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In Silico Studies and Histopathological Analysis on the Bioremediation Effect of Spirulina in Various Tissues of Diclofenac Treated *Pangasius* Sps

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Abstract

The aquatic biome is an integral part of Earth's survival. Humans, with an attitude to lead a comfortable life, have invaded the ecosystem to a larger extent owing to many direct and indirect catastrophes in the longer run. Our aim was to establish the link between the genes and compounds that turn toxic to the organisms over due course of time. With the selected compound as diclofenac, Comparative Toxicogenomics database was used to identify the diseases related to the compounds, the top expressing genes, and the common genes of the compounds. By incorporating the coalescence of spirulina bioremediation under histopathological studies, the effect of the algae was observed. From these two studies, we were able to conclude that there was a significant link between the effects of the compound on the organisms.

Keywords: Diseases, Remediation, Spirulina, Toxicity

1. Introduction

Pharmaceutical compounds and their active metabolites are one of the significant emerging environmental toxicants. The major routes of entry of these compounds into the environment are industries, hospitals, or direct disposal of unwanted or expired drugs made by the patient[1]. The current scenario has called for new strategies to mitigate the pollution caused by man-made processes. With the advent of recent technologies, the rate of pollution has increased. Be it land, water, air, the level is increasing ten folds every day. The toxicities due to pharmaceutical industries have caused developmental defects in both aquatic animals and amphibians thereby affecting the growth[2]. The sources of pollution, such as industrial wastes, are recognized as long-term and widespread pollutant sources to aquatic systems[3]. The pharmaceutical industry in India stands 14th rank in terms of value and is the world's third-largest in terms of volume[4]. According to the CPhi Global Pharma Index, India ranks third most competitive nation. Aquatic plants and animals contribute to 1/3rd of the ecological balance. Pharmaceuticals are intentionally designed to have a specific mode of action and exert an effect on specific organs, tissues, cells, or biomolecules in humans, mammals, or other vertebrates, and many of them are persistent in the body[5]. In the context of how drugs intervene in Drug-Toxicity Signaling Paths (DTSP), they hinder the Protein-Protein Interaction (PPI) and cause certain discrepancies such as non-immune neutropenia in the long run over increased concentrations[6]. On the similar standards of scrutinizing the necessary influences of Trace elements and Toxicants on fish; it is quite evident that potentially they do wharf sublethal effects such as endocrine disruptors, seemingly affecting the reproductivity of fish either directly or indirectly alongside the environmental defects. There are umpteen numbers of researches and findings coming up every day to curb the toxic effects. At the same time, the failure is also high

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in these experiments. Bioremediation is the process of removal of harmful chemicals and toxic substances from the environment using microorganisms. The pharmaceutical effluent treatment involves the usage of a costly processing system for the treatment processes, hence to reduce the capital we have tried to exploit the bioremediation action using spirulina as a mediator. Besides reducing the cost, Spirulina possesses sublime antioxidant properties which increases the longevity of the fish when exposed to a toxic environment.

To palliate the labour cost and the time spent, the field of Bioinformatics efficiates the concern and has been doing wonders. We have used the Comparative Toxicogenomics Database to identify the top expressing genes based on our previous studies[7]. Non-Alcoholic Fatty Liver Disease(NAFLD) is often considered as the hepatic manifestation of the metabolic syndrome (insulin resistance, obesity and hyperlipidemia), comprising hepatic steatosis and Non-Alcoholic SteatoHepatitis (NASH). Diclofenac is the Non-Steroidal Anti-Inflammatory Drug (NSAID) most often associated with hepatic side effects. NASH is a combination of lipid accumulation, hepatocyte death, inflammation and fibrosis, making this pathology a significant risk factor for hepatic cirrhosis, hepatocellular Carcinoma (HCC) and ultimately Liver-related mortality. DNA damage-inducible transcript 3, also known as C/EBP Homologous Protein (CHOP), is a pro-apoptotic transcription factor that is encoded by the DDIT3 (DNA Damage Inducible Transcript 3) gene[8]. It is a member of the CCAAT/enhancer-binding protein (C/EBP) family of DNA-binding transcription factors. Also, in our wet lab experiments carried out on the fish model *Pangasius* Sps., it was found that there were noteworthy changes in the liver tissues (by histopathological studies and antioxidant activities).

