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HEPATOPROTECTIVE EFFECT OF *Artemisia pallens* ON *Pangasius* sp. TREATED WITH CHLOFIBRATE AND PHENOL

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Aim. The key motive was to investigate the toxicological upshot of clofibrate and phenol prescribed under human medicine, having potential in water and sediments contamination. This is via input from sewage treatment plants as active pharmaceutical ingredients' discharge into the environment had kindled present catastrophic effects upon the aquatic ecosystem.

Methods. The present study involved, exposing the fish model, *Pangasius sp.* after acclimatizing them at a suitable LC_{50} concentration of selective drugs. Their toxic effects were studied in terms of oxidative stress markers, antioxidant status, and protein damage levels in the occupancies under the bioremediation source, *Artemisia pallens* and further supported by histopathological and cortisol level studies.

Results. The results' comparison between fish maintained under the bioremediation source, when exposed to clofibrate and phenol resulted in severe oxidative stress (significant $^{*}P < 0.001$, $^{\#}P < 0.001$) with significant alterations in antioxidant enzyme activities (significant $^{*}P < 0.001$, $^{\#}P < 0.001$), histopathological changes and cortisol levels. In the fish exposed to clofibrate and phenol, the significant increase in cortisol level (significant $^{*}P < 0.05$, $^{\#}P < 0.05$) may confer distinctive effects on the cell survival by protecting against oxidative stress-induced changes.

Conclusion. Since these results varied with the dwelling of the bioremediation source, determination of oxidative stress biomarkers in *Pangasius* along with *Artemisia pallens* may serve as a convenient approach for pollution biomonitoring.

Key words: bioremediation, *Pangasius*, *Artemisia pallens*; LC_{50}.

Pharmaceutical compounds present in water bodies, directly or indirectly affect the user's health through various dialects of exposure; insinuating physical and psychological abnormalities, and also detriment the productivity of agricultural land, altering agricultural infrastructure leading to massive overwhelming instances of livestock and fish persistence and their respective livestock. As under their inclusion of normal drinking water amenities; in long term consumption, these chemicals can in turn harbour an overall hazard to the subjected systems that undergo their consecutive iterative consumption. Moreover, phenol and its derivatives have noxious effects on fish; they are known to induce carcinogenic, immunotoxic, and physiological effects and have an increased bioaccumulation rate along the food chain due to its lipophilicity [1, 2]. Consumption of water containing higher concentrations of phenol and its derivatives have been known to cause various detrimental effects to humans such as necrotic lesions in the mouth, throat, esophagus. Phenols (Fig. 1, *a*) also have the propensity to cause DNA damage and arylation thereby destroying some proteins and disrupting the transportation of electrons in the energy transducing membranes [3]. Clofibrate (Fig. 1, *b*) is an antilipidemic component similar to gemfibrozil. It works to lower elevated serum

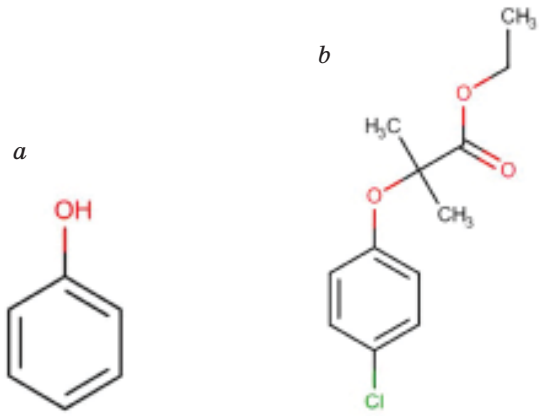

Fig. 1. **Structure of Phenol (*a*) and Clofibrate (*b*)**

lipids. In animals, cholesterol biosynthesis prior to mevalonate formation was interrupted by clofibrate [4, 5]. This ROS formation can intervene within the biochemical cascades of energy production and the overall felicity of the organism's equilibrium; alongside the energy production linked with the mitochondrial membrane efficiency, subsequently, the increasing ROS production can thereby affect the membrane potentials.

In recent times, international environmental regulation has focused on the risk posed by emerging contaminants released into the aquatic environment. The evidence against phenol toxicity and clofibrate toxicity at the ecosystem level is limited. Therefore, there is a need to investigate the biological toxic effects on exposure to clofibrate and phenol and its bioremediation action using *Artemisia pallens* in *Pangasius* sp. fish model. The species of *Artemisia* are widely used in traditional medicine all over the world where there anti-inflammatory, antitumor, antioxidant, antispasmodic, antimicrobial, insecticidal, antimalarial, antifungal, and antioxidant activities have been of significant importance possessing a broad spectrum of bioactivity. In a relative study conducted by Kamel [6], the essential oils from the species of *Artemisia* (wormwood) exhibited inhibitory effects towards certain phytopathogenic organisms under the targeted basis of their speciation thereby providing a better establishment about the antioxidative characteristics of *Artemisia* as antiradical activity towards polyphenols of the targeted microorganisms [6]. *Artemisia* Sp *is* known to be a very rich source of various types of biological compounds which find usage in the field of pharmacology [7]. *Artemisia pallens* (commonly referred to as 'davana') an aromatic plant native to south India has been used to produce various types of aromatic oils with various health benefits. The plant due to its enormous health benefits have been used to treat diabetic mellitus, microbial infections, etc [8]. The free radical scavenging and the immunomodulating properties of the plant would enable the organism to survive and prolong its life when exposed to toxic conditions. Hence these qualities would make it a very good bioremediation and improve its longevity.

