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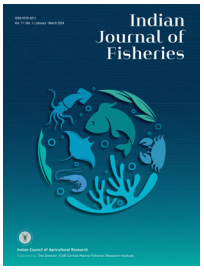
Effect of turmeric on amelioration of arsenic induced toxicity in grass carp (*Ctenopharyngodon idella*) fingerlings

Manoj Kumar Tripathi, Rajni Kumari, Vivekanand Bharti*, P. K. Ray, Jyoti Kumar, P. C. Chandran, S. Dayal, Tarkeshwar Kumar, Saurabh Kumar, A. Dey, Kamal Sarma and Anup Das

ICAR-Research Complex for Eastern Region, Patna-800 014, Bihar, India



Abstract

The present study evaluated the protective effects of turmeric against arsenic (As) toxicity in grass carp (*Ctenopharyngodon idella*) fingerlings. Four experimental groups were maintained for the study: Control (commercial feed only), T1 (turmeric powder @ 1.2% of daily feed), T2 (sodium meta-arsenite @ 3200 µg l^{-1} + commercial feed), and T3 (sodium meta-arsenite @ 3200 µg l^{-1} + turmeric powder @ 1.2% of daily feed). The fish fingerlings were randomly assigned to the four groups, each comprising two replicate tanks containing ten fish per tank; and the experiment was conducted over a period of three months. At the end of the experiment, growth performance was assessed, and blood samples were collected for haemoglobin and haematocrit estimation. Liver samples from the Control, T2, and T3 groups were analysed for the expression of metallothionein, tumour necrosis factor-α (TNF-α), and NF-κB p65 genes, which are key markers of detoxification, immunity, and inflammation. Total RNA was extracted andreverse-transcribed into cDNA for gene expression studies. Results showed that arsenic exposure significantly reduced weight gain, haemoglobin, and haematocrit levels. Turmeric supplementation partly restored these parameters. Arsenic exposure also led to significant upregulation of metallothionein, TNF-α, and NF-κB p65, indicating oxidative and inflammatory stress. Interestingly, turmeric supplementation downregulated the expression of these genes, highlighting its protective role. Overall, the findings of the study indicated that turmeric has a potential ameliorative role in mitigating arsenic-induced toxicity in grass carp.

*Correspondence e-mail:
bhartivivekanand24@gmail.com

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Introduction

Arsenic (As) is a highly toxic and bio-accumulative element that poses a serious threat to humans, animals and aquatic life throughout the world including India (Maji *et al*., 2016; Chandel *et al.,* 2024). Arsenic occurs in the environment in both organic and inorganic forms, predominantly in its trivalent and pentavalent states. Aquatic organisms largely absorb arsenic in its inorganic form (Zhang *et al.,* 2022), with the trivalent form being more toxic as compared to pentavalent state (Byeon *et al*., 2021). Arsenic exposure has been reported to adversely affect growth, immune system and reproduction in fish and can cause tissue damage including necrosis of gills (Rabbane *et al*., 2022). It is also involved in causing oxidative stress, carcinogenesis, apoptosis, antiproliferative activity and inhibition of angiogenesis (Zhang *et al*., 2025).

The liver is the primary target organ for water pollutants due to its central role in detoxification and biotransformation processes (Pandey and Bhatt, 2015; Malik *et al*., 2023). Chronic arsenic exposure causes liver injury, hepatoportal sclerosis, as well as liver cancer and makes the host immuno-compromised (Banerjee *et al*., 2015; Duan *et al.,* 2022). The concentrations of arsenic have been found higher in liver, kidney and gills of fishes (Malik *et al.,* 2023). Histopathological studies have demonstrated that arsenic exposure induces significant alterations in the liver of the tilapia including congestion, focal infiltration of lymphocytes and macrophage, focal necrosis,

hepatocytes shrinkage, vacuolisation and dilation of sinusoids, vacuolar degeneration, nuclear hypertrophy and chromosome fragmentation (Malik *et al*., 2023). Following absorption, the inorganic arsenic is bio-transformed through a series of reactions comprising reduction, oxidation, and methylation to generate organic arsenicals such as monomethylarsonic acid (MMA) and dimethylarsinic acid (DMA) (Zhang *et al.,* 2022; Tang *et al.,* 2024). Fish can convert trivalent arsenic into the less toxic pentavalent arsenate *via* enzymatic and oxidative mechanisms (Tang *et al.,* 2024).

