THERAPEUTIC EFFECT OF THE URTICA DIOICA LEAVES ON ADENINE-INDUCE CHRONIC RENAL FAILURE IN MALE RATS

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ABSTRACT

Anemia is a common feature of chronic kidney disease (CKD). It is a process related to shortened erythrocyte survival, uremic erythropoiesis inhibitors, erythropoietin deficiency, and disordered iron homeostasis. Therefore, the study was conducted to investigate protective and therapeutic effect of Urtica dioica leaves on anemia induced by renal failure in adult male rats, and evaluated the effects on some complete blood count, kidney function test (creatinine, urea) and other hormones such as free erythrocyte protoporphyrine (FEP), hepcidin parameters. Forty-five adult male rats used in the present study and divided into five groups (9/group), first group (G1) consider as control group gave Dimethyl sulfoxide (DMSO) intraperitoneal for 4 weeks, the second group (G2) gave Urtica dioica leaves powder (4% mg/kg bw) for 4 weeks with diet, while the third group (G3) gave adenine (100 mg/kg) intraperitoneal for 4 weeks, the fourth group (G4) adenine is administering interaperitoneally 100mg/kg,bw for 4 weeks for induction of renal failure and then give Urtica dioica leaves powder at dose (4% mg/kg bw) for 3 weeks with diet and the fifth group (G5) received adenine interaperitoneally100 mg/kg,bw for 4 weeks for induction of renal failure + Urtica dioica leaves powder at dose 4% bw. for 3 weeks with diet. In the present study when treated by adenine group were determined that the CBC count profile results showed significance decrease (p≤0.01) in RBCs count and PCV (%) w the results showed significance (p≤0.01) increase in RBCs count, Hb concentration and PCV (%) in the group of Urtica dioica showed significance enhancement in the most of parameters which studied to resumption near to the control group.

Keywords: Urtica dioica, anemia, adenine–induced chronic renal failure.

I. INTRODUCTION

In patients with CKD, anemia is usually normocytic, normochromic, and hypoproliferative. The identification of a circulating substance that stimulates erythropoiesis, as well as the kidney as the primary source of erythropoietin (EPO) in patients with CKD, EPO insufficiency is the most common cause of anemia (1). Anemia due to iron deficiency (IDA) is a serious health issue that affects people all over the world (2). Due to relative EPO insufficiency, uremic-induced inhibitors of erythropoiesis, and shorter erythrocytes, CKD anemia is a complex process. Iron homeostasis is disrupted, resulting in survival. Hepcidin has recently been discovered. It is excess as a major cause to anemia and disturbed iron homeostasis. CKD impairs iron absorption from the diet and the mobilization of iron from the body’s reserves. The formation of red blood cells in the bone marrow is dependent on iron and EPO. Hepcidin, a liver hormone that affects dietary iron absorption and macrophage iron recycling from senescent red blood cells, regulates iron availability hepcidin levels are controlled by numerous feedback loops, including iron and EPO. Hepcidin levels have been observed to be considerably raised in CKD patients (especially in end-stage kidney disease patients on hemodialysis), possibly due to impaired renal clearance and induction by inflammation, resulting in iron-restricted erythropoiesis. CKD also stops the kidneys from producing EPO, which can lead to anemia (3). Free erythrocyte protoporphyrin (FEP), one of the outcomes of iron deficiency is an increase in erythrocyte protoporphyrin (EP) levels. The ideas basis for the measurement quantification measurement of protoporphyrin is a reduction of iron in the bone marrow for integration into newly synthesized globin and the protein porphyrin as the haemoglobin molecule is arriving its definitive steps in synthesis (4). Increased EP concentrations, as well as the ratio of EP to haemoglobin (Hb), are good indicators of a lack of iron to meet the normal demands of the bone marrow (5). Urtica dioica (UD) leaves belongs to the family Urticaceae commonly known as ‘stinging nettle’. It is an annual plant, the leaf of the UD has a long history as an herbal
medicine and a nutrient-dense food (6). *Urtica dioica* is widely utilized by the traditional medicinal practitioners for treating different pharmacological properties like blood purifier, diuretic, nasal and menstrual haemorrhage, rheumatism, eczema, anaemia, antibacterial, nephritis, antioxidant, analgesic, anti-inflammatory (7). Aims of study the present study was undertaken to observe and test the preventive and therapy effects from the medicinal herbs *Urtica dioica* leaves in anemia of adult rat’s males that induction of renal failure.

