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Full Length Research Paper

Modulation of renal redox status by garlic based on route of administration in rat

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Several controversies persist about the beneficial or toxic effects of garlic. The present study was undertaken to determine the effects of high dosage garlic according to its mode of administration in rat. We studied the ability of high dosage garlic to modulate kidney antioxidant status when administered either orally (PO) or by intraperitoneal (IP) route. In renal tissue homogenate, PO garlic was antioxidant as it decreased malondialdehyde (MDA), protein carbonyl, free iron and hydrogen peroxide (H_2O_2) and increased antioxidant enzyme activities as peroxidase (POD) and superoxide dismutase (SOD). IP garlic was pro-oxidant as it increased protein carbonyl and H_2O_2, has no effect on MDA but unexpectedly increased catalase (CAT) activity and decreased POD and SOD activities. In plasma compartment, PO garlic has no effect on creatinine and urea levels whereas IP treatment increased them. High garlic dosage is safer when PO administered.

Key words: Garlic, kidney, redox status, administration mode, lipoperoxidation, protein carbonylation, free iron, hydrogen peroxide, antioxidants enzymes.

INTRODUCTION

Garlic (*Allium sativum L.*) has been usually used as a flavoring compound and in traditional medicine as a healing agent. Health effects of garlic extract were mainly attributable to organosulfur compounds (Berginc et al., 2010) and to a lesser extent to flavonoids and phenolics (Shirzad et al., 2011). Allicin (allyl-2-propenethiosulfinate) exhibited antibacterial (Fujisawa et al., 2009) and anticancer activity by inducing apoptosis in a caspase -3-dependent way (Padilla-Cambero et al., 2010). Diallyl monosulfide (DAMS) and diallyl-trisulfide (DATS) exhibited antioxidant activity *in vivo* and *in vitro* (Ponnusamy and Pari, 2011) whereas diallyl-disulfide (DADS) and S-allyl-L-cysteine sulfoxide (SACS) improved heart activity and hyperlipidemia (Yeh and Liu, 2001; Rai et al., 2009; Asdaq 2010). Recently thiacremonone a novel sulfur compound isolated from garlic was shown to exert anti-inflammatory properties (Ban et al., 2009). Flavonoids and phenolic compounds have been implicated in anticancer activities of fresh garlic (Shirzad et al., 2011) whereas fructosans exhibited immunomodulatory activity by stimulating lymphocyte proliferation and macrophage activation (Puthanapura et al., 2011); however numerous works were opposite according to experimental length, garlic dose and also the way of administration (Banerjee and Maulik, 2002). Garlic is usually administered either orally or by intraperitoneal (IP) way. IP route which avoids the gastric barrier was earlier shown to be more successful than gastric gavage particularly concerning the hypo-

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cholesterolemic effect of garlic (Alnaqueeb et al., 1996). In a prior study, we showed that high garlic dose administered by IP way could be detrimental inducing a prooxidant effect and ultimately blood toxicity (Hamlaoui-Gasmi et al., 2011a).

In the present work, we investigated the antioxidant effect of aqueous garlic extract when orally (PO) or by (IP) route administered on renal tissue homogenate by evaluating malondialdehyde (MDA), protein carbonyl, free iron, hydrogen peroxide and antioxidant enzyme activities as peroxidase (POD), catalase (CAT) and superoxide dismutase (SOD). We also evaluated plasma creatinine and urea levels. Data support an efficient antioxidant effect of garlic when orally administered. Furthermore the supposed link between pro or antioxidant effect of garlic and free iron overload is discussed.

MATERIALS AND METHODS

Chemicals

Thiobarbituric acid (TBA); 2,6,-di-tert-butyl-4-hydroxy-toluene (BHT); trichloroacetic acid (TCA); hydrogen peroxide (H_2O_2); 2-methoxyphenol (gaiacol); bovine catalase, 4-(1-Hydroxy-2-methylamino-ethyl)-benzene-1,2-diol (epinephrine), and 2,4,dinitrophenyl hydrazine (DNPH) were obtained from Sigma-Aldrich Co (Germany).

Preparation of garlic extract

One kilogram garlic (*Allium sativum* L.) was purchased from local market, peeled and grounded for 30 min with an electric mincer (FP3121 Moulinex) until an aqueous suspension was obtained. It was diluted in double distilled water at 4 g/ml on the basis of the weight of the starting material and centrifuged (Beckman J20, 15 min at 10000 g and 4°C). Supernatant was aliquoted into 1 ml fractions and stored at -80°C until use.