Fishes respond to arsenic toxicity by increasing the synthesis of metallothioneins, which play a crucial role in metal detoxification. Metallothioneins are cysteine rich cytosolic proteins containing abundant sulfhydryl groups which can bind, transport and store heavy metals through thiolate bonding (Yang *et al*., 2024). These proteins are found in liver, gills, kidney and other organs of aquatic vertebrates and can be induced by a variety of environmental factors especially heavy metals (Wang *et al*., 2014). Metallothionein play important roles in essential metal homeostasis, detoxification, binding of excess and non-essential metals for removal, maintaining proper redox potentials, protection against oxidative stress, and exhibit protective roles against the toxicity of heavy metals in fishes (Wang *et al*., 2014; Castaldo *et al*., 2020; Yin *et al*., 2020; Rabbane *et al*., 2022). MT is a potent antioxidant; and exhibit high affinity for heavy metals like As. During As^{+3} toxicity, MT binds with As^{+3} to release Zn ion, which causes reduction in As^{+3} induced ROS production (Lu *et al*., 2021). The change in metallothionein gene expression is used as biomarker in fish for detecting water pollution caused by heavy metals (Sturve *et al.,* 2005).

Arsenic is immunotoxic and causes perturbation of defence systems in different fish species (Banerjee *et al.,* 2015). Nuclear factor-κB (NF-κB) and tumor necrosis factor-α (TNF-α) are master regulators of immune system (Arkoudi *et al*., 2025). The inflammatory response is a crucial part of fish immune function and is mediated by cytokines (Ming *et al*., 2020; Kong *et al*., 2021). NF-κB is a family of inducible transcription factors, that regulates genes involved in immune and inflammatory responses (Liu *et al*., 2017). It plays pivotal role in a variety of classical inflammatory pathways (Li *et al*., 2022). NF-κB is involved in regulating the production of different cytokines including TNF-α (Ming *et al*., 2020; Kong *et al*., 2021). Arsenic exposure could damage the functions of immune cells and alter the expression of inflammatory cytokines, such as TNF-α (Zhang *et al*., 2022). TNF-α is important in regulating the inflammation, necrosis and apoptosis in fishes (Li *et al*., 2021). It enhances migration of immune cells and is required for the proliferation and development of T cells in fish thymus (Hino *et al*., 2006). Various herbs like turmeric, cinnamon, ginger, and garlic have been tried to study the ameliorative effect of water pollutants like lead, cadmium, zinc, and copper in fishes (Fatima *et al*., 2024). However, the effect of turmeric on ameliorative effect on arsenic induced toxicity has not been studied in fishes. Turmeric (*Curcuma longa*), is a perennial herb widely grown in India (Li *et al*., 2022). Curcumin, is the most active component of turmeric (Rajabiesterabadi *et al*., 2020). Curcumin constitutes multiple beneficial health effects and exhibits anti-inflammatory, antioxidative, anti-neoplastic, anti-bacterial, anti-viral as well asanti-apoptosis effects, and exerts a protective effect against liver damage (Li *et al*., 2022). The toxicity and carcinogenicity due to arsenic occurs as a result of the oxidative stress caused by the generation of reactive oxygen species such as the hydroxyl and superoxide radical or hydrogen peroxide (García-Niño and Chaverrí, 2014). Curcumin has shown protective effect against arsenic-induced genotoxicity, angiogenesis, skin disorders, reproductive toxicity, neurotoxicity, immunotoxicity, nephrotoxicity and hepatotoxicity in rodents as well as in *in vitro* models (García-Niño and Chaverrí, 2014). Curcumin ameliorates arsenic induced hepatomegaly in rats (El-Demerdash *et al.,* 2009). The ameliorative effect of curcumin on the heavy metals induced toxicity has been attributed to its scavenging and chelating properties (García-Niño and Chaverrí, 2014).