II. MATERIALS AND METHODS:

The study was conducted on 45 adult males' rats and these rats were classified into five groups to study each group separately, the rats’ ages ranged between (2-3 months) and their weights ranged between (150-200) gm per body weight, the experiment lasted about during 7 weeks the rats were administration as the following groups, the first group, which include control negative group 9 rats, theses rats was given only Dimethyl sulfoxide (DMSO) by interaperitoneally for 4 weeks, while the second group the control positive also contained 9 rats, theses rats was given the *Urtica dioica* leaves powder at dose 4% mg/kg bw. for 4 weeks with diet, the third group, which also consisted of 9 rats, adenine is administrating interaperitoneally 100mg/kg-bw for 4 weeks for induction of renal failure, the fourth group, which also consisted of 9 rats, adenine is administrating interaperitoneally 100mg/kg.bw for 4 weeks for induction of renal failure and then give *Urtica dioica* leaves powder at dose 4% bw. 3 weeks with diet, and the remainder of the 9 rats fifth group, adenine is administrating interaperitoneally100 mg/kg.bw for 4 weeks for induction of renal failure + *Urtica dioica* leaves powder at dose 4% bw. for 4 weeks with diet.

Blood samples were collected from all rats, and samples were coded to avoid the possibility of bias. Experimental animals (rats) get anaesthetized by putting them in covered jar include cotton rinsed with chloroform to be sedated for the next step which is blood via cardiac puncture in sterile syringes by needle prick in the heart draining 5ml of blood carefully, then separation of the blood collected into 1 ml drained in EDTA tube for the analysis of iron homeostasis tests quickly separate the blood in the centrifuge at 3500 rpm in 15 minutes and then set at Eppendorf tube, while the rest of the blood drained into two separated parts; about 2ml set in gel tube it is left about half hour at room temperature for properly agglutinated, then it would be separated at centrifuge at 3000 rpm for fifteen minutes to get the serum apart in Eppendorf tube, both of samples are hold in freezer at -20°C, while the remaining of the blood drained into EDTA-tube for hematological test

Preparation of *Urtica dioica* leaves powder. The ready-made dried *Urtica dioica* leaves were obtained from Bensina center and then the samples were then grained by the electric grinder into powder 0.04 of *Urtica dioica* leave powder was mixed with the usual nutrition ration of crushed feed diet mixed with tap water then made in shape of cookies then bake in the oven for 15 minutes 180°C.

III. PARAMETERS OF STUDY

**Blood Count Test**

Blood samples were collected with EDTA, and blood counts were performed using ABX Micros ABC Vet® equipment.

**Iron Homeostasis**

Iron concentration in serum was determined by using also, the mind ray device was used to determine the percentage of iron, and the instructions of the producing company were followed in the method of examination.

**Estimation of rate Free erythrocyte Protoporphyrin (FEP)**

We used ELISA Kit. This examination was done by preparing process from Biocellular Company (China) by using a kit specific to measures rapidly and reliably free erythrocyte porphyrmins in a blood sample.

**Estimation of Rat Serum Hepcidin**

Hepcidin ELISA Kit, this examination was done by preparing process from Biocellular Company (China) by using enzyme-linked immunosorbent assay method to determine the concentrations hepcidin in rat serum (9).

**Statistical Analysis**

Data were analyzed using the software package SPSS version 24.00 where one way (ANOVA) was used to assess the significant changes between the groups’ results. The data were expressed as mean ± standard Error (SE) (10).
IV. RESULTS

Effect of adenine and *Urtica dioica* leaves on some blood parameters of male rats

The reduction (p≤0.01) in RBCs count were recorded in adult male rats group three (3.145±0.501) was injected by adenine in comparison with other groups (Table 1). While, the RBCs count showed a significant increased (6.312±0.822) in the value of adult male rats treated by *Urtica dioica* leaves in comparison with control group (7.598±0.34), it was evident from (Table 1).