Animals and treatment

Male Wistar rats (180 to 200 g) from Pasteur Institute (Tunis) were maintained in animal facility for one week at room temperature 22 ± 1°C and a 12 h/12 h dark/light cycle. They were supplied with standard chow and top water *ad libitum*. Procedures with laboratory animals and their care were conducted in conformity with institutional guidelines of Tunis University of Medical Sciences and in accordance with the NIH guidelines (NRC, 1985). Animals were randomly divided into four groups of 10 animals each: Group I received standard diet (control). Group II received standard diet supplemented with aqueous extract of garlic (5 g/kg bw). Group III was IP injected with 9% NaCl (control). Group IV was IP injected with garlic (5 g/kg bw). Animals were treated daily for one month and checked for weight gain or loss. Twenty-four hours after the last injection, animals were sacrificed; their kidney rapidly excised, weighted and homogenized in phosphate buffer saline pH 7.4 with an ultrathurax T25 homogenisator at a 2 ml/g ratio. After centrifugation at 10000 g for 10 min at 4°C, supernatant was used for the determination of lipoperoxidation, carbonylation, antioxidant enzyme activities and intracellular mediators as free iron and H_2O_2. Blood was also collected and plasma processed for urea and creatinine determination.

Kidney function assessment

Plasma urea and creatinine analysis were performed using an auto blood analyser (Coulter).

Lipid peroxidation determination

Lipid peroxidation was determined by MDA measurement according to the double heating method (Draper and Hadley, 1990). Briefly, aliquot from kidney homogenate was mixed with BHT-TCA solution containing 1% BHT (w/v) dissolved in 20% TCA (w/v) and centrifuged at 1000 g for 5 min at 4°C. Supernatant was blended with 0.5 N HCl, 120 mM TBA in 26 mM Tris and then heated at 80°C for 10 min. After cooling, absorbance of the resulting pink chromophore was determined at 532 nm using a BIORAD UV-visible spectrophotometer. MDA levels were determined by using an extinction coefficient for MDA-TBA complex of 1.56×10^5 $M^{-1}cm^{1}$.

Protein carbonylation

Oxidative damage to proteins was evaluated by quantifying protein carbonylation in kidney homogenates according to Levine et al. (1990). After proteins precipitation with 20% TCA and centrifugation at 11000 g during 3 min at 4°C (Beckman J20), pellet was reprised in 10 mM DNPH-containing buffer and vortexed every 10 min at room temperature for 1 h. After 3 washings with ethanol-ethylacetate (1:1), pellet was dissolved in 20 mM potassium phosphate (pH 2.3) containing 6 M guanidine chloride and absorbance measured at 366 nm using the molar extinction coefficient of 22000 $M^{-1}cm^{-1}$. Results were expressed as nmol carbonyl residues/mg protein.

Protein determination

Total soluble proteins were determined according to the biuret method (Ohnishi and Barr, 1978). Briefly, at acidic pH, soluble proteins constituted with copper a colourful complex measurable at 546 nm using a SmartSpec 3000 BIORAD UV-visible spectrophotometer (Germany).

Analysis of antioxidant enzyme activities

All spectrophotometric analyses of kidney antioxidant enzyme activities were performed with a SmartSpec 3000 BIORAD UV-visible spectrophotometer (Germany).

Catalase

CAT activity was assayed by measuring the initial rate of H_2O_2 disappearance at 240 nm (Aebi, 1984). The reaction mixture contained 33 mM H_2O_2 in 50 mM phosphate buffer pH 7.0 and renal extract. CAT activity was calculated using the extinction coefficient of 40 $mM^{-1}cm^{-1}$ for H_2O_2.

