometry, and the total hemocyte count was determined by direct microscopic examination and cell count plate enumeration. Control (A), PVP-I (B), PVP-I+EGCG (C), Column chart of apoptosis rate (D) and THC (E). All data are presented as the means $\pm$ SEM, and the asterisks indicate significant differences (ns $P \geq 0.05$, * $P < 0.05$, *** $P < 0.001$).