Materials and Methods

Sample collection. Pangasius sp. were bred along the coastal areas and they were purchased from the local dealers in Chennai. The fish were fed with pellets rich in protein, consisting of fish meal mixture and worms at a regular interval. They were allowed to acclimatize in the laboratory environmental conditions for about 15 days. The scrutiny for acclimatization was assessed in minutes and/or days. After this, they were treated with different concentrations of clofibrate and phenol.

Preparation of Clofibrate and Phenol for toxicity studies. Clofibrate and phenol of chemical purity ≥ 98.0% were purchased as a commercial powder from Sigma Aldrich. It was dissolved partially in water and a few drops of glacial acetic acid were added to dissolve it completely after which they were added to the tanks containing fish. For the test group, different concentrations of clofibrate and phenol in the range of 10 mg, 20 mg, 30 mg, 40 mg, and 50 mg were used.

Determination of Lethal Concentration LC_{50}. The LC_{50} was determined after a series of exposure to clofibrate and phenol at various concentrations in the range of 7 mg, 14 mg, 21 mg, 28 mg and 35 mg of clofibrate and 250 ml, 500 ml, 750 ml, 1000 ml and 1250 ml of phenol for ~ 12 litres of water in a fish tank (12×6×8) inches in dimension. These concentrations were chosen based on the previous toxicity studies where the fish were subjected to LC_{50} of 21 mg/L and 1000 mg/L for phenol and clofibrate respectively. In this acclaim, the fishes were intoxicated with clofibrate and phenol at the lethal concentrations of 1.18 mg/ml and 22.7 mg/ml discreetly.

Artemisia pallens preparation include 1% preparation. For the preparation of 1% solution: The plant, *Artemisia pallens* (commonly called as davana) is widely grown in humid habitats in the plains. It can be easily identified based on their aromatic odour and

grey/white leaves. The leaves of *Artemisia pallens* were shade dried and grinded to obtain fine powder, 6 grams of *Artemisia pallens* powder was dissolved in 600 ml of water and further 600 ml of *Artemisia pallens* solution was dissolved in 60 litres of water.

Oxidative stress parameters. The oxidative stress assay was carried out for different parameters such as lipid peroxide (LPO), conjugated diene (CD), protein sulfhydryl and protein carbonyl.

Assay of Lipid Peroxide (LPO). TBA method [9] was used to estimate LPO. In this assay, to 100 µL of tissue homogenate, the supernatant was added to Tris–HCl buffer solution of pH 7.4 until a volume of 1 mL was reached. The samples were incubated at 37 °C for just about 30 min; with 2 mL TBA–TCA reagent [0.375% thiobarbituric acid (TBA) in 15% trichloroacetic acid was added and samples were vortexed]. They were heated and boiled for 45 min, and allowed to cool. The emanate precipitate was removed by centrifugation at 3000 rpm for 10 min with the absorbance read at 535 nm against a reaction Blank.

Estimation of Conjugated Diene. For the quantification of conjugated dienes, test samples (tissue fractions) exposed to oxidative stress were treated with chloroform: methanol mixture (2:1). Subsequently, they were subjected to vigorous vortexing and centrifugation at 2,000 rpm for 10 min [10]. The proteins were removed along with the supernatant, while the lower chloroform layer was dried under a stream of nitrogen at 45 °C. The residue obtained was dissolved in cyclohexane and absorbance was taken at 233 nm against a cyclohexane blank.

Estimation of protein carbonyl. The carbonyl content of whole protein extracts were measured using the Levine method. The soluble protein (0.5 mL) was treated with 10 mM DNPH in 2 M hydrochloric acid for 1 hour at room temperature and precipitated with 6% trichloroacetic acid (TCA). Following, the pelleted protein was washed thrice by resuspension in ethanol/ethyl acetate (1:1). Protein was then solubilized in 6 M guanidine hydrochloride, 50% formic acid, and centrifuged at 16,000x g for 5 min to remove any feasible trace of insoluble materials. The carbonyl contents were measured spectrophotometrically at 336 mm and expressed as nanomoles of DNPH incorporated/mg protein based on the molar extinction coefficient of 21,000 1/M cm.

Estimation of protein sulfhydryl. Protein sulfhydryl was estimated using the DTNB method [11]. Diluted tissue homogenate suspension of 0.5 ml was added to 1.5 ml of 0.2 M Tris HCl and 0.1 ml of 0.01 M DTNB in methanol. The mixture was constituted up to 10 ml using methanol and incubated at room temperature for 15 min. The sample was centrifuged at 300 x g for 10 min and the absorbance of the supernatant was read at 412 nm.

Assay of inorganic phosphorus. The Inorganic phosphorus contents were estimated using an ANSA reagent [12]. Following a protocol of 1 ml test sample solution mixed with 0.6 ml of 2.3% molybdate reagent (2.3% of Ammonium molybdate in 10 N Sulfuric acids). Then, 0.2 ml of ANSA solution (0.25 g of 8-Anilino-1- naphthalene sulfonic acid (ANSA) in 15% sodium bisulphite solution and 5 ml of 20 % sodium sulfite solution) were added to it and the observed absorbance was read at 660 nm after incubation for a period of 10 min at room temperature.

Extraction of inorganic phosphorus from the fish liver and muscle tissue. The tissue (liver and muscle) samples were collected from the toxin-induced fish after sacrificing. They were homogenized for 3 min in 5 ml of extraction buffer (1.25 ml of conc. Perchloric acid, 0.2 ml conc. Phosphoric acid diluted to 100 ml with distilled water). The homogenate was centrifuged at 6000 rpm for 15 min at room temperature with the supernatant, separated and filtered and stored till usage.

