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Utilization of ursolic acid methyl ester in the treatment of *Streptococcus pyogenes* in Nile tilapia, concerning physiological, genetic, immune-oxidative, stress, and neuro-ethological parameters

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ABSTRACT

The present work is an innovative approach for application the *Streptomyces misakiensis S. misakiensis* metabolite called ursolic acid methyl ester (UAME) in treating *Streptococcus pyogenes* of Nile tilapia. A total of 110 fish was used for assessment of the therapeutic dose of UAME. In addition, the therapeutic efficacy of UAME was tested *in vivo* against *S. pyogenes* infection (after determination of LC_{50}) using 200 Nile tilapia. Fish (30.00 ± 1.5 g) were divided into four groups: CON (control), UAME (1.0 mg/L), *S. pyogenes*, and UAME (1.0 mg/L) + *S. pyogenes* groups. The inoculations of 0.2 mL of *S. pyogenes* (1×10^7 CFU/mL) were administered to the fish in the *S. pyogenes* and UAME + *S. pyogenes* groups and continued for ten days. The minimal inhibitory concentration of UAME was 0.5 μg/mL. Moreover, irregularity and bacterial cell shape distortion were evident after treatment with UAME under a scanning Electron Microscope. Challenged groups in *S. pyogenes* exhibited significant increases in surfacing frequency, swimming, laterality and aggression behavior ($P < 0.05$). Along with this, the hepatorenal metrics (ALT, ALP, AST, creatinine, total bilirubin, and urea.), MDA (an oxidant biomarker), (8-OHdG), glucose and cortisol (stress indicators) were all significantly elevated in the diseased fish ($P < 0.05$). Contrariwise, the infected fish showed a significant drop ($P < 0.05$) in survival %, red blood cells, hemoglobin, total proteins, total globulin, albumin, AchE activity, and the immune-antioxidant indices (IgM, complement factor 3, lysozyme activity, nitric oxide, hepatic CAT, SOD, GSH, and GPx). Analysis of genes in response to *S. pyogenes* infection demonstrated an up-regulation of the pro/anti-inflammatory genes (*il1b, tnfa, il8, tgfb, il10*). Surprisingly, enriched groups in UAME modulated all the measured parameters. Overall, UAME is a promising antibacterial candidate that can efficiently retrieve stress and enhance behavioral, biochemical, immune-antioxidant, and genetic changes endowed by *S. pyogenes* infection.

1. Introduction

Aquaculture is a steady-promising food sector but is overwhelmed by an excess of bacterial pathogens that adversely infect fish (Irshath et al., 2023). *Streptococcus* has been documented to occur in fresh, marine, and brackish water fish, making Streptococcus spp. one of the most harmful

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bacteria in the world. Streptococcus has inflicted significant economic losses in the aquaculture industry worldwide, amounting to millions of dollars (Economou and Gousia, 2015). Additionally, it is believed that tilapia is the perfect host for an infection caused by Streptococcus (Nasr-Eldahan et al., 2022). Among bacterial infections is the pyogenic group which belongs to the genus Streptococcus and involves many species, most of them are commensal of the flora of humans and animals, but under particular circumstances may induce localized and systemic infections which in turn negatively influence both the public health sector and the veterinary industry (Alves-Barroco et al., 2020; Peters et al., 2017). There have been at least 517,000 fatalities worldwide due to illnesses caused by the *streptococcus pyogenes* bacteria, which has infected more than 700 million people (Carapetis et al., 2005). Such an organism induces an extensive diversity of acute infections through the body of the host (Hirose et al., 2023). It induces a wide range of human illnesses, from less serious ones like impetigo and superficial pharyngitis to more serious ones like streptococcal toxic shock syndrome and necrotizing fasciitis with their invasive structures (Walker et al., 2014). *Streptococcus pyogenes* was isolated from tilapia fish in Egypt at the rate of 39.47 % and resulted in diminished total protein, albumin, and globulin levels in blood serum, while total cholesterol, urea, creatinine levels, and the activity of AST, and ALT considerably increase (Ali et al., 2020). In addition, it results in skin lesions, hemorrhaging on the body surface, redness on the fins, as well as optic eye damage in Tilapia fish. Also, the internal examination revealed a liver and spleen that seemed pale and swollen, accompanied by the presence of bloody fluid in the abdominal cavity (Nasr-Eldahan et al., 2022). Caused a mortality rate of up to 60 % in all fish that were afflicted (Abou El-Gheit, 2005).

The global dissemination of multidrug-resistant bacteria and resistance genes, which are now considered emerging environmental contaminants, has been triggered by the inappropriate and excessive use of antibiotics in both people and food animals. To combat antimicrobial resistance, researchers were compelled by this circumstance to create novel and improved antimicrobial compounds (El-Houseiny et al., 2021). Therefore, many recent therapeutic approaches, such as probiotics and secondary metabolites, are applied successfully in aquaculture practices which have antibacterial activity beside environmentally-friend, safe, and easily applicable (Almarri et al., 2023; El-Houseiny et al., 2023; Mansour et al., 2022). Streptomyces, a prominent bacterial genus, belongs to the Actinobacteria, a Gram-positive, extensively distributed filamentous bacterial phylum that is a major producer of many naturally occurring antibiotic chemicals (Barka et al., 2016). The bioactive metabolites produced by actinobacteria have a wide range of antimicrobial, antiviral, antifungal, anti-inflammatory, and insecticidal effects (Rangseekaew and Pathom-Aree, 2019). These metabolites have a substantial influence on the expansion of the pharmaceutical industry and the control of several infectious diseases (Abdelaziz et al., 2023).

Among the promising secondary metabolites, the ursolic acids are plant-based pentacyclic triterpenoid consisting of 30 carbon atoms and are broadly disseminated in nature (Kvasnica et al., 2015; G. Liu et al., 2023). Ursolic acid is commonly identified in great quantity from plants and exerts stimulating biological characteristics such as antibacterial, antitumor, anti-inflammatory, and antiviral activities (Ghaffari Moghaddam et al., 2012; Hussain et al., 2017; Kazakova et al., 2010). It has been reported to have a strong antibacterial action against the bacteria *Staphylococcus aureus, Bacillus cereus, Salmonella typhimurium, Escherichia coli,* and the yeast *Candida albicans* (Innocente et al., 2014; Pereira et al., 2022). With every aspect considered, the current study aims to assess the antimicrobial activity of ursolic acid methyl ester (UAME) against pathogenic *S. pyogenes* to Nile tilapia fish *in vitro.* Additionally, an *in vivo* investigation is conducted to assess the therapeutic effectiveness of the potent antibacterial biomaterial in *O. niloticus* with emphasis on neuro-behavior, hematology, biochemical, immune-antioxidant, and gene expression indices.

2. Material and methods

2.1. Ursolic acid methyl ester source

An earlier work described the isolation and identification of the *Streptomyces misakiensis* strain (GenBank accession number OP168477) (Abdelaziz et al., 2023). After five days of incubation at 28 °C, the broth from *Streptomyces misakiensis* was transferred to a conical flask. This was done to create the mycelium-free culture broth. Coarse cotton and Büchner porcelain funnels from Stonylab Egypt were used to filter out the mycelium. Centrifugation was used for 15 minutes at 13,000 rpm to separate the cells of the *S. misakiensis* mycelium. The solution containing the metabolites was prepared through centrifugation and filtering. The ethyl acetate extract derived from *S. misakiensis* metabolites was subjected to thin-layer chromatography (TLC) analysis. Six fractions were identified by TLC analysis of the *S. misakiensis* metabolite extract. The studied bacterial species were all susceptible to the antibacterial effects of portion R3. At 3.5 cm, R3 had a resistance value. The HPLC preparative, which produces six peaks, validated the TLC findings. The chemical that had been isolated from TLC was subjected to GC-MS analysis. The compounds were recognized by calculating their molecular formula, molecular weight, and peak area. A substance's concentration in the active band is directly proportionate to its area. UAME (Urs-12-en-28-oic acid, 3-hydroxy-, methyl ester, (3á)) is a newly discovered antibacterial metabolite derived from the metabolites of *S. misakiensis*. The IR spectrum of UAME exhibited functional group absorptions at 32982, 1748, 1377, 1243, 1051, 929, 847, 620, and 456.18 cm^{-1} corresponding to (OH) stretching in alcohol, (C-H aliphatic), (C═O), (ester), and (C═C), respectively. The 1 H NMR spectrum was at 3.502, showing that this antibiotic predominantly consists of UAME (Urs-12-en-28-oic acid, 3-hydroxy-, methyl ester, (3á)) (Abdelaziz et al., 2023) (Fig. S1).