2. Materials and Methods

2.1 Identification of Top Interacting Genes

Using Comparative Toxicogenomics Database (http://ctdbase.org/): The top expressing genes, with it's related diseases linking to diclofenac were found using this database and using the same Comparative Toxicogenomics Database, we were able to claim a gene which is common to both human and *Pangasius* Sps. For the analysis of pairwise sequencing, FASTA sequences of DDIT3 (DNA Damage Inducible Transcript 3) gene were accessed using NCBI and were provided as input to the EMBOSS needle webtool software. This includes both the sequences as well as their local alignment with their respective algorithms.

2.2 Selection and Acclimatization of the Fish Model

Pangasius Sps., a freshwater fish was chosen for the study due to its similarity with the human model and reproducibility. In this study, the fish that had been used were 12-15 cm long, with a relative weight of nearly 14-15 grams. Obtained from a local dealer, raised from the state of Andhra Pradesh, these fish were attuned to the tank conditions for a period of 15 days. During this period, they were fed with appropriate fish food mostly consisting of proteins as well as other nutritionary such as worms. The potent toxin diclofenac was introduced into the fish tank as a solution prepared by the addition of powdered diclofenac with HPLC grade water. After the addition of the solution, the Lethal Concentration LC_{50} was determined. Based on previous work the value of LC_{50} was found to be 264 mg for 11 liters[7]. After the identification of the lethal concentrations, the histopathological analysis was carried out on the toxin-infested fish and the addition of spirulina bioremediator was added.

2.3 Preparation of the Bioremediator

In this study, we recount the hazards of toxic chemicals' ascendancies on the animal biomes' well-being and their consecutive curatives are to be assayed as well. The bioremediator for the study, Spirulina, was prepared by dissolving 1 ml of spirulina solution in 500 ml of water.

2.4 Histopathological Analysis

The liver samples were fixed at 10% formalin solution in 10ml tubes. Fish-livers were sliced using a microtome(5µm), embedded in paraffin and stained with hematoxylin and eosin[8]. Histological changes in liver samples were examined using the Multihead microscope Olympu zs(200x).

3. Results

In order to procure the most contrived genes that are affected by the continuous exposure to toxic chemicals, by employing this database we were able to find that a number of diseases were related to diclofenac by effectuating the usage of Comparative Toxicogenomics Database (CTD). A few of them are Acute kidney injury, Heart failure, Cholestasis, Liver Cirrhosis, Ulcer, Multiple Organ Failure, Nausea, Hepatomegaly, Stomach Ulcer, Vomiting, Headache, Urticaria, Nephrotic Syndrome, Anemia, Necrosis, Proteinuria, Fatigue etc.[10–14].

The genes that are responsible for metabolizing these compounds when consumed or ingested in the form of water or food are revoked from (http://ctdbase.org/).

The most expressive genes for diclofenac observed are as follows:

1. CYP2C9 - It is a gene that is directive in the synthesis of enzymes found in cell structure and plays a vital role in protein processing and transport. This gene reduces the metabolism of diclofenac and alters the extent of drug-drug interaction.
2. IL1B – The IL1B, Interleukin-1 beta is a potent proinflammatory cytokine. Initially discovered as the major endogenous pyrogen, it induces prostaglandin synthesis, neutrophil influx and activation, T-cell activation and cytokine production, B-cell activation and antibody production, and fibroblast proliferation and collagen production. It promotes Th17 differentiation of T-cells. On the subsequent analysis, it was incurred that the common diseases observed were tabulated.

Based on results from the EMBOSS Needle webtool (Figure 1), Pairwise alignment was claimed to reveal nearly 39.9 % similarity with the human genes to certain localized regions of *Pangasius* Sps. Thereby, we can vindicate a remedial action on *Pangasius* Sps. providing a possible scale up exhibiting a similar remedial in humans as such. DDIT3 (DNA Damage Inducible Transcript 3) gene is commonly associated with fatty liver, drug induced liver injury and other liver related disorders (Figure 2).