Peroxidase

POD activity was measured at 25°C using guaiacol as hydrogen donor. The reaction mixture contained 9 mM guaiacol, 19 mM H_2O_2 in 50 mM phosphate buffer pH 7 and renal extract in 1 ml final volume. The reaction was initiated by the addition of H_2O_2 and monitored by measuring the increase in absorbance at 470 nm

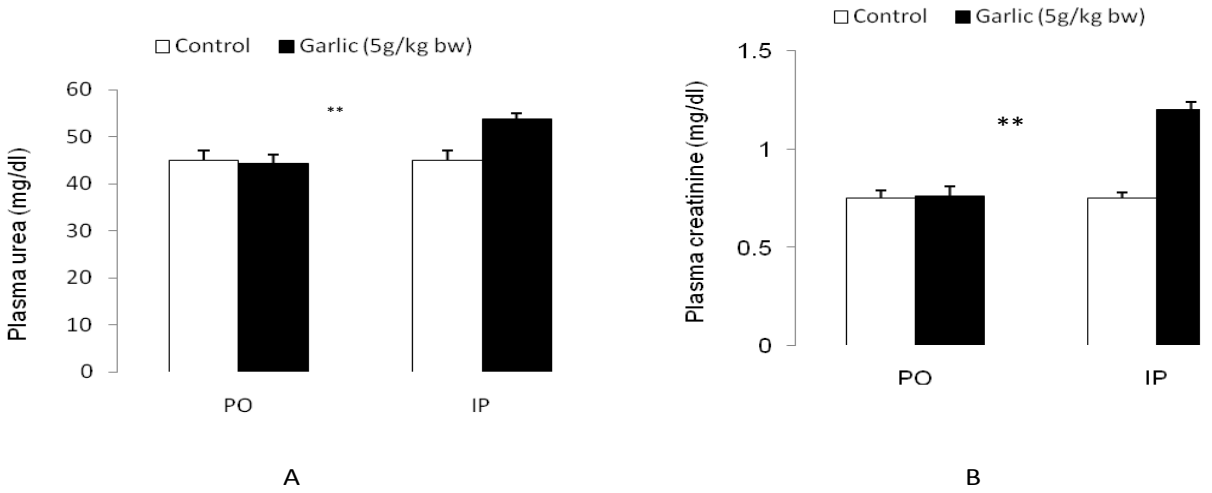

Figure 1. Effect of garlic way of administration on plasma creatinine and urea levels. NaCl 9‰ (C □) or garlic (G ■) were IP or PO administered to rats during one month and plasma creatinine (A) and plasma urea (B) determined. Results are expressed by mean ± SEM of 10 rats per group and assays done in triplicate. *, $p < 0.05$; **, $p < 0.01$.

each 30 s during 3 min. Peroxidase activity was expressed as nmol of guaiacol oxidized per min with a molecular extinction coefficient of 26.2 mM^{-1} for calculation (Chance and Maehly, 1955).

Superoxide dismutase

SOD activity was determined by using modified epinephrine assay (Misra and Fridovich, 1972). At alkaline pH, superoxide anion (O_2^-) causes the autoxidation of epinephrine to adenochrome. One unit of SOD is defined as the amount of extract that inhibits the rate of adenochrome formation by 50%. Enzyme extract was added in 2 ml reaction mixture containing 10 µl bovine catalase (0.4 U/µl), 20 µl epinephrine (5 mg/ml) and 62.5 mM sodium carbonate/sodium bicarbonate buffer pH 10.2. Changes in absorbance were recorded at 480 nm. Characterization of SOD isoforms was performed using KCN (3 mM) which inhibited Cu/Zn-SOD or H_2O_2 (5 mM) affecting both Cu/Zn-SOD and Fe-SOD. Mn-SOD was insensitive to both inhibitors.

Free iron determination

Renal free iron was determined according to Leardi et al. (1998) using a commercially available kit from Biomaghreb, Tunisia. The principle of this method is as follows: At acidic pH 4.8, all Fe^{3+} released from transferrine were reduced by ascorbic acid into Fe^{2+}, which constituted with ferrozine a purple colourful complex measurable at 560 nm. Briefly renal extract was added to reaction mixture containing ascorbic acid (5 g/L) and ferrozin (40 mM) and incubation was performed at 37°C for 10 min.

H_2O_2 determination

Renal H_2O_2 level was determined enzymatically according to Kakinuma et al. (1979) using a commercially available kit from Biomaghreb (Tunisia). Briefly in the presence of peroxidase, H_2O_2 reacts with 4-amino-antipyrine and phenol to give a red colored quinoeimine which absorbed at 505 nm. Results are expressed as mmol H_2O_2/mg protein.