2.2. Streptococcus pyogenes strain and determination of LC_{50}

Streptococcus pyogenes strain with established biochemical and pathogenicity profiles were generously supplied by the microbiological repository of the Department of Microbiology, Animal Health Research Institute, Dokki, Egypt. The identification of isolate was carried out using colony morphology, β-hemolysis, and biochemical properties with the commercial identification system rapid ID 32 STREP (BioMerieux, Marcy L'Étoile, France including catalase activity, oxidase testing, growth at 6.5 % NaCl, arginine decarboxylase detection, hippurate hydrolysis, bile esculin testing, and sugar fermentation (Bergey, 1994). Biochemical verification was additionally validated through sequencing of the 16S rRNA gene. Amplification of the 16S rRNA gene using the forward primer (5′-TTTCACCAGCTGTATTAGAAGTA-3′) and the reverse primer (5′-GTTCCCTGAACATTATCTTTGAT-3′) was used to molecularly confirm the active isolate. Up until usage, the bacterial cultures were stored at a temperature of −80 °C in brain-heart infusion (BHI) broth (Oxoid, USA) with 15 % glycerol added. The isolates were recovered in BHI medium and left to incubate for the night at 37 °C. In order to ascertain the lethal dose of 50 (LD_{50}) from *S. pyogenes*, several bacterial concentrations were examined. The fish were organized in five distinct groups in triplicates (30 fish per group; 10 fish per replica) weighing an average of 30 ± 1.5 g (N = 150). Each of the 10 fish was kept in a 60 L tank with good ventilation, and the water was kept at a temperature of 24 ± 1.5 °C. A day before to infection, the fish were allowed to starve. Benzocaine solution 100 mg/L was used to anesthetize fish (Lugo et al., 2008). 0.2 mL of sterile saline was administered to the first group. The other four groups received 0.2 mL of various *S. pyogenes* microbiological dispersion dilutions with 10^6–10^9 CFU/fish intraperitoneally (IP) at the same time. Four days after the inoculation, the mortality rate was noticed. The LD_{50} was 1.5 × 10^7 CFU/mL, as reported by the Probit Analysis Programme version 1.5 (U.S. Environmental Protection Agency). For the treatment study, a sub-lethal dose (1 ×10^7 CFU/mL)

was used.

2.3. Evaluating the UAME's minimal inhibitory concentration

For the determination of minimum inhibitory concentrations (MIC value) of UAME against *S.pyogenes* under investigation, 96-well polystyrene microtiter plates (Costar, Corning Inc., USA) were utilized in conjunction with the broth microdilution method. In each well of the 96-well microtiter plate, 100 μL of a new bacterial culture suspension (5×10^5) was added. Then, UAME, which stands for vacuum-dried ethyl acetate metabolites of *Streptomyces misakiensis,* was dissolved in tween 20 (1 gm/1 mL). The mixture was then diluted in Mueller-Hinton broth, with a concentration range of 0.125 μg/ mL to 512 μg/ mL. The next step was to incubate the plate at 37 °C for a period of one to two days. Negative and positive controls were both incorporated. The MIC is the lowest concentration at which bacterial proliferation is inhibited.

2.4. Determination of antibacterial activity of UAME against Streptococcus pyogenes using scanning electron microscope (SEM)

To identify the morphological alterations in *S. pyogenes* treated with UAME, scanning electron microscopy (SEM) experiments were performed following the described procedure with a few modifications. After the *S. pyogenes* strain had grown to the logarithmic stage in a new MH liquid medium, it was incubated for 8 hours in a shaker at 28 °C and treated with UAME. Centrifugation at 5000 rpm for 10 minutes at 4 °C and three washes with sterile PBS were followed by SEM (SU8100, Hitachi, Ltd., Japan) analysis of the lower layer precipitation, which was fixed with 2.5 % glutaraldehyde (Shanghai Aladdin Biochemical Technology Co., Ltd.) at 4 °C.

2.5. Bioactive metabolite efficacy in treating streptococcosis in Nile tilapia

The effective ursolic acid methyl ester discovered *in vitro* was tested *in vivo* in *Streptococcus pyogenes* infection.

2.5.1. Fish and culturing conditions

In the current study, 310 healthy Nile tilapia (mean body weight 30.00 ± 1.5 g) were recruited. The Fish Research Unit at Zagazig University's Faculty of Veterinary Medicine was the source of the fish. For two weeks before testing, the fish were carefully monitored and allowed to adapt. In addition, the fish underwent a clinical test to rule out any potential diseases. Throughout the acclimation and testing periods, the fish were maintained in 40 × 30 × 80 cm glass aquariums with 60 L of dechlorinated tap water per 10 fish. The controlled day-to-night cycle (12 h dark: 12 h light) was adopted to maintain the laboratory's water quality parameters, which included dissolved oxygen (6.5 ± 0.40 mg/L), pH (6.7 ± 0.3), temperature (24 ± 1.5°C), and ammonia (0.02 ± 0.01 mg/L). The fish were fed a basic diet accounting for 3 % of their biomass twice daily, at 9:00 a.m. and 4:00 p.m. On a daily basis, the excretory wastes were removed by the process of siphoning. All of the procedures and protocols that were utilized in this research project were authorized by the Animal Usage in Research Committee (ZU-IACUC/2/F/418/2023) of Zagazig University, Egypt. These procedures and protocols were in accordance with the National Institutes of Health's recommendations for the use and care of laboratory animals.

2.5.2. Assessing the therapeutic dose of ursolic acid methyl ester

The recommended dose of UAME was calculated using the protocol of Ibrahim et al. (2023), with certain modifications. About 110 fish (10 fish per tank) were exposed to 11 various concentrations of UAME as a bath treatment for ten days to ascertain the initial dosage (Table 1). The freshly extracted UAME solution was incorporated to maintain its level following water exchange (3 times weekly). Each and every day, the mortality rate as well as the clinical signs were recorded. The chosen therapeutic dose of UAME was 1.0 mg/L.

Table 1
Assessment of mortality and clinical observations in Nile tilapia after ten days of exposure to varying doses of ursolic acid methyl ester.

Conc. (mg/L)	Clinical signs				mortality
	Abnormal swimming	**Loss of reflexes**	**External skin lesion**	**Postmortem changes**	
0.00	-	-	-	-	0/10
0.2	-	-	-	-	0/10
0.4	-	-	-	-	0/10
0.6	-	-	-	-	0/10
0.8	-	-	-	-	0/10
1.0	-	-	-	-	0/10
1.2	-	-	-	-	1/10
1.4	+	-	-	+	1/10
1.6	+	+	-	+	2/10
1.8	++	++	+	+++	4/10
2.0	++	++	++	+++	4/10

Absence of signs or lesions (-), mild signs or lesions (+), moderate signs or lesions (++), severe signs or lesions (+++)

2.5.3. Experimental layout

Two hundred fish were randomly allocated into four groups for ten days (n = 50 fish/group; 5 tanks/group; 10 fish/tank). There were four groups: CON (no addition of UAME or challenge with *S. pyogenes*), UAME, *S. pyogenes*, and UAME + *S. pyogenes* groups. IP inoculations of 0.2 mL of *S. pyogenes* (1×10^7 CFU/mL) were administered to the fish in the *S. pyogenes* and UAME + *S. pyogenes* groups. Following the emergence of clinical symptoms on the second day of the trial, the UAME was added to the tank water at a dose of 1.0 mg/L and continued for ten days. To dispose of the waste products, syphoning was done every day. After three weekly water exchanges, the freshly made UAME solution was added to maintain the 1.0 mg/L UAME level. Recordings were taken of clinical manifestations, postmortem lesions, behavior patterns, and mortality during the trial, which lasted for ten days and was conducted consistently. The experimental setup and the parameters assessed are illustrated in Fig. 1.