Histopathological studies [13]. Fish tissue slices collected from the fish exposed to clofibrate, clofibrate and *Artemisia pallens*, phenol, phenol and *Artemisia pallens* were immediately fixed in a 10% formalin solution. The tissues were sliced with the help of microtome (5 µm) and were embedded in paraffin wax. Hematoxylin and eosin staining protocol was followed and the histopathological changes of tissues were visualized under a multi-head microscope.

Antioxidative stress parameters. The antioxidative stress assay was carried out using different parameters such as the measurement of superoxide dismutase and catalase enzymes.

Assay of superoxide dismutase. The activity of SOD was estimated by observing the epinephrine oxidation according to the procedure of Laville et al., [14] 2.5 mL of carbonate-bicarbonate buffer (0.3 m, pH 10.2) and 0.5 mL of EDTA (0.6 mm) solution were added to the tubes. Suitably diluted sample 1 and sample 2 were added and the absorbance changes were observed after adding 0.5 mL of epinephrine (1.8 mm) at 420 mm for 2 min at 15 s interval using an UV spectrophotometer. The activity was exhibited as units/minutes/mg of protein.

Assay of catalase. The activity of catalase was estimated by the method of Beers [15], 23 ml of H_2O_2 — phosphate buffer was pipetted out, a vital amount of tissue supernatant (cytosolic fraction) was added as enzyme source, and the contents were mixed completely. The decrease in absorbance at 240 nm was made noted for every 30 s for 3 min. The results were exhibited as units/mg protein.

Estimation of Cortisol. Employing the protocol by Yohana et al. [16]. Cortisol was estimated and measured using enzyme-linked immunosorbent assay (ELISA) with the blood samples taken from caudal peduncle using heparin-induced syringes to obtain plasma after centrifugation at 10.000xg for a duration of 5 min. It was later maintained on ice until the cortisol concentrations were determined. For the assay, 20 µl of fish plasma samples were annexed to the plate alongside 200 µl of the enzyme conjugated to horseradish peroxidase, added into each well. Finally, the wells were gently mixed on a plate mixer at 200 beats/min for 10 min and were incubated at 1 hour under room temperature. The well contents were briskly annihilated to avoid any residual content, the solution of each well was later removed by washing the plate three times with 400 µl of PBS and shaking out the content onto an absorbent paper, with the aim of removing any residual drops that could possibly affect the accuracy and precision of the underwent assay. Subsequently, 100 µl of TMB enzyme substrate was added to each well and incubated at room temperature for 15 min. The ensued enzymatic reactions were visualized by colour change and were stopped by addition of 100 µl of 0.5 M phosphoric acid (H_2PO_3). The resulting intensity of colour is inversely proportional to the concentration of cortisol in the samples, with this substantial; the absorbance values were read in a spectrophotometer at 450 nm on a microtiter plate reader within 10 min after the addition of stop solution.

Statistical Analysis

The observed data were probed using a commercially available statistical software package (GraphPad software). By employing Students 't', the significance of variations between the control and fish exposed to clofibrate and phenol was attested. The results were presented as mean ± SD along with a P value less than 0.01 and 0.05 considered to be statistically significant.

Results and Discussion

The changes in lipid peroxidation led to the destruction of membrane lipids as well as the production of lipid peroxides and their by-products such as aldehydes. The medium lethal concentrations were perceptibly altering the rate of lipid peroxidation and the consecutive activities of antioxidant systems in various organs of the test fish, harbouring nearly 50% of the oxidative stresses, LPO is accountable for the mere survival of the fish; On a comparative study carried out by Laville et al. [14] on the effects human pharmaceuticals on the cytotoxicity on fish hepatocytes; the 7-ethoxyresorufin-*o*-deethylase (EROD) assays were procured as well as MTT assays to evaluate the extent of their long-term toxicities. Amongst the chemicals tested, CF along with FX and FF had higher cytotoxicity in PLHC-1 cell line of rainbow trout; which were readily involved in oxidative stresses of the cell lines with specific EROD inhibition in addition to the loss of cell viability by FF and CF by overproduction of ROS through Peroxisome proliferator-activated receptors (PPARs). The application of *Artemisia pallens* decreases significantly the ill effects of the pharmaceuticals onto the cell membranes and the effect on conjugated dienes. In the graphical representation (Fig. 2, *a*). It is very evident indicating the increase in the expression for LPO and CD, and its subsequent result by the remedial inclusive. The toxicity factors as the fish were subjected to clofibrate and phenol increased initially, which reduced subsequently by 7- and 2-fold reduction respectively upon treatment with *Artemisia pallens* on LPO. Similarly, on the parameters of CD; the increased toxicity got suppressed by 6.45% and 82.31% for Fish treated with clofibrate and phenol upon *Artemisia pallens* was observed. Hence this would be providing a collective benefactor to an overall $P < 0.001$ significance of remedial action.

