Protective role of *Cardiospermum halicacabum* against the cypermethrin effect on the haematological parameters of *Cirrhinus mrigala* (Hamilton)

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Abstract

The aim of study was to assess the effect of cypermethrin, on the haematological parameters of *Cirrhinus mrigala* (Hamilton) fish were subjected to haematological investigation. The haematological analysis showed significant reduction in red blood cells (RBCs) count, haemoglobin (Hb) values, packed cell volume (PCV), mean corpuscular haemoglobin (MCH), mean corpuscular haemoglobin concentration (MCHC), and mean corpuscular volume (MCV), while total white blood cells (WBCs) count were significantly increased in the fish *Cirrhinus mrigala, Cardiospermum halicacabum* plant supplementary feed influence to recover the toxic effects.

Key words: *C. mrigala, C. halicacabum*, RBC, WBC, Hb, PCV, MCH, MCV and MCHC.

Introduction

Pesticides are one of the most potentially harmful chemical introduced into the environment, though they have contributed considerably to human welfare, their adverse effects on non target organisms are significant [1]. The contamination of surface water by pesticide used in agriculture is a problem of worldwide importance [2].

Cypermethrin is a synthetic pyrethroid insecticide used to control many pests, such as moth pests of cotton, fruit and vegetable crops, including structural pest control, lands cape maintenance, for residential and garden use. This has resulted in its discharge into the aquatic environment and consequently several laboratory studies have been performed which evidenced that cypermethrin is extremely toxic to fish at very low concentrations [3].

Blood parameters are considered to be pathophysiological indicators of the whole body and are important in diagnosing the structural and functional status of fish exposed to toxicants and medicaments [4]. A number of haematological indices such as haemoglobin (Hb), Red blood cells (RBCs) are used to assess the functional status of the oxygen carrying capacity of the blood stream and have been used as indicator of heavy metal pollution in the aquatic environment [5].

Evaluation of the hemogram involves the determination of the total erythrocyte count (RBC), hematocrit (Ht) Packed cell volume (PCV), haemoglobin concentration (Hb), erythrocyte indices MCV, MCH, MCHC, WBC differential count and the evaluation of stained peripheral blood films.

*Cardispermum halicacabum* linn. (Sapindaceae) is an herbaceous climber found through the plains of India [6]. Ballon wine in English name which annually spread with tendril hooks it is used in ayurveda and folk medicine for the treatment of rheumatism, lumbago, earache and fever [7]. Experimental pharmacological studies have shown analgesic, anti-inflammatory and vasodepressant activates of this plant, [8].

Biological studies are very much essential to substantiate the therapeutic properties of medicinal plant and drugs mentioned in Ayurveda on scientific lines. The plant kingdom represents an extraordinary reservoir of novel molecules. The potential of higher plants, as source for new drug is thus still largely unexplored [9].
Traditional aboriginal knowledge of plants is responsible for the medicine and food used in modern society; we are only beginning to understand the depth of this knowledge and the consequences for society at target. For example, it is hypothesized for that by considering several different mechanisms, traditional cultures have found several sub-taxa (cryptic ethnotaxa) not apparent to a linnean approach based mainly on morphologic characteristics. The multi mechanistic approach is discussed by [10]. There is no much work available in the haematological parameters influence of cypermethrin and also the protective activities of *Cardiospermum* in the fresh water fish. Hence an attempt has been made to investigate the protective effect of *C. halicacabum* against the toxic impact of cypermethrin in the fresh water fish *C. mrigala*.

**Materials and methods**

The fish *Cirrhinus mrigala* of size 8 to 12 cm and 50 to 70g weight were collected from a local fish farm in Pinnaloor, at Navarathna form. Fish collected and acclimatized at 28°C in the large sized aquarium tank disinfected with potassium permanganate and washed thoroughly prior to conduction of fish to prevent the fungal disease for acclimatization in the laboratory condition for 15 days. During laboratory condition fishes were fed with artificial feed, water was renewed on every day to maintain water quality. The LC50 concentration of cypermethrin was noted at 120 hrs. Fishes were exposed in 4 groups. Group-1 fish exposed to tap water Group- 2 fish exposed to cypermethrin Group-3 Fish exposed to cypermethrin along with *Cardiospermum halicacabum* Group-4 Fish exposed to *Cardiospermum halicacabum* alone

**Plant preparation**

Healthy disease free leaves of *Cardiospermum halicacabum* were collected from Villipuram district Nallavur Village in January-2011 and plant was identified. The leaves were washed in running tap water for 10 minutes leafs were dried, aerial parts (1kg) of *Cardiospermum halicacabum* were macerated thrice at room temperature and prepared in powdered condition and equal amount of rice brane mixed well and small amount water added and prepared small pellet for used in treated fish.

