

## Benefic Interactive Effects between Garlic Consumption and Serum Iron Excess

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### Abstract

Hemochromatosis is the most common form of iron overload disease. In case of pathological serum iron increase, kidney is the first organ directly affected. Moreover, anemia is the major symptom of most cases of kidney failure and oxidative stress is thought to be a significant factor in the pathogenesis of iron-overload disease. Garlic, was recently demonstrated as inhibitor of intestinal iron absorption. The purpose of this study was to show that crude garlic consumption in case of serum iron increase could prevents biochemical and histological kidney perturbations and ameliorates hematological parameters. For this, four groups of young rats were treated for forty five days: control group C, iron overload group I, garlic overload group G and both garlic and iron overload group IG. In iron treated rats (group I) all haematological parameters showed a decrease. Moreover, kidneys showed a significant increase in protein and malondialdehyde (MDA) concentrations associated to total antioxidant capacity important decrease and deep histological changes. For group G rats, we had found a significant decrease in red blood cells and hemoglobin concentrations and in the kidney an important increase in total antioxidant capacity and in creatinine levels. After association between iron and garlic consumption (group IG) we had found positive interactive effects and important regulation of all modified parameters. In conclusion, for a great part of its effects, garlic protects from iron increase problems and could have important clinical relevance in case of hemochromatosis disease.

**Keywords:** Kidney; Iron excess; Anemia; Garlic; Total antioxidant capacity; MDA

### Introduction

Iron, the most abundant transition metal in the body, is required by all mammalian cells for growth and survival. Iron overload has been shown to result in several structural and functional changes in various tissues of patients with primary or secondary increased iron load [1-3]. The toxic effect of iron is mediated mainly by reactive oxygen species (ROS) which formation is catalyzed by iron in the Haber-Weiss reaction and which cause inflammatory response reflected in the kidney among other organs. In fact, Zager [4] studied the potential nephrotoxic effect of iron compounds given intravenously in vitro experiments on isolated mouse proximal segments or cultured proximal tubular cells and he found variable cytotoxicity of these substances. Oxidative stress is thought to be a significant factor in the pathogenesis of iron-overload disease. Iron catalyzed lipid peroxidation which promotes the formation of highly reactive aldehydes, such as malondialdehyde (MDA) in several organs and particularly in the kidney [5]. Reactive oxygen species (ROS) form covalent links to proteins, phospholipids, and DNA which cause considerable kidney tissue damage [6].

Recent trends in controlling and treating oxidative stress tend to favor natural antioxidant compounds rather than synthetic ones [7]. The human diet, which contains large number of natural compounds, is essential in protecting the body against the development of diseases, and garlic (*Allium sativum*) is one of the well known plant with remarkable hypolipidemic [8], hypoglycemic [9], anti-atherosclerotic

[10], antioxidant [11] and anti-carcinogenic [12] properties. Garlic is a commonly worldwide used food. In mediterranean cooking, is regularly consumed at various doses both crude and cooked, and its potential medical properties have been recognized for thousands of years [13]. Garlic in different forms has antioxidant properties. These properties are shown to be due to the existence of compounds such as water soluble organosulfure compounds, S-allylcysteine, and lipid soluble compounds like diallyl sulfide [7, 12].

Many studies had demonstrated that iron intoxication induced severe kidney damage which progress to failure [14-15]. Moreover, it has been approved that secondary hemochromatosis causes sever aplastic anemia and the complete hematopoietic recovery is possible after continuous iron chelation therapy [16]. Other studies have improved that the consumption of garlic gives renal protection and accelerates red blood cell turnover and splenic erythropoietic gene expression [17,18]. However, there are no previous investigations dealing with the curative garlic effects in case of iron overload, kidney failure and hematological disorders. In this study we state the hypothesis that crude garlic consumption has positive interactive effects with iron excess which could protects kidney and hematological parameters from iron increase.

### Materials and methods

#### Animals

According to the European convention for the protection of vertebrate animals used for experimental and other scientific purposes (Council of Europe No. 12, Strasbourg, 1985) and according to the

review committee of our institution, rats rearing and experiments of this work were approved. In the present study, Suisse strain male rats, aged 2 months were purchased from the Central Pharmacy (SIPHAT, Tunisia). They were housed at 22 ± 3°C with light/dark periods of 12 h

and a minimum relative humidity of 40%. They had free access to water and commercial diet (SICO, Sfax, Tunisia). The standard diet contained 73.44 mg of iron by gram of diet.

