Evaluation The Iron Homeostasis and Erythropoietic Activity in Male Rats with Anemia Related with Induced Chronic Renal Failure

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Abstract

The present study conduct to evaluation the iron homeostasis and erythropoietic activity in male rats with anemia related with induced chronic renal failure. Sixty adult male rats were divided into four groups, the first group administrate with DMSO as a control negative, while second group administrate DMSO and then moringa leaf powder, third group adenine were administrated only and fourth group adenine then given moringaoleifera leaf powder the experiment were lasted for forty-four days. At the end of the study , blood was collected via the heart puncyure and measured the following biomarkers (Urea , creatinine , BBC count ,Erythropoietinerythoferrone ,, ferritin, Serum iron and hepcidin).The results revealed that the rats treated adenine caused significant increase in (creatinine ,urea ,ferritin and hepcidin concentration), one the other hand there was significantly decrease in RBC count ,PVC and HB in adenine group in addition to Erythropoietin,erythoferrone and serum iron levels , however combined adenine with moringa results in return these biochemical criteria to their normal levels in control group .

Keywords: *moringaoleifera ,Erythroferrone, renal failure, Adenine*

Introduction

Anemia commonly occurs in people with chronic kidney disease (CKD)—the permanent, partial loss of kidney function. Healthy kidneys produce a hormone called erythropoietin (EPO). A hormone is a chemical produced by the body and released into the blood to help trigger or regulate particular body functions prompts the bone marrow to make red blood cells Which then carry oxygen throughout the body. [1] ,When kidneys are diseased or damaged, they do not make enough EPO. As a result, the bone marrow makes fewer red blood cells, causing anemia. When blood has fewer red blood cells, it deprives the body of the oxygen it needs [2] Erythroferrone is a hormone that control iron metabolism through its actions on hepcidin [3] it is produced in erythroblasts, which proliferate when new red cells are synthesized. This process is governed by the renal hormone, erythropoietin [4] The mechanism of action of Erythroferrone is to inhibit the expression of the liver hormone, hepcidin. By suppressing this, ERFE increases the function of the cellular iron export channel (Ferroportin), This then results in increased iron absorption from the intestine and mobilization of iron from stores**,** [5] which can then be used in the synthesis of hemoglobin in new red blood cells. Moringa have variously biological activities such as reducing hyperglycemia, anti-inflammatory, anti-diabetic, antimicrobial, anticancer and antioxidant. in fact, it is believed in that the Moringa has many benefits based on its nutrition. The ratio of grams per gram, Moringa leaves dry powder contains 25 times more iron than spinach [6], in which iron is one of the therapeutic agent for anemia which can compensate for the loss of the hemoglobin [7] The leaves are the most nutritious part of the plant, being a significant source of B vitamins, vitamin C, provitamin A as beta-carotene, vitamin K, manganese, and protein. [8] The present study was designed to investigated the rule of Erythroferrone (ERFE)in iron homeostasis and erythropoietic activity in anemia related with induced

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chronic renal failure in male rats

Materials and Methods

Preparation of MoringaOleifera leaves powder

Fresh moringaoleifera leaves are collected from Alkafeel Nurseries Group Washed and dried at room temperature then grained by the electric grinder into powder. (9) ,5% of moringaoleifera leave powder were mixed with the usual nutrition ration of crushed feed diet (10)

Chemicals:

Adenine was obtained from Sigma Aldrich. all ELIZA kit like, (Erythropoietin erythoferrone, Ferritin, Serum iron and hepcidin) obtain from (Bio cellular Company) (china), Urea and creatinine obtain from (SPECTRUM company) (Egypt) measured by spectrophotometer.

Experimental design

Sixty male rats(Rattus norvegicus) aged (2 -3month) weighting (170-200) gm were divided randomly into four groups (15/group)

First group (GI) control negative were administrate with DMSO by interaperitoneally for 4 weeks. Second group (GII) is the control positive were administrating with DMSO by interaperitoneally for 4 weeks and then moringa leaf powder given at dose rang 5% for 2 weeks with diet [10]. While rats included in third group (GIII) adenine were administrated interaperitoneally 100mg/kg.bw for 4 weeks for induction of renal failure [11] .finally the fourth group (GIV) adenine were administrated interaperitoneally 100mg/kg.bw for 4 weeks for induction of renal failure [11] and the given moringaoleifera leaf powder at dose 5% for 2 weeks with diet [10]

At the end of the study, blood was collected via the heart puncture and measured the following biomarkers (Urea, creatinine, BBC count, Erythropoietin, erythoferrone ,