With these genes that are expressed and influenced by this potent chemical, we can infer their prevalence in the health of an organism when exposed to increasing concentrations duly over time with repeated exposition. Upon screening the common mundane, the relative abundance of the chemical diclofenac was chosen for its paramount prevalence in general administered drugs; at certain dosage-dependent and had relatively higher dose to impart biochemical changes; such as necrosis, increased induced hepatotoxicity, along with fatty changes imbibed. As diclofenac is included within the subcategory of non-steroidal anti-inflammatory drugs (NSAIDS); in liver, harbouring as a site for gluconeogenesis and urea production during amino acid catabolism, fluctuations were caused into increased lactate production. Along with this, diclofenac also orchestrated the uncoupling of oxidative phosphorylation and mitochondrial dysfunction. Along with these outcomes, upon subsequent higher doses of diclofenac, more adverse effects could be emanated. This, was thus, considered as the aspects to be restrained by the bioremediator.

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Aligned_sequences: 2
1: c57520517-57516588
2: 11517494-11520910
Matrix: EDNAFULL
Gap_penalty: 10.0
Extend_penalty: 0.5

Length: 4813
Identity:    1920/4813 (39.9%)
Similarity:  1920/4813 (39.9%)
Gaps:        2279/4813 (47.4%)
Score: 2717.0
```

Figure 1. DDIT3 (DNA Damage Inducible Transcript 3) pairwise alignment between human and *Pangasius* Sps. were generated using Emboss Needle Webtool (https://www.ebi.ac.uk).

DDIT3	Fatty Liver	*114 chemicals:* 2,2',4,4'-tetrabromodiphenyl ether | 2,3',4,4',5-pentachlorobiphenyl | 2,4,5,2',4',5'-hexachlorobiphenyl | 4-methylene-2-octyl-5-oxofuran-3-carboxylic acid | Acetaminophen | Acetylcysteine | Amiodarone | Arsenic | Arsenic Trioxide | arsenite | Aspirin | Benzo(a)pyrene | bisphenol A | Butyric Acid | Cadmium Chloride | Capsaicin | Carbon Tetrachloride | Chloroform | Chloroquine | Chlorpromazine | Cholesterol | Choline | Clodronic Acid | Clofibrate | cobaltiprotoporphyrin | Copper | Curcumin | cyanoginosin LR | Cyclosporine | Decitabine | Dexamethasone | Dichlorodiphenyl Dichloroethylene | Diclofenac | Dietary Fats | Diethylnitrosamine | Diethylstilbestrol | Estradiol | Ethanol | Ethinyl Estradiol | Exenatide | fatostatin | Fatty Acids, Nonesterified | Fenofibrate | Fenretinide | Flame Retardants | Fluorouracil | Galactosamine | geraniol | Glutathione | hexabromocyclododecane | Hydralazine | Hydrogen Peroxide | Ibuprofen | Irinotecan | Iron | Isoniazid | Ketoconazole | Lamivudine | Leflunomide | Linoleic Acid | Lipopolysaccharides | Melatonin | Mercuric Chloride | Metformin | Methionine | Methotrexate | Nicotine | Nifedipine | nonylphenol | obeticholic acid | olaparib | Oleanolic Acid | oleuropein | Orlistat | Oxaliplatin | Palmitates | Palmitic Acid | Particulate Matter | Penicillamine | pentabromodiphenyl ether | perfluorohexanesulfonic acid | Phenobarbital | pirinixic acid | Plant Extracts | potassium bromate | Quercetin | Resveratrol | Rifampin | Rimonabant | Rosiglitazone | sibutramine | sodium arsenite | Streptozocin | Sulindac | Superoxides | Tacrolimus | Tamoxifen | tauroursodeoxycholic acid | Telmisartan | Tetrachlorodibenzodioxin | Tetracycline | Thioacetamide | thymoquinone | TO-901317 | tributyltin | Trichloroethylene | trigonelline | triptolide | Troglitazone | Ursodeoxycholic Acid | Valproic Acid | Wortmannin | Zidovudine | Zinc