The altered enzyme activity and DNA damage are widely used as biomarkers to reduce the genotoxicity and oxidative stresses of natural and/or man-made chemical materials [17]. It was also noted that heavy metals and other pollutants findings concluded that pollution of aquatic environments affected physiologically in fish and leading to various imbalances and triggering the oxidative damage resulting in certain detrimental effects such as lipid peroxidation, DNA damage etc; and antibiotic defences disturbances. During protein carbonyl assay, the DNPH involved in protein oxidation indicates the level to which the protein content has been subjected to the oxidative process, coming under oxidative marker parameters. As observed in the scrutiny, the *Artemisia pallens* imbibed into the fish under the influencing subjected

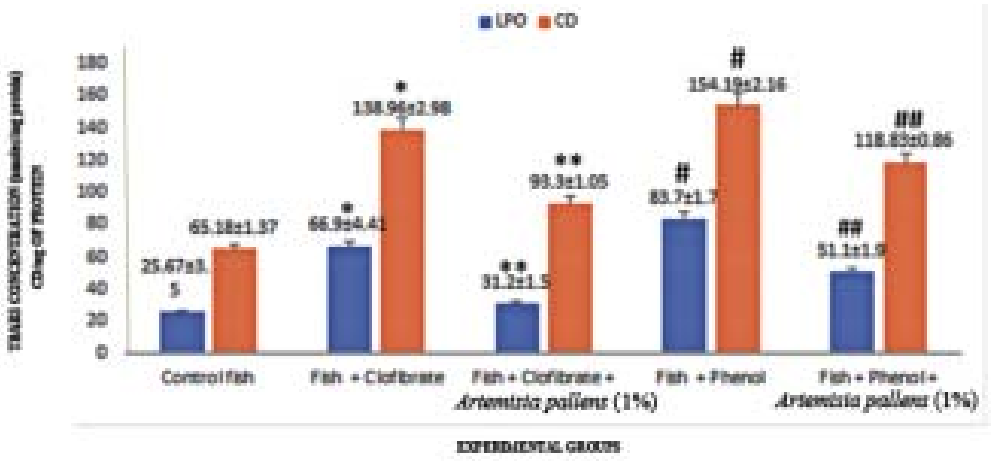

Fig. 2, a. **LPO and CD values of clofibrate and phenol treated *Pangasius* fish liver sample in the presence and absence of aqueous extract of *Artemisia pallens* (1% concentration)**

(*P* < 0.001 gives the concordance of the positive indication about the implementations of *Artemisia pallens* on LPO and CD parameters)

* Clofibrate exposed fish compared with control fish.

Phenol exposed fish compared with control fish

** Clofibrate exposed fish along with bioremediation agent (*Artemisia pallens*) when compared with control fish

Phenol exposed fish along with bioremediation agent (*Artemisia pallens*) when compared with control fish.

Results are represented as mean — standard deviation.

chemicals, indicated the level of oxidation inclining towards the normal control value as it is illustrated, A remediated measure for 13.7% of toxicity by clofibrate was reduced to 6.45% under *Artemisia pallens* influence, by two folds. Though observably in the graphical result; it is quite evident for the control synonymity (Fig. 2, *b*). Thiols are organic compounds containing a sulfhydryl group, among all the antioxidants that are prevailing in the body; thiols constitute a major portion of the total body antioxidant precedence. They play a significant role in the defences against ROS, they are very specific and reactive in nature possessing fluorescent properties. Protein thiols are primordial targets of oxidative stresses. A variety of reductive pathways ensure that the cytosolic thiol groups are maintained in their reduced states and preclude the formation of stable disulphide bonds in the majority of organisms. In the carbonyl sulfhydryl assay as clofibrate and phenol detriment the overall cell stature as the ROS concentration increases, the remedial *Artemisia pallens* effect (Fig. 2, *b*) reducing the overall toxicity indications of clofibrate and phenol nearly a suppression by 2 folds, thereby controlling the positive effects under the significance of $P < 0.001$.

Inorganic phosphates are attested to provide a basis for the level of ADP involved during ATP production. As the cells are manifested to the chemical stresses, this, in turn, increases the demand for more inorganic phosphate requirements, hence, posing a threat for energy production. Upon the manifestations of these chemicals under *Artemisia pallens* influence, they assist in the inorganic phosphate level mediation, thereby decreasing the ill effect; the overall toxicity of clofibrate and phenol influences respectively were repressed to 99.16% and 24.20% under *Artemisia pallens* regulatory (of possible significance $P < 0.001$ control). In (Fig. 2, *c*) Inorganic phosphate calibration, with the indicative effects of phenol and clofibrate increased repercussions were discernible to the implication of *Artemisia pallens.*

As depicted in the images of the histopathological deduction, in accordance to the control liver tissues of fish (not subjected to phenol and clofibrate) it were found to be healthy and appeared normal and the fish hepatocytes exposed to clofibrate and phenol showed substantial changes. In contrast with the control fish, the liver of the fish exposed to clofibrate showed large-sized vacuoles (highlighted in yellow) and peripheral inflammation (Fig. 3, *b*). However, on treatment with *Artemisia pallens* the size of the vacuole reduced and scattered inflammation was observed (Fig. 3, *c*). Similar remarks were observed in the treatment of *Artemisia pallens* with the phenol intoxicated histopath with the reduction in the subsequent inflammatory indications. (Fig. 4, *b*, *c*). In

