



## Beneficial effects of dietary supplementation of dragon fruits (*Hylocereus undatus*) extract on hematological changes against paracetamol induced *Labeo rohita* fingerling

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### Abstract

Possible effects of Dragon fruits (*Hylocereus undatus*) were observed in a freshwater fish *Labeo rohita* Juveniles of test fish were treated with paracetamol and analysis the Hematological parameters including Red Blood Cell (RBC) count, white blood cell (WBC) count, haemoglobin (Hb), Packed Cell Volume (PCV), Mean Corpuscular Volume (MCV), mean corpuscular haemoglobin (MCH), Mean Corpuscular Haemoglobin Concentration (MCHC). The results of the present study concluded that paracetamol treated fish has drastic hematological changes in the fish blood indicated the toxicity of paracetamol. On supplementation of Dragon fruit to paracetamol treated group restored the hematological parameters indicated the Dragon fruit nullify the toxicity of paracetamol.

**Keywords:** hematological parameters, dragon fruits (*Hylocereus undatus*), paracetamol

### Introduction

Continuous release of xenobiotic and their metabolites into the aquatic environment is a matter of serious concern all over the world. Pharmaceuticals a diverse group of substances can enter the aquatic environment from wastewater treatment plants in the form of non-metabolized parent compounds or their metabolites (Fent *et al.*, 2006; Ramesh *et al.*, 2018) [1, 2]. The occurrence of pharmaceuticals has been detected in aquatic recipients such as sewage treatment plants, municipal wastewater, agricultural run-off, natural surface water, ground water, drinking water (Corcoran *et al.*, 2010; Liao *et al.*, 2015) [3, 4], and also in aquatic organisms (Mottaleb *et al.*, 2016) [5]. Studies have reported that the concentrations of these pharmaceutical medications in various aquatic matrices are in µg or mg levels (Ramaswamy *et al.*, 2011; Shanmugam *et al.*, 2014) [6, 7].

Among various pharmaceuticals, one of the most important discoveries in field of medicine was synthesis of Paracetamol which is one of the most commonly used medications worldwide. Paracetamol which is commonly used as analgesic and antipyretic shows some strange and life threatening effects like liver damage which leads to fulminant liver failure and also death. Paracetamol is now the most common drug in self-poisoning, with a high rate of morbidity and mortality (Ghaffar and Naser Ashraf Tadvi, 2014) [8]. Keeping in view in the present study to investigate the beneficial effects of dietary supplementation of Dragon fruit (*Hylocereus undatus*) extract on hematological parameters as haemoglobin (Hb), red blood Cell (RBC) count, white blood cell (WBC) count, Packed Cell Volume (PCV), Mean Corpuscular Volume (MCV), mean corpuscular haemoglobin (MCH) and Mean Corpuscular Haemoglobin Concentration (MCHC) against Paracetamol induced *Labeo rohita* fingerling.

### Materials and Methods

Fingerlings of *Labeo rohita* (Average weight  $4.70 \pm 1.10$  g) were procured from Fish farm, Thittai, Thanjavur District, Tamil Nadu, India, using cast net and maintained in the laboratory in a glass aquarium tank and acclimated in aerated tap water with continuous aeration for two weeks prior to experimentation.

### Collection of *Hylocereus undatus* (Dragon fruit)

The *Hylocereus undatus* or white-flesh with red peel dragon fruit purchased in Nilgiris fruit shop, Thanjavur, Tamil Nadu. The collected fruits were washed in water, cleaned well to remove all traces of insects, dust and other kinds of pollution. Cut off the crown of the dragon fruit and collected the white flesh. The collected flesh was used it in the next experiments.

### Experimental design

The fishes were divided into four groups (one control and three experimental groups) of ten individuals each and introduced into the trough containing dechlorinated tap water. All the experimental fishes were administered orally with the respective materials: group I (control) with 0.09% saline, group II with a single dose of Paracetamol (500 mg/kg body weight), group III with Paracetamol (500 mg/kg body weight)+ *Dragon fruits* (250 mg/kg body weight) and group IV with *Dragon fruits* (250 mg/kg body weight) alone. *Dragon fruits* were administered after 30 min of Paracetamol administration. Paracetamol was first dissolved in water at 70°C and then cooled to 37°C before administration. The fishes were killed at 24 h by decapitation. Blood samples were collected with anticoagulant and prepared for hematological analysis. A duplicate was run simultaneously and the pooled samples analysed.