Adenine group (4.35±1.48) had a lowest Hb concentration as compared with the control group. There were no significant differences observed in Hb values in group two, four and five respectively when compare with control group.

The PCV (%) was showed account significant decrease (p≤0.01) in adenine group compared with the control group, but the treatment group showed significant increase in PCV account of group two treated by *Urtica dioica* leaves approximately reach near the normal value of control group (Table 1).

Table 1. Effect of adenine alone and in combination with *Urtica dioica* leaves on some blood parameters in male rats (Mean±SE).

<table>
<thead>
<tr>
<th>Groups</th>
<th>Parameters</th>
<th>GI Negative Control</th>
<th>GIH positive Control+ <em>Urtica dioica</em> L.</th>
<th>GIH Adenine</th>
<th>GIH Adenine + <em>Urtica dioica</em> L.</th>
<th>GV Adenine + <em>Urtica dioica</em> L.</th>
<th>Level of Significance</th>
</tr>
</thead>
<tbody>
<tr>
<td>RBCs</td>
<td>Cell(10¹²/l)</td>
<td>7.598 ± 0.34</td>
<td>6.312 ±0.822</td>
<td>3.145 ± 0.501 B</td>
<td>5.385 ± 0.602 B</td>
<td>4.97 ± 0.411 B</td>
<td>0.01</td>
</tr>
<tr>
<td>Hb</td>
<td>mg/dl</td>
<td>14.15 ±0.71</td>
<td>12.56 ± 2.18</td>
<td>4.35 ± 1.48 C</td>
<td>11.78 ± 1.73 B</td>
<td>10.88 ± 0.59 AB</td>
<td>0.01</td>
</tr>
<tr>
<td>PCV</td>
<td>%</td>
<td>50.33 ± 0.83</td>
<td>46.13 ± 2.34</td>
<td>35.27 ± 1.801 B</td>
<td>40.33 ± 0.714 B</td>
<td>35.418 ± 3.77 B</td>
<td>0.01</td>
</tr>
</tbody>
</table>

-Values = Mean±SE -Different letters represent significant (p≤0.05) differences between groups. -Number of rats in each group = 9

Effect of adenine alone and in combination with *Urtica dioica* leaves on iron homeostasis parameters in male rats with induced CRD

Free Erythrocyte Protoporphyrine (FEP)

The present study table 2 was exhibited a higher significant differences in free erythrocyte protoporphyrine in adenine treaded group (6.74±0.214) as compare with other groups. Moreover, the free erythrocyte protoporphyrine lacked to significance among two, four and five groups as compared to the control group (Table 2).

Serum Iron

A significant (p≤0.02) reduction in table (2) of serum iron was demonstrated in adenine treaded group (134.98±54.15) comparing to the other groups. Also the same table revealed a significant elevation in the group treated adenine plus *Urtica dioica* leaves (group four) comparing to adenine group but not significant as compared to the control group.

Hepcidin

Higher significant differences in adenine treated group as compared with other groups (Table 2). Adenine + *Urtica dioica* leaves (group four) lead to the reduction of the value of hepcidin but that not significant differences was showed to value of control group.
Table 2. Effect of adenine alone and in combination with *Urtica dioica* leaves on iron homeostasis parameters in male rats with induced CRD

<table>
<thead>
<tr>
<th></th>
<th>GI Negative Control</th>
<th>GII Adenine</th>
<th>GIII Urtica dioica L.</th>
<th>GIV Adenine + Urtica dioica L.</th>
<th>GV Adenine + Urtica dioica L.</th>
<th>Level of Significance</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>FEP (mg/dl)</strong></td>
<td>2.109±0.103</td>
<td>1.701±0.813</td>
<td>6.74±0.214</td>
<td>1.57±0.921</td>
<td>1.77±0.24</td>
<td>0.86</td>
</tr>
<tr>
<td><strong>Fe (mg/dl)</strong></td>
<td>354.5±61.51</td>
<td>241.17±80.13</td>
<td>134.98±54.15</td>
<td>308±68.81</td>
<td>215.01±127.13</td>
<td>0.02</td>
</tr>
<tr>
<td><strong>Hepcidin</strong></td>
<td>12.37±3.39</td>
<td>13.66±1.93</td>
<td>16.03±5.11</td>
<td>12.35±5.31</td>
<td>14.66±2.31</td>
<td>0.022</td>
</tr>
</tbody>
</table>