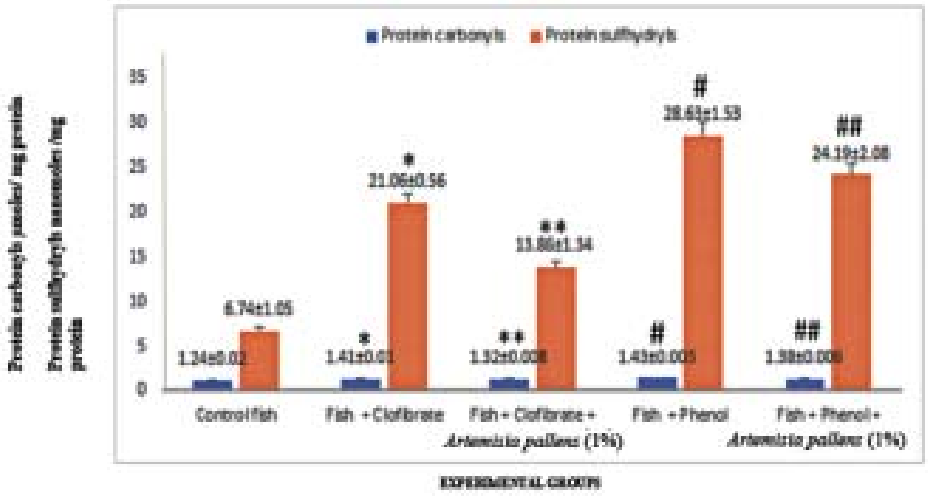

Fig. 2, b. **Protein sulfhydryl and protein carbonyl levels of clofibrate and phenol treated *Pangasius* fish liver samples in the presence or absence of aqueous extract of *Artemisia pallens* (1% m/v)**
For both the assays of Protein sulfhydryl and Protein carbonyls of $P < 0.001$ (level of significance)
* Clofibrate exposed fish subjected to bioremediation agent and compared with control fish.
# Phenol exposed fish subjected to bioremediation agent and compared with control fish.
** Clofibrate exposed fish along with bioremediation agent (*Artemisia pallens*) and compared with control fish.
Phenol exposed fish along with bioremediation agent (*Artemisia pallens*) and compared with control fish

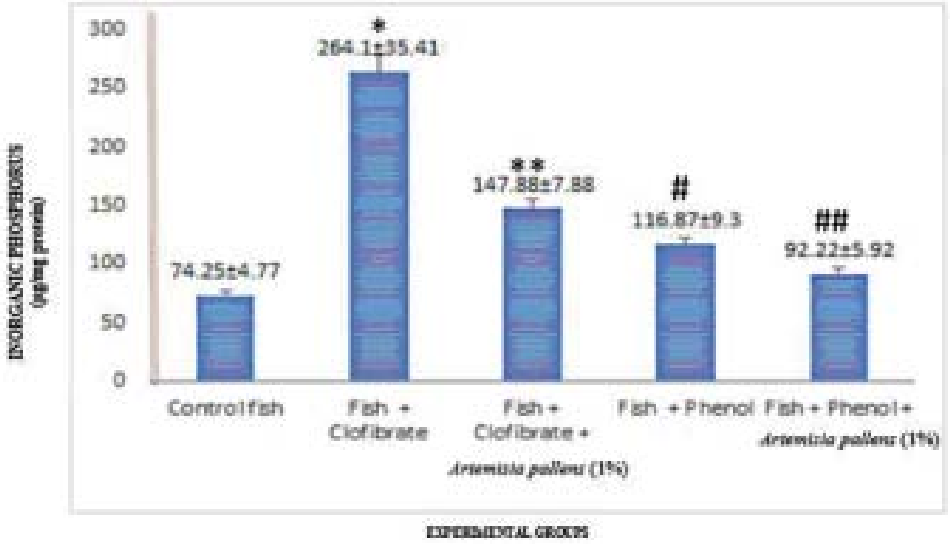

Fig. 2, c. **Inorganic Phosphates values of clofibrate and phenol treated *Pangasius* fish liver sample in the presence and absence of aqueous extract of *Artemisia pallens* (1% concentration)**
At the level of significance $P < 0.001$
* Clofibrate exposed fish subjected to bioremediation agent and compared with control fish.
# Phenol exposed fish subjected to bioremediation agent and compared with control fish
** Clofibrate exposed fish along with bioremediation agent (*Artemisia pallens*) when compared
Phenol exposed fish along with bioremediation agent (*Artemisia pallens*) when compared with control fish

case of phenol exposed fish, degeneration of vacuoles was prominent, and in trying to remedy the effect/lessen the effect on exposure to the phenol and bioremediation combination, reduction in inflammation was observed. In another research carried out by Reza et al., [13] hepatocytes morphological observations of *Pangasius sanitwongsei* were compared with mammals. Lipid-like vacuolization was observed in *C. idella* liver cells exposed to BPA for 14 days [18]. In our own study Vijaya Geetha [19] carried out in *Pangasius* species exposed to diclofenac, the hepatocyte damage was significant and similar vacuolar degeneration

were observed. On a more detailed insight; based on the work of Triebskorn [20], on the ultrastructural effects of the pharmaceuticals on rainbow trout and common carp; amongst the chemicals of diclofenac, metoprolol, and clofibric acid (derivative of clofibrate), it was noticed on exposure to a specific exposure of nearly 100 µg/L showed effects of blood cells dilation and occurrences of membrane materials within the intercellular junctions on the hepatocytes of the cell lines. On the other hand, over the likely deliverance on the primary filaments and the secondary lamellae of the gills, it was epithelial lifting, hyperplasia and hypertrophy of mucus and Cl^- cells indicating a strong reaction over kidney cells and moderate reaction on hepatocytes at above a control value of 5 µg/L. A further approach on how far normal drugs affect the wellbeing of fishes were studied by Eva [21] where the varied diclofenac concentrations had varied mortality effects. Though some of them did not indulge in the histopathological differences, the chemical infested a decrease in the TBARS concentration due to oxidative stresses.

