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ORIGINAL ARTICLE

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Investigation of oxidative effects of physiological doses of metformin on liver of *Labeo rohita*

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ABSTRACT

Metformin (Glucophage) is an orally administered antidiabetic drug. It is commonly prescribed as the first-line therapy for managing type 2 diabetes. Additionally, it is used to treat gestational diabetes, cancer, and polycystic ovary syndrome. The imbalance between the production of free radicals and the body's ability to neutralize their harmful effects is known as oxidative stress. In this study, we investigated the potential oxidative effects of metformin on the liver of *Labeo rohita* at physiological doses of 0.6 µg/ml and 1.2µg/ml. The liver of *Labeo rohita* was exposed to metformin for a period of 5 days, after which we evaluated the protective effects of enzymatic antioxidants such as superoxide dismutase, catalase, and glutathione peroxidase. In conclusion, the findings suggest that the physiological doses of metformin may induce oxidative stress, as evidenced by the increased activity of glutathione peroxidase and decreased activities of catalase and superoxide dismutase in the liver of *Labeo rohita*. These changes suggest a potential disruption of the antioxidant defense system in the liver of the fish, highlighting the need for further investigation into the potential effects of metformin on aquatic organisms.

KEYWORDS

Oxidative stress; Diabetes; Enzymatic antioxidants; Metformin

ARTICLE HISTORY

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Introduction

Rohu (*Labeo rohita*) is a fresh water, living bony fish which is extensively available in Asian Regions like Pakistan, India and Nepal. Young Species of Rahu feed on zooplanktons while after transitioning into mature stage they rely primarily on plant matter as food So, generally known as bottom-feeding herbivore [1].

Metformin is anti-diabetic drug procured from Galega officinalis, also recognized as goat's rue. Galega is a perennial leguminous shrub rich in guanidine, a chemical compound helps to alleviate the Blood glucose level [2]. Metformin is considered as potent and generally safe medication for diabetic patients yet it can induce oxidative stress by generating the Free Radicals However, the underlying mechanism by which metformin may trigger oxidative stress remains obscure. Metformin stimulates the AMP-activated protein kinase (AMPK) pathway by repressing the Nrf2 expression leading to hamper the downstream signaling of Nrf2 [3-6]. This repression of Nrf2 expression can disturb the regulation of antioxidants, metabolic genes and cyto-protective genes directing toward oxidative stress and ultimately leading to cellular damage [7]. Certain factors such as duration and dose of metformin treatment along with specific cell types also influence the effects of metformin on Nrf2 expression and ultimately oxidative stress. Further study has become vital to entirely understand the mechanism behind metformin stimulating oxidative stress and to forge potential strategies to mitigate this menacing effects.

Material and Methods

The experimental work was performed with the approval of Directorate of Graduate Studies, Faculty of Sciences, and University of Agriculture Faisalabad.

Experimental fish

Eighteen males *Labeo rohita*, weighing between 70-100g and 15.24cm long, were collected from Satayana Road Fish Farm in Faisalabad, Pakistan. The fish were classified into three glass aquariums, each aquarium containing 6 fish in 20 liters of water. The water constitutes on 0.62mM $CaCl_2$ and 0.31mM $MgCl_2$ having a pH of 7.1. The water was changed regularly to get rid of nanoparticles as well as food residues and oxygen level was also maintained by using a continuous system of water aeration.

For 5 days the behavior and condition of the fish were noted after every 24 hours. The first and second groups were exposed to concentrations of 1.2271ug/mL and 0.614ug/mL of metformin, respectively, while the third group acted as a control group.

Physico-chemical parameter

To guarantee the well-being of the fish in an aquarium, certain physico-chemical parameters were kept in check. The temperature was settled at 28 °C, pH at 7.0, and dissolved oxygen levels were maintained at 7.0 mg/L. A thermometer and a portable dissolved oxygen meter were used to keep a check on aquarium.

Fish dissection

The fish after being exposed to metformin for 5 days were anesthetized with ice. The fish were subjected to a dissection process to acquire internal organs, including the liver, kidney, and gills. To dissect the fish an incision was done in the anal (vent) area of the fish with the help of a scalpel after that the bone attached to the ventral fins was cut through using

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scissors. Two walls of the body cavity were split by lacerating the operculum and gut to gain access to internal organs.

Sample collection and processing

Liver sample was extricated by dissecting the fish and stored in a phosphate buffer at -4 °C for further biochemical analysis.