**Blood collection**

Blood samples were collected at the end of toxic treatment by severing the caudal peduncle of both the control and experiment fishes, by using EDTA as anticoagulant. Erythrocytes (RBC), leucocytes (WBC) was counted by the method of [11] using haemocytometer. The haemoglobin concentration of blood was estimated by the method of Shalis haemoglobin meter [11], the blood collected was used for the estimation of PCV using in heparin covered microhaematocrit tubes, using haematocrit centrifuge and standards reading devices. The mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCH) and (MCHC) were calculated by following the standard formula [12].

**Statistically analyses**

The data obtained in the present work were expressed as means ± SE, percentage changes and were statistically analyzed using student t-test [13], to compare means of treated for the various haematological parameters studies data against their control ones and the result were considered significant at (P <0.05), (P<0.01) level.

**Results and Discussion**

In the present investigation the fishes were exposed to cypermethrin group-2 when compared to control group-1 the RBC content was decreased gradually, for the period of 24, 48, 72, 96 and 120hrs. The observed value of group-3 alone with *C. halicacabum* exposed fish shows gradually recovered. When compared with group-2. The observed value of group-4 *C. halicacabum* fish when compared to controls, the RBC content increased gradually compared to control group-1. The recorded RBC contents were statistically significant at 5%, 1% levels (Table-1).

The reduction of RBC is mainly due to development of hypoxic condition during the treatment which intern leads to increased in destruction of RBC or decrease in rate of formation of RBC due to non availability of Hb content in cellular medium [14]. The damaging of toxicant on erythrocyte may be secondary, as resulting from a primary action of toxicant on erythroipoietic tissues on which there exists a failure in red cell production and or due to increase in the rate of erythrocyte destruction, [15]. From the recent studies, it is revealed that the RBC count and Hb concentration decreased may depend upon age of animal, stress condition, sex and availability of food in a particular medium [16]. Changes in haematological parameters might have been brought about by cypermethrin as an anemic condition due to decreased synthesis of Hb and RBC number in bone marrow cells. Significant reduction in haemoglobin in experimental animals might be destruction in haemoglobin has been reported by [17].

In the present study, WBC values also increased after exposure of cypermethrin to various concentrations, compared to control. The WBC values group-3 exposed to gradually recover when compared to group-2, the group-4 *C. halicacabum* compared to control group-1 for the period of 120 hrs. The increased WBC contents were statistically significant at 5%, 1% levels (Table-1).

In the present study, Hb and PCV values also decreased after exposure of cypermethrin to various concentrations, compared to control. The Hb and PCV values group-3 exposed to gradually recover when compared to group-2, the group-4 *C. halicacabum* compared to control
Table-1 Variations of RBC, WBC, Hb and PCV content in the C.mrigala exposed to cypermethrin and C. halicacabum for the period of 120 hours