Grass carp (*Ctenopharyngodon idella*) is a major farmed freshwater fish worldwide (Ming *et al*., 2020). Groundwater inorganic arsenic is high at several pockets in many districts of Bihar (Kumari *et al*., 2021). Groundwater is still used on a large scale in commercial fish ponds (Chandel *et al.,* 2024). The toxic effects of As are largely mediated through oxidative stress and inflammatory responses. Therefore, the present study aimed to investigate the impact of arsenic exposure on metallothionein gene expression and important inflammatory markers including NF-κBp65 and TNF-α in the liver of grass carp. Furthermore, the turmeric having potent anti-inflammatory, anti-oxidative as well as metal chelating properties was studied for its potential ameliorative effects against arsenic-induced toxicity. To the best of our knowledge, no studies have been so far been conducted to evaluate the protective role of turmeric against arsenic toxicity in fish.

Materials and methods

Experimental fish

The experiments were performed following the approved guidelines of Institute Animal Ethical Committee (IAEC/ICAR-RCER/20/09). The study was conducted on grass carp (*Ctenopharyngodon idella*) at ICAR-Research Complex for Eastern Region (ICAR-RCER), Patna, Bihar, India. The fingerlings were acclimatised for a period of four weeks in dechlorinated freshwater tanks before starting the experiment. Grass carp fingerlings (initial weight: 2.10±0.73 g) were randomly divided into four equal groups, each with two replicate tanks containing ten fish per tank. The control group was provided only commercial feed, while the treatment groups received additional interventions as follows: T1 (turmeric powder @1.2% of daily feed), T2 (sodium meta-arsenite @ 3200 µg l^{-1} of water), and T3 (sodium meta-arsenite @ 3200 µg l^{-1} of water along with turmeric powder @ 1.2% of daily feed). Turmeric powder was prepared as an aqueous solution, applied evenly to pelleted feed, and air dried, before feeding. The fishes were provided commercial fish feed (ABIS floating, containing crude protein 20%, crude fat 3%, fibre 8%, moisture 11%) @3% of body weight. The initial water quality parameters such as: pH (6.61±0.04), dissolved oxygen (8.98±0.49 mg l^{-1}), total dissolved solids (655.06±1.06 ppm) and water conductivity (610.3±3.35 ms cm^{-1}) were recorded. The experiment was conducted for three months and water in each tank was changed at weekly intervals. Aerators were connected to all the water tanks to maintain dissolved oxygen level around 6 ppm and a constant source of power supply and light were also provided.

Sample collection and analysis

Clove oil dissolved in ethanol at 1:10 ratio was used to anesthetise the sampled fishes @50 mg l^{-1} (Nambiar *et al.,* 2024). The weight of

all experimental fishes was recorded. Blood from caudal vein (10 µl) was directly used for estimation of haemoglobin and haematocrit using test strips in hemoglobinometer (AccuSure HB 101). The liver tissues of the fishes were sampled for gene expression studies.

RNA isolation and cDNA synthesis

Total RNA was extracted from liver tissue (control, T2, and T3 groups) using TRIzol reagent (Invitrogen, USA). Approximately 10-15 mg of liver tissue was homogenised in 600 µl of TRIzol, and phase separation was carried out by adding 200 µl of chloroform. After centrifugation at 12,000 *g* for 15 min at 4°C, the aqueous phase was collected and RNA was precipitated using an equal volume of isopropanol. The RNA pellet was washed with 75% ethanol, air-dried, and resuspended in RNase-free water. The concentration and purity of RNA were determined by nanodrop spectrophotometer (Thermo Scientific), at 260 nm and 280 nm. RNA integrity was checked using agarose gel (1%) electrophoresis. Two intact bands of 28 S and 18 S with smearing indicated good quality and intactness of RNA. cDNA was synthesised from 1 µg of total RNA in a 20 µl reaction using Quantitect Reverse Transcription Kit (Qiagen Cat. No./ID: 205311) according to manufacturer's instructions.