Measurement of oxidative stress

Labeo rohita, possess highly efficient and sensitive enzymatic antioxidant defense system, comprising on GPx, SOD, and CAT. The impact of metformin on these enzymatic antioxidants were noted by exposing the fish to certain low and high concentration of drug at physiological doses.

Estimation of Superoxide dismutase (SOD)

Superoxide dismutase activity was measured applying the protocol of [8]. The reaction solution consisted of methionine (0.222 g) in 15 mL of water, NBT (0.015 g) in 17.5 mL of water, Triton-X (0.0375 mL) in 17.5 mL of water, riboflavin (0.0132 g) in 17.5 mL of water, and a 0.2 M buffer.

Estimation of Glutathione peroxidase (GPx)

Glutathione peroxidase activity was measured by adding 0.1 mL of enzyme extract to a reaction mixture consisting of phosphate buffer (50 mM, pH 5), guaiacol (20 mM), and H2O2 (40 mM), according to the protocol of [9]. The enzyme activity was monitored at 470 nm after every 20 seconds.

Estimation of Catalase (CAT)

The catalase enzymatic activity was evaluated by following the protocol described by 0.1 mL of enzyme extract was added to a reaction mixture containing phosphate buffer (50 mM, pH 7) and H_2O_2 (5.9 mM) [10]. The reaction was observed at 240 nm.

Statistical Analysis

The data were presented as the mean ± standard error of the mean. Statistical analysis was performed using analysis of variance using Tukey test as a post-test as appropriate. A significance level of P<0.05, P<0.01, or P<0.001 was specified by *, **, or ***, respectively, presenting a significant difference from the absence of metformin [11].

Results and Discussion

This study was planned to evaluate the oxidative effects of physiological doses of metformin on *Labeo rohita's* liver. Two groups of *Labeo rohita* were exposed to different metformin concentration 0.6µg/ml and 1.2µg/ml respectively for 5 days and compared with control group (No drug exposure) (figure 1-3). After exposure of 5 days, the fish liver was isolated by dissection to estimate the metformin effect on superoxide dismutase, catalase and glutathione peroxidase. Ultimately the enzymatic activity of SOD, CAT and Gpx in metformin exposed fish was compared with control group fish (Table 1-3).

Table 1. Impact of metformin on Superoxide Dismutase.

Sr. No.	0 µg/ml	0.6 µg/ml	1.2 µg/ml
1	3.17	2.19	1.22
2	1.78	2.89	1.445
3	2.675	1.665	1.15
4	1.97	2.035	1.585
5	2.82	2.64	0.98
6	2.755	2.33	1.36
7	2.175	1.87	1.155
8	2.77	2.96	0.96
9	1.8	1.88	1.17
10	3.105	1.285	1.625
11	2.775	2.14	0.975
12	1.765	1.615	1.41
13	3.175	1.655	1.36
14	2.83	2.695	1.455
15	1.845	2.38	0.86
Mean	2.494	2.148667	1.247333
N	15	15	15
SD	0.54048	0.500501	0.238094
SEM	0.14	0.13	0.06

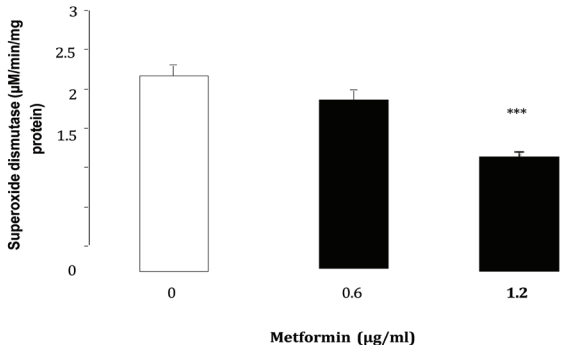

Figure 1. Enzymatic variation of superoxide dismutase levels.

Arithmetic means ± SE of *Labeo rohita* (n=15) with metformin (0.6-1.2 µg/ml) indicate black bar and without metformin white bar while y-axis bar indicating standard mean error ***, *** (P<0.001), (P<0.001) show significant decreases the super oxidase dismutase activities in metformin-induced fish liver (ANOVA).

Alleviation in superoxide dismutase activity was detected in this study which exhibits the detrimental effects of metformin on the antioxidant defense system in liver tissues of *Labeo rohita*. However, further studies are the crucial requirement to confirm these findings and to evaluate the underlying mechanisms involved in suppression of SOD level.

Table 2. Impact of metformin on Catalase.