<table>
<thead>
<tr>
<th>RBC ($\times 10^9$/mm$^3$)</th>
<th>24</th>
<th>48</th>
<th>72</th>
<th>96</th>
<th>120</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group-1</td>
<td>1.248 ± 0.031</td>
<td>1.071 ± 0.026</td>
<td>0.980 ± 0.032</td>
<td>0.933 ± 0.028</td>
<td>0.898 ± 0.022</td>
</tr>
<tr>
<td>Group-2</td>
<td>0.986**±0.029</td>
<td>0.828**±0.025</td>
<td>0.735**±0.026</td>
<td>0.705**±0.024</td>
<td>0.571***±0.033</td>
</tr>
<tr>
<td>Group-3</td>
<td>0.695**±0.032</td>
<td>0.758**±0.028</td>
<td>0.883**±0.017</td>
<td>1.038**±0.020</td>
<td>1.061***±0.029</td>
</tr>
<tr>
<td>Group-4</td>
<td>1.310**±0.027</td>
<td>1.428**±0.028</td>
<td>1.554**±0.030</td>
<td>1.600**±0.027</td>
<td>1.622***±0.028</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>WBC ($\times 10^3$/mm$^3$)</th>
<th>24</th>
<th>48</th>
<th>72</th>
<th>96</th>
<th>120</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group-1</td>
<td>1.256 ± 0.025</td>
<td>1.303 ± 0.032</td>
<td>1.355 ± 0.027</td>
<td>1.368 ± 0.031</td>
<td>1.421 ± 0.029</td>
</tr>
<tr>
<td>Group-2</td>
<td>2.103**±0.028</td>
<td>2.221**±0.029</td>
<td>2.263**±0.031</td>
<td>2.280**±0.028</td>
<td>2.318**±0.031</td>
</tr>
<tr>
<td>Group-3</td>
<td>2.170**±0.038</td>
<td>2.121**±0.028</td>
<td>2.071**±0.030</td>
<td>2.040**±0.025</td>
<td>1.986**±0.027</td>
</tr>
<tr>
<td>Group-4</td>
<td>1.326**±0.036</td>
<td>1.480**±0.025</td>
<td>1.496±0.031</td>
<td>1.524±0.031</td>
<td>1.572±0.037</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Hb (g/dL$^2$)</th>
<th>24</th>
<th>48</th>
<th>72</th>
<th>96</th>
<th>120</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group-1</td>
<td>3.345 ± 0.025</td>
<td>3.221 ± 0.027</td>
<td>3.205 ± 0.028</td>
<td>3.071 ± 0.031</td>
<td>2.970 ± 0.028</td>
</tr>
<tr>
<td>Group-2</td>
<td>2.436**±0.028</td>
<td>2.383±0.030</td>
<td>2.261**±0.031</td>
<td>2.150**±0.027</td>
<td>1.971**±0.033</td>
</tr>
<tr>
<td>Group-3</td>
<td>2.345**±0.036</td>
<td>2.460±0.030</td>
<td>2.543**±0.028</td>
<td>2.681±0.039</td>
<td>2.825**±0.058</td>
</tr>
<tr>
<td>Group-4</td>
<td>3.384**±0.029</td>
<td>3.418±0.031</td>
<td>3.496±0.036</td>
<td>3.533±0.032</td>
<td>3.600±0.042</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>PCV (% coc)</th>
<th>24</th>
<th>48</th>
<th>72</th>
<th>96</th>
<th>120</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group-1</td>
<td>30.161 ± 0.331</td>
<td>29.783 ± 0.230</td>
<td>29.460 ± 0.374</td>
<td>28.938 ± 0.429</td>
<td>28.523 ± 0.234</td>
</tr>
<tr>
<td>Group-2</td>
<td>28.870±0.246</td>
<td>27.370±0.348</td>
<td>26.561±0.133</td>
<td>26.105±0.424</td>
<td>25.898±0.488</td>
</tr>
<tr>
<td>Group-3</td>
<td>26.503**±0.397</td>
<td>-12.13</td>
<td>-7.04</td>
<td>-6.47</td>
<td>-1.13</td>
</tr>
<tr>
<td>Group-4</td>
<td>31.250**±0.427</td>
<td>31.965**±0.330</td>
<td>32.603**±0.477</td>
<td>32.907**±0.348</td>
<td>33.796**±0.432</td>
</tr>
</tbody>
</table>

Values are mean ± SE of six replicates, percentage changes and student t- test. Significant at *P<0.05; ** P<0.01 levels and NS- Non significant.

group-1 for the period of 120 hrs. The increased Hb and PCV contents were statistically significant at 5%, 1% levels (Table-1).

The decrease in haematological variables (PCV, Hb and RBC) of the exposed fish may be due to haemolysis of red blood cells by cypermethrin leading to significant decrease in haematocrit value which results in fish anaemia. Similar observations were reported for juvenile C. gariepinus separately treated with Lambdacyhalothrin, Cypermethrin and Deltamethrin pesticides [18]. Reduction in haematological indices may also be due to an appreciable decline in the haematopoesis. Similar reduction in RBC was reported for Cypermethrin treated Labeo rohita [19], and African cat fish (C. gariepinus) treated with diazinon [20]. Pesticides are known to alter the blood parameters of fishes. A significant decrease in RBC, Hb and PCV has been observed earlier in fishes exposed to different pesticides [21].

The decreased in MCV values after exposed of cypermethrin to various concentration, when compared to control group. The observed value of group-3 along with cypermethrin with C. halicacabum exposed to gradually recover when compared to treated group-2, the group-4 slightly decreased compared to control group-1 for the period of 24, 48, 72, 96 and 120 hrs. The recorded of MCV contents were statistically significant at 5%, 1% levels (Table-2).