-Values = Mean±SE -Different letters represent significant (p≤0.05) differences between groups. –Number of rats in each group = 9

V. **DISCUSSION**

Chronic kidney disease (CKD) negatively affects the health status of the patient (11). According to the Global Burden of Disease Study, CKD was the 12th leading cause of mortality worldwide in 2015. (12,13). As a result, the current study looked into the potential therapeutic benefits of UD on CKD damage in rats. Anemia control targets were determined using the Kidney Disease Improving Global Outcomes (KDIGO) recommendations. Therefore, animal models are of considerable importance to investigate and find targets and new pharmacological approaches (14).

**Effect of adenine and *Urtica dioica* L. on some blood parameters of male rats**

The reduction (p≤0.01) on some blood parameters were recorded in adult male rats group three was injected by adenine in comparison with other groups (Table 1), in red blood cells count (RBCs), hemoglobin (Hb) levels and packed cell volume (PCV). The red blood cell indices, reticulocyte count (new red blood cell formation), and iron parameters are contributory to detect the cause of several anemias which are not due to EPO deficiency (15). Several confirmations in patients with CKD, was noted with a low concentration of hemoglobin (16,17). Adenine produces lipid peroxidation in experimental animals after administration. These reactive free radicals initiate cell damage through the mechanisms of covalent binding and lipid peroxidation (18). Increased ROS production occurs in inflammation, during radiation, or during metabolism of hormones, drugs, and environmental toxins (19). This might be one of the reasons for decreased RBC count, PCV, and Hb level. Anemia occurs in individuals with acute and chronic renal failure. It is traditionally defined as a drop in haemoglobin concentration in the blood this result agrees with (20,21,22,23). Another reason might be the erythropoietin level. The formation of RBCs (erythropoiesis) is controlled primarily by the kidney and liver (24). It has been suggested that the ability to secrete erythropoietin in response to anemia is defective in many patients (25). The reduction in percentage of packed cells volume (PCV), agree with the study that showed between 1994 and 1997, a survey of 200,000 patients participating in a health maintenance organization found that percent of patients with chronic renal disease had hematocrit levels below 30 percent (26). Also the result of study agreement with (27). In this study, we demonstrated that UD treatments increased the rising RBC, Hb value, and PCV. This result indicated that UD treatments might ameliorate the anemia and some mineral disturbances and increase the body’s defense mechanism in adenine-treated rats. The fresh leaves contain high concentrations of vitamins A, C, D, E, F, K and P, as well as of vitamin B-complexes (28), that may aid in the improvement of anemia in particular, as well as a general element that may be related to similar situations. In rats treated with CCl4, (29) found that *Urtica dioica* treatments might decrease the effects of Chemokine (C-C motif) ligand 4 (CCl4)-induced anemia, minerals, and the body's defensive mechanism. The results of (30) led us to conclude that nettle probably neutralizes Cisplatin induced damage hepatotoxicity, nephrotoxicity, and hepatic oxidative stress through its effects and antioxidant-free radical-defusing. These characteristics make nettle’s idea for a variety of uses, including functional foods, dietary supplements, and pharmaceutical formulations. Yet, following UD administration, we can find a definite result that indicates an increase in RBC and Hb count with a little increase in PVC count in the blood.
Effect of adenine alone and in combination with *Urtica dioica* leaves on iron homeostasis parameters in male rats with induced CRF

Free Erythrocyte Protoporphyrine (FEP)

The present study table 2 was exhibited a higher significant differences in free erythrocyte protoporphyrine in adenine treaded group (6.74±0.214) as compare with other groups.