, Ferritin, Serum iron and hepcidin). these parameters were measured according to the

instruction of the manufacture company by using ELIZA

Results

Table (1) Effect of MoringaOleifera Leaves powder on Some Serum Kidney Function Tests in Male rats with Induced CRF

groups / parameters	GI Control negative	GII Control positive + moringa leaves	GIII Adenine	GIV Adenine+ Moringa
Urea Mg/dl	45.00±2.921 C	55.50 ±3.403 BC	99.68±6.135 A	69.20±5.75 B
Creatinine Mg/dl	0.45±0.084 B	0.60± 0.057 B	2.56±0.209 A	0.86±0.091 B

- Mean ±SE(n=6 rat / group)

-Different letters represent significant($p \leq 0.05$) difference between groups

The results in table (1) clarified there were a significant ($p \leq 0.05$) elevation in the serum urea in (GIII) adenine treated group in comparison with other groups

On the other hand, combined adenine with moringa (GIV) in the same table showed a significant ($p \leq 0.05$) reduction comparing to (GIII) as compared to control group (GI)

Also Table (1) illustrated there were statistically significant ($p \leq 0.05$) increment in serum creatinine in

adenine treated group (GIII) as compare to other groups

As the same table showed combined adenine plus moringa (GIV) caused a significant ($p \leq 0.05$) decrement of serum creatinine comparing to (GIII)

Table (2) effect of MoringaOleifera leaves powder on Complete Blood Count in male rats with Induced CRF

Parameters groups	RBC Count (cell*1012/ l)	PCV %	Hb (gm/dl)
Control	7.43±0.43 B	40.83±0.82 AB	15.25±0.45 A
Moringa	8.49±0.25 A	42.66±0.66 A	15.72±0.41 A
Adenine	5.69±0.23 C	35.66±0.33 C	9.06±0.34 C
Adenine +moringa	7.29 ±0.32 B	39.50±0.67 B	13.65±0.64 B

Mean ± ES (n=6 rat / group)

-Different letters represent significant ($p \leq 0.05$) difference between groups

The results showed significant ($p \leq 0.05$) decrease in RBC count, PVC and HB in adenine group (GIII) in comparison with the other groups. Table (2) Also combined moringa with adenine (GIV) caused significant ($p \leq 0.05$) increase in RBC count, PVC and HB comparing to (GIII)

Table (3) Effect of MoringaOleifera leaves powder on Iron Homeostasis Parameters in male rats with induced CRF

Groups / Parameters	GI Control negative	GII Control positive + moringa leaves	GIII Adenine	GIV Adenine+ Moringa
Erythropoietin ng/ml	9.88 ±0.302 A	11.07±0.602 A	4.10 ±0.367 C	7.50 ±0.439 B
erythoferrone ng/ml	19.66±.1.516 A	18.04±1.593 AB	10.23±0.426 C	15.52±1.025 B
Ferttin ng/ml	6.60 ±0.174 B	6.80 ±1.133 B	9.98 ±0.837 A	7.49 ±.0183 B
Serum iron µmol/L	8.19 ±0.090 A	9.59 ±0.264 A	6.41 ±0.495 B	8.26 ±0.454 A
hepcidin ng/ml	19.25 ±0.456 B	19.60±0.622 B	23.09±1.101 A	19.64±1.363 B

-Mean ± ES (n=6 rat / group)

-Different letters represent significant ($p \leq 0.05$) difference between groups

There were statistically significant ($p \leq 0.05$) decrease of serum erythropoietin in adenine treated group (GIII) in comparison with the other treated groups. Table (3) Also combined moringa with adenine (GIV) ameliorate the serum erythropoietin. The serum erythoferrone showed a significant decrease ($p \leq 0.05$) in adenine treated group (GIII) as compared to other groups. The results also revealed that rat treated adenine plus moringa (GIV) caused significant ($p \leq 0.05$) increase comparing to (GIII). Depending on the results clarifies in table (3) there were a significant ($p \leq 0.05$) elevation in serum ferritin in adenine treated group (GIII) comparing to the other groups, Also the combine adenine with moringa group (GIV) caused a significant ($p \geq 0.05$) decrease comparing to (GIII). A significant ($p \leq 0.05$) reduction in table (3) of serum iron in (GIII) adenine treaded group (GIII) comparing to the other groups, also there was revealed a significant ($p \leq 0.05$) elevation in the group treated adenine plus moringa (GIV) Comparing to (GIII). Table (3) illustrated there were a significant ($p \leq 0.05$) increment in this parameter in adenine treated group (GIII) Comparing to other groups, Combined adenine with moringa (GIV) in the same table caused significant ($p \leq 0.05$) decrement of serum hepcidin comparing to (GIII) group,