As an organism made to undergo the indicative oxidative stress, it thereby increases the production of ROS, which simultaneously decreases the level of antioxidative activity including the SOD and catalase enzymes in this study. SOD is a key enzyme, providing the first line defense against the pro-oxidants and the catalyzation of superoxide radicals transformation to H_2O_2 and O_2. On the other hand, CAT belongs to the cellular antioxidant system that counteracts the reactive oxygen species (ROS) toxicity. They are the heme-containing enzymes facilitating H_2O_2 removal, which are metabolized to O_2 and water [22]. Subsequently, it is liable that the antioxidative effect would be decreasing as the chemicals phenol and clofibrate are infested within. But, under the mediatory action of the assistance provided by *Artemisia pallens* the antioxidative measures are evidently elevated to a discrete level as in the tabulated observations. The oxidative stresses in fish by environmental factors were explained by the work of Anton [23] wherein the oxidative stress is occurring to a subjective imbalance between the production of oxidants and antioxidants. Though antioxidants protect the enzymes from increased oxidative damage by free radicals; the increased enzyme activity can indicate oxidative stress. For the conclusion arrived at their study on the effects by the heavy metals

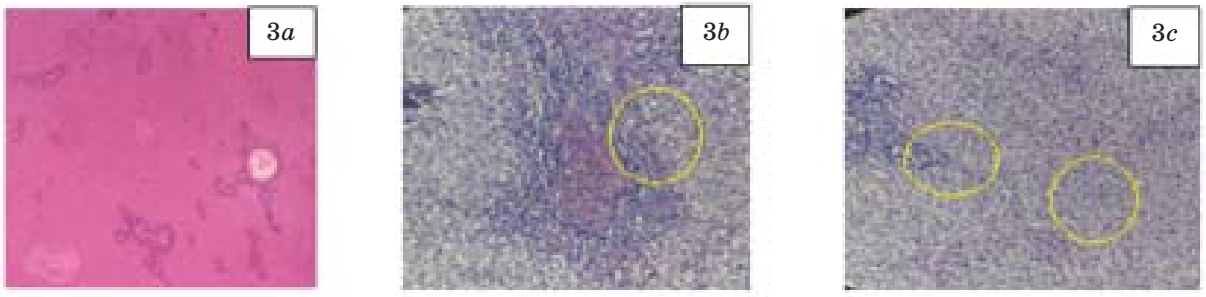

Fig. 3. **Histological microphotographs of *Pangasius* liver tissue; normal healthy control fish, fish exposed to clofibrate and fish exposed to clofibrate + *Artemisia pallens***

3*a* — control (Scale bar = 50 µm); 3*b* — clofibrate exposed fish showed large sized vacuoles and peripheral inflammation (Scale bar = 50 µm); 3*c* — clofibrate + *Artemisia pallens* exposed fish showed small sized vacuoles and scattered inflammation (Scale bar — 50 µm)

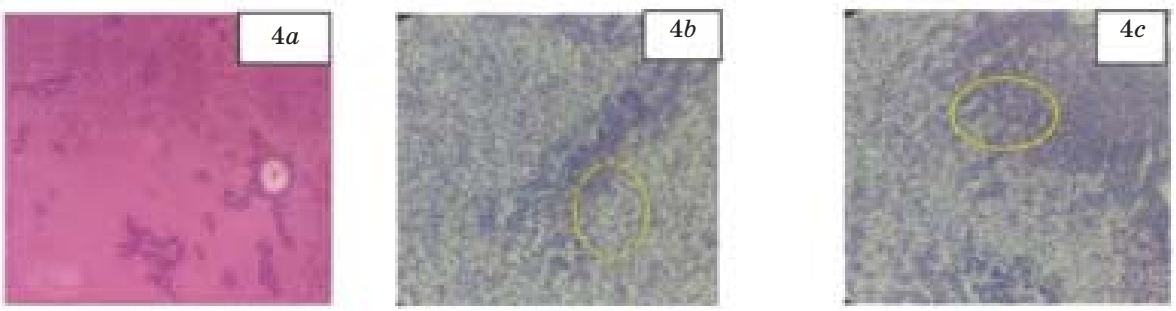

Fig. 4. **Histological microphotographs of *Pangasius* liver tissue; normal healthy control fish, fish exposed to phenol and fish exposed to phenol + *Artemisia pallens***

4*a* — control (Scale bar = 50 µm); 4*b* — phenol exposed fish showed vacuolar degeneration and inflammation (Scale bar = 50 µm); 4*c* — reduction in inflammation (Scale bar — 50 µm)

(Cd, Pb, and Hg) the induced effects such as SOD, catalase, Glutathione-S-transferase, GR, GSH, and MT on previous studies; provide us the substantiate how the increased ROS deteriorate lipids, proteins, and DNA. With reference to the chart (Fig. 5), in the scenario of SOD, the subsequent effect is clearly controlled to the control value, as in reference to catalase. The expression is well deduced for the suppression to the reprisal effects. Under the indication for the remedials of *Artemisia pallens* upon clofibrate and phenol treated fish, the toxicities were reduced by 2 folds under SOD parameter scrutiny. By the same notion for CAT the toxicities were reduced by *Artemisia pallens* under similar folds for clofibrate and phenol respectively under a positive significance of $P < 0.001$.

Cortisol assays substantiate as stress hormone biomarkers that indicate the level to which the organism is made to undergo the stress factors. Under the consideration where the control and the subjected fish underwent the respective chemicals dosage; phenol and clofibrate, the stress factors indicated by the cortisol assay were observed to be increasing. In the previous monitories as observed so far where we had inculcated the remediative aspect of *Artemisia pallens* within, it did help in controlling the level of stress inducers that affected the scrutinised chemical parameters. With the similar arch of further upgradation of environmental bioremediations, upon the application of *Artemisia pallens,* it seemingly decreased the overall value to a level where ($P < 0.05$) (Fig. 6) indicated the instance where the control of these chemicals was observed by the application of *Artemisia pallens.* As an additional marker value of ($P < 0.001$) this gives a conclusion that the overall stress impact is reduced and controlled to the ideal control values, hence bestowing a further extrapolation of the bio remedial activity of *Artemisia pallens*. Apart from the intrinsic factors that influence the oxygen balances in organism; the emerging threats such as pollutants and land use changes provide a base for more radical species exposure, as they bear the end reservoir for receiving higher rates of pollution, in accordance with the article by Kim et al. [24].