Statistical analysis

Data were analyzed by unpaired Student's t-test or one-way analysis of variance (ANOVA) and expressed as means ± standard error of the mean (SEM). Assays were carried out in triplicate. All statistical tests were two-tailed, and a p value of 0.05 or less was considered significant.

RESULTS

Kidney function assessment

We reported in Figure 1 data of garlic dosage administered either by IP or PO way on plasma creatinine (Figure 1A) and urea (Figure 1B). When administered by PO route, garlic has no effect on plasma creatinine and urea. However, when IP administered garlic increased plasma creatinine (+60%) and urea (+20%).

Kidney lipoperoxidation and carbonylation

Figure 2 show data of garlic dosage administered either by IP or PO way on kidney lipoperoxidation (Figure 1A) and protein carbonylation (Figure 1B). When administered by PO route, garlic decreased renal MDA (-60%) and protein carbonylation (-20%). However, garlic administered by IP route increased MDA (+7%) and protein carbonylation (+50%).

Kidney antioxidant enzyme activities

Figure 3 dealt with the effect of garlic mode of administration on kidney antioxidant enzyme activities. PO garlic has no effect on CAT (Figure 3A) but increased

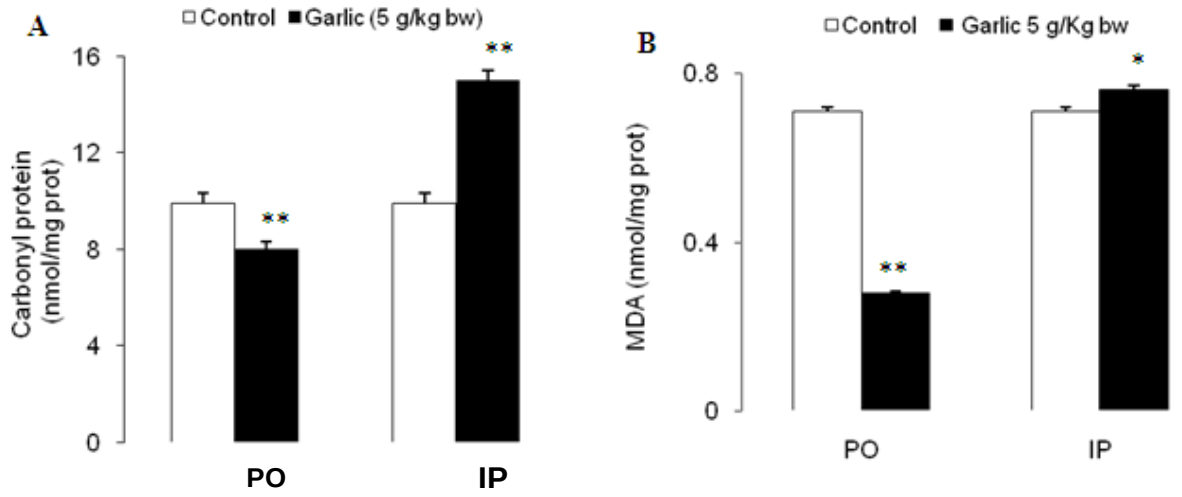

Figure 2. Kidney lipoperoxidation and carbonylation. NaCl 9‰ (C) or garlic (G) were IP or PO administered to rats during one month and renal MDA (A) and protein carbonylation B) determined. Results are expressed by mean ± SEM of 10 rats per group and assays done in triplicate. *, $p < 0.05$; **, $p < 0.01$.