Quantitative real time PCR and relative quantification

Primers used in the study for analysing the expression of metallothionein (MT), TNF-α, and NF-κB p65 gene were selected based on previously published studies. The sequences and expected PCR product lengths are presented in Table 1.

Transcriptional abundance of the target genes were analysed for the following group comparisons: (a) Control (C) *versus* T2, and (b) T2 *versus* T3. For each group, three biological samples were analysed, and each sample was subjected to qRT-PCR in technicaltriplicates for each gene. Reactions were carried out in a final volume of 20 µl, containing 2 µl of cDNA (equivalent to 100 ng RNA input), 10 µl of 2× SYBR Green/ROX qPCR Master Mix (Qiagen), 0.5 µl each of forward and reverse primers (final concentration 0.5 µM), and 7 µl of nuclease-free water. Amplification was performed using a real-time PCR system under the following thermal cycling conditions: Initial denaturation at 95°C for 10 min, followed by 40 cycles of denaturation at 95°C for 15 s, and annealing/extension at 57°C for 40 s. A melt curve analysis was performed at the end of each run to confirm amplification specificity and exclude primer–dimer formation, followed by agarose gel electrophoresis to verify the expected size of the PCR products. No-template controls and no-RT controls were included in each qPCR run. No amplification was observed in these controls confirming the absence of genomic contamination.PCR efficiency for each target and reference genes was determined prior to relative quantification by generating standard curves from serial dilutions of cDNA. The Ct values of the housekeeping gene (β-actin) were stable across all samples, indicating consistent expression. The mean Ct values for β-actin, MT, TNF-α, and NF-κB p65 were 18.48, 17.06, 21.58, and 25.35, respectively. Gene expression levels were normalised to the endogenous control gene β-actin, and relative quantification was performed using the 2^−ΔΔCt method (Livak and Schmittgen, 2001) as implemented in the Applied Biosystems 7500 Real-Time PCR System (SDS Software). For comparative analysis, Ct values from the control group were used as calibrators for the T2 *vs*. C comparison, and Ct values from the T2 group were used as calibrators for the T3 *vs*. T2 comparison.

Table 1. Details of primers used in the study

Primer code	Primer sequence (5' - 3')	Reference/Length
β- actin F(5'-3')	GACCTGACTGACTACCTCAT	121 bp (Liu, 2022)
β - actin R(5'-3')	CGAAGTCAAGAGCCACATAG	
MT-F (5'-3')	ATGGATCCTTGCGATTGCG	182 bp (Jabeen, 2022)
MT-R (5'-3')	CATTGACAGCAGCTGGAGCC	
TNFα (Forward)	CTTCCACTGCAGTCACACAC	142 bp (Ming *et al.*, 2020)
TNFα (Reverse)	ATGCCCATGTTTTCACCCAC	
NF-κB p65 F	ATTTACAGGAGCGGCGGATA	101 bp (Ming *et al.*, 2020)
NF-κB p65 R	TTGTGTTTGCAGTCGACAGG	

Relative quantification (RQ) values, including upper and lower confidence limits (RQ max and RQ min), were obtained directly from the Applied Biosystems 7500 Real-Time PCR System using SDS Software. For each gene and treatment, fold changes were averaged over technical triplicates.

Statistical analysis

Data are presented as mean±SE which are compared using students t-test at 5% level of significance. Body weight and haematogical data were compared using one way analysis of variance (ANOVA) at 5% level of significance. The treatment mean difference were analysed by Dunkan's multiple range test (DMRT) using agricole package in R(R-4.3.1) software. Prior to analysis, normality of the data and homogeneity of variances were assessed using the Shapiro–Wilk and Levene's tests, respectively.