DDIT3	Non-alcoholic Fatty Liver Disease	*50 chemicals:* 2-methyl-2H-pyrazole-3-carboxylic acid (2-methyl-4-o-tolylazophenyl)amide | 4-hydroxy-2-nonenal | 7,14-dihydroxydocosa-4,8,10,12,16,19-hexaenoic acid | Acetaminophen | Allopurinol | alpha-naphthoflavone | Arsenic Trioxide | Benzo(a)pyrene | bisphenol A | Blood Glucose | Cadmium | Cholesterol | Choline | Chromium | cobaltiprotoporphyrin | Copper | cyanoginosin LR | Dietary Fats | Diethylnitrosamine | Dust | Ethanol | Fenofibrate | Folic Acid | Genistein | geraniol | GW 4064 | Irinotecan | Iron | Linoleic Acid | Methionine | obeticholic acid | Orlistat | Palmitates | perfluorohexanesulfonic acid | perfluorooctanoic acid | Phenylbutyrates | pirinixic acid | Plant Extracts | Quercetin | Resveratrol | Rosiglitazone | sodium arsenite | Streptozocin | Tamoxifen | Tetrachlorodibenzodioxin | Thioctic Acid | Tobacco Smoke Pollution | tributyltin | Tunicamycin | Valproic Acid

Figure 2. DDIT3 (DNA Damage Inducible Transcript 3) gene is associated with Liver related disorders. The Figure was claimed from the Comparative Toxicology Database.

4. Discussion

On the account of environmental changes, the alteration in high blood antioxidant enzyme activities in the fish caught in 2012 compared to 2003 was observed by Skuratovskaya[9]. Using Comparative Toxicogenomics Database, we were able to confer the cross-linking of the genes that were responsible for the action of these chemicals, to the subsequent related diseases. As observed above, the genes identified though not held accountable for acting as the sole reason for the diseases they cause, draws a reasonable insight for us to provide a new understanding of how these can involve themselves as a block in the long-term harms. They are likely to induce these diseases in a longer run with increased concentrations accordingly. Spirulina has long been recognized as a potential immunostimulant. It augments the components of the mucosal and systemic immune system through the activation of non-specific immune systems[15] as well as latent antioxidant properties and higher anti-inflammatory aspects, along with its greater viability in the surroundings; this could provide us a latitude to deduce the possible remedial actions these algae can be procured under. Vacuolation is an indication within cells that harbours the existence of a xenobiotic effect that incurs a detriment in the normal functioning of the cell structure as well as the tissue as a whole. Apace with the repudiating effects of diclofenac, other pharmaceuticals such as phenol and clofibrate also exhibit the misconstruing effect alongside the rupture in the histopathological cells. Wherein, the bile duct cells' rupturing and the retrogressive action around the central vein in the histopathic studies indicate the inimical effects of pharmaceutical effects along with the oxidative stress persuasion[16]. As observed under histopathological studies, it was evident that the cells that were incited with the chemical Diclofenac showed increased vacuolation amongst the cells causing rupture and imbalances thereby affecting mitochondria and reducing the normal functioning of the cells (Figures 3a, b)

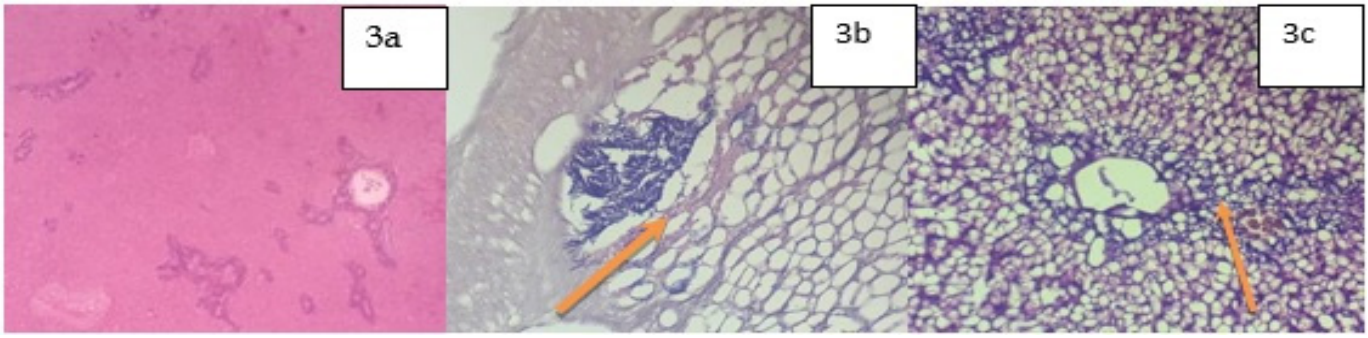