Discussion

The results in table (1) clarified there were a significant ($p \leq 0.05$) elevation in the serum urea in (GIII) adenine treated group in comparison with other groups. This study was agreeing with result conducted by Ikizler, et al. [12]

Urea nitrogenous compounds usually released from the blood by the kidney into the form of urine, renal dysfunction results in accumulation of urea in blood circulation as it is obvious in chronic renal failure conditions in high levels. [13]

On the other hand, combined adenine with moringa (GIV) in the same table showed a significant ($p \leq 0.05$) reduction comparing to (GIII) as compared to control group (GI).moringaoleifera leaves administration works on develop of the results that show the fall in urea concentrations better than before a significant decrease in serum urea in group (GIV) combined adenine with moringa in comparison with adenine group . [14].

Also Table (1) illustrated there were statistically significant ($p \leq 0.05$) increment in serum creatinine in adenine treated group (GIII) as compare to other groups ,this our data agrees with results of [15] ,Creatinine is a product released by muscles from the degradation of a compound named creatine. Creatinine is cleared from the body by the kidneys, which filter high levels of it from the blood and cleared it into the urine(16).As the same table showed combined adenine plus moringa (GIV) caused a significant ($p \leq 0.05$) decrement of serum creatinine comparing to (GIII) ,that the disturbance of plasma electrolyte imbalance may influence the pH, osmolality, and blood volume which may lead to renal dysfunction or anther body disturbances .*M. oleifera* has appear to own diuretic effect (17). Table (2) , this agree with (18)Which occur Through dialysis HB concentrations are decreased to critical levels ,that results in anemia , [19] happen during anemia condition in patients with acute and chronic renal failure results revealed reduction in RBC count and HB levels and [20] It is occur due to pathogenesis of renal anemia . Also combined moringa with adenine (GIV) caused significant ($p \leq 0.05$) increase in RBC count , PVC and HB comparing to (GIII), after moringaoleifera leaves administration we can see clear result that describes by increasing in RBC count value with little extent for PVC count and HB count in (GIV) group moringaoleifera is rich with high nutritional supplements such as (iron ,calcium and vitamins) which is one of the essential component in synthesis of hemoglobin in red blood cells [21]

There were statistically significant ($p \leq 0.05$) decrease of serum erythropoietin in adenine treated group (GIII) in comparison with the other treated groups .Table (3) The present study agree with results conducted by [22]

Generally cases with kidney disease will posses anemia which occur in early in the course of kidney disease and grow worse as kidneys fail and can no longer make EPO. [23] .Also combined moringa with adenine (GIV) ameliorate the serum erythropoietin ,moringaoleifera may assist on keeping further homeostatic balance by support Erythropoietin to fasten its effects by linking to the erythropoietin receptor EpoR [24]

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The serum erythoferrone showed a significant decrease ($p \leq 0.05$)in adenine treated group (GIII) as compared to other groups, the results of present study agree with result of [25] since one of the most essential element of red blood cell manufacture is hemoglobin that is obtained from iron stores in the body [26] ,yet anemia occur because erythoferrone release is restricted and become in low concentrations that because of hepcidin hormone release from it which stimulate the absorption of iron from alimentary track [27]. The results also revealed that rat treated adenine plus moringa (GIV) caused significant ($p \leq 0.05$) increase comparing to (GIII), moringaoleifera leaves provide a natural source for iron (28) with addition to other supplements calcium, minerals, vitamins which assist on maintenance of biological homeostasis for iron insufficiency. Depending on the results clarifies in table (3) there were a significant ($p \leq 0.05$) elevation in serum ferritin in adenine treated group (GIII) comparing to the other groups, these results agree with [29] In fact chronic renal failure resulting in disturbances in hormonal released from kidney which is later may effect on erythropoiesis ,when erythropoietin is the chief regulator of the red blood cells synthesis so as a result their would be reduction in this process yet anemia occur [30] . Also the combine adenine with moringa group (GIV) caused a significant ($p \geq 0.05$) decrease comparing to (GIII),this due to it may diminish before been used by the target cells whither it execrated with waste materials or not fully absorbed from alimentary tract or other related conditions [31].

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Conflict of Interest: None to declare.

Ethical Clearance: All experimental protocols were approved under the University of Baghdad and all experiments were carried out in accordance with approved guidelines.

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