Sr. No.	0µg/ml	0.6 µg/ml	1.2 µg/ml
1	34.94	33.22	32.49
2	34.31	33.21	32.12
3	35.82	32.61	32.36
4	32.64	32.05	33.05
5	32.51	32.78	32.79
6	34.18	36.8	32.31
7	34.45	33.28	32.51
8	35.46	33.27	33.12
9	32.64	32.56	33.23
10	32.97	32.97	32.65

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11	35.81	36.23	32.39
12	32.75	35.32	32.32
13	35.76	33.52	32.19
14	34.97	32.35	33.2
15	33.75	36.34	32.15
Mean	34.19733	33.76733	32.592
N	15	15	15
SD	1.253404	1.576911	0.392141
SEM	0.323627	0.407157	0.10125

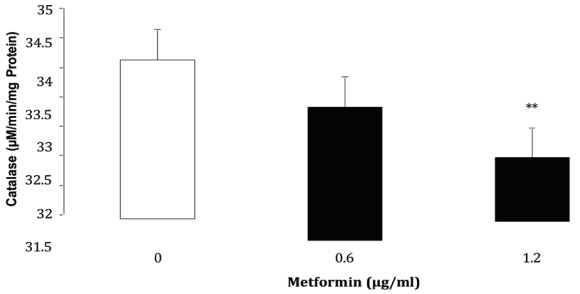

Figure 2. Enzymatic variation of catalase levels.

Arithmetic means ± SE of *Labeo rohita* (n=15) with metformin (0.6-1.2 µg/ml) indicate black bar and without metformin white bar while y-axis bar indicating standard mean error **, * (P00<0.01), (P<0.05) show significant decreases the catalase activities in the metformin-induced fish liver (ANOVA).

Significant oxidative stress is observed as a result of metformin exposure as the reduction in catalase activity is also noted in the liver of *Labeo rohita* on drug treatment.

Table 3. Impact of metformin on glutathione peroxidase.

Sr. No.	0 µg/ml	0.6 µg/ml	1.2 µg/ml
1	4.05	7.32	15.27
2	3.99	9.3	19.62
3	2.79	3.42	17.28
4	2.58	12.9	17.91
5	2.49	10.17	20.79
6	3.48	8.61	18.63
7	4.11	4.38	12.06
8	3.84	9.39	13.68
9	2.91	6.48	17.04
10	2.94	4.38	14.85
11	2.58	7.23	14.88
12	3.57	11.49	16.92
13	2.64	10.89	19.71
14	26.7	7.41	17.16
15	4.29	10.11	12.66
Mean	4.864	8.232	16.564
N	15	15	15
SD	6.074599	2.774746	2.614377
SEM	1.568455	0.716436	0.675029

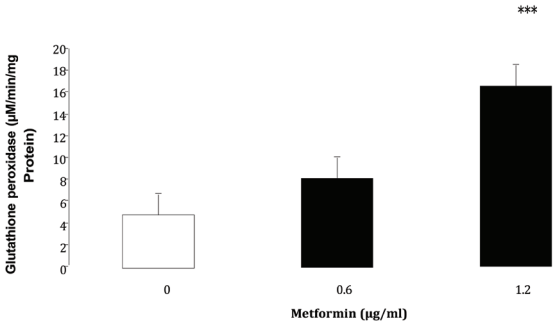

Figure 3. Enzymatic variation of glutathione peroxidase levels.

Arithmetic means ± SE of *Labeo rohita* (n=15) with metformin (0.6-1.2 µg/ml) indicate black bar and without metformin white bar while y-axis bar indicating standard mean error ***, *** (P<0.001), (P<0.001) show significant increases the glutathione peroxidase activities in metformin-induced fish liver (ANOVA).

Results indicate that metformin is potential inducer of oxidative stress as the glutathione peroxidase activity in the liver of *Labeo rohita* was found to be reduced.

Conclusions

The findings of this study suggest that metformin (an antidiabetic drug), a commonly used to manage type 2 diabetes. Metformin even at physiological doses, may interfere with the fish body's ability to produce antioxidants such as catalase, glutathione, and superoxide dismutase which could lead to oxidative stress in the *Labeo rohita* liver. The level of superoxide dismutase and catalase were significantly decreased while glutathione significantly increased in the liver of metformin-treated fish. The elevated levels of free radical and commonly reduced enzymatic antioxidant activities revealed a disturbance in redox within hepatic tissue. These findings raise concerns about oxidative stress can dysfunction of fish liver and can also boost up the risk of liver cancer. Further research is needed to determine the underlying mechanisms and the long-term effects of metformin on *Labeo rohita* liver health.

Disclosure statement

No potential conflict of interest was reported by the authors.

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