Anthocyanin–Rich Red Dye of *Hibiscus sabdariffa* L. Calyx Modulates CdCl$_2$-Induced Hypochromic Microcytic Anaemia and Oxidative Stress in Rat Red Blood Cells

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**Abstract**

Protective efficacy of anthocyanin–rich dye of *Hibiscus sabdariffa* L. were studied against cadmium chloride (CdCl$_2$)-induced hypochromic microcytic anaemia and oxidative stress in rat blood cells. Male albino rats were pretreated with red dye of HS [250 mg/kg] daily for one week, after seven days these animals were injected with CdCl$_2$ (4 mg/kg) intraperitoneally (i.p.) 6 h after the last treatment and blood samples were collected for the evaluation of haematological parameters, oxidative stress, red blood cells counts, haematocrit, haemoglobin concentrations and other blood indices (MCV, MCH, MCHC), lipid peroxidation level (MDA) and reduced glutathione content (GSH). The current results showed that CdCl$_2$ significantly ($P < 0.05$) decreased the blood picture compared to control group, whereas the level of oxidative marker malondialdehyde (MDA) was significantly ($P < 0.05$) elevated in rat erythrocytes. Supplementation of anthocyanin–rich red dye of HS counteracts the toxic effect on cadmium chloride on haematological pictures and oxidative stress parameters. The results of the present study indicated that anthocyanin of HS possess hematopoietic potential and prevent oxidative stress.

**Keywords**: Roselle Anthocyanin, CdCl$_2$, Hematopoietic, Oxidative Stress Markers

1. **Introduction**

Cadmium is one of the most dangerous environmental and occupational toxins. It is found in drinking water in food and atmospheric air due to its use in industry (Buchet et al, 1990). Cadmium is reported to be very toxic to biological system. The erythrocytes, liver, kidney, brain, and testes are considered, to the most susceptible in the case of exposure to cadmium become this heavy metal accumulate in these cells of these organs (Jarup et al, 1998).

Free radicals are evolved at the early stages of Cd-intoxication (Gutterdige et al, 1995 and Sarker et al, 1995). *Hibiscus sabdariffa* L. is a member of the malvaceae family, which is a perennial plant native to Africa and Asia. It has different ethnomedical properties. Its calyx is red coloured and sour to the taste. *H. sabdariffa* L. has been used extensively in traditional medicine in Arab countries, China, Turkey and Nigeria to treat different diseases such diabetes ulcer and jaundice (Yesilada et al, 1995). It has been reported to be anticancer (Duke, 1985), anti-inflammatory (Daffallah & Mustafa, 1996), anti mutagenic (Farombi & Fakoya, 2005) and hypolipidemic effects have been reported (Hirunpanich et al, 2006). Other studied properties of flower extract of *H. sabdariffa* include cytotoxicity and genotoxicity (Rosa et al, 2007), significant immunoprotective (Okoko & Freb, 2012) and antibacterial activities (Al-Hashim, 2012).

The flower extract of *H. sabdariffa* are known to be rich in polyphenol, flavonoids, anthocyanin, protocatechuic acid and vitamin C. Anthocyanins, which found to possess wide
biological actions especially potent free radical scavenging and antioxidant (Al-Ismail et al, 2006 and Oluso la et al, 2012). Moreover, Shwagfeha & Al-Kubaisy (2013) found that anthocyanins were proved to be an effective hepatoprotective agent against CdCl₂ induced liver injury (Liu et al, 2006).

It was also reported that anthocyanins exhibited significant protection against acetaminophen-induced oxidative damage of rat liver (Liu et al, 2010). Ademiluyi et al (2013) showed that anthocyanins of H. sabdariffa modulates cisplatin, induced nephrotoxicity and oxidative stress in rat.


Nevertheless, the aim of this study was to determine the effects of flower anthocyanins of HSE on CdCl₂-induced hypochromic microcytic anemia and oxidative stress in rat blood cells.

2. Material and Method
2.1. Preparation of Aqueous Flower Extract

Dried flowers of H. sabdariffa L. (Roselle) was obtained from a local herbal store in Amman, Jordan. The sample of plants specimen was identified by a Botanist from biological sciences –University of Jordan-Amman. The dried flowers were round into fine powder using an electrical dry mill. A total of 100 g of the round powder was soaked in 500 ml distilled water for 24 hours at 40 °C. The mixture was filtered with Whatman filter paper No. 1. The filtrate was dried at 40 °C. temperature.

The yield of HSE was around 20%. Previous phytochemical investigation of flowers; this plant shows the presence of anthocyanin, phenolic compounds, flavonoids protocatechuic acid and vitamin C. (Al-Ismail et al, 2006 and Ademiluyi et al, 2013).

Appropriate concentration of the HSE was then subsequently made by dilution with distilled water into 250 mg/kg body weight and administered to the animals; this concentration was more effective than higher concentrations, which was well comparable with standard drug silymarin (20 mg/kg) according to Sunilson et al (2008).