Results and discussion

Trivalent salt of arsenic is more toxic than other forms and hence, sodium meta arsenite was selected as the test toxicant. The doses of the sodium meta arsenite and turmeric were decided on the basis of previous studies (Al-Faragi and Hassan, 2017; Rajabiesterabadi *et al*., 2020; Rabbane *et al*., 2022).

Body weight, haemoglobin and haematocrit

The mean body weight (g) for control, T1, T2 and T3 were 2.834±0.280, 2.682±0.413, 2.155± 0.172 and 2.394±0.255 respectively. The haemoglobin level (g dl^{-1}) for control, T1, T2 and T3 were 6.62±0.904, 5.35±0.534, 3.5±0.690 and 5.53±0.422 respectively. Similarly the haematocrit values (%) for control, T1, T2 and T3 were 19.5±2.97, 15.6±1.59, 10.2±2.26 and 185±2.54 resectively as presented in Fig. 1.

A significant decrease in haemoglobin (Hb) levels in arsenic treated group was recorded, as compared to control which could be attributed to interference of arsenic in erythropoiesis. Decrease in haemoglobin content has also been reported in earlier studies in *Clarius batrachus* and rainbow trout (Oladimeji *et al.*, 1984; Kumar and Banerjee, 2016).

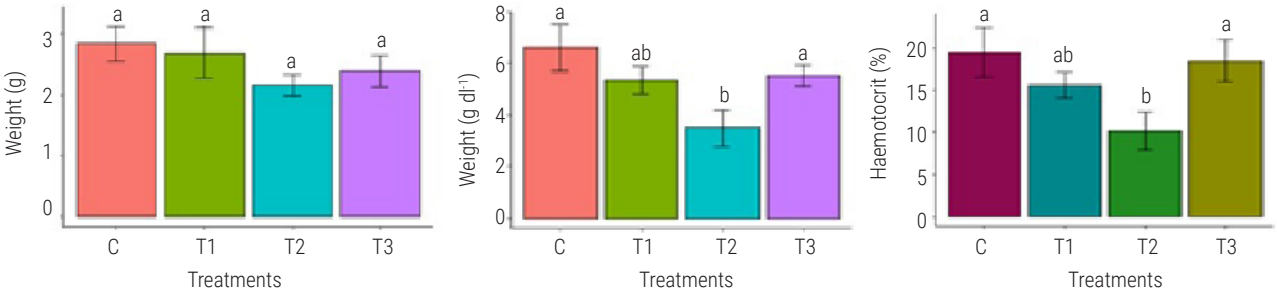

Fig. 1. Effect of dietary turmeric on body weight, haemoglobin and haematocrit levels in grass carp fingerlings recorded in different treatment groups. Values are presented as mean±SE. Significant differences are indicated by different letters ($p<0.05$)