All these results were found to be in accordance with the study carried out by Vijaya Geetha and colleagues [25] (2021) on the toxic effects of clofibrate and phenol, the significant changes in the lipid peroxidation and total antioxidant capacity were observed proving the fact that there are noteworthy alterations in the oxidative potential of *Pangasius* sp.s

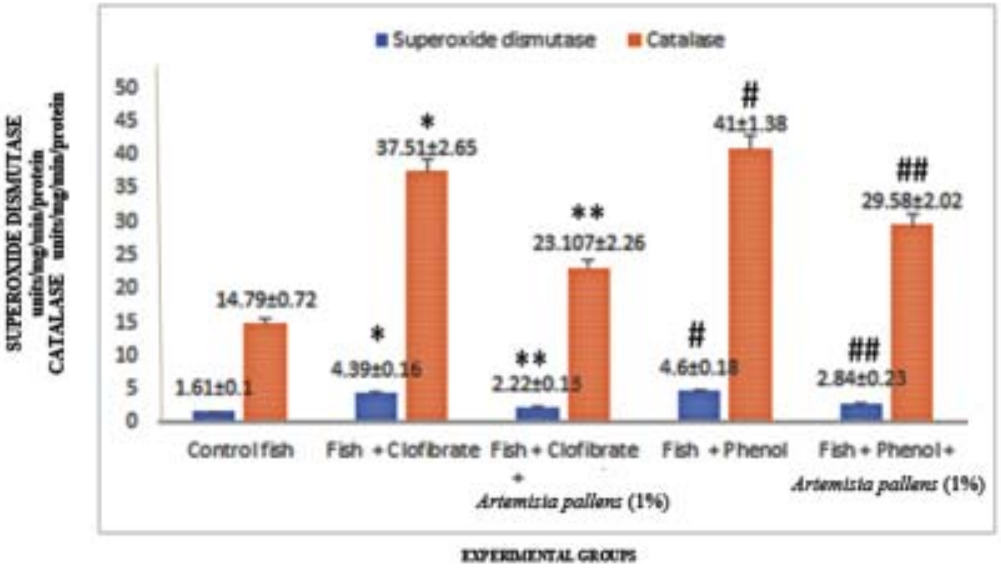

Fig. 5. **Superoxide dismutase and catalase activity values of clofibrate and phenol treated *Pangasius* fish liver samples in the presence or absence of aqueous extract of *Artemisia pallens* (1% m/v)**

At $P < 0.001$,

* Clofibrate exposed fish subjected to bioremediation agent when compared with control fish;

Phenol exposed fish subjected to bioremediation when compared with control fish;

** Clofibrate exposed fish along with bioremediation agent (*Artemisia pallens*) when compared with control fish;

Phenol exposed fish along with bioremediation agent (*Artemisia pallens*) when compared with control fish

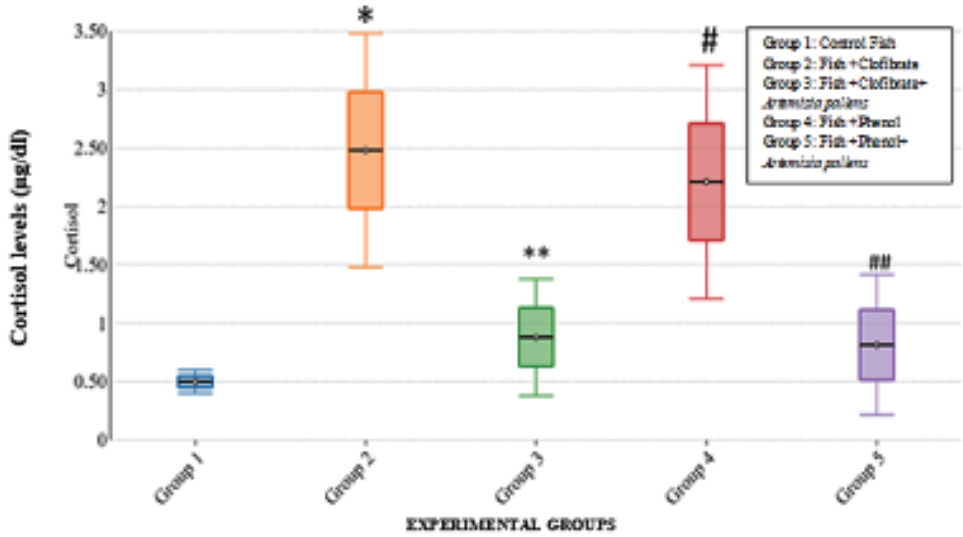

Fig. 6. **Cortisol levels in the liver samples obtained from clofibrate and phenol treated *Pangasius* fish in the presence or absence of aqueous extract of *Artemisia pallens* (1% m/w)**
At $P < 0.001$,
* Clofibrate exposed fish when compared with control fish;
# Phenol exposed fish when compared with control fish;
** Clofibrate exposed fish along with bioremediation agent (*Artemisia pallens*) when compared with control fish;
Phenol exposed fish along with bioremediation agent (*Artemisia pallens*) when compared with control

Based on the histopathological studies of *Pangasius* sps. exposed to longer duration and specific concentrations of phenol and clofibrate (CF), the likely deliverance resulted in inflammation and other detrimental. As it was observed that the effect was reduced by the influence of *Artemisia pallens*, a plant used often by the abode of our natural medicine. By this we can provide a venture to pursue many such possibilities about the extent to which the habitual plants that are observed in the common livelihood can be used and establish further importance of natural medicine and other restoratives. With the concordant results providing the reduction of the potent chemical influences by *Artemisia pallens*, reduced the chemical action under similar folds; yet, relatively manifesting a higher inclination upon clofibrate at certain measures. In this study as the descry of the cytotoxicity of these chemicals have substantiated a stricture by the plant used as such; we can further extrapolate the possibilities and cognizance of these remedies to further research approach.