## Results and Discussion

Table I represents the Hematological profile Hb, WBC, RBC, PCV, MCV, MCH and MCHC in blood of normal and experimental rats. The content of Hb, WBC and RBC count in blood of normal and experimental fishes were investigated. Group II Paracetamol treated fishes showed a significant decreased Hb content and RBC count while increased WBC count when compared to Group I fish. Group III and IV Paracetamol intoxicated fishes treated with *Dragon fruits* and standard silymarin significantly restored

Hb content, RBC and WBC count when compared to group II.

The hematological parameters as PCV, MCV, MCH and MCHC in blood of normal and experimental fishes were investigated. Group II Paracetamol treated fishes showed a significant decreased PCV, MCH and MCHC count while increased MCV when compared to Group I fish. Group III and IV Paracetamol intoxicated fishes treated with *Dragon fruits* and standard silymarin significantly restored PCV, MCV, MCH and MCHC when compared to group II.

**Table 1:** Effect on hematological changes in dragon fruit (*Hylocereus undatus*) extract against paracetamol induced *Labeo rohita* fingerling

Parameters	Group I	Group II	Group III	Group IV	Group V
Hb (g/dl)	4.62±0.10 <sup>a</sup>	2.47±0.06 <sup>b</sup>	4.47±0.17 <sup>a</sup>	4.64±0.07 <sup>a</sup>	4.51±0.09 <sup>a</sup>
RBC (Million/cu. mm)	1.17±0.04 <sup>a</sup>	0.87±0.05 <sup>b</sup>	1.14±0.05 <sup>a</sup>	1.18±0.04 <sup>a</sup>	1.15±0.06 <sup>a</sup>
WBC (cu. mm)	6743.33 ± 35.11 <sup>a</sup>	8146.66 ± 45.09 <sup>b</sup>	6746.66 ± 55.07 <sup>a</sup>	6796.66 ± 61.10 <sup>a</sup>	6760.00 ± 122.88 <sup>a</sup>
PCV (%)	15.37±0.26 <sup>a</sup>	12.30±0.18 <sup>b</sup>	15.38±0.12 <sup>a</sup>	15.54±0.08 <sup>a</sup>	15.48±0.20 <sup>a</sup>
MCV (Cu. micron)	131.12±3.66 <sup>a</sup>	141.89±11.20 <sup>a</sup>	134.82±7.56 <sup>a</sup>	131.14±4.88 <sup>a</sup>	134.89±8.40 <sup>a</sup>
MCH (Pictograms)	39.39±0.97 <sup>a</sup>	28.44±1.17 <sup>b</sup>	39.10±0.45 <sup>a</sup>	39.16±1.55 <sup>a</sup>	39.28±2.04 <sup>a</sup>
MCHC (%)	30.04±0.18 <sup>a</sup>	20.08±0.79 <sup>b</sup>	29.05±1.34 <sup>a</sup>	29.86±0.52 <sup>a</sup>	29.13±0.53 <sup>a</sup>

Values are expressed as Mean ± SD for three experiments (each group 10 fish). Data were analyzed by one-way ANOVA followed by post-hoc DMRT test using SPSS ver. 20. Mean values are within the row followed by different letters Superscript (homogeneous subsets) are statistically significant ( $P < 0.05$ ) and same letter was statistically non-significant ( $P > 0.05$ ) from each other groups, significant level alpha 0.05. Group I, Group III, Group IV and Group V were non-significant compare with Group II Negative control (Paracetamol only 500mg).

Group I: Normal control; Group II: Negative control (Paracetamol only); Group III: Negative + Dragon Fruit extract 250mg; Group IV: Dragon Fruit extract 250mg only; Group V: Negative + Silymarin 50mg.

In the present investigation, Paracetamol exposed to fishes presented a significant decrease in RBC count, haemoglobin, PCV values, MCH and MCHC and an increase WBC over control. These results express a probable condition of anemia, since it is known that many chemical pollutants, including pesticides can induce anemia in fish (Min and Kang, 2008)<sup>[9]</sup>. This anemia may be due to ROS-induced oxidative injury via oxidation of haemoglobin or other cellular components (Bloom and Brandt, 2008)<sup>[10]</sup>. The reduction in RBC count either by haemolysis or erythropoietic disorders and also the reduction in Hb contents by haemopoietic disorders lead to anemic condition in fish. The anemia may affect the general well-being of the fish.