Zhou *et al*. (2020) reported that arsenic disrupts the function of zinc finger transcription factor GATA-1 which is the key regulator of normal erythropoiesis. They elaborated that arsenic interacts with zinc finger motifs of GATA-1, and replaces the zinc within the zinc fingers causing zinc loss and inhibits the DNA and protein binding pursuit, leading to dyserythropoiesis and interference in hematopoietic differentiation. Kumar and Banerjee (2016) observed that the metal may directly affect hematopoietic centres; enhance erythroclasia as a result of altered membrane permeability or mechanical fragility, and defective intestinal uptake of iron due to mucosal lesions leading to impaired erythropoiesis. Interestingly arsenic has strong binding affinity with thiol groups present in the reactive cysteine residues of the α and β chains of Hb (Lu *et al.*, 2004; Singh and Rana, 2010). Our results indicate that turmeric itself has negative effect on Hb level. The negative impact of turmeric on Hb level has also been observed by Smith and Ashar (2019), who reported that turmeric binds to ferric iron in the gut and causes iron deficiency in mice. In contrast, Anene *et al*. (2021) reported that there is significant increase in Hb levels in *Clarias gariepinus* fingerlings at 1% feeding of turmeric powder in basal diet. However; in the T3 group of our experiment, turmeric by virtue of its antioxidative properties, contributed to the decrease in free radical damage to blood cells thereby compensating the haemoglobin loss to some extent. Our findings gain support from the finding of Kumari and Paul (2020) who reported that turmeric used against the fenvalerate (pyrethroid) induced toxicity in *Clarias batrachus,* exhibiting ameliorating and rejuvenating property and caused significant regain in the haematological parameters including, haemoglobin. Haematocrit values varied in a similar fashion as Hb in our experiment. The decrease in body weight is directly linked with the decrease in Hb. Decreased haemoglobin leads to hypoxia and inefficient metabolism resulting in decrease in body weight in arsenic treated fingerlings which are partially resumed by turmeric treatment in our experiment. Similar findings have been reported by El-Demerdash *et al*. (2009), wherein sodium arsenite treatment in rat, decreased body weight as compared to control and curcumin treatment alleviated its toxic effects.

Metallothionein (MT) gene expression

In our study, the gene expression of metallothionein (MT), increased (1.684±0.113 fold) in arsenic treated group (Fig. 2). Liver is one of the most sensitive organs for increased MT synthesis following exposure to metals (Bhattacharya *et al*., 2007). The increase in MT gene expression in our study indicates that the fish liver may be producing more metallothionein protein to bind with arsenic. As^{+3} enters the nucleus and attaches with the metal response element leading to enhanced MT expression (Lu *et al*., 2021). Our results are supported by an *in vivo* study carried out by Albores, *et al*. (1992) who reported that As^{3+} injection increased MT protein in rat liver. Kovendan *et al* (2013) also reported that in fishes, MT expression in liver and kidney was increased by arsenic trioxide. Similar finding was reported by Rabbane *et al.* (2022) wherein they found that expression of MT gene in gill and muscle tissue of rohu fish was increased due to arsenic induced stress. In addition, there are other reports wherein arsenical has been shown to induce MT activity in mice and fish (Kreppel *et al*., 1993; Kovendan *et al.,* 2013). We observed that the co-treatment of turmeric and arsenic caused decrease (0.524±0.09 fold) in the gene expression of metallothionein (Fig. 3) indicating beneficial effect of turmeric which could be attributed to reduction in the toxic load of arsenic in the liver or of the antioxidative properties of turmeric. The literature available on the effect of turmeric feeding on the arsenic induced gene expression of MT is scanty. However, Smirnova *et al.* (2023) reviewed that supplementation of curcumin helped to reduce the total toxic load of arsenic in the liver and to increase the arsenic excretion through the urinary tract in human.

TNF-α and NF-κB p65 genes expression

In our study, we found that the gene expression of TNF-α and NF-κB p65 increased (2.06±0.186; 1.319±0.10 fold respectively) in arsenic treatment group (Fig. 2). Arsenic induced oxidative stress can also directly activate the NF-κB inflammatory signalling pathway. Activation of the NF-κB inflammatory signalling pathway led to increased secretion of downstream cytokines including TNF-α which further exacerbate the inflammatory response (Lu *et al*., 2021). In an *in vivo* study, Duan *et al.* (2022) reported that $NaAsO_2$ causes inflammatory infiltration in the liver of mice. They reported that there was increased expression of the cytokine (TNF-α) in the liver as well as kidney and concluded that arsenic enhanced the expression of MAPK/Nrf2/NF-κB signalling molecules. Arsenic has a generalised immune-suppressive effect and leads to downregulation of both Th1 and Th2 cytokine genes (Banerjee *et al*., 2015). We observed that the co-treatment of turmeric in arsenic exposed group caused decrease (0.342±0.04; 0.274±0.04 fold) in the gene expression of TNF-α and NF-κB p65 respectively (Fig. 3) indicating the role of