2.5.4. Gross observation and behavioral outcomes

Using a controlled camera with a programmable timer, fish were examined every day from 8:00 a.m. to 3:00 p.m. during the treatment period to record mortalities, clinical diagnostic symptoms, and behavioral changes (Altmann, 1974). According to Noga (2010), Chen et al. (2001), Scheurmann (2000), Ismail et al. (2009) and De Boer (1980) respectively, the observed behavioral alterations were surfacing, poor swimming, resting, laterality and aggression.

2.5.5. The collection of blood and tissue samples

After the therapy study, multiple physiological specimens were collected from the fish. The fish were anesthetized with 100 mg/L of benzocaine solution after the exposure time (10 days) to reduce handling stress. For the hematobiochemical tests, whole blood samples (ten fish from each group) were collected from the caudal vasculature and put into test tubes with sterile EDTA coating. An additional ten blood samples were obtained, with two samples collected for each replication. The samples were collected in sterile tubes that were devoid of anticoagulants. The serum obtained from the blood samples was subsequently isolated using centrifugation at a speed of 3000 ×g for 10 minutes. The isolated serum was then stored until it could be subjected to a physiological profile study. Following the collection of blood samples, five fish from each group underwent an aseptic necropsy, during which liver samples were obtained to assess the oxidant-antioxidant markers. Moreover, five samples of spleenic tissue were subjected to the TRIzol treatment (Thermo Scientific) and stored in a deep freezer at a temperature of −80 °C for RNA separation. Additionally, five brain tissues per group were gathered in order to calculate the

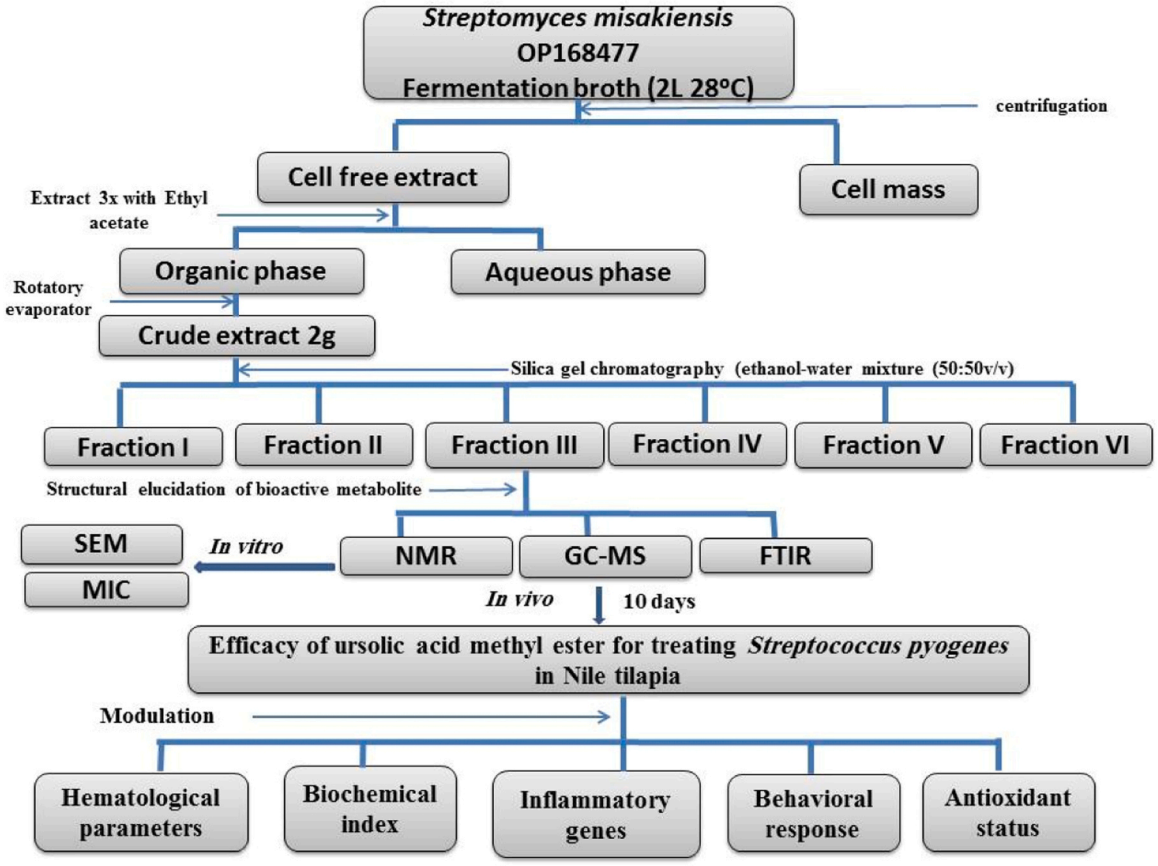

Fig. 1. The experimental setup and the parameters assessed.

neuro-related indicators.

2.5.6. Blood hematological assessments

In accordance with Bain et al. (2016), the blood cell profile was evaluated using an automated cell counter (Hospitex Hema screen 18, Italy), taking into account the total and differential leukocyte counts as well as the counts of erythrocytes, haemoglobin, packed cell volume, mean corpuscular volume, mean corpuscular haemoglobin, and mean corpuscular haemoglobin concentration (RBCs, Hb, PCV, MCV, MCH, and MCHC, respectively).

2.5.7. Protein profile and hepato-renal function indicators

Following the procedure described by Badawi (1971), serum proteins, such as total proteins, total globulin, and albumin, were separated by electrophoresis on cellulose-acetate.

Aalanine aminotransferase (ALT), Alkaline phosphatase (ALP) and aspartate aminotransferase (AST) serum activity were assessed using the methods described by Burtis and Ashwood (1994), Murray (1984), and Wenger et al. (1984) respectively. The values of urea and creatinine were also calculated (Ajeniyi and Solomon, 2014).

2.5.8. Immunological indices reactions

The levels of serum immunoglobulin M (IgM) and complement factor 3 were assessed via fish-specific ELISA kits, Cat. Nos. MBS282651 and MBS005953, following the instructions provided by the manufacturer. Furthermore, the levels of lysozyme activity and nitric oxide were assessed through the Lee and Yang (2002) and Bryan et al. (2007) methods, respectively.

2.5.9. Hepatic oxidant/ antioxidant assay

Antioxidant activity in liver homogenate was assessed using colorimetric commercial kits purchased from Biodiagnostic Co. in Cairo, Egypt. The method developed by Aebi (1984) was used to track catalase (CAT) activity. Superoxide dismutase (SOD) activity was evaluated using the Nishikimi et al. (1972) technique. According to Beutler (1963) quantitative colorimetric glutathione dehydrogenase (GSH) was carried out. Quantitative colorimetric glutathione peroxidase (GPx) assays were conducted using Bio-Assay Systems' (Hayward, CA, USA) Enzy-ChromTM Glutathione Peroxidase Assay Kit (EGPX-100) (Paglia and Valentine, 1967). The method developed by Uchiyama and Mihara (1978) was used to monitor malondialdehyde.

2.5.10. Stress-linked and brain neurostress indicators

Using the methods outlined by Trinder (1969) and Saliu et al. (2017), we measured serum glucose and cortisol levels using calorimetric techniques. The spectrophotometric assessment of acetylcholine esterase (AchE) activity in brain tissues was conducted using acetylthiocholine iodide, as described by (Ellman et al., 1961). According to Setyaningsih et al. (2015), an ELISA commercial kit (My-Biosource Inc., San Diego, California, USA) was employed to evaluate the activity of 8-hydroxy-2-deoxyguanosine (8-OHdG), an indicator of oxidative DNA damage.

2.5.11. Analysis of spleenic immunity and inflammatory-related genes

The TRIzol solution (easyREDTM, iNtRON Biotechnology, Korea) was employed to extract total RNA from the frozen splenic samples. The first-strand cDNA was synthesized from the extracted RNA using the Quantitect® Reverse Transcription kit (Qiagen, Germany). Table 2 comprises the *β-actin* gene, which is used for housekeeping purposes, along with the sequences of the forward and reverse primers. The Rotor-Gene Q device was used for qPCR analysis with the QuantiTect SYBR Green PCR kit (Qiagen, Germany). The thermocycler conditions

Table 2
Oligonucleotide primer sequences.