Parameters and Treatments	C	I	G	IG
Initial body weight	181 ± 6	183 ± 4	181 ± 9	185 ± 8
Final body weight (g)	230 ± 10	263 ± 16***	233 ± 18	254 ± 6 * +++xx
Food consumption (g/day/rat)	8.3 ± 0.6	3.7 ± 0.3 ***	7.8 ± 0.4	4.4 ± 0.5 *** +++xxx
Water consumption (ml/day/rat)	2.8 ± 0.4	1.7 ± 0.5***	3.1 ± 0.8	2.1 ± 0.6 *** +++xxx
Ingested iron (g/day/rat)	0.59 ± 0.08	3.18 ± 0.78***	0.62 ± 0.12	3.27 ± 0.46 ***
Ingested garlic (g/day/rat)	-	-	0.39 ± 0.09	0.22 ± 0.02

Treated I, G and IG vs controls C: \*: p ≤ 0.05, \*\*\*: p ≤ 0.001; Treated IG vs treated I: ++: p ≤ 0.01, +++: p ≤ 0.001; Treated IG vs treated G: XX: p ≤ 0.01, XXX: p ≤ 0.001

**Table 1:** Body weight (n=10), Daily food (n=30), water (n=30), iron and garlic intake (n=30) by 2 months aged rats: controls (group C), treated by FeCl<sub>2</sub> (group I), by garlic (group G) or by both FeCl<sub>2</sub> and garlic (group IG) for 45 days.

### Experimental protocol

The initial number of young rats (weighing~180g) was 40, equally divided into four groups of ten individuals each one: controls (group C; n=10), overload with FeCl<sub>2</sub> at a dose of 150 mg/100ml of drinking water (group I; n=10), overload by garlic at a dose of 5g/100g of dampen standard diet (group G; n=10) and overload with FeCl<sub>2</sub> and garlic respectively at doses of 150mg/100ml of drinking water and 5g/

100g of dampen standard diet (group IG; n=10). Sacrifice of all groups' rats was done 45 days after beginning treatments. The daily consumed food, water and supplemented iron and garlic quantities' were precisely measured during all the treatment period (Table 1). In this study, the used doses of iron and garlic were precisely determined after a serious of experiments in which we have obtained the most important effects.

Parameters and Treatments	C	I	G	IG
RBC	7.77±0.46	7.25±0.20*	6.91±0.07**	7.36±0.59
Hb	1.51±0.28	1.51±0.15*	1.61±0.54*	1.07±0.51
HCT	40.63±0.47	3.01±0.07**	40.08±.16	4.71±.03+x
T Bil	0.55±0.03	0.78±0.18*	0.64±0.03*	0.44±0.15+
Iron	1.71 ± 0.08	1.93 ± 0.17*	.66±0.08	1.58 ± 0.05+

Number of determinations (n=10 ); Treated I, G and IG groups versus controls C: \*: P ≤ 0.05, \*\*: P ≤ 0.01; Treated IG versus treated I: +: P ≤ 0.05; Treated IG versus treated G: x: P ≤ 0.05

**Table 2:** Red blood cells (RBC) (1012/L), hemoglobin (Hb) (g/L), hematocrite (HCT) (L/L), total bilirubin (T Bil) (µmol/l) and iron serum levels (µmol/L) of young rats: controls (group C), treated by FeCl<sub>2</sub> (group I), treated by garlic (group G) and by both FeCl<sub>2</sub> and garlic (group IG) for a period of 45 days.

### Samples extraction

After anaesthesia with chloral hydrate by intraabdominal way, kidneys (twenty/group) were carefully dissected out for weight, biochemical, oxidative stress and histological analysis. All blood samples were withdrawn from the brachial artery of young rats. Some parts of them were collected on EDTA treated tube for haematological parameters determination using a Coulter Maxem machine and the rest others were centrifuged at 2200 g for 15 min. All kidneys and serum samples were kept at -80°C until analysis.