2.2. Experimental Animals

A total of 18 healthy adult, make albino rats weighing between (160-180g) obtained from animal house university of applied sciences – condition (Temperature 25±1) with a 12/12 h. light/dark cycle. All rats were allowed free access to food and water ad libitum. The animals were divided randomly into 3 groups of six rats each as follows:

- Group I: It makes control group receiving normal saline as placebo.
- Group II: Rats were administered CdCl₂ 4 mg/kg body weight of HSE for one week and subcutaneously.
- Group III: Rats were treated with 250 mg/kg body weight of HSE for one week and subsequently exposed to a single injection of CdCl₂ for 12 hours after the last HSE-treatment.

All experimental animals were handled according to the guidelines of the institution animal ethical committee. All chemicals used were of analytical grade, purchased locally in Amman, Jordan.

2.3. Blood Collection and Analyses

At the end of the experimental period, animals were subjected to ether anaesthesia and blood was collected by cardiac puncture, into two sets:

One set the blood was transferred into EDTA anticoagulant tubes and allowed to clot and centrifuged at 3000 g for 10 minutes and plasma was removed by Pasteur pipette. Then the erythrocytes were washed three times with normal saline and were haemolysed by addition of Tris-HCl buffer (pH 7.2). Haemoglobin (Hb) values of the samples were measured by a counter i.e. Haematology analyzer.

The samples were kept in -60 °C until biochemical determinations.

The other set, the whole blood collected into EDTA tube, were used for to determine haematological indices using a fully automated coulter counter.

2.4. Biochemical Assay

* Malondialdehyde (MDA) determination: RBCs-MDA was determined as a measure of lipid peroxidation according to Stocks & Dormandy's method (1971).
* Total reduced glutathione (GSH) determination: GSH was determined in erythrocytes according to Beutler et al (1963) method.

2.5. Statistical Analysis

The results obtained from this study were analysed by one-way analysis of variance (ANOVA). The results are expressed as means ± SD of 6 animals/group. The level of significance taken as p < 0.05.
3. Results and Discussion

The results of this study are shown in Tables 1 and 2.

Table 1. The Effect of the Anthocyanin of H. Sabdariffa on Hematological Parameters in Rats after CdCl₂ Administration

<table>
<thead>
<tr>
<th>Groups</th>
<th>RBcs (10¹³/mm³)</th>
<th>Hb (g/dL)</th>
<th>PCV(%)</th>
<th>MCV (%)</th>
<th>MCHC</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>6.3 ± 1.4</td>
<td>14.5 ± 0.7</td>
<td>42.8 ± 0.80</td>
<td>53 ± 0.9</td>
<td>29.5 ± 0.6</td>
</tr>
<tr>
<td>CdCl₂</td>
<td>4.1 ± 1.8</td>
<td>11.2 ± 0.5</td>
<td>36.7 ± 1.33</td>
<td>44 ± 0.8</td>
<td>22.3 ± 0.4</td>
</tr>
<tr>
<td>Cd + HSE</td>
<td>5.2 ± 1.8</td>
<td>13.0 ± 0.4</td>
<td>39.2 ± 1.52</td>
<td>91 ± 0.9</td>
<td>24.1 ± 0.7</td>
</tr>
<tr>
<td>P. Value</td>
<td>Significant</td>
<td>Significant</td>
<td>Significant</td>
<td>Significant</td>
<td>Significant</td>
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</table>

The data are presented as mean ± SD, n = 6. p. value = < 0.05 compared Group, with Group 2 and Group 3 with Group 2

Table 1 shows the effect of CdCl₂ on haematological parameters clinical variables in exposed rats. Administration of anthocyanin of flower H. sabdariffa provides significantly protection to these altered clinical variables. The red blood counts, haematocrit, haemoglobin concentration and other blood indices improved significantly (p < 0.05).

Table 2 indicates toxic effects on some blood oxidative stress variables upon CdCl₂ exposure and pretreatment with anthocyanin. We found a significant elevation in the oxidative stress marker malondialdehyde (MDA) and decrease in total glutathione (GSH) content in erythrocytes after CdCl₂ exposure. Supplementation of anthocyanin (HS) resulted in a significant recovery in the lipid peroxidation (LPO).

Table 2. Effect of HSE Supplementation 250 mg/kg Body Weight on Oxidative Stress Parameters (MDA & GSH) of Blood Rats Intoxicated with CdCl₂

<table>
<thead>
<tr>
<th>Groups</th>
<th>MDA (nmol/g Hb)</th>
<th>GSH (nmol/g Hb)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>9.8 ± 4.2</td>
<td>452 ± 38.3</td>
</tr>
<tr>
<td>CdCl₂</td>
<td>367.3 ± 23.81*</td>
<td>367 ± 41.7*</td>
</tr>
<tr>
<td>CdCl₂ + HSE</td>
<td>249.2 ± 14.45*</td>
<td>408 ± 36.6*</td>
</tr>
</tbody>
</table>

Results are given as means ± SD for six rats. Statistical significance taken as P < 0.05. In comparison between the normal group and tested groups 1 and 2

CdCl₂ intoxication imposes deleterious effect on the total glutathione in erythrocytes as evidenced by a significant depletion suggesting impaired antioxidant defence system and inhibited reduced glutathione showed significant protection pretreatment with anthocyanin. The results of this study showed, that administration of anthocyanin (HS) at dose 250 mg/ kg significantly reversed the blood picture and oxidative stress markers to their near normal.