Gene name	Primer sequences		NCBI accession no.	Product size (bp)	References
actb	F	TGACCTCACAGACTACCTCATG	XM_003455949.2	89	(Abarike et al., 2020)
	R	TGATGTCACGCACGATTTCC			
tnfa	F	CCAGAAGCACTAAAGGCGAAGA	NM_001279533.1	82	(Standen et al., 2016)
	R	CCTTGGCTTTGCTGCTGATC			
il1b	F	TGGTGACTCTCCTGGTCTGA	XM_005457887.3	86	(Standen et al., 2016)
	R	GCACAACTTTATCGGCTTCCA			
tgfb	F	GTTTGAACTTCGGCGGTACTG	NM_001311325.1	80	(Standen et al., 2016)
	R	TCCTGCTCATAGTCCCAGAGA			
il10	F	CTGCTAGATCAGTCCGTCGAA	XM_013269189.3	94	(Standen et al., 2016)
	R	GCAGAACCGTGTCCAGGTAA			
il8	F	GCACTGCCGCTGCATTAAG	NM_001279704.1	85	(Wangkaghart et al., 2021)
	R	GCAGTGGGAGTTGGGAAGAA			

Beta actin (*actb*, house-keeping gene), interleukin 1beta (*il1b*), interleukin 10 (*il10*), interleukin 8 (*il8*), transforming growth factor beta (*tgfb*), tumor necrosis factor-alpha (*tnfa*).

consisted of an initial denaturation step at 95 degrees for 10 minutes, followed by 40 cycles of denaturation at 95 degrees for 15 seconds, annealing at 60 degrees for 30 seconds, and extension at 72 degrees for 30 seconds. The Livak and Schmittgen (2001) comparative $^{2-\Delta\Delta}$CT analysis was employed to ascertain the relative expression of the mRNA pattern for each gene.

2.6. Statistical analysis

Prior to performing the data analysis, Bartlett's test and the Kolmogorov-Smirnov test were used to confirm the variance homogeneity and normality of the collected data's distribution. A one-way analysis of variance (ANOVA) was used to compare data from all groups, and Duncan's multiple-range test was performed afterward using SPSS 21.0 (Chicago, IL, USA). All data were expressed as follows: means ± standard errors (SE). For the statistical difference, the significance limit was established at $P < 0.05$.

3. Results

3.1. Antibacterial activity of UAME and its effects on S. pyogenes shape and structure using SEM

UAME exhibited antibacterial activities against *S. pyogenes* with (MIC= 0.5 μg/mL). As shown in Fig. 2, UAME treatment could cause morphological alterations in *S. pyogenes*. The non-treated control group exhibited spherical bacterial cells that tended to join in chains and did not exhibit motility or sporing (Fig. 2, A.B&C). The modification of *S. pyogenes* surfaces by UAME treatment could greatly reduce a total number of bacteria as shown in (Fig. 2,D) and more severe morphological changes were appeared as irregularity and cell shape distortion as shown in (Fig. 2,E.F).

3.2. Clinical assessment and behavioral indices

The CON and UAME groups of uninfected fish had 98.00 and 100 % survival and displayed no unusual indications (Fig. 3 A,B) or behaviours when exposed to 0 and 1.0 mg/L UAME (Table 3). Without therapy, fish

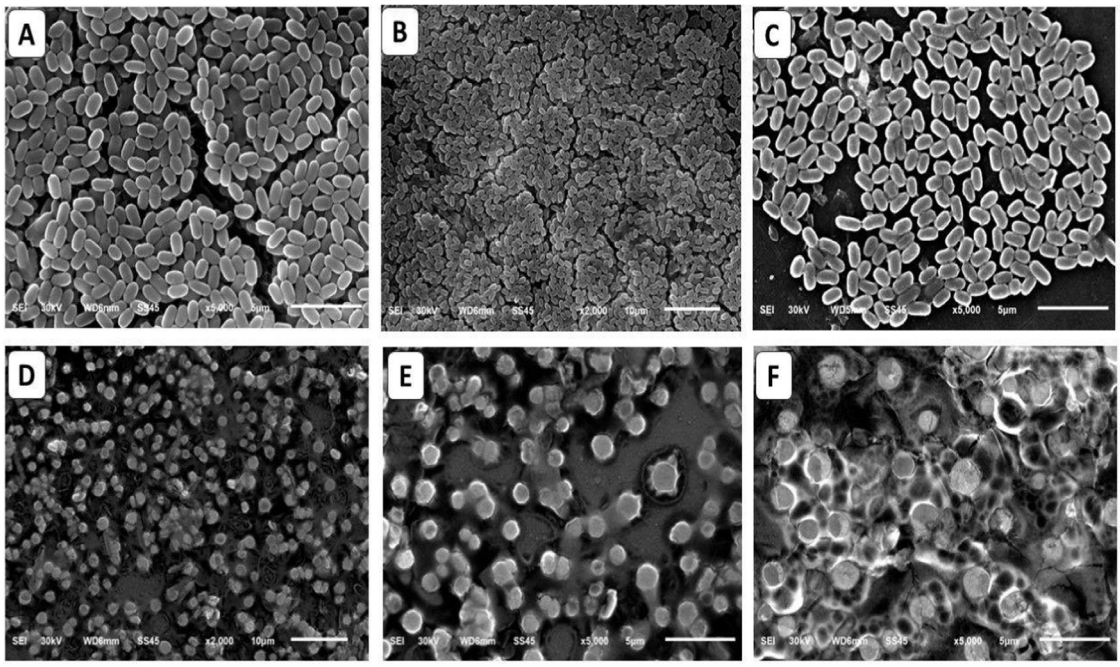

Fig. 2. Micrograph of *S. pyogenes* culture after being treated with ursolic acid methyl ester (UAME by scanning electron microscope (SEM). The adverse control group was A-C, the colonies emerge as spherical cells that like to join in chains; they are not sporing and are not motile. The UAME-treated group was (D-F), decrease in the total number of bacteria and more severe morphological changes were appeared as irregularity and cell shape distortion.

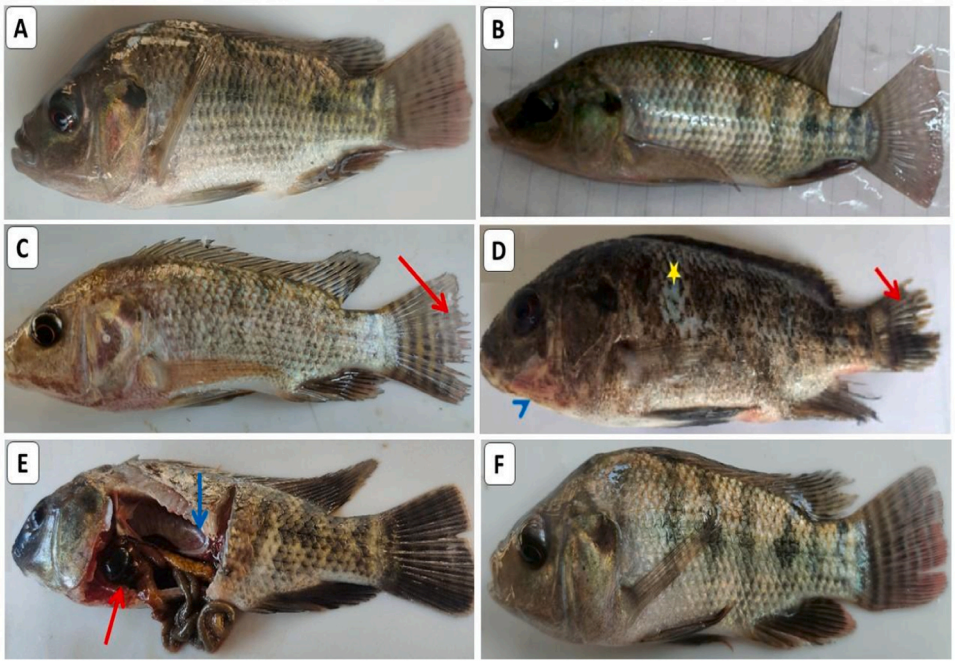

Fig. 3. Clinical observation of *O. niloticus* experimentally infected with *S. pyogenes* and treated with UAME (1.0 mg/L) for ten days. (A) Fish of the control group. (B) UAME group exhibited normal appearance. Fish of the *S. pyogenes* group exhibiting fin rot (red arrow) (C), body hemorrhages (arrow head), scale loss (star), and sever fin rot (red arrow) (D). Inflammation of swim bladder (blue arrow), internal organ congestion, and gall bladder enlargement (red arrow) in postmortem examination (E). Fish of OHM + *S. agalactiae* group exhibiting normal appearance with mild hemorrhage on caudal fin (F).

