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Evaluation of oxidative stress induced by physiological doses of metformin in the kidney of Labeo rohita

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ABSTRACT

Metformin (Glucophage) is an orally administered antidiabetic drug and the preferred first-line treatment for type 2 diabetes. It also used to treat gestational diabetes, polycystic ovary syndrome and cancer. In this study, we aimed to evaluate the potential oxidative effects of metformin on the kidney of labeo rohita at physiological doses of 0.6 µg/mL and 1.2µg/mL. Kidney of labeo rohita exposed to physiological doses of metformin for a period of 5 days, subsequently; we measured the defensive response of enzymatic antioxidants like catalase (CAT), superoxide dismutase (SOD) and glutathione peroxidase (GPx) to determine their role in counteracting metformin- induced oxidative stress. In conclusion, this study explore the physiological doses of metformin might be induced oxidative stress, as increased activity of glutathione peroxidase, catalase and superoxide dismutase in the kidney of labeo rohita. Significant changes in enzymatic antioxidants activities suggest a potential disruption in the kidney of Labeo rohita. Further research, needed to elucidate the underlying mechanisms and assess the long-term effects of metformin exposure on renal health in Labeo rohita.

KEYWORDS

Metformin toxicity; Oxidative stress; Labeo rohita; Renal antioxidant response; Aquatic toxicology

ARTICLE HISTORY

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Introduction

Labeo Rohita is a dominant and commercially significant species among freshwater carps, which has been cultivated globally. Labeo Rohita confers 15% of global aquaculture production, composing 72% of total carp production [1]. It has high market demand in South and Southeast Asia due to its nutritional value. It is highly susceptible to certain xenobiotics present in the water, serving as a water quality indicator, which makes it a potent bioindicator to assess the contamination caused by industrial waste, heavy metals, and pesticides. Aquatic organisms, along with Labeo Rohita are under the influence of different environmental stressors [2].

Among emerging aquatic contaminants, metformin ($C_4H_{11}N_5$) has secured special attention due to its ubiquitous use and environmental persistence. Metformin is a first-line anti-diabetic drug extracted from the herb galega officinalis, which is enriched with guanidine that helps to reduce the blood glucose level. Other than diabetes, metformin is being prescribed to manage various chronic diseases such as certain types of cancer and polycystic ovary syndrome [3]. Unlike other pharmacological compounds, metformin passes unaltered from the body as it is quite stable and resistant to biodegradation, which is the prime reason for its persistence in nature. Medical use in humans, veterinary applications and industrial waste are the main reasons for metformin presence in water bodies [4]. Metformin and its metabolites have been detected in water bodies in different concentrations, leading to global contamination. Conventional wastewater treatment is not designed particularly to remove pharmaceutical products [5]. Even the partially degraded metformin forms bioactive metabolites such as guanyl-urea, which can be more toxic than the parent compound [6].

Metformin is known to modulate the immune response by increasing expression of the inflammatory mediator interleukin-6 (IL-6) and interleukin-1β (IL-1β) in clarias gariepinus [7]. High doses damage DNA, leading to genotoxicity [8]. Metformin can lead to impaired growth and malformations in pimephales promelas, orchestrated as skeletal or ocular abnormalities and cardiac or yolk sac edema [9]. Long-term exposure to metformin, even at low concentrations, may lead to oxidative stress, inflammation, endocrine disruption, as well as accumulation in organs such as the liver or kidneys, initiating a toxic response.

Xenobiotic-induced toxicity manifests differently across organs such as the liver, gills, or kidneys, depending upon permeability, vascularization and metabolic activity. The kidneys act as one of the major bio-marker to study the toxicity due to vascularization, permeability and major excretory organ [10].

Materials and Methods

This study was conducted with the approval of the Directorate of Graduate Studies, Faculty of Sciences, University of Agriculture, Faisalabad, Pakistan.

Experimental fish

Eighteen males, Labeo Rohita weighing 70-100g and length of

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were 15.24 cm, provided by satayana road fish farm in Faisalabad, Pakistan. Three glass aquariums were prepared, as each contains 20 liters of water along with 0.62 mM $CaCl_2$ and 0.31 mM $MgCl_2$. The pH was maintained at 7. These eighteen fish were randomly classified into three aquaria, as each aquarium contains 6 fish. To remove excessive food particles and nanoparticles, the water was changed daily. By continuous aeration, the oxygen level was maintained. For 5 days, the conditions and behavior of the fish were monitored every 24 hours. The first group was taken as control, 0µg/mL, while the second group was treated with 1.2271µg/mL and the third group with 0.614µg/mL concentration of metformin, respectively. The physiological concentrations of metformin, 1.2 µg/mL and 0.6 µg/mL were taken to mimic realistic environmental exposures faced by aquatic organisms. These doses were used to validate the dose-dependent oxidative potential of metformin, ensuring environmental relevance and biological significance of the findings.