A research was conducted to determine the sensitivity of iron status markers. The prevalence of iron deficiency was much greater when the EP concentration was used to assess the prevalence than when the ferritin concentration was utilized as the only indication of iron deficiency, according to the researchers. Reticulocyte production in response to iron treatment was associated to iron stores in the bone marrow, although ferritin concentration, EP, and the serum transferrin receptor (sTfR) to ferritin ratio all showed a substantial predictive value in distinguishing iron deficiency anemia (IDA) from non-IDA. (31) found that EP can be used to assess the degree of reduced iron transport to the marrow in a limited sample of patients with chronic inflammatory diseases. The bone marrow response is blocked or diminished by inflammatory or renal disease (32). The nature, intensity, and duration of this inflammatory response appear to be contributing factors in the lack of consensus on the use of detecting EP concentration in clinically complicated settings. Because it cannot discriminate between the many causes of a shortage of iron in the bone marrow, the EP concentration does not offer a precise indication of iron insufficiency. In situations when iron shortage occurs along with inflammatory diseases and infection, a field experiment was recently undertaken to see if EP, sTfR, or ferritin concentrations were better predictors of iron status (33).

Since EP is not sensitive to acute inflammation and it is not time consuming or expensive to measure the concentration, there is some strong appeal for its use in screening people. Mei and colleagues (34) recently conducted a study that looked at the sensitivity and specificity of Hb and EP concentrations in children and adult women for diagnosing iron deficiency. Meanwhile, in the *Urtica dioica*-treated adenine-induced CRF group four, FEP levels are higher see in table 2. Numerous studies have proved *Urtica dioica*‘s beneficial properties over the world. Understanding the mechanisms that underpin positive effects can lead to a new therapeutic approaches.

**Serum Iron**

A significant (p≤0.02) reduction in table (2) of serum iron was demonstrated in adenine treaded group comparing to the other groups. Iron is critical for Hb synthesis. Consequently, patients should be carefully evaluated for the availability of iron, by measuring the serum iron. The serum iron reflects the amount of iron immediately available for hemoglobin synthesis.

Iron stores in the body range from 800 to 1,200 mg (Council on Food and Nutrition, 1968). If the original Hct is reduced by 25% and the target Hct is raised to 35%, the amount of additional iron necessary is required. Both iron and erythropoietin are required for effective erythropoiesis. Anemia occurs when CKD patients do not have enough of one or both of these substances. In absolute iron deficiency, functional iron deficiency occurs when the quantity of iron required to maintain hemoglobin production exceeds the amount available from iron stores (reticuloendothelial cells). In the presence of appropriate iron stores, this situation, which can be produced by pharmacological stimulation of erythropoiesis by erythropoietin, might occur. Iron is required for several critical physiological activities, including oxygen transport, cellular respiration, and DNA synthesis, due to its capacity to donate and absorb electrons. Excess iron, on the other hand, is harmful because it produces free radicals, which can damage or kill cells. As a result, systemic and cellular iron levels must be closely monitored. So several CKD patients have functional iron deficiency, which is defined as impaired iron release from body stores that is insufficient to meet the demand for erythropoiesis (also called reticuloendothelial cell iron blockade). These individuals have low serum transferrin saturation (a measure of circulating iron) and normal or high serum ferritin (a marker of body iron stores). Based on existing evidence, supplementary iron treatment should be given to patient with chronic disease anemia and absolute iron deficiency (35-36). Phytochemical researches showed the existence of several beneficial chemical compounds such as minerals (especially iron), manganese, potassium, and calcium, and vitamins, including pro-vitamin A and vitamin C (37-38-39).

**Hepcidin**

Higher significant differences in adenine treated group as compared with other groups (Table 2). Increased hepcidin levels are linked to low ferroportin expression on duodenal enterocytes and macrophages, as well as impaired dietary iron absorption and iron retention in macrophages (40) resulting in decreased iron delivery for
erythropoiesis in animal models of AI and patients with inflammatory diseases (41). The endocrine regulating role of hepcidin in iron balance was addressed (42) in a recent review, during acute and chronic inflammation, proinflammatory cytokines like interleukin-6 raise hepcidin levels, according to their study. This results in iron-restricted erythropoiesis caused by inflammatory anemia. In addition to anemia, particularly in people with iron-loading anemias or chronic anemia: hepcidin levels are low, the inhibition of, or control is lost. *Urtica dioica* leaves may help in improvement of the results. This finding suggested that UD therapies might help adenine-treated rats with anemia and mineral imbalances, as well as boost their defensive mechanisms.

**REFERENCES**