### Biochemical and histological studies

**Serum biochemical analysis:** Serum samples were collected for iron and total bilirubin analysis using Hitachi 912 kits analyser. Commercial kits from Roche laboratories were respectively used for iron (ref: 11970747 216) and total bilirubin (ref: 11877976 190) analysis. Serum creatinin, urea and acid uric determination was realized using photometric method.

**Kidney cytosol extraction:** Kidneys (ten/group) were utilized for cytosol extraction. Cells fraction was realized after adding 10 ml of KCl (1.15%) to 1g of kidney by using ultra-turrax at 4°C temperature.

**ABTS assay in kidney cytosol samples:** The Trolox equivalent antioxidant capacity (TEAC) assay is measuring the reduction of the ABTS radical cation by antioxidants. ABTS radical cation (ABTS<sup>+</sup>) was produced by reacting 7mM ABTS stock solution with 140 mM potassium persulfate and allowing the mixture to stand in the dark at room temperature for 12-16 h before use. For the study, ABTS<sup>+</sup> solution was diluted with ethanol to an absorbance of 0.70 ( $\pm 0.02$ ) at 734 nm. After addition of 1ml of diluted ABTS<sup>+</sup> solution to 50 $\mu$ l of kidney cytosol, or Trolox standard, the reaction mixture was incubated for 2 min in a glass cuvette at 30°C. The decrease in absorbance was recorded at 734 nm. All measurements were performed in triplicate. The free radical scavenging capacity of the biological sample, calculated as inhibition percentage of ABTS<sup>+</sup>, was equated against a Trolox standard curve prepared with different concentrations (40-200  $\mu$ mol/l). The results are expressed as mM of Trolox equivalents.

**Kidney TBARS determination:** As a marker of lipid peroxidation, the TBARS (thiobarbituric acid-reactive substances) concentrations were measured in kidney homogenates using the method of Park and his collaborators [19]. For this, 200 ml of a 10% (w/v) tissue homogenate solution was mixed with 600 ml of distilled H<sub>2</sub>O and 200 ml of 8.1% (w/v) SDS, vortexed, and incubated for 5 min at room temperature. The reaction mixture was heated at 95°C for 1 h after the addition of 1.5ml of 20% acetic acid (pH 3.5) and 1.5ml of 0.8% (w/v) TBA. After cooling the reaction, 1ml of distilled water and 5ml of butanol:pyridine (15:1) solution were added and vortexed. The mixture was centrifuged at 1935 $\times$ g for 15min and the resulting colored layer was measured at 532 nm using malondialdehyde (MDA) made by the hydrolysis of 3-tetramethoxypropane as standard.

**Bradford kidney cytosol analysis:** For protein determination samples were brought to a volume of 800  $\mu$ l with water. Next, 200  $\mu$ l of Bradford reagent (Bio-Rad laboratories catalog number 500-0006) was added to each sample to bring it to a volume of 1 ml. The samples were then analyzed in a Beckman spectrophotometer to determine their absorbance at 595 nm. Kidney protein concentrations were calculated using a standard serum albumin bovine (BSA) curve prepared with different concentrations (0-20  $\mu$ g/ml).

**Kidney histopathological analysis:** Three kidneys were randomly selected from each group for light microscopy. They were taken and immediately fixed in a Bouin solution, embedded in paraffin and serially sectioned at 5  $\mu$ m. Then, the sections were stained with hematoxylin eosin (HE) for routine histological examination, with PAS for glycoprotein revelation and with Perls' Prussian blue for iron sedimentation.

**Statistical analysis:** Comparisons of mean values between rats treated groups (I and IG) and control group (C) or between treated rats (group IG) and (group I). Statistical differences were calculated using a one-way analysis of variance (ANOVA) using SPSS13 logiciel, followed by Student's t-test. Statistical significance was defined as a P value of less than 0.05. Values were expressed as the means followed by ecartype.

## Results

### Growth and feeding

**Body growth rate variation:** Animals used for all groups have, at the beginning of the experience; no body weights significant differences (Table 1). During the studied period a regular increase in body growth rate was noted. However, the body growth rates of groups I and IG

were more important than that of controls (Table 1). Indeed, at the sacrifice day, we have obtained respectively a significant increase by  $12.5 \pm 0.06$  % and  $6 \pm 0.6$  % in group I and IG rats' body weights comparatively to controls (Table 1). For group G, no difference was obtained comparatively to control rats.