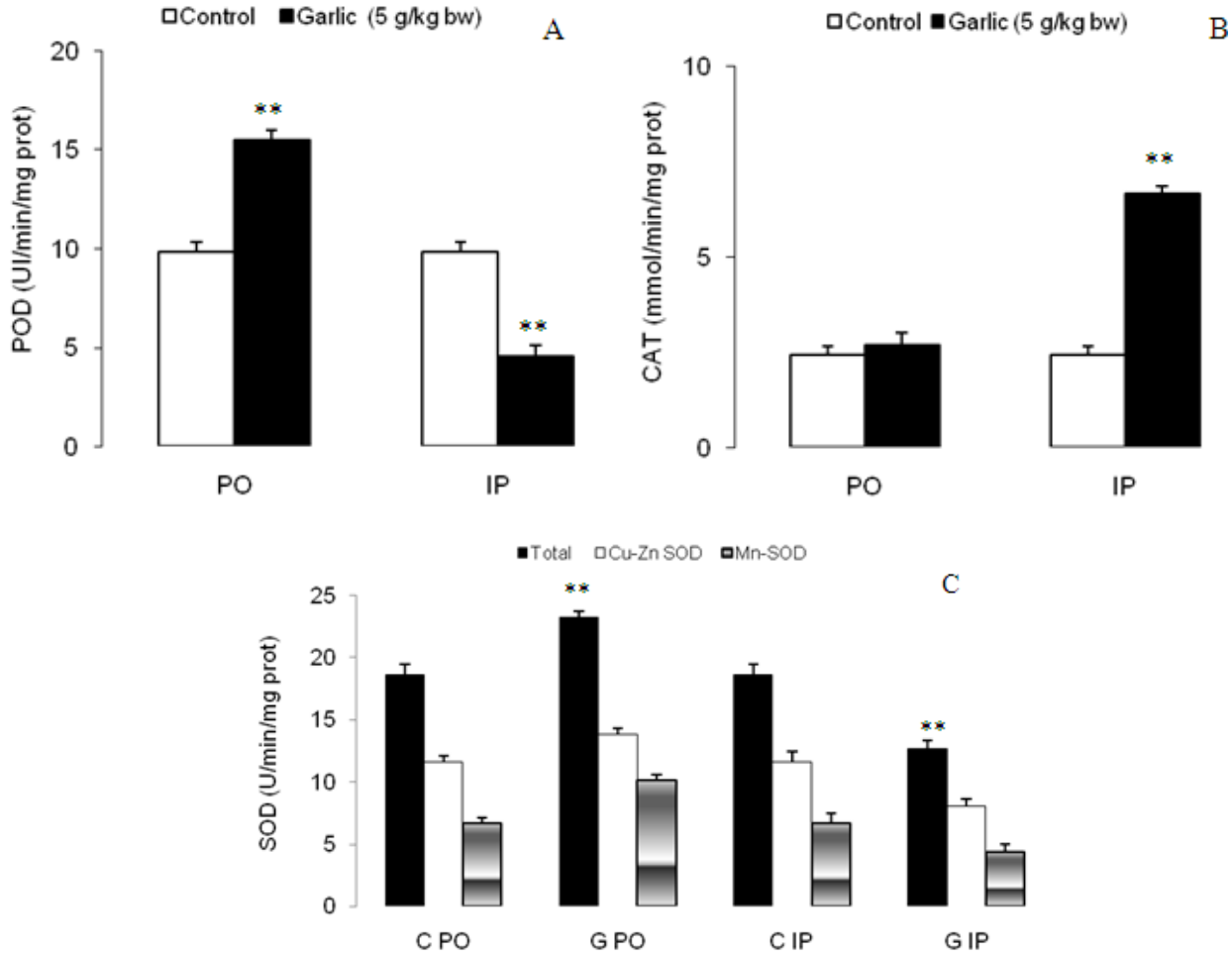

Figure 3. Effect of garlic way of administration on renal antioxidant status. NaCl 9‰ (C) or garlic (G were IP or PO administered to rats during one month and renal CAT (A), POD (B) and SOD (C) activities determined. Results are expressed by mean ± SEM of 10 rats per group and assays done in triplicate. *, $p < 0.05$; **. $p < 0.01$.

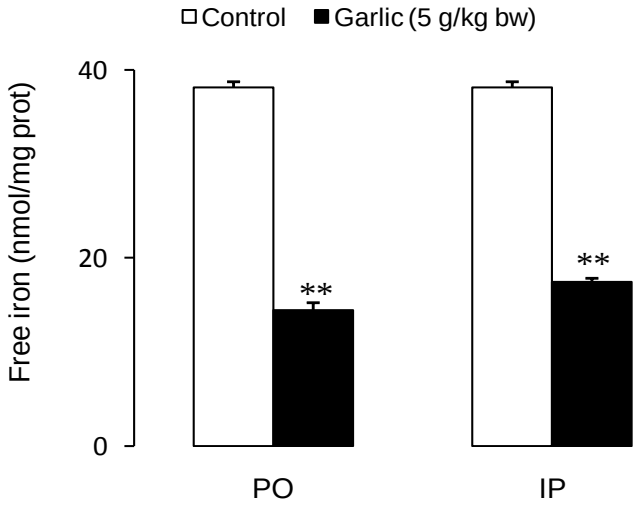

Figure 4. Effect of garlic way of administration on kidney free iron level. NaCl 9‰ (C □) or garlic (G ■) were IP or PO administered to rats during one month and renal free iron determined. Results are expressed by mean ± SEM of 10 rats per group and assays done in triplicate. *, indicated $p < 0.05$. **, indicated $p < 0.01$.