Recently, extensive application of medicinal plants has been reported to be employed in folk medicine in the treatment of anaemia (Dina et al, 2006). Haematological analysis of plant extract in animals is one of the important methods of assessing the toxicity of plant extract (Ashafa et al, 2009). It is possible that the extract contain constituent that can stimulate the hematopoietic systems (Ita et al, 2007; Aka et al, 2009; Odesanm & Lawal, 2010; Kolawoles et al, 2011 and Al-Kubaisy et al, 2012).

Cadmium (Cd) is one of the most dangerous environmental and occupational toxins due to its use in industry (Buchet et al, 1990). Cd is reported to be very toxic to biological systems. The erythrocytes, liver, kidney and brain are considered to the most susceptible when exposure to Cd (Jarup et al, 1998). Cd is associated with significant toxicity due to over production of reactive oxygen species (ROS) resulting in increased levels of oxidative stress (OS) (Gutterdige et al, 1995 and Sarker et al, 1995).

In the present study the rat treated with CdCl₂ showed significant changes in the values of blood picture (Table 1). This may have resulted from the action of CdCl₂ which affected the erythropoiesis in the bone marrow.

The determination of haematological indices provides physiological information on a proper blood assessment. Anaemic condition is characterised by a decrease in the level of circulating haemoglobin less than 12g/dl (Table 1). The data in Table 2 indicates that the experimental CdCl₂ treated rats, a significant elevation in the oxidative stress marker malondialdehyde (MDA) level and decrease in total glutathione (GSH) content in erythrocytes after CdCl₂ exposure was noted when compared with the control group.

Phytochemical analysis of Hibiscus flowers products, revealed the presence of four major flavonoids, phenolic acids. Vitamin C and anthocyanins which possess potent free radical scavenging and antioxidant properties (Tseng et al, 1997; Lin et al, 2011 and Usoh, 2011). The potential of the flower extract of the HS may be related to its antioxidant activity and free radical scavenging. In our routine laboratory studies, we found that aqueous extract of H. sabdariffa flowers exhibited the protective effect on liver function in rats. Torell et al (1986) Factino et al (1990) and Shwagfeha & Al-Kubaisy (2013) reported that flavonoids inhibit peroxidation of polyunsaturated fatty acids in cell membranes, and the formation of superoxide ions and hyd-
roxyl radicals, which are two strong peroxidation agents. HSE inhibited the MDA production in CdCl₂ treated erythrocytes in rats. These observations are consistent with those reported on lipid peroxidation of rat hepatocytes and human erythrocytes respectively (Tseng et al, 1997 and Suboh et al, 2004).

Oxidative stress is a major cause of Cd induced toxicity (Dong et al, 1998) found that toxic effect of CdCl₂ stimulates inflammatory cytokines in hepatocytes through an oxidative stress mechanism. On other hand CdCl₂ declines glutathione and protein bound sulphhydryl groups leading to increased lipid peroxidation and enhanced intracellular oxidized states (Koyu et al, 2006). HSE showed protective effects against Cd induced toxicity in rat erythrocytes. In this study, we confirmed the protection of flower extract of H. sabdariffa against Cd induced oxidative damage in rat erythrocytes. This may be due to its antioxidant and free radical scavenging character and may possibly serve as an acceptable blood booster in anaemic condition. This study confirmed the improvement in haematological indices. In this study, we confirmed the protection of flower extract of H. sabdariffa against Cd induced oxidative damage in rat erythrocytes. This may be due to its antioxidant and free radical scavenging character and may possibly serve as an acceptable blood booster in anaemic condition. This study confirmed the improvement in haematological indices.

The action is assumed to be a direct effect of the HSE on the hematopoietic systems. It can be possible that HSE contains constituents that can interact and stimulate the formation of erythropoietin and hematopoietic growth factors committed stem cells. Stimulations of these factors have been reported to enhance rapid synthesis of blood cells (Murry, 2000). Moreover, the hematopoietic potential of HSE may be related to its antioxidant activity. This anti-oxidant activity may protect the hematopoietic systems and formation of blood cells from the attack of the reactive free radicals in the body of rats. The ability of anthocyanins to scavenge free radicals has been demonstrated in other studies i.e. Gabrielska, et al (1999) and Noda, et al (2002) as well. These results indicate that HSE has a protective action against CdCl₂ and suggest that HSE may find clinical application against a variety of toxins where cellular damage is consequence of reactive oxygen species (ROS).

4. Conclusion

Results of the present study indicated that Hibiscus sabdariffa as possess hematopoietic potential and prevent oxidative stress of red blood cells which is properly due at least partly to its antioxidant properties, scavenging CdCl₂ associated free radicals.

Acknowledgement

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References


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