Physico-chemical parameter

Certain physico-chemical parameters were governed in an aquarium to ensure the comfort of the fish. The temperature of 28°C, pH of 7 and dissolved oxygen concentration of 7.0 mg/L were maintained. A portable dissolved oxygen meter and a thermometer were used to monitor the parameters in an aquarium. A specified photoperiod of 12 hr light and 12 hr dark was maintained. Other water quality parameters, such as nitrate-N and ammonia-N, and nitrite-N, were kept at the optimum rearing conditions for Labeo Rohita.

Fish dissection

The fish were exposed to metformin for 5 days and were later anesthetized with ice. To acquire the internal organs and specifically the kidney, the fish was subjected to dissection. The dissection was done with the help of scalpel by making an incision in the anal area of fish. The bone associated with ventral fin was removed by scissors. By lacerating the operculum, the body cavity was split and internal organs were acquired [11].

Sample collection and processing

After dissection of the fish, samples were collected and stored at -40C in 0.2 M phosphate buffer having pH 6.5. For further biochemical analysis, specimen were subjected to homogenization, which was followed by centrifugation at 10,000 rpm for 15 minutes.

Measurement of oxidative stress

Metformin is known to induce oxidative stress, which can unbalance the level of these enzymatic antioxidants (GPx, SOD and CAT). The fish was exposed to selected physiological doses of metformin to study its effect on the enzymatic antioxidants. Future research should aim to answer several open questions. For example:

Estimation of Superoxide dismutase (SOD)

To evaluate the activity of superoxide dismutase, the protocol explained by Bradford was utilized [12]. For the enzyme assay, the reaction mixture comprises 0.2 M buffer, methionine (0.222 g) in 15 mL of water, NBT (0.015 g), riboflavin (0.0132 g), and Triton-X (0.0375 mL) in 17.5 mL of water.

Estimation of Glutathione peroxidase (GPx)

To investigate the activity of glutathione peroxidase, the protocol explained by Paglia and Valentine was used [13]. For the enzyme assay, the reaction mixture consists of 40 mM of H_2O_2, 20 mM of guaiacol, and 50 mM of phosphate buffer at pH 5. After every 20 seconds, the enzyme activity was recorded at 470nm.

Estimation of Catalase (CAT)

To inquire about the activity of catalase, the protocol explained by Chance and Maehly was followed [14]. For the enzyme assay, the reaction mixture included 5.9 mM of H_2O_2, 50 mM of phosphate buffer at pH 7, along with 0.1 mL of enzyme extract. The enzymatic activity was observed at 240nm.

Statistical analysis

The data were arithmetically presented as ± (SEM) (n=15). Analysis of variance (ANOVA) was used for statistical analysis, along with Tukey's multiple comparison test (t-test) as a post-test. In the absence of metformin $^*p < 0.05$, $^{**}p < 0.01$, and $^{***}p < 0.001$ ensure the significant differences. GraphPad Instat 3.1 was used for statistical analysis [15].

Results

Three groups were randomly made, two of them were exposed to different concentrations of metformin (0.6µg/ml and 1.2µg/ml) while one was taken as a control group with no exposure to the drug. After the exposure of five days, the fish were subjected to dissection and selected organ was collected for further analysis. The enzymatic activity of superoxide dismutase, catalase, and glutathione peroxidase was assessed in the kidneys exposed to metformin and compared with the control.

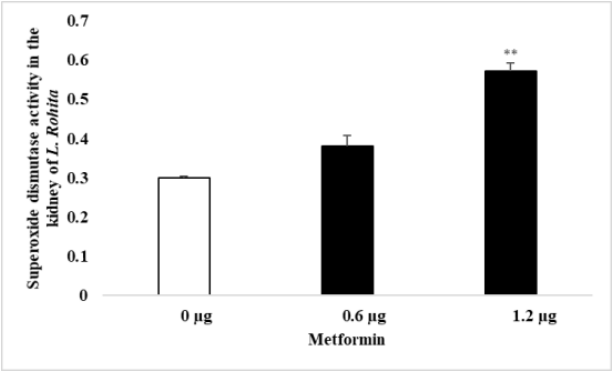

Figure 1. The effect of metformin on the activity of superoxide dismutase in L. rohita.

Arithmetic mean ± standard error mean (SEM) (n=15) with metformin 0.6- 1.2 µg/mL is represented in black bars, while the white one is taken as control with 0 µg/mL metformin. The y-axis reflects the SEM indicate the elevation in the SOD activity upon treating with metformin's different concentration, as the concentration of the metformin increase the activity of the SOD also increase which is the potential indicator of the aggravation of free radical species. Statistically significant difference (**; $P < 0.01$) with a confidence interval of 99% was observed by ANOVA, which is indicated by asterisks.