Figure 3. Histological pictures of the liver of *Pangasius* Sps., normal healthy fish, fish exposed to diclofenac and fish exposed to diclofenac and spirulina treated. **(a)** Control (Scale bar = 50µm). **(b)** diclofenac (LC50 12 mg/500 ml)) exposed fish showed Vacuolation (Scale bar = 10µm). **(c)** Liver healing observed due to spirulina treatment (Scale bar = 20µm).

A similar effect was also seen in the degeneration and vacuolation of heart, harbouring along with the lung cells (Figure 4 a, b).

The intestinal tract that was treated with Diclofenac as well, showed the vacuolation effects of indicating similar regressions, as the rupturing of the cells within the cell matrix spaces stipulated the harbouring lethal effects (Figure 5a).

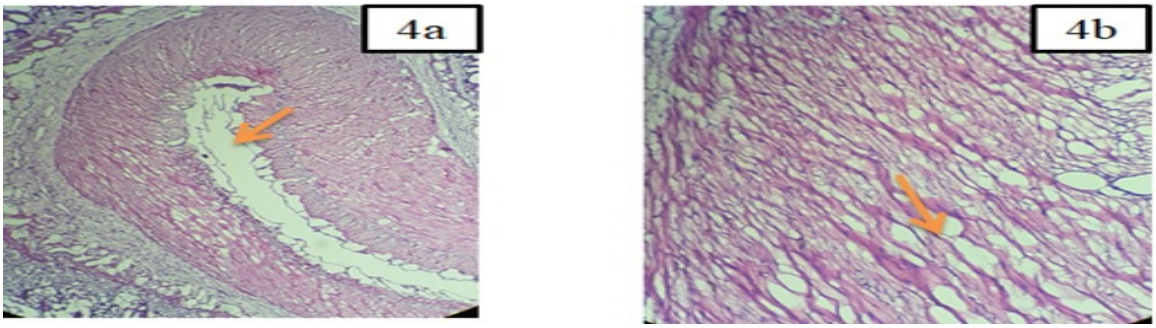


Figure 4. Histological pictures of the heart surrounded by lung degeneration of *Pangasius* Sps., fish exposed to diclofenac and fish exposed to diclofenac and spirulina treated. **(a)** Vacuolation of the fish heart cells upon exposure to diclofenac for nearly a couple of days. (Scale = 20µm). **(b)** Similar vacuole formation observed in the lung cells with diclofenac (LC50 12 mg/500 ml). (Scale = 10µm).

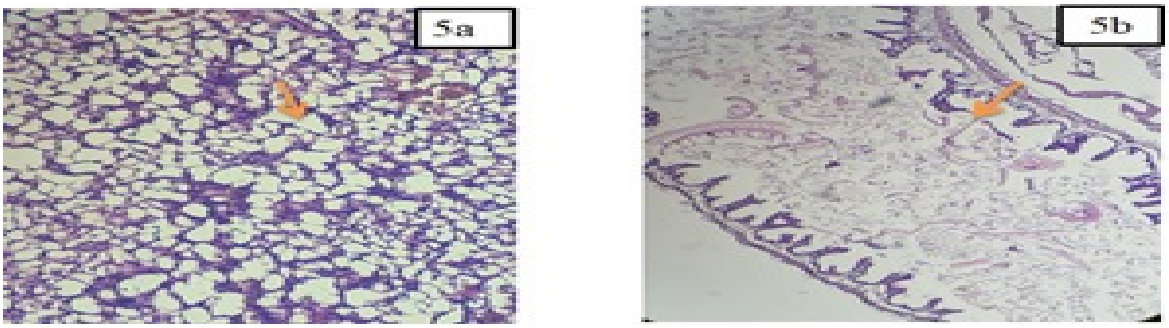


Figure 5. Histological pictures of the intestines of *Pangasius* Sps., fish exposed to diclofenac and fish exposed to diclofenac and spirulina treated. **(a)** Reduction of vacuolation along with the implication of Spirulina into diclofenac exposed fish (Scale bar = 10µm). **(b)** A similar reduction in vacuole formation and observable indications of adherence from rupture by the algae along with the exposed intestinal tissues (Scale bar = 20µm).

In a comparative contrast to the control, it was very evident that the indifferences were observed. Once the algae spirulina were introduced within the cells, in the case of liver cells; the remedial actions were espied as the algae began to substantiate limited vacuole spaces comparatively to the chemicals exposition (Figure 4c). By this, it was also to be noted that the nucleation, unlike the rupture caused in the chemical exposure, was rather controlled in the Spirulina action and thus, vindicated the bioremediation action of Spirulina. Furthermore with the note of similar remedial effect by the algae subsidence, (Figure 6b). In the intestinal tissues as well the reduction in vacuole generation was configured and had a better effect by the implication of spirulina. In the account of the plant arbitrated action, the substantial of bioremediative descents from plants have a superlative response on the antagonistic action of microbes as well as toxicity ubiety in the environment. The elicitation and the consuetude of the antibacterial effects frequented by the plants provide the consequential auxiliaries for endorsement of natural remediatives[17].

With the data illustrated, we infer that the link to elucidate a wholesome view on how the genes involved within, would serve as a counter reason to come up with a solution to suppress the increased expression of these chemicals. With this known knowledge of the interconnection of the pathways they were included within; we can subject separate restoratives in order to overcome the increased harmful expression in future[17]. Upon substantiating the useful links between the common top expressing genes; the possibility of the subsequent reduction or suppression of the effects can be countered. As in the study