Table 3
Effect of ursolic acid methyl ester (UAME) on the Behavioral responses and neuro-stress biomarkers of experimentally infected *O. niloticus* with *S. pyogenes* for ten-days.

Behavioral patterns Items		Experimental groups			
		CON	UAME	*S. pyogenes*	UAME+ *S. pyogenes*
Surfacing frequency		0.29^{c} ± 0.023	0.28^{c} ± 0.011	0.50^{a} ± 0.020	0.37^{b} ± 0.008
Swimming behavior		0.51^{bc} ± 0.017	0.44^{c} ± 0.012	1.33^{a} ± 0.072	0.65^{b} ± 0.029
Resting activity		0.22^{a} ± 0.014	0.23^{a} ± 0.012	0.08^{c} ± 0.011	0.17^{b} ± 0.011
Laterality		0.07^{c} ± 0.014	0.03^{c} ± 0.005	0.49^{a} ± 0.020	0.15^{b} ± 0.011
Aggression		0.18^{bc} ± 0.011	0.10^{c} ± 0.008	0.57^{a} ± 0.040	0.26^{b} ± 0.014
Brain	AChE (ng/mg)	0.52^{a} ± 0.011	0.52^{a} ± 0.014	0.20^{c} ± 0.011	0.45^{b} ± 0.014
	8-OHdG (mg/mg)	37.06^{c} ± 1.072	36.55^{c} ± 0.476	88.01^{a} ± 0.725	42.33^{b} ± 0.437

Values are represented as the mean ± SE. The means within the same row carrying different superscripts are significant at $p < 0.05$. (N = 5/group)

infected with *S. pyogenes* had the lowest survival rate (54.00 %), the least amount of resting activity, the highest surfacing frequency, surface swimming, laterality, and aggression behavior (Table 3). Moreover, the *S. pyogenes* groups displayed fin rot (Fig. 3, C), scale loss, and severe hemorrhages on multiple body parts (Fig. 3, D), along with congestion in internal organs (Fig. 3, E). However, treatment with 1.0 mg/L UAME (UAME + *S. pyogenes* group) enhanced survival (84.00 %) and reversed the previous clinical symptoms, with the exception of a mild caudal fin hemorrhage (Fig. 3, F). Also, there was an improvement in behavior pattern (Table 3).

3.3. Brain acetylecholine etrase (AChE) and DNA damage marker

Table 3 exhibits that *O. niloticus* infected with *S. pyogenes* had significantly ($P < 0.05$) lower brain AChE content than those present in non-infected groups. On the other hand, infected fish that were treated with 1.0 mg/L UAME revealed a significant ($P < 0.05$) boost in AChE activity in their brain tissue. Furthermore, compared to the other groups, infected fish with *S. pyogenes* exhibited a significantly greater 8-OHdG. On the opposite hand, non infected groups showed significantly ($p < 0.05$) lower levels of 8-OHdG than other groups. The levels of 8-OHdG were decreased in the UAME + *S. pyogenes* group but still significantly different from CON or UAME groups ($p < 0.05$).

3.4. Hematological parameters

The effects of UAME treatment or *S. pyogenes* infection on hematological indicators in *O. niloticus* are discussed in Table 4. The erythrogram showed that fish infected with *S. pyogenes* had significantly ($p < 0.05$) lower mean values for RBCs, Hb, and PCV than fish in the other groups. However, the anemic indices that bacteria induced were successfully averted through the provision of 1.0 mg/L UAME in UAME + *S. pyogenes* group. Leukograms revealed that fish infected with *S. pyogenes* had considerably higher levels of total WBCs, heterophils, eosinophils, and monocytes with lower levels of lymphocytes than other groups. In comparison to values in fish infected with *S. pyogenes*, UAME treatment significantly diminshed the levels of total WBCs, heterophils, eosinophils, and monocytes.

3.5. Parameters linked to immunity

Table 5 displays a substantial spike in the immunological markers (IgM, Lysosyme, NO, and C3) in the UAME group compared to the control fish. These parameters were significantly lower in the S. *pyogenes* group than in the control group. The treated group (UAME+ *S. pyogenes*) showed noticeable improvements in these parameters compared to the infected group.

Table 4
Effect of ursolic acid methyl ester (UAME) on the hematological indices of experimentally infected *O. niloticus* with *S. pyogenes* for ten-days.

Estimated parameters	Experimental groups			
	CON	UAME	S. *pyogenes*	UAME+ S. *pyogenes*
Erythrogram				
RBCs (10^6/mm^3)	2.15^a ± 0.023	2.16^a ± 0.034	1.68^b ± 0.044	2.03^a ± 0.034
Hb (gm/dL)	8.32^b ± 0.072	8.87^a ± 0.053	6.54^d ± 0.061	8.03^c ± 0.044
PCV (%)	26.47^b ± 0.236	28.29^a ± 0.176	20.59^d ± 0.201	25.50^c ± 0.146
MCV(fL)	122.75^a ± 0.294	130.64^a ± 2.697	122.58^a ± 4.470	125.70^a ± 1.747
MCH (%)	38.60^a ± 0.103	40.99^a ± 0.837	38.91^a ± 1.439	39.58^a ± 0.553
MCHC (%)	31.44^b ± 0.008	31.37^c ± 0.006	31.78^a ± 0.015	31.49^b ± 0.008
Leukogram				
WBCs (10^3/mm^3)	4.34^c ± 0.050	4.54^c ± 0.043	7.31^a ± 0.052	4.86^b ± 0.087
Lymphocytes (10^3/mm^3)	2.28^b ± 0.015	2.34^b ± 0.011	1.67^c ± 0.012	2.56^a ± 0.029
Heterophils (10^3/mm^3)	1.06^c ± 0.014	1.130^c ± 0.011	4.06^a ± 0.014	1.44^b ± 0.032
Eosinophils (10^3/mm^3)	0.32^b ± 0.008	0.35^b ± .005	0.68^a ± 0.008	0.27^c ± 0.014
Monocytes (10^3/mm^3)	0.67^b ± 0.012	0.72^b ± 0.014	0.89^a ± 0.017	0.58^c ± 0.011

Values are represented as the mean ± SE. The means within the same row carrying different superscripts (a, b, c, and d) are significantly different at $p < 0.05$ (N = 10 /group). RBCs: red blood cells; Hb: hemoglobin; PCV: packed cell volume; MCV: Mean corpuscular volume; MCH: mean corpuscular hemoglobin; MCHC: mean corpuscular hemoglobin concentration; WBCs: white blood cells.

3.6. Criteria pertaining to oxidative stress

Fish infected with *S. pyogenes* exhibited significantly higher levels of MDA, a consequence of lipid peroxidative degradation, than control fish, but significantly lower levels of SOD, CAT, GPx, and GSH antioxidants, as shown in Fig. 4. Interestingly, liver tissues from fish given 1.0 mL/L UAME showed a considerable decrease in MDA level but a large improvement in SOD, CAT, GPx, and GSH contents when compared to CON groups. The previously mentioned variables were improved in the UAME+ *S. pyogenes* groups when compared to the *S. pyogenes* group, although they were still significantly lower than those observed in the CON group.

3.7. Hepato-renal variables and the profile of proteins

Table 5 reveals that there were no significant differences in creatinine, urea, T bilirubin, AST, ALT, or ALP levels between the UAME and CON groups. The *S. pyogenes* group exhibited notably ($P < 0.05$) higher levels of these variables when compared to the CON group. The UAME+ *S. pyogenes* group exhibited an improvement in hepato-renal variables but still lower than CON groups. The protein profile was substantially increase in UAME group followed by CON groups then UAME+ *S. pyogenes*, as Table 5 illustrates. Following the *S. pyogenes* challenge, there was a appreciable drop in these biomarkers in comparison to the other groups.