turmeric in mitigating arsenic induced inflammatory response in the liver of fish. Our study gain support from the findings of Kong *et al*., (2021) wherein it was reported that dietary supplementation with curcumin attenuated the deltamethrin-induced inflammatory response by downregulating the expression of pro-inflammatory genes (NF-κB p65, and TNF-α) in the liver of fish. They reported that curcumin could attenuate deltamethrin-induced cellular oxidative stress, inflammation and apoptosis *via* the Nrf2 and NF-κB signalling pathways in *Channa argus*. Perveen *et al*. (2019) reported that there was increased level of NF-κB in uterus, TNF-α in serum and metallothionein-1 in liver of As^{+3} treated rats and feeding of curcumin in the arsenic-fed group significantly suppressed their levels. Giri *et al*. (2019) communicated that curcumin reduced the mRNA expression of TNF-α in the liver of common carp. There are other reports wherein the role of curcumin in reducing mRNA levels of TNF-α and NF-κBp65 in liver of different fish species challenged with various inflammatory inducers like *Aeromonas hydrophila*, CCl4, lipopolysaccharide (LPS) and chromium plus ammonia has been published (Cao *et al*., 2015; Ming *et al*., 2020; Li *et al.,* 2022; Mulla *et al*., 2025) and suggested that dietary curcumin could attenuate inflammation, oxidative stress, cell apoptosis and improve the growth performance.

High level of chronic arsenic exposure causes cellular damage due to activation of the NF-κB signalling pathway (Wei *et al.,* 2016). NF-κB pathway plays an important role in the host's defence against metal poisoning (Lu *et al*., 2021). Normally the NF-κB proteins are sequestered in the cytoplasm due to action of inhibitory proteins

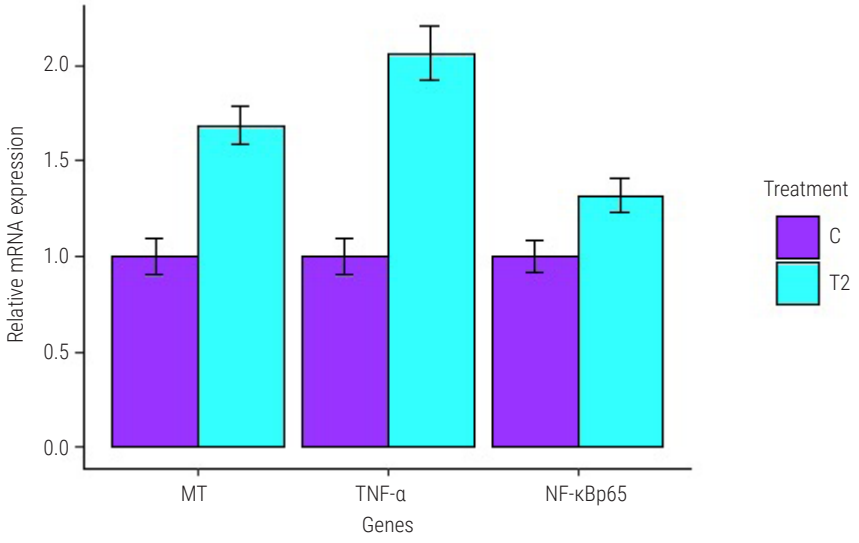

Fig. 2. Effect of arsenic exposure (T2) on relative mRNA expression of metallothionein, TNF-α and NF-κBp65 in the liver of grass carp. A significant increase ($p<0.05$) in relative expression of all three genes in the arsenic exposed group is evident, as compared to control