orchestrated by[18] by means of bioremediation by bacterial consortium of *Enterobacter, Citrobacter* etc. as such; in order to procure a substantiated remedial for pharmaceuticals' effects wherein Diclofenac, Ibuprofen and Sulfamethoxaole was controlled to a relative measure, can be implemented accordingly in order to retrieve from any long term determinants. Upon the suffusement of Spirulina on the gut microbiome and the overall reduction in the oxidative stress levels along with the lipid levels; surmised a controlled effect on the relative abundance of certain gut microbiota thereby, indicating the temporal shift in biological responses as well as disease prevention, in ode to the remedies over the Xeno chemicals. Within the manifestation of microbiota treatment as well as the chemical imbalances stabilization the algal treatment can be provided as a new restorative. HDLs and LDLs have an intricate relationship procured both with the functioning of the liver as well as the Cardiovascular tissues. Upon the stresses that the liver cells may face along with the changing degeneracies, we could secure a better understanding between the affected organs; In accordance to the work of Mostafa[19] stipulating that Spirulina effects that reduce the levels of LDLs, CHO, and TRI, in turn, increasing the HDL levels, this curative can also be included along with the Xeno chemical remediation aspect by virtue of which we can substantiate a betterment from the pre-existing malign effects of the potent chemicals.

5. Conclusion

By identifying the top expressive genes and diseases fostered within diclofenac using an *in silico* approach, we can decipher a collective measure that can be elucidated to simultaneously reduce the effect involved. Both dry and wet lab studies do provide an eminent basis as the water containing these compounds once infested on the organisms prevalent amongst them, if consumed by human beings, might cause repercussions on a long-term basis. Spirulina has always served as a source of valuable benefactors, by inculcating the measures of chemical remediation as well as the notion of therapeutics, this can harbour as an extrapolation of natural bio remedial usage on affected organs and synchronized pathways accordingly. Hence

this study can serve as an ideal, to provide a new insight to further understand the cross-linkage of the compounds, and as an awareness to the suitable sustainability that is required in order to keep a concurrent balance of the effects, likely to occur.

6. References

1. Roy K, Kar S. In silico models for ecotoxicity of pharmaceuticals. Methods. Mol Biol. 2016; 1425:237–304. http://dx.doi.org/10.1007/978-1-4939-3609-0_12
2. Severtsova EA, Severtsov AS. Comparison of variability of *Rana temporaria (Amphibia, anura)* gastrula from different populations developing under the conditions of antropogenic pollution. Russ. J Dev Biol. 2005; 36:82–93. https://doi.org/10.1007/s11174-005-0015-9
3. Padmini E, Vijayageetha B. Seasonal influences on water quality parameters and pollution status of the Ennore estuary, Tamil Nadu, India. J Environ Hydrol. 2015; 15: 1–9.
4. Patneedi CB, Prasadu KD. Impact of pharmaceutical wastes on human life and environment. Rasayan J Chem. 2015; 8(1):67–70.
5. Santos LH, Araújo AN, Fachini A, Pena A, Delerue-Matos C, Montenegro MC. Ecotoxicological aspects related to the presence of pharmaceuticals in the aquatic environment. J Hazard Mater. 2010; 175(1–3):45–95. https://doi.org/10.1016/j.jhazmat.2009.10.100