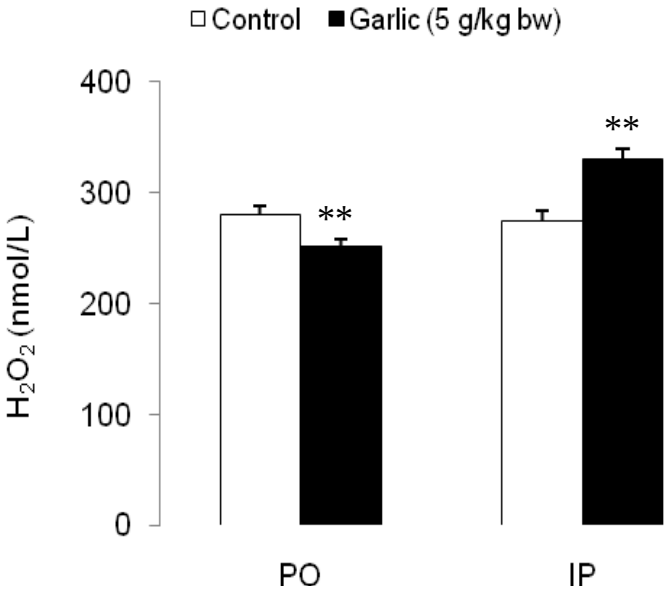

Figure 5. Kidney hydrogen peroxide level. NaCl 9‰ (C□) or garlic (G■) were IP or PO administered to rats during one month and renal H_2O_2 level determined. Results are expressed by mean ± SEM of 10 rats per group and assays done in triplicate. *, $p < 0.05$; **, $p < 0.01$.

POD (+ 80%) (Figure 3B) and SOD (+25%) (Figure 3C) activities; in this latter case, Mn-SOD was strongly up-regulated (+ 50%) when compared to Cu/Zn isoform (+19%). When IP administered, garlic also up-regulated CAT (+ 170%) but decreased POD (-50%) and SOD (-30%); the two isoforms, that is, Mn and Cu/Zn were decreased to the same extent respectively by -35 and -30%.

Effect of garlic mode of administration on renal free iron level

We further looked at renal free iron level (Figure 4) and data showed that PO and IP garlic significantly decreased this parameter at -62 and -55%, respectively.

Effect of garlic mode of administration on kidney hydrogen peroxide level

We also assessed the effect of garlic mode of administration on kidney hydrogen peroxide level (Figure 5). As expected, PO garlic route decreased H_2O_2 level in kidney (-0%) but IP route increased it (+20%).

DISCUSSION

This work was undertaken in order to bring some clues to several discrepancies about the effectiveness of garlic beneficial health effects (Agarwal, 1996). We used garlic in subchronic experiments of 1-month duration at 5 g/kg dosage previously shown to exert cholesterol and transaminases lowering activities (Hamlaoui-Gasmi et al., 2011a) and compared IP versus PO mode of administration. Our data first showed that in kidney PO garlic decreased MDA, protein carbonylation and hydrogen peroxide. However garlic had opposite effect that is increasing plasma urea and creatinine as well as MDA, protein carbonylation and H_2O_2 when IP administered. Elevated levels of thiobarbituric acid reactive species (TBARS) in kidney can be an indicator of increased lipid peroxidation in renal cells membranes that suggests the participation of free-radical induced oxidative cell injury in mediating the toxicity of IP route. The formation of MDA in kidney is a sign of lipid membrane degradation involving the deterioration of cellular integrity. As an affirmation, PO garlic induced antioxidant effect was further confirmed by its positive effects on POD and SOD, while it increases these antioxidant enzymes activities but unexpectedly has no effect on CAT activity. PO garlic-induced antioxidant effect was further confirmed by the positive role it exerted on SOD isoforms especially the Mn isoform. First this isoform could correspond to the secreted form of the enzyme (Marklund, 1982). Second, this apparent discrepancy could be interpreted in light of the deleterious increase in ROS production exerted by Mn-SOD (Goldstein et al., 2008) especially in such a free iron overload setting.