Anaemic condition in fish is a consequence of inductive haemolytic effects on exposure to paracetamol, due its high affinity to membrane phospholipids, which accounts for its lytic activity. In addition paracetamol, with a slightly higher lipophilicity and is known to partition extensively into the phospholipid component of biological membranes. Hence it is suggested, that anemia found in paracetamol exposed fish in the present study is a consequence of an interaction between paracetamol and the erythrocyte membrane. The reduction in RBC, Hb and Hct in test fish can be interpreted as a compensatory response that reduce the oxygen carrying capacity to maintain gas transfers and indicates a change in the water blood barrier for as exchange in the gill lamellae (Jee *et al.*, 2005)<sup>[11]</sup>. The results indicate that paracetamol causes Hematological changes that can interfere in oxygen

uptake, which may jeopardize the animal's overall health. The results are in accordance with earlier reports stated that a significant decrease in RBC's, haemoglobin and packed cell volume of freshwater fish exposed to chemical toxicant (Kannan *et al.*, 2014; Akinrotimi *et al.*, 2012; Velisek *et al.*, 2011)<sup>[12, 13, 14]</sup>.

The increase in WBC is considered as an adaptive mechanism. This may be due to the direct stimulation of the immunological defense mechanism against stress (Henry *et al.*, 1978)<sup>[15]</sup> which may be associated with the tissues damage. Such increase in WBC count may be due to lymphocytosis and immune response in paracetamol exposed fish. Increase in the number of WBC (leucocytes) of treated fish under sub lethal concentrations reflects a general state of toxemia exhibiting impairment of the defense mechanism, and is manifested into leucocytosis to cope with such a situation. Similar results were reported in *Catla catla* (Vani *et al.*, 2012)<sup>[16]</sup>, *Labeo rohita* (Adhikari *et al.*, 2004)<sup>[17]</sup>, *Channa orientalis* (Shinde *et al.*, 2014)<sup>[18]</sup> and *Cyprinus carpio* (Masud and Singh, 2013)<sup>[19]</sup>. Since the leucocyte cells are important cells in the immune system play a major role in the defense mechanism of the fish. Thus increasing or decreasing numbers of leucocyte cells are a normal reaction to a chemical such as paracetamol as in the present investigation. Declining trend in packed cell volume or haematocrit values may be due to impaired oxygen supply to various tissues, resulting in slow metabolic rate and low energy production. The decline in PCV might shrink cell size due to intoxication. Similar results were noted in *Channa punctatus* following chemical toxicant treatment (Saxena and Seth, 2002)<sup>[20]</sup>. Recently, Khatun *et al.* (2014)<sup>[21]</sup> also opined that the decrease in the PCV of *Cyprinus carpio* exposed sub lethal concentration of paracetamol was the result of either rapid oxidation of haemoglobin to methaemoglobin or release of oxygen radical brought about by toxic stress of the pesticide. According to Adedjei, 2009<sup>[22]</sup>, decrease in PCV in the test fish could be ascribed to reduction in erythrocyte lifespan and/or a suppressive effect of the active substance of the pesticide on the erythropoietic tissues, resulting in the failure of erythrocyte production. Increased MCV may be caused due to endosmosis which leads to the passage of solvent from less concentrated solution to more

concentrated one. This results in haemodilution, further increasing the MCV value as suggested by Anandkumar (1994) [23]. As MCH and MCHC are derived from Hb and RBC, any sort of alteration in the levels of Hb and RBC would result in the alteration of MCH and MCHC. Decrease levels of MCH and MCHC recorded in the present investigation were in accordance with the findings of Parma *et al.* (2007) [24] and Atamanalp *et al.* (2002) [25].

On supplementation of *Dragon fruit* to paracetamol treated group restored the hematological parameters. Present study agreement with earlier study by Anjusha *et al.*, (2019) [26]. Ginger and garlic supplemented diet enhanced hematological parameters (Red blood cells, hemoglobin, and white blood cells) of Husohuso at 1% inclusion level (Kanani *et al.*, 2014) [27]. Similarly, rainbow trout (*Oncorhynchus mykiss*) fed with a diet containing 1% powdered ginger rhizome (*Zingiber officinale*) showed a significant increase in immunostimulatory effect by inducing phagocytic activity of leucocytes and it increases the number of red blood cells and hematocrit value compared to the control group (Haghighi *et al.*, 2013) [28].

### Conclusion

The results of the present study concluded that paracetamol treated fish has drastic hematological changes in the fish blood indicated the toxicity of paracetamol. On supplementation of *Dragon fruit* to paracetamol treated group restored the hematological parameters indicated the *Dragon fruit* nullify the toxicity of paracetamol.

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