6. Desai K, Brott D, Hu X, Christianson A. A systems biology approach for detecting toxicity-related hotspots inside protein interaction networks. J Bioinform Comput Biol. 2011; 9:647–62. http://dx.doi.org/10.1109/HISB.2011.61.
7. Geetha BV, Sujata KR, Shreenidhi S, Sundararaman TR. Histopathological and HPLC analysis in the hepatic tissue of *Pangasius* sp. exposed to Diclofena. Pol J Environ Stud. 2018; 27(6):2493–8. http://dx.doi.org/10.15244/pjoes/75829
8. Hardy T, Oakley F, Anstee QM, Day CP. Nonalcoholic fatty liver disease: Pathogenesis and disease spectrum. Annu Rev Pathol. 2016; 11:451–96. https://doi.org/10.1146/annurev-pathol-012615-044224
9. Skuratovskaya EN, Kovyrshina TB, Rudneva II. A comparative study of long-term pollution effects of marine waters on the blood biomarkers of two benthic fish species: The scorpion fish *Scorpaena porcus* (Linnaeus, 1758) and the round goby *Neogobius melanostomus* (Pallas, 1814) from the Black Sea. Russ. J Mar Biol. 2017; 43:232–8. https://doi.org/10.1134/S1063074017030105
10. Shafeek F, Abu-Elsaad N, El-Karef A, Ibrahim T. Gum Acacia mitigates diclofenac nephrotoxicity by targeting monocyte chemoattractant protein-1, complement receptor-1 and pro-apoptotic pathways. Food Chem Toxicol. 2019; 129:162–8. https://doi.org/10.1016/j.fct.2019.04.050
11. Ryu B, Kim CY, Oh H, Kim U, Kim J, Jung C, Lee B, Lee S, Chang S, Lee JM, Chung H, Park J. Development of an alternative zebrafish model for drug-induced intestinal toxicity. J Appl Toxicol. 2018; 38:259–73. https://doi.org/10.1002/jat.3520
12. Hirashima R, Itoh T, Tukey RH, Ryoichi F. Prediction of drug-induced liver injury using keratinocytes. J Appl Toxicol. 2017; 37:863–72. https://doi.org/10.1002/jat.3435
13. Chang X, He H, Zhu L, Gao J, Wei T, Ma Z, Yan Y. Protective effect of apigenin on Freund's complete adjuvant-induced arthritis in rats via inhibiting P2X7/NF-κB pathway. Chem Biol Interact. 2015; 236:41–6. https://doi.org/10.1016/j.cbi.2015.04.021
14. Shi F, Zhou D, Ji Z, Xu Z, Yang H. Anti-arthritic activity of luteolin in Freund's complete adjuvant-induced arthritis in rats by suppressing P2X4 pathway. Chem Biol Interact. 2015; 226:82–7. https://doi.org/10.1016/j.cbi.2014.10.031
15. Kim S, Kiyosawa N, Burgoon LD, Chang CC, Zacharewski TR. PPARα-mediated responses in human adult liver stem cells: In vivo/in vitro and cross-species comparisons. J. Steroid Biochem Mol Biol. 2013; 138:236–47. https://doi.org/10.1016/j.jsbmb.2013.06.004
16. Geetha BV, Shreenidhi KS, Sundararaman TR. A study on the oxidative stress status of *Pangasius* sp. exposed to phenol and clofibrate. Zeichen Journal. 2021; 7(1):23–32.
17. Baba E. Antibacterial effects of seven essential plant oils on fish pathogens. Bol Inst Pesca. 2020; 46(3). https://doi.org/10.20950/1678-2305.2020.46.3.565.
18. Aissaoui S, Ouled-Haddar H, Sifour M, Beggah C, Benhamada, F. Biological removal of the mixed pharmaceuticals: diclofenac, ibuprofen, and sulfamethoxazole using a bacterial consortium. Iran J Biotechnol. 2017; 15(2):135–42. https://doi.org/10.15171/ijb.1530
19. Al-Ahmad MM, Amir N, Dhanasekaran S, John A, Abdulrazzaq Y, Ali RB, Salema M. Genetic polymorphisms of cytochrome P450-1A2 (CYP1A2) among Emiratis. PLoS ONE. 2017; 12:1–13. https://doi.org/10.1371/journal.pone.0183424\