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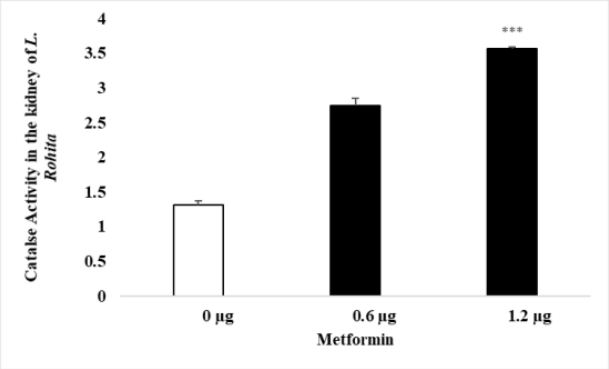

Figure 2. Effect of metformin on the catalase activity in L. rohita.

Arithmetic mean ± standard error mean (SEM) (n=15) with metformin 0.6,1.2 µg/mL is represented in black bars, while the white one is taken as control with 0 µg/mL metformin. Statistically significant difference is described along the y-axis in the form of asterisks (***; P < 0.001) with the confidence interval of 99.9%, which were studied by ANOVA. Figure 2 exhibits that the catalase activity increases as the metformin concentration is enhanced.

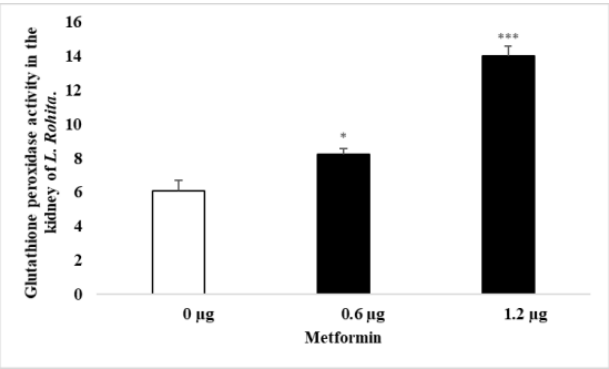

Figure 2. Effect of metformin on the glutathione peroxidase activity in L. rohita.

Arithmetic mean ± standard error mean (SEM) (n=15) of glutathione peroxidase (Gpx) in the kidney of L. rohita after being exposed to metformin's different concentrations (0.6,1.2 µg/mL) is depicted in black bars, while white bars are without metformin. SEM is demonstrated on the y-axis, which reflects the significant difference expressed in the form of asterisks (*; P < 0.05) with the confidence interval of 95% and (***; P < 0.001) with the confidence interval of 99.9% perceived by ANOVA analysis.

Discussion

Metformin, despite being the first-line antidiabetic drug, is associated with certain deleterious effects, oxidation is one of them. Metformin is known to cause oxidative stress even under physiological doses, which have been observed in this study. Oxidative stress directly influences the concentration of naturally occurring antioxidant enzymes, which act as a biomarker of oxidative stress. GPx activity was increased in the kidneys of L. Rohita upon treatment with metformin concentration (0.6 µg/ml, 1.2 µg/ml) [16-19]. Superoxide dismutase activity was also significantly increased in kidney L. Rohita with metformin concentration (0.6 µg/ml, 1.2 µg/ml) [20,16]. Catalase activity was also accelerated in the kidney of L. Rohita upon treatment with metformin (0.6 µg/ml, 1.2 µg/ml) as the redox status of the cell changed, as catalase activity altered [21-23]. The elevation in the activity of antioxidant enzymes due to metformin suggests the accelerated production of free radicals, leading to oxidative stress, which might be compensated by an adaptive cellular response to maintain the redox balance.

Conclusion

This study investigated the oxidative role of metformin even at physiological doses in the renal tissue of L. Rohita. A significant upregulation in the activity of antioxidant key enzymes such as superoxide dismutase, catalase and glutathione peroxidase were observed. The elevation in the activity of enzymes indicated oxidative stress, generated due to excessive free radical production due to metformin. Chronic exposure to pharmaceutical residues such as metformin will not only induce deleterious effects in L. Rohita but also in higher organisms such as humans due to excessive consumption of L. Rohita. This study highlights the ecotoxicological risks raised by the imperishable nature of metformin in the aquatic ecosystem and its bioaccumulation potential and trophic transfer. The cumulative effects of pharmaceutical residues on the ecosystem from physiological disturbance to impaired population dynamics raise concern about eco-toxicological risk assessments as well as the regulation of pharmaceutical effluents.

Disclosure statement

No potential conflict of interest was reported by the author.

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