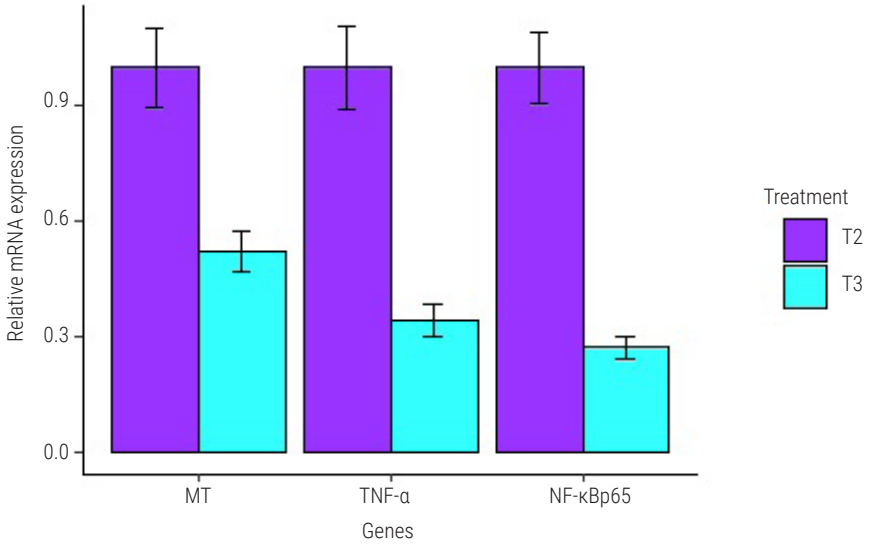

Fig. 3. Ameliorative effect of turmeric on arsenic induced relative mRNA expression of metallothionein, TNF-α and NF-κBp65 in the liver of grass carp. A significant decrease ($p<0.05$) in the relative expression of all three genes is evident in the turmeric treated arsenic exposed group (T3) as compared to the arsenic treated group (T2)

like IκBα (Liu *et al*., 2017). The NF-κB activation occurs through the degradation of IκBα which is triggered through the site-specific phosphorylation by IκB kinase (IKK) complex. IKK can be activated by various stimuli,including cytokines and stressors (Liu *et al*., 2017). High dose of arsenic, increases cytoplasmic IKK levels, thus more IKK phosphorylation, resulting in increased IKK activity (Wei *et al.,* 2016). The activated IKK phosphorylates the inhibitory proteins IκBα and, triggers their degradation, resulting in nuclear translocation of NF-κB members (Liu *et al*., 2017). The nuclear translocation of NF-κB triggers transcriptional activation of the inflammatory cascades (Liu *et al*., 2017). The DNA-binding activity of NF-κB enhances the expression of NF-κBp65 (Wei *et al.,* 2016). Activation of the NF-κB increased the expression of downstream cytokines including TNF-α (Lu *et al*., 2021; Kong *et al*., 2021; Li *et al*., 2022).

The co-treatment of turmeric in arsenic exposed group in our experiment, showed a decrease in the gene expression of TNF-α and NF-κB p65 which can be explained by documentation that curcumin inhibits NF-κB activation by preventing the degradation of IκBα and reducing the phosphorylation levels of NF-κB subunits (p65 and p50) (Liu *et al.,* 2025). Giri *et al*., (2019) also suggested that the metabolites of curcumin, covalently bind to and inhibit proteins in the inflammatory NF-κB signalling pathway and curcumin may be involved in inhibition of the NF-κB p65 translocation to the nucleus and thereby down-regulates the gene expression of pro-inflammatory cytokines. Turmeric supplementation which contains curcumin might exhibit its ameliorative effects by down-regulating the expression of TNF-α and NF-κB p65.

Our experimental results indicated that arsenic at 3.2 mg l^{-1} causes adverse effects in grass carp, including reduced body weight, haemoglobin and haematocrit, along with increased expression of genes associated with detoxification (metallothionein) and immune response (TNF-α and NF-κB p65) in lthe iver. Dietary supplementation of turmeric at 1.2% of daily feed improved growth performance in terms of body weight and haematological parameters (haemoglobin and haematocrit) and significantly downregulated the expression of these genes, demonstrating its protective and ameliorative effects.

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