3.8. Stress-related parameters

Table 5 conveyed that there were discernible variations in the cortisol and glucose levels between the UAME and CON groups. When comparing the *S. pyogenes* group to the CON group, the *S. pyogenes* group had significantly higher ($P < 0.05$) levels of cortisol and glucose, followed by the UAME+ *S. pyogenes* group.

Table 5
Effect of ursolic acid methyl ester (UAME) on the immunological and biochemical indices of experimentally infected *O. niloticus* with *S. pyogenes* for ten-days.

Estimated parameters	Experimental groups			
	CON	UAME	*S. pyogenes*	UAME+ S. *pyogenes*
IgM (μg/mL)	173.96^b ± 1.510	195.83^a ± 1.894	127.86^d ± 2.400	165.46^c ± 1.334
Lysozyme activity (units/L)	15.42^b ± 0.164	21.45^a ± 0.332	8.80^d ± 0.160	13.76^c ± 0.208
Complement 3 (ug/mL)	74.06^b ± 1.373	95.86^a ± 1.675	44.30^d ± 1.274	65.66^c ± 1.105
Nitric oxide (μmol/L)	30.83^b ± 13.217	61.83^a ± 1.481	27.86^b ± 1.041	40.00^{ab} ± 1.154
ALT (U/L)	21.45^b ± 0.104	21.20^b ± 0.152	35.23^a ± 1.361	24.06^b ± 0.520
AST (U/L)	25.53^c ± 0.290	25.20^c ± 0.208	60.33^a ± 1.452	31.16^b ± 0.440
ALP (U/L)	48.20^c ± 0.404	47.36^c ± 0.272	87.63^a ± 0.272	52.26^b ± 1.386
creatinine (mg/dL)	0.80^{bc} ± 0.026	0.75^c ± 0.011	1.24^a ± 0.031	0.88^b ± 0.011
T bilirubin (mg/dL)	1.25^b ± 0.011	1.23^b ± 0.014	2.04^a ± 0.034	1.32^b ± 0.011
urea (mg/dL)	3.71^b ± 0.066	3.60^b ± 0.057	6.96^a ± 0.260	4.10^b ± 0.152
Survival %	98.00^a ± 2.000	100.00^a ± 0.000	54.00^c ± 2.449	84.00^b ± 2.449
Cortisol (ng/dL)	67.65^{bc} ± 1.726	61.63^c ± 0.952	90.93^a ± 2.370	73.46^b ± 1.068
Glucose (mg/dL)	58.16^b ± 1.166	53.00^c ± 1.154	72.00^a ± 1.154	62.66^b ± 0.881
T protein (g/dL)	4.46^b ± 0.202	4.99^a ± 0.017	3.13^d ± 0.044	3.94^c ± 0.029
Albumin (g/dL)	1.85^b ± 0.075	2.15^a ± 0.032	1.33^d ± 0.030	1.61^c ± 0.003
T globulin (g/dL)	2.61^{ab} ± 0.127	2.84^a ± 0.027	1.80^c ± 0.014	2.33^b ± 0.028

Values are represented as the mean ± SE. The means within the same row carrying different superscripts are significant at $p < 0.05$ (N = 10 /group).

3.9. Gene expression profile

Fig. 5 conveyed that there were no discernible variations in the inflammation (*il-1β, tnf-α, il-8*) and anti-inflammation (*tgf-β, il-10*) gene expression between the UAME and CON groups. Fish infected with *S. pyogenes* showed a significant increase ($P < 0.05$) of inflammatory genes while, anti-inflammatory genes were significantly upregulated in UAME+ *S. pyogenes* group followed by *S. pyogenes* groups.

4. Discussion

The future of the aquaculture business depends on the development of novel approaches to the control of bacterial infections. As an alternative to antibiotics, antimicrobial substances produced by some bacteria can be valuable. Streptomyces can produce novel enzymes, bioactive compounds, and antibacterial agents; they can also stimulate immunity and enhance bacterial resistance to aquatic pathogens (James et al., 2023). The pyogenic streptococcus bacterium includes virulent species for humans and animals and has been allied with morbidity and higher mortality (Barnett et al., 2015). The basic reason for the failure of the treatment of streptococcal infection is the amplified resistance to antibiotics (Alves-Barroco et al., 2020). Hence, the present work is a pioneering trial that aims to verify the antibacterial activity of UAME from *S. misakiensis* against the pathogenic fish bacterium *in vitro*. Then, an *in vivo* trial was done for the treatment of *S. pyogenes* by evaluating some biomarkers including ethological, blood picture, biochemical, immune-antioxidant trait, and expression of genes in *O. niloticus*.

The current perspective verified the antibacterial activity of

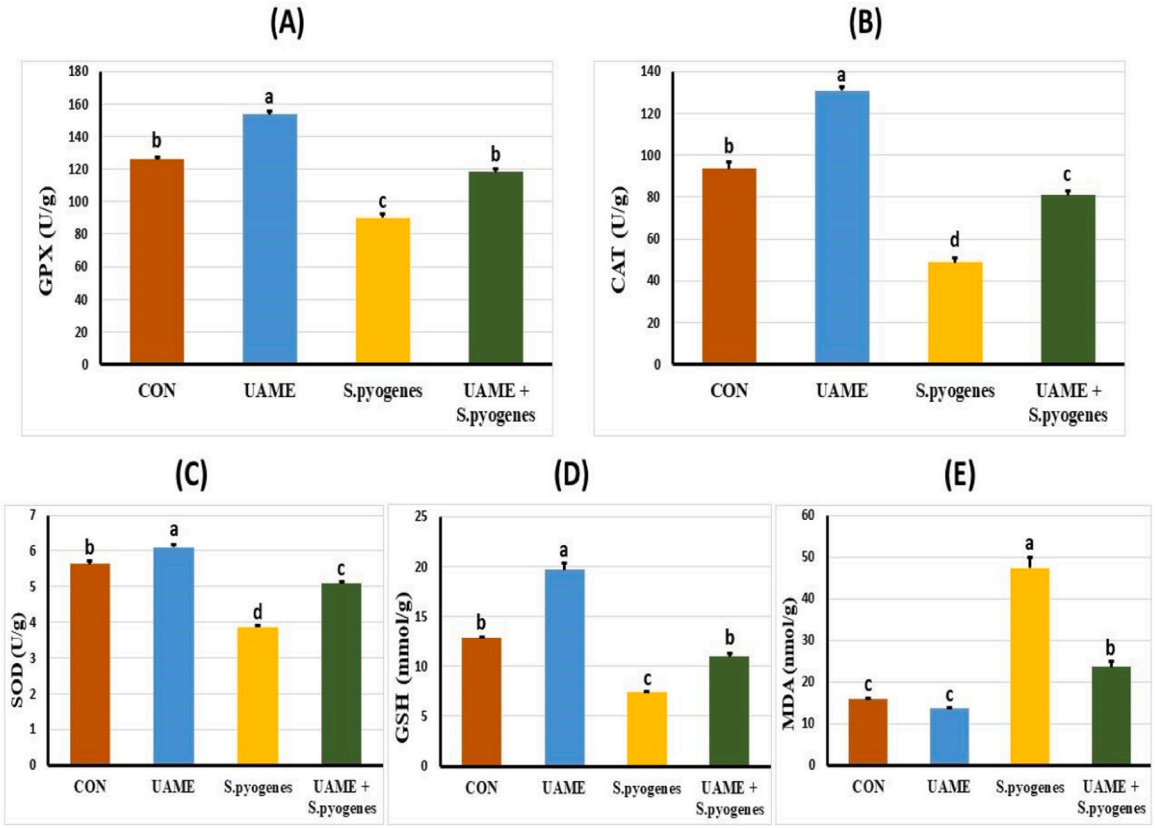

Fig. 4. Effect of *S. pyogenes* challenge and/or ursolic acid methyl ester (UAME) treatment for 10 days on superoxide dismutase (SOD), catalase CAT, glutathione peroxidase (GPx), reduced glutathione (GSH), and malondialdehyde levels MDA in liver of *Oreochromis niloticus*. Data expressed as mean ± SE, n = 5 for each group. Each bar carrying different letters (a, b, and c) significantly differed at $P < 0.05$.