The increased in MCH and MCHC values after exposed of cypermethrin to various concentration, when compared to control group. The observed value of group-3 along with cypermethrin with C. halicacabum exposed to gradually recover when compared to treated group-2, the group-4 slightly decreased compared to control group-1 for the period of 24, 48, 72, 96 and 120 hrs. The recorded of MCH and MCHC contents were statistically significant at 5%, 1% levels (Table-2).

Blood cell indices like mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCH) and mean corpuscular haemoglobin concentration (MCHC) seem to be changes that are more sensitive and can cause reversible changes in the homeostatic system of fish. Fluctuations in
A similar response was noted in common carp and other freshwater fish exposed to cypermethrin and *M. trigerla* study a significant increase in these indices was noticed in haemoglobin concentration and packed cell volume. In this study, these indices correspond with values of RBC count, MCV, MCH, and MCHC. In our experiments, the haematological parameters neither altered with the different concentrations nor exposure periods, except for the haemoglobin content and MCHC after 120 hours of treatment. Similar results were obtained except for the haemoglobin content and MCHC after exposure to cypermethrin. In common carp and other freshwater fish exposed to acute toxic level of pesticides [22].

<table>
<thead>
<tr>
<th>Table-2 Variations of MCV MCH and MCHC values in the <em>C. mrigala</em> exposed to cypermethrin and <em>C. haliacabum</em> for the period of 120 hours</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group</td>
</tr>
<tr>
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<tr>
<td></td>
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<tr>
<td>Group-1</td>
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<td>Group-2</td>
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<td>Group-3</td>
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<tr>
<td>Group-4</td>
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<td>Group-1</td>
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<td>Group-3</td>
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<td>Group-2</td>
</tr>
<tr>
<td>Group-3</td>
</tr>
<tr>
<td>Group-4</td>
</tr>
</tbody>
</table>

Values are mean ± SE of six replicates, percentage changes and student t-test. Significant at *P*<0.05; **P*<0.01 levels and NS- Non significant.
haematological values seem contradictory [24]. Significant decrease in total erythrocyte count, haemoglobin content, PCV, MCHC have been reported in air breathing fish, Channa punctatus exposed to cadmium [25].

The increase in MCH perhaps is due to toxic substances in the medium causing differences in haemopoietic activity [26]. Have stated that the fluctuation observed in both MCV and MCHC in aluminium exposed fish Oreochromis mossambicus for different periods result in a microcytic hyochromic type after 8 weeks. Similar pattern has been detected in Lebeo umbratus after exposure to various pollutants [27].

The chemical profile of Cardiospermum halicacabum L. is relatively complete; there is some variability in the content of specific chemicals. [28] reported the chemical profile: specified fatty acids 98.8 % of lipids; Oil content 31.60% by weight; Iodine value 71% by weight. However, noticed that leaves contain considerable amounts of saponins, alkaloids, (+)-pinitol, apigenium, luteolin and chrysoeriol. The major cyano lipid (49%) is a diester having two fatty acid moieties esterified with 1-cyano-2-hydroxymethyl-prop-2-ene-1-ol followed by a diester derived from 1-cyano-2-hydroxymethyl-prop-2-ene-3-ol (6%). Of the fatty acids, 11-eicosenoic acid is the major component (42%), other chief components of the oil include oleic acid (22%), arachidic acid (10%), linolenic acid (8%), palmitic acid (3%) and stearic acid (2%) including small proportions (1-2%) of a low-molecular weight acid, and several C22 acids [29]. Other minerals such as Ca (1.30%), K (4.01%), Mg (0.43%), P (0.83%), Organic-N (5.19%), Total-N (7.16%), and C (48.1%) were recorded by [30].

The plant C. halicacabum has been used as anti-inflammatory [8], an antipyretic [31]. Extracts of this plant have been reported to contain different triterpenoids, glycosides, and a range of fatty acids, [32], investigated the antioxidant potency. The multiple antioxidant activity of this plant was evident, as it also possessed reducing power, superoxide scavenging ability, nitric oxide scavenging activity, and also ferrous ion chelating potency. Further research is needed to substantiate these medicinal claims.

Conclusion
From the results obtained, can be concluded that the cypermethrin is toxic to Cirrhus risgala. The 120 hrs LC50 to sub-Lethal concentrations of cypermethrine result in significant haematological alterations. These suggest that the treated fish are faced with a serious metabolic crisis. The elevated values of RBC, WBC, Hb, PCV, MCV, MCH, and MCHC by the fish. Haematological level were recovered control of plant C. halicacabum can percent of slow down the haematological changes induced in fish C. mirigala.

Reference


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