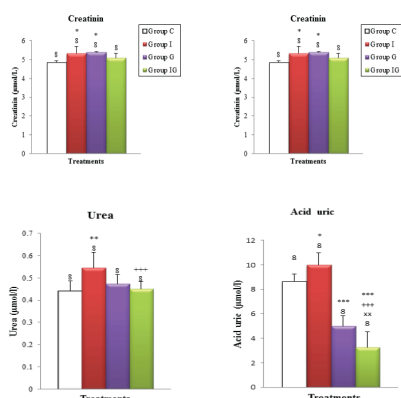
**Food and water consumption:** Food and water consumptions were decreased in group I and IG rats. In fact, we have obtained a decrease in food consumption respectively by  $55 \pm 0.2$  % and  $48 \pm 0.12$  % and in water by  $39 \pm 0.22$  % and  $23 \pm 0.14$  % (Table 1). For group G, food and water consumptions were at the same order of magnitude comparatively to controls (Table 1).

**Haematological parameters:** For group I, all haematological parameters showed a decrease comparatively to control group C. Indeed, red blood cells, haemoglobin and hematocrite significantly decreased by  $5 \pm 0.02$ ;  $6 \pm 0.08$  and  $19 \pm 1.02$ % respectively. In rats of group G, we had found a decrease by  $11 \pm 0.12$  and  $7 \pm 0.08$ % in red blood cells number and in haemoglobin levels in comparison to control rats and a partial recovery of hematocrite (Table 2). After both iron and garlic treatment (group IG), a partial recovery, without reaching control levels, in red blood cells and haemoglobin was noted and an increase by  $6 \pm 0.06$  and  $23 \pm 0.12$  % in hematocrite comparatively to group I and group G (Table 2).

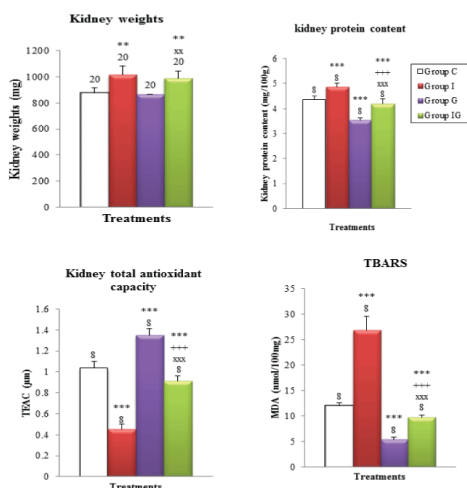
**Serum biochemical parameters:** After iron treatment, group I rats showed a significant increase by  $11 \pm 0.09$ %;  $33 \pm 0.13$  %  $10 \pm 0.12$ % and  $19 \pm 0.20$ % in serum iron, total bilirubin, creatinine and urea levels, respectively (Table , Figure 1). After garlic treatment and comparatively to control group, serum iron total bilirubin and urea levels showed no changes but creatinine increased by  $11 \pm 0.13$ %. However, for group IG these parameters reached those of control rats (Table, Figure 1). Acid uric, in group I increased by  $13 \pm 0.13$  and in G and IG groups, decreased by 23;  $42 \pm 0.68$  and  $68 \pm 0.15$ %, respectively, comparatively to control group C (Figure 1).

**Kidney weights:** We have obtained an increase in kidney weights by  $14 \pm 0.12$  and  $11.5 \pm 0.08$ % of treated rats of groups I and IG respectively comparatively to control group C. For rats treated by iron and garlic (group IG), kidney weights were  $26.5 \pm 0.05$ % less than those of group I rats and  $13 \pm 0.16$ % superior than those of group G. Group G kidney weights, show no difference comparatively to control ones' (Figure 2).

**Kidney biochemical analysis:** In kidneys of rats of group I, a significant increase by  $10 \pm 0.10$  and  $55 \pm 0.07$ % of protein and MDA concentrations was obtained but total antioxidant capacity showed an important decrease by  $56 \pm 0.11$ % comparatively to control group C (Figure 2). After garlic treatment (group G) we had obtained a decrease ( $19 \pm 1.67$  and  $54 \pm 0.85$ %) in protein and MDA concentrations and an increase ( $24 \pm 0.29$ %) in total antioxidant capacity comparatively to controls. For rats of group IG, protein and MDA kidney concentrations decreased by  $4 \pm 0.05$ ;  $19 \pm 0.08$ % and by  $14 \pm 0.03$  and  $64 \pm 0.05$ % comparatively to controls and group I, respectively and increased by  $15 \pm 0.09$  and  $44 \pm 0.45$ % comparatively to group G rats. However, kidneys total antioxidant capacity significantly increased by  $51 \pm 0.09$ % comparatively to group I without reaching that of control group C and decreased comparatively to group G by  $33 \pm 0.75$ % (Figure 2).