S. misakiensis metabolite, UAME against *S. pyogenes* via recording the minimum inhibitory concentration and SEM profile. This could be dominated by biological activities allied with UAME. UAME could inhibit fatty acid biosynthesis, and cause bacterial cell death and reduced infectivity (Meng et al., 2024). Recently, reactive oxygen species (ROS) have gained recognition as potential bactericidal targets for microorganisms that have developed resistance to pesticides (H.-W. Liu et al., 2023). The antibacterial mechanism demonstrated that ursolic acid derivatives caused an substantial accumulation and generation of reactive oxygen species in bacteria, compromising cell membrane integrity and destroying them (Yang et al., 2023). A previous report supported our finding and documented the inhibitory activity of UAME and reported a strong antibacterial action against *Escherichia coli* and *Staphylococcus aureus* and the minimum inhibitory concentration (MIC) recorded 64 and 32 μg/mL (Do Nascimento et al., 2014). In addition, Mallavadhani et al. (2004) verified the effective activity of UAME against Gram-negative bacteria *Pseudomonas syringae* with a range of MIC value from 20 to 30 μg/mL. A complete shift in bacterial shape and abundance was noted as a result of UAME action.

The *in vivo* therapy with the substance revealed that *S. pyogenes* had detrimental effects on *O. niloticus* by disrupting its behavioral response, decreasing its survivability by 54 %, and causing severe disease symptoms such as hemorrhagic skin, fin rot, internal inflammation, and congestion of internal organs. Comparable findings were documented in Nile tilapia that were afflicted with *S. pyogenes* (Nasr-Eldahan et al., 2022). These destructive outcomes may have a connection to the virulence elements of *S. pyogenes*, which are the factors that are responsible for the pathogenicity of the bacteria in fish. The presence of hyaluronic acid in bacterial capsules makes them resistant to phagocytosis. Bacterial adhesion to host cells is accomplished via M protein, lipoteichoic acid, and protein F (Kanwal and Vaitla, 2020). The pyrogenic (erythrogenic) toxin that causes scarlet fever and toxic shock syndrome is one of the exotoxins produced by *S. pyogenes*. Invasion of tissues is aided by additional pathogenic agents such as streptokinase, streptodornase, hyaluronidase, and streptolysins (Nasr-Eldahan et al., 2022). The body's immune system was ultimately compromised due to these toxins. It is possible that the UAME antibacterial activity that was demonstrated in this study by the *in vitro* assay is responsible for the remarkable recovery of the prior clinical indications and the improvement in the survival of the infected fish, which was 84 percent.

Regarding monitoring ethological changes and brain function, the present work implies to occurrence of behavioral changes and neurological disturbance upon exposure to *S. pyogenes*. Such alterations could be accredited to the ability of *S. pyogenes* to stimulate sensory neurons to create pain and suppress the function of immune cells, endorsing bacterial survival, and in turn results in ethological alterations and a decrease in AchE as clarified by (Pinho-Ribeiro et al., 2018). Previous studies discussed the virulence route of *S. pyogenes* via activating iModulons which involves the immunity factor, the nga-ifs-slo72 operon encoding a NAD-glycohydrolase, and the pore-forming cytolytic toxin streptolysin O that all enhance the pathogenic activity (Finn et al., 2021; Paluscio et al., 2018; Zhu et al., 2015a, 2015b). Furthermore, the virulence of *S. pyogenes* could be dominated to the nature of the bacterium in utilization of dextrin this in turn activates iModulons as well as the carbon sources that regulate the expression of hemolytic toxins as recently clarified by (Hirose et al., 2021). In contrast, the UAME-water-borne treatment resulted in palliating ethological alterations and modulating level of AchE. It is assumed that the protecting effect of UAME could be attributed to its constituents of phenolic that have the power to activate immune cells and suppress inflammatory cells. As a naturally occurring triterpenoid, ursolic acid possesses notable pharmacological properties, including anti-inflammatory,

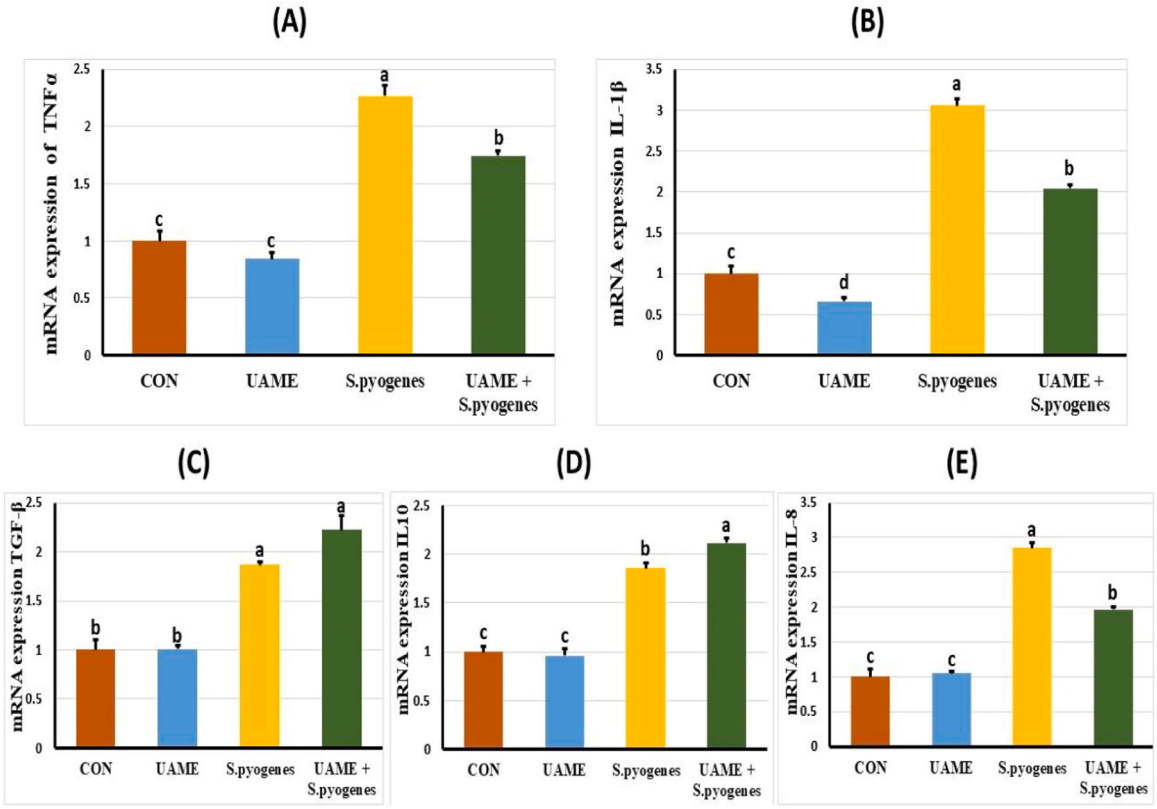

Fig. 5. Effect of *S. pyogenes* challenge and/or ursolic acid methyl ester (UAME) treatment for 10 days on tumor necrosis factor-alpha (*tnf-α*), transforming growth factor-beta (*tgf-b*), interleukin 1 beta (*il-1b*), interleukin-10 (*il-10*), and interleukin-8 (*il-8*) mRNA expression of the spleen of *Oreochromis niloticus*. Data expressed as mean ± SE, n = 5 for each group. Each bar carrying different letters (a, b, and c) significantly differed at $P < 0.05$.

antioxidant, neuroprotective, and nontoxic properties (N. Wang et al., 2022). Concurrently, Zhang et al. (2022) reported the potential activity of UAME to hinder the evolution of the inflammatory response via obstructing cyclooxygenase 2 (COX-2) activities. The capacity of UA to alleviate oxidative stress, purinergic dysfunctions, and cholinergic dysfunctions in isolated rat brains, while simultaneously suppressing proteolytic activity, demonstrating its neuroprotective action against oxidative damage (Salau et al., 2021).