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The authors state that they have no conflict of interest.

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ГЕПАТОПРОТЕКТОРНИЙ ВПЛИВ ВОДНОГО ЕКСТРАКТУ ЛИСТЯ *Artemisia pallens* НА ПРІСНОВОДНИХ РИБ *Pangasius* sp., ОБРОБЛЕНИХ ХЛОФІБРАТОМ І ФЕНОЛОМ

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Мета. Вивчити токсикологічні ефекти клофібрата і фенолу, які входять до складу фармакологічних препаратів і виявляються в стічних водах і донних відкладеннях, що надходять з очисних споруд.

Методи. Було створено експериментальну модель з використанням риб *Pangasius* sp., які після акліматизації вирощували в середовищі, що містить досліджувані сполуки в концентраціях, відповідних величинам LC_{50}. Токсичні ефекти впливу препаратів оцінювали за вмістом в тканинах риб маркерів окисного стресу, антиоксидантного статусу та пошкодження протеїнів при біоремедіації з використанням засобу, отриманого з *Artemisia pallens*. Гістопатологічні дослідження і визначення рівнів кортизолу проводили з метою отримання додаткових токсикологічних даних.

Результати. Порівняльний аналіз досліджуваних параметрів показав значну різницю між рибами, що містяться в середовищі з присутністю токсикантів, і за умов біоремедіації. Встановлено, що клофібрат і фенол індукували в тканинах риб окислювальний стрес ($*P < 0{,}001$, $\#P < 0{,}001$, відповідно), який супроводжувався значними змінами активності антиоксидантних ензимів ($*P < 0{,}001$, $\#P < 0{,}001$ відповідно) і гістопатологічними змінами. У риб, які містилися за умовах біоремедіації з використанням *Artemisia pallens*, спостерігалося значне зниження рівня кортизолу, підвищення вмісту якого зазначалося під впливом клофібрату і фенолу ($*P < 0{,}05$, $\#P < 0{,}05$, відповідно). Ця обставина може позитивно впливати на виживаність клітин за рахунок захисної дії за умов окисного стресу.

Висновки. Визначення декількох біомаркерів, включаючи показники окисного стресу і рівні кортизолу, може бути використано для біомоніторингу водного середовища. Водні екстракти *Artemisia pallens* — як ефективний засіб для біоремедіації.

Ключові слова: біоремедіація, *Artemisia pallens*, риби, біомаркери.

ГЕПАТОПРОТЕКТОРНОЕ ВОЗДЕЙСТВИЕ ВОДНОГО ЭКСТРАКТА ЛИСТЬЕВ *Artemisia pallens* НА ПРЕСНОВОДНЫХ РЫБ *Pangasius* sp., ОБРАБОТАННЫХ ХЛОФИБРАТОМ И ФЕНОЛОМ

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Цель. Целью работы было изучение токсикологических эффектов клофибрата и фенола, которые входят в состав фармакологических препаратов и обнаруживаются в сточных водах и донных отложениях, поступающих с очистных сооружений.

Методы. Была создана экспериментальная модель с использованием рыб *Pangasius* sp., которые после акклиматизации выращивались в среде, содержащей изучаемые соединения в концентрациях, соответствующих величинам LC_{50}. Токсические эффекты воздействия препаратов оценивали по содержанию в тканях рыб маркеров окислительного стресса, антиоксидантного статуса и повреждения протеинов при биоремедиации с использованием средства, полученного из *Artemisia pallens*. Гистопатологические исследования и определение уровней кортизола проводили с целью получения дополнительных токсикологических данных.

Результаты. Сравнительный анализ изучаемых параметров показал значительную разницу между рыбами, содержащимися в среде с присутствием токсикантов, и в условиях биоремедиации. Установлено, что клофибрат и фенол индуцировали в тканях рыб окислительный стресс ($* P < 0{,}001$, $\#P < 0{,}001$ соответственно), который сопровождался значительными изменениями активности антиоксидантных энзимов ($*P < 0{,}001$, $\#P < 0{,}001$, соответственно) и гистопатологическими изменениями. У рыб, которые содержались в условиях биоремедиации с использованием *Artemisia pallens*, наблюдалось значительное снижение уровня кортизола, повышение содержания которого отмечалось под воздействием клофибрата и фенола ($*P < 0{,}05$, $\#P < 0{,}05$, соответственно). Данное обстоятельство может оказывать позитивное влияние на выживаемость клеток за счет защитного действия в условиях окислительного стресса.

Выводы. Определение нескольких биомаркеров, включая показатели окислительного стресса и уровни кортизола, может быть использовано для биомониторинга водной среды. Водные экстракты *Artemisia pallens* — как эффективное средство для биоремедиации.

Ключевые слова: биоремедиация, *Artemisia pallens*, рыба, биомаркеры.