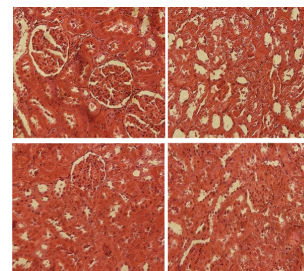


**Figure 1:** Creatinin ( $\mu\text{mol/l}$ ), urea ( $\mu\text{mol/l}$ ), and acid uric ( $\mu\text{mol/l}$ ) serum contents of young rats: controls (group C) and treated for a period of 45 days by  $\text{FeCl}_2$  (group I), by garlic (group G) or by both  $\text{FeCl}_2$  and garlic (group IG). Treated I, G and IG vs controls C: \*:  $P \leq 0.05$ ; \*\*:  $P \leq 0.01$ ; \*\*\*:  $P \leq 0.001$ , Treated IG vs treated I: +++:  $P \leq 0.01$ , Treated IG vs treated G: xx:  $P \leq 0.01$



**Figure 2:** Kidney weights (mg), protein content (mg/100g), TEAC ( $\mu\text{mol}$ ) and TBARS (nmol/l) kidney contents of young rats: controls (group C) and treated for a period of 45 days by  $\text{FeCl}_2$  (group I), by garlic (group G) or by both  $\text{FeCl}_2$  and garlic (group IG). Treated I, G and IG vs controls C: \*\*:  $P \leq 0.01$ ; \*\*\*:  $P \leq 0.001$ , Treated IG vs treated I: +++:  $P \leq 0.01$ , Treated IG vs treated G: xx:  $P \leq 0.01$ ; xxx:  $P \leq 0.001$

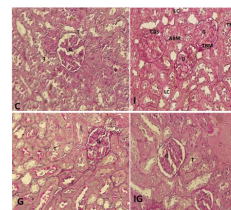
**Kidney histology:** After hematoxylin-eosine and PAS staining, kidney glomeruli tissue group C rats showed normal cellularity, cell nuclei are not clustered or overlapping. The tubules are almost back to back and the tubular basement membranes are almost touching with a very little interstitium in the cortex (Figures 3C and 4C). Whereas, in iron treated group, we had observed some shrunken proximal tubules and hydropic epithelial cell damages (Figure 3I). Some tubules and glomeruli are collapsed (Figure 3I); others are surrounded by thickened tubular and glomeruli basement membranes (Figure 4I).



**Figure 3:** Haematoxyline eosin kidney-stained sections of young rats: controls (C) and treated for a period of 45 days by  $\text{FeCl}_2$  (group I), by garlic (group G) or by both  $\text{FeCl}_2$  and garlic (group IG). G: glomeruli, T: tubules, SPT: shrunken proximal tubules, CT: collapsed tubules, CG: collapsed glomeruli, LC: Large cavities, CBS: closed bowman space, (Gx400)

Note the laminated appearance of some tubular basement membrane segments as well as the abrupt attenuation of others in the same tubule (Figure 4I). Distal tubules delimited large clear cavities (Figure 3I, 4I). Glomeruli sizes, were little than those observed in control group with closed bowman space (Figures 3I and 4I). For rats of group G and IG, hematoxylin-eosine and PAS staining kidney sections showed the same histological aspect of controls ones' (Figures 3G; 3IG and 4G; 4IG).

The Perls' Prussian blue kidney stained sections of iron treated rats (group I) showed iron deposition in tubules comparatively to control and garlic treated groups (Figures 5C and 5I). For groups G and IG no iron deposition was observed (Figures 5G and 5IG).