However IP garlic exerted a strong prooxidant effect also by having just an opposite result, while it decreases POD and SOD activities but unexpectedly increased CAT

activity. This last data is reminiscent of the described pro-oxidant effect of catalase (Heck et al., 1998).

We further looked at renal free iron level and data showed that whatever the mode of administration, garlic significantly decreases renal free iron level. This decrease is more important with PO way than IP. These data which fully corroborated our recent work (Hamlaoui-Gasmi et al., 2011b) add some new information on the relationship between garlic mode of administration and its effect on iron homeostasis. Both iron deficiency and iron excess can lead to cellular dysfunction, hence maintaining normal iron homeostasis is therefore crucial (Andrews, 1999). Our work adds some new information on the relationship between garlic mode of administration and the extent in free iron overloading. Excess free divalent iron can be highly toxic, mainly via the Fenton reaction producing hydroxyl radicals leading to cellular dysfunction (Andrews, 1999).

Iron homeostasis is a highly complex and finely regulated network, involving several regulatory proteins. Garlic mode of action might convey through iron shuttling proteins as hepcidin (Papanikolaou et al., 2005) or lipocalin 2 (Deviredly et al., 2005). Interestingly, hepcidin has been described in various organs as liver (Park et al., 2001), heart (Merle et al., 2007), brain (Wang et al., 2008) and pancreas (Kulaksiz et al., 2008) and lipocalin 2 in the liver (Sunil et al., 2007). Moreover, hepcidin which exerted a pivotal role in the pathogenesis of iron overload (Papanikolaou et al., 2005) was even shown to be implicated as a key regulator of anemia of inflammation (Ganz, 2003) and high levels of hepcidin caused intracellular iron sequestration and decreased level in the plasma (Pigeon et al., 2001). Thus one can speculate about PO garlic inducing up-regulation of hepcidin and decreased iron deposition into kidney cells and conversely IP garlic inducing down-regulation of hepcidin and iron. Further work about garlic effect on hepcidin level is warranted. It is tempting to speculate about IP garlic inducing upregulation of hepcidin and drastic renal iron shortage thus leading to increased oxidative stress.

Garlic-induced iron excess or deficiency seems to be organ specific. For instance we previously showed that in the liver, slight iron deficiency is associated with antioxidant effect (PO garlic treatment), although, high iron deficiency is rather associated with prooxidant effect (IP garlic) (Hamlaoui-Gasmi et al., 2011b).

Regarding to kidney functions, our results show that PO garlic has no effect on plasma creatinine and urea levels but IP garlic induces kidney toxicity by increasing these parameters. Garlic and its various components are postulated to have an important cytoprotective role through their antioxidant and anti-inflammatory properties. In fact, AGE partially prevented the increase in plasma creatinine and blood urea induced by gentamycin-treatment (Maldonado et al., 2003). The protective effect of AGE could be related with its ability to scavenge O_2^- (Borek, 2001; Kim et al., 2001) and H_2O_2 (Borek, 2001; Ide and Lau, 1999), and/or with its ability to totally prevent the decrease in Mn-SOD activity (Maldonado et al., 2003). Also in kidney transplant recipients serum, creatinine and urea increased, but the patients who took one clove of garlic (1 g) by chewing or swallowing for two months, serum levels of urea and creatinine did not increase (Bagheri et al., 2011). Moreover, S-allylcysteine, a water-soluble nontoxic garlic compound, having antioxidant properties both *in vivo* and *in vitro*, was able to ameliorate the increase in serum urea and creatinine and to decrease the kidney histopathological damage (Bagheri et al., 2011). S-allylmercaptocysteine is another water soluble organosulfur compounds found in garlic extract. The protective effect of S-allylmercaptocysteine on gentamicin-induced nephrotoxicity was associated with decrease in serum urea and increase in creatinine clearance (Bagheri et al., 2011). Inside garlic oil showed a clear improvement in kidney functions, perhaps due to the antioxidant properties of garlic in scavenging free radicals leading to reduced levels of lipid peroxidation (Bagheri et al., 2011).

In conclusion, we showed that garlic could modulate oxidative stress in rat according to its administration route. The underlying mechanism seems to involve disturbances in iron homeostasis and likely iron shuttling proteins.

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