Two of the most important stress markers in aquatic environments are glucose and cortisol (El-Houseiny et al., 2022a). Based on this study's findings, fish infected with *S. pyogenes* exhibited a considerable stress reaction by having increased levels of cortisol and glucose. The infected group that got treatment with 1.0 mg/L UAME noticed a reduction in the stress state, which was demonstrated by a decrease in cortisol and glucose levels throughout the body. The UA treatment resulted in a considerable reduction in both plasma glucose and insulin levels. The improvement in glycemic control can be attributable to processes involving insulin action rather than insulin secretion (Sundaresan et al., 2012). A previous study had shown that the hypoglycemic impact of UA appears to be mediated by changes in the activity of hepatic glucose-regulating enzymes in the animals that were used in the experiments (Jayaprakasam et al., 2006). By suppressing cortisol release, blocking the CRHR1 receptor, and lowering the activities of 11β-hydroxysteroid dehydrogenase type 1 and catechol-O-methyltransferase, ursolic acid considerably suppressed cortisol levels in the forskolin-induced cortisol release experiment (Jothie Richard et al., 2016).

Investigating oxidative stress in response to bacterial infection is crucial for indicating the physiological status of fish (El-Houseiny et al., 2021). Herein, we revealed the occurrence of oxidative damage in response to *S. pyogenes* infection indicated by a reduction in the antioxidant biomarkers (CAT, SOD, GSH, and GPx) and an elevation in the oxidant indicator MDA, 8-hydroxy-2-deoxyguanosine (8-OHdG), and hepato-renal biomarkers. Such oxidative stress could be dominated by the ability of *S. pyogenes* to secrete two strong cytolytic toxins including streptolysin S (SLS) and streptolysin O that are considered primary inflammatory activators by inducing pore formation in cell membranes, tissue damage, and activating pro-inflammatory responses as recently documented by Richter et al. (2021). On the other hand, an obvious improvement was noticed in the antioxidant indices and a modulation in hepato-renal indices in the case of supplemented groups in UAME, implying its anti-inflammatory and antioxidant-protective activity. A recent report by Al-Kuraishy et al. (2022) supported our findings and documented that UAME has anti-inflammatory and antioxidant properties via lessening the production of pro-inflammatory cytokines, enhancing anti-inflammatory cytokines, and suppressing the release of reactive oxygen species (ROS). Also, Yang et al. (2023) discovered that UAME has an effective antimicrobial activity via a ROS-controlled apoptosis mechanism. Moreover, a previous study showed that UAME has the ability to attenuate inflammation and oxidative stress by suppressing the expression of AngII type 1 receptor-associated protein (Ma et al., 2019). C. Wang et al. (2020) added that UAME can suppress inflammation by obstructing the inflammasome signaling pathway (nod-like receptor pyrin 3, NLRP3) inflammasome. Dietary UA-treated rats exhibited markedly decreased 8-OHdG production proposing that UA may have therapeutic potential in the treatment of diabetic nephropathy (Ling et al., 2013).

Fish rely on their innate immune system as their first line of defense against invading organisms (El-Houseiny and Khalil, 2020). Lysozyme activity, in particular, is a primary indicator of fish innate immune response (El-Houseiny et al., 2025). Additionally, NO is recognized as a marker of activated innate immune response (Mansour et al., 2024). According to Holland and Lambris (2002), complement proteins such as C3 play an essential role in the innate immune system of fish. It is

well-known that antibodies play an adaptive function in neutralizing invading pathogens in fish, and they are an essential element of the humoral immune system. Also, one of the most reliable ways to evaluate the physiological and stress levels of fish is monitoring their hematological indices (El-Houseiny et al., 2022b). The current study implied to occurrence of immune dysfunction upon exposure to *S. pyogenes* reflected in a clear reduction in the measured hematological and immunological biomarkers (IgM, lysozyme activity, C3, and nitric oxide) with a decline in markers of the protein profile. This could be returned to the production of proteases by *S. pyogenes* which can split and counteract vital signaling molecules of the immune system as mentioned by Potempa and Pike (2009). The mechanism of action of *S. pyogenes* on altering blood profile is recently described by Brouwer et al. (2023) who verified that secreted associated protein (S protein) has the ability to conjugate with red blood cell membranes or result in lysed of red blood cell membranes as mentioned by Wierzbicki et al. (2019). Moreover, Brouwer et al. (2023) attributed the negative effect of *S. pyogenes* on immune function to the production of gases in a massive amount in cells and the virulence factors that influence the constituents of the immune response. A decline in fish immunity may be indicated by lower total protein levels, which may be caused by decreased protein synthesis and absorption and protein outflow (Shah et al., 2015). Surprisingly, UAME has an immune-modulating effect indicated by significant enhancement of blood picture and immune parameters. In line with a previous report, Hussain et al. (2017) established that UAME has an immunomodulatory role via regulating apoptotic pathways and mitochondrial function by inhibiting nuclear factor kappa B (*NF-κB*), besides stimulating caspase activity.

Assessment of inflammatory genes is essential to reflect the antioxidant status (Alzahrani et al., 2022). The current work revealed up-regulation of the pro/anti-inflammatory genes (*il-1β, tnf-α, il-8, tgf-β, il-10*) in response to exposure to *S. pyogenes* infection. The genetic alteration endowed by *S. pyogenes* infection could be returned to its ability to secrete two types of proteases including C5a peptidase (ScpA) and *S. pyogenes* cell envelope proteinase (SpyCEP) which have the power to split the chemokine *IL-8* and complement component 5a (C5a) resulting in immune dysfunction and altered gene expression (Edwards et al., 2005). Focusing on the significance of UAME, the present perspective demonstrated enhanced anti-inflammatory gene expression and suppression of the expression of inflammatory genes upon UAME-water-supplementing. This could be attributed to the potential impact of UAME as an anti-inflammatory via suppressing NF-κB nuclear translocation, thus minimizing the expression of inflammation-derived factors, such as cyclooxygenase-2, *TNF-α*, and inducible nitric oxide synthase (iNOS) as recently clarified by G. Liu et al. (2023). Moreover, it has a confirmed role in regulating nuclear factor-kappa B (*NF-κB*) (Seo et al., 2018), and in evading cell dysfunction by constraining the expression of C-reactive protein (CRP) and *IL-6* (Lv et al., 2012). Taken together, the application of UAME in the aquatic environment is a successful unique approach that demonstrates promising results in relieving stress and improving ethological, biochemical, immunological, antioxidant, and genetic alterations induced by *S. pyogenes* infection.

5. Conclusion

Based on the study consequences, *S. pyogenes* is a pathogenic bacterium that induces higher morbidity and mortality. UAME is a novel useful metabolite extracted from *S. misakiensis* and exerts potent antibacterial activity against *S. pyogenes*. It is considered an efficient natural antibacterial agent for safe application in the aquatic environment for sustaining the health, performance, immunity, and production of fish. UAME could be efficiently utilized in aquaculture practices as a natural, safe, aqueous antibacterial agent besides its role as anti-inflammatory, immune enhancer, gene-modulator, and antioxidant agent. Such study will be applied in the treatment of *S. pyogenes* infected-tilapia. Future studies are urgently needed for analyzing other actions of ursolic acid in aquaculture practices as well as testing other fish species.

CRediT authorship contribution statement

Conceptualization: R.A., M.A.S., W.E., A.A.A., E.M.Y., H.H.M., S.Z., R.A.A.W., B.A.E., S.J.D., A.O.S. Methodology: R.A., M.A.S., W.E., A.A.A., E.M.Y., H.H.M., S.Z., R.A.A.W., B.A.E., S.J.D., A.O.S. Z.H. Software and data curation: R.A., M.A.S., W.E., A.A.A., E.M.Y., H.H.M., S.Z., R.A.A.W., B.A.E., S.J.D., A.O.S. Writing-Original draft preparation: W.E., R.A., H. H.M., Writing- Reviewing and Editing: H.H.M., R.A., W.E.

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Declaration of Competing Interest

The authors declare no competing interests.

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Consent for publication

All authors review and approve the manuscript for publication.

Informed consent statement

Not applicable.

Institutional review board statement

All of the procedures and protocols that were utilized in this research project were authorized by the Animal Usage in Research Committee (ZU-IACUC/2/F/418/2023) of Zagazig University, Egypt. These procedures and protocols were in accordance with the National Institutes of Health's recommendations for the use and care of laboratory animals.

Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.aqrep.2025.102776.

Data availability

The datasets generated or analyzed during the current study are not publicly available but are available from the corresponding author upon reasonable request.

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