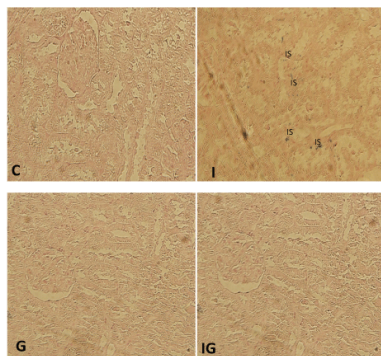
**Figure 4:** PAS kidney-stained sections of young rats: controls (C) and treated for a period of 45 days by  $\text{FeCl}_2$  (group I), by garlic (group G) or by both  $\text{FeCl}_2$  and garlic (group IG). TBM: thickened basement membranes, ABM: attenuation basement membranes, LC: Large cavities, CBS: closed bowman space, (Gx400).

## Discussion

Iron is an integral part of a diverse array of biologically active molecules, which form key components of homeostatic processes that are central to life [20]. Iron homeostasis must be maintained so that cells have sufficient iron for cell growth, but not excess due to its toxicity [21].

In this study, group I treated rats showed increased serum iron concentration. This result could be explained by a total iron passage across the enterocytes apical membrane transporters divalent metal transporter 1 (DMT1) to the blood [22]. This important passage of iron via intestine provoked iron accumulation in many organs

especially kidney. Indeed, acute kidney injuries after iron excess has been confirmed by several studies [23,24] but little is known about iron kidney functional impairment and garlic protective effects.



**Figure 5:** Perls' Prussian blue kidney-stained sections of young rats: controls (C) and treated for a period of 45 days by FeCl<sub>2</sub> (group I), by garlic (group G) or by both FeCl<sub>2</sub> and garlic (group IG). IS: Iron sedimentation, (Gx400).

The human diet, which contains large number of natural compounds, is essential in protecting the body against the development of diseases, and garlic is one of the well-known plants with remarkable antioxidant properties [7,12] and inhibitory effects on iron availability [25]. For group IG treated rats, the consumption of garlic decreased serum iron levels. This result was confirmed by the study of Ma and collaborators [26] suggesting that the Bioactive garlic polyphenols inhibit iron absorption in a dose-dependent manner in human intestinal Caco-2 cells. Moreover, Tuntipopipat and his collaborators [25] confirmed that garlic polyphenolic compounds are able, in a dose-dependent manner, to inhibit iron absorption by forming iron complexes in the intestine, making dietary iron less available for absorption.

At the beginning of our experiments, no significant body weight differences between control and treated group I rats were observed. But after forty-five days (sacrifice day) of iron treatment we had obtained a significant increase in body weights comparatively to controls. This result could not be explained by food consumption because we had found a significant decrease in daily food and water intakes. But, it may be explained, as demonstrated by previous studies, by the disturbance of endogenous insulin glucose and lipid metabolism [27,28] or by a general stimulation of collagen production [29]. However, at the sacrifice day of garlic treated rats (group G), we had found no changed body growth rate, food and water consumptions comparatively to those of controls'. In comparison, to group IG rats, for which we have obtained a decrease in body weights despite the relative increase in daily food and water consumptions. The decrease in body weights of rats of group IG comparatively to rats' of group I could be explained by a decrease in collagen production or by endogenous insulin glucose and lipid metabolism equilibration [30].

For kidney function, we had found in group I treated rats important increase in serum creatinine and urea comparatively to control group C. These results are similar to those found by Kadkhodae and collaborators [31] who had demonstrated that rats iron dextran treatment produced kidney injuries and elevation of plasma creatinine

and urea levels. As well, Petrak and collaborators [15] identified increased levels of three enzymes of urea cycle (carbamoyl-phosphate synthase, ornithine carbamoyl transferase and arginase) in mice iron overload. These perturbations of biochemical renal function biomarkers may be explained by the important increase of oxidative stress resulted from excess iron filtration and transport via nephron. Indeed, our results showed that in iron treated group I, lipid peroxidation was importantly increased and total antioxidant capacity was significantly decreased suggesting a negative pool of antioxidant factors in kidney tissue. This result could be explained by the direct implication of iron in kidney injuries and nephrotoxicity via the formation of hydroxyl radicals [32], which play a critical role in acute as well as chronic renal diseases [33]. In fact, oxidative stress and intracellular iron metabolism share the same metabolic pathways and gene products regulated by iron and stress play a crucial role in the maintenance of cellular homeostasis [34]. Moreover, in group I, kidneys were deeply affected by iron transport which induced weights and protein content increase associated to deep histological changes. Indeed, we had observed tubular iron sedimentation, hydropic epithelial cell degeneration, modified tubule aspects and atrophic glomeruli sizes with closed bowman spaces. All these disorders could not only due the oxidative stress induced by iron transport but they also may be a result of intralysosomal storage of iron in the kidney [33].

In group G kidneys', we had found, comparatively to controls, a little increase in creatinine and urea levels and a decrease in protein content and in acid uric levels. Theses biochemical perturbations were obtained in absence of any histological changes. In fact, the histological aspect of group G kidneys' hadn't changed comparatively to control ones'. This result could be explained by improvement of the antioxidant status. Indeed, MDA concentration was significantly decreased and total antioxidant capacity was importantly increased. According, to this experimental design, the daily ingested quantities of garlic seem to have some negative effects on kidney histological aspect.

After garlic adjunction to iron treated rats (group IG), the daily ingested quantities of garlic were sufficient to protect kidney from iron increase damages. Indeed, we had obtained decreased creatinin, urea and MDA levels associated with increased renal total antioxidant capacity suggesting improvement of total renal antioxidant status and normal histological aspect. These results confirmed previous data of Pedraza-Chaverri and collaborators [35] who have demonstrated that garlic, by its antioxidant power, has nephroprotective properties, which have been attributed to the active compound S-allyl cysteine (SAC) [36]. According to Hassan and collaborators [37], garlic oil treatment induced a clear improvement of kidney function, due to its antioxidant properties in scavenging free radicals and reducing levels of lipid peroxidation. SAC is reported to suppress the formation of superoxides, while diallyldisulfide (DADS) and diallylsulfide (DAS) scavenge hydroxyl radicals, thus enhance in vivo endogenous antioxidant system and prevent oxidative stress [38].

For the haematological parameters, we had found, in group I treated rats, an increase in serum acid uric and total bilirubin associated to haematological parameters impairment. Indeed, the reduction in red blood cell (RBC), haemoglobin (Hb) and hematocrite (Ht) obtained in group I treated rats, is a situation of haematological system failure which could be a result of kidney failure. Recently, Weiss and his collaborators [39] showed that chronic renal failure anemia is due to insufficient production of renal erythropoietin. In fact, erythropoietin has been shown to increase transferrin receptor

synthesis and cell surface expression in erythroid cells by activating the iron regulatory protein 1. Without sufficient erythropoietin stimulation of the erythroid cell, the number of erythroid cell surface transferrin receptor is probably down-regulated, increasing the likelihood of iron uptake by nonerythroid tissues [40]. The increase of total bilirubin obtained in group I rats may be explained by hemolysis of red blood cells induced directly by serum iron increase. In fact, De Gobbi and his collaborators [41] demonstrated that juvenile hemochromatosis is associated to anemia and  $\beta$  thalassemia. As well, Park and Han [16] have obtained a complete hematopoietic recovery after continuous iron chelation therapy in a patient with severe aplastic anemia with secondary hemochromatosis induced by multiple packed red blood cell transfusions. So, kidney failure status obtained in group I could be explained by the association of free heme release after hemolysis and iron excess. Indeed, increased levels of free red blood cell constituents together with an exhaustion of their scavengers induced renal tubular damage [42].

In group G, the daily consumed doses of garlic induced a decrease in red blood cells and in hemoglobin concentrations and an increase in total bilirubin. These results showed that the consumption of crude garlic alone induced anemia. This disease was obtained by different mechanisms to those previously demonstrated by serum iron increase. In fact, according to Munday and his collaborators [43] and Oboh [44] crude garlic could either induces hemolytic anemia or shortens the half-life of red blood cells than the controls.

For garlic and iron treated rats (group IG), the daily consumed doses of garlic were able to induce an amelioration in all erythrocyte parameters associated to decreased total bilirubin serum levels. These results could be explained by the fact that garlic consumed quantities were appropriate to prevent hematological parameter disorders and to protect from kidney failure as it was previously demonstrated.

In conclusion, the results obtained in this study confirmed our hypothesis and showed the positive interactive effects between crude garlic consumption and iron increase in protecting rats from hematological disorders and chronic kidney failure.

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