African Journal of Biotechnology Vol. 11(4), pp. 912-918, 12 January, 2012
Available online at http://www.academicjournals.org/AJB
DOI: 10.5897/AJB11.1696
ISSN 1684–5315 © 2012 Academic Journals

Full Length Research Paper

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

Sonia Hamlaoui-Gasmi[1]*#, Nadia Limam[1#], Meherzia Mokni[1], Ferid Limam[2], Ezzedine Aouani[2], Mohamed Amri[1] and Lamjed Marzouki[1]

[1]Laboratoire de Neurophysiologie Fonctionnelle et Pathologies, Faculté des Sciences de Tunis, Campus Universitaire Manar II 2092 Tunis, Tunisia.
[2]Laboratoire des Substances Biologiquement Actives, Centre de Biotechnologie, Technopole Borj-Cedria, BP 901, 2050 Hammam-Lif, Tunis, Tunisia.

Accepted 9 September, 2011

We analyzed the ability of high dosage garlic administered per orally (p.o.) or via intraperitoneal (i.p.) route to act on heart antioxidant status in rats. In this organ, p.o. garlic is an antioxidant as it decreased H_2O_2 and lactate dehydrogenase (LDH) levels and increased free iron level. However, it had no effect on malondialdehyde (MDA), catalase (CAT) and superoxide dismutase (SOD) but decreased peroxidase (POD) activity. Intraperitoneal garlic is pro-oxidant as revealed by high MDA, LDH and H_2O_2 levels. It also induced an increase in free iron deposition and in CAT and SOD activities, but this way of treatment has no effect on POD activity. It can be concluded therefore that high garlic dosage is safer when orally administered. These effects are free iron mediated and organ specific.

Key words: Garlic, heart, redox status, administration way, lipoperoxidation, free iron, hydrogen peroxide.

INTRODUCTION

Garlic (*Allium sativum L.*) was shown to exert a wide range of pharmacological effects such as antiviral, antibacterial (Taratinsev et al., 1992), antitumor (Schaffer et al., 1996), anti-atherosclerotic (Campbell et al., 2001), antianemic and hypolipidemic activity (Hamlaoui-Gasmi et al., 2011a) and antioxidant (Hamlaoui-Gasmi et al., 2011b). However, several reported effects were deviating and conflicting and depended on experimental duration, garlic dosage and mode of administration (Banerjee and Maulik, 2002). Garlic is generally administered orally (p.o.) or by intraperitoneal (i.p.) route. This latter way of administration which avoids the gastric barriere was previously shown to be more effective than gastric gavage especially concerning the hypocholesterolemic effect of garlic (Alnaqeeb et al., 1996; Hamlaoui-Gasmi et al., 2011a). In a recent study, we could establish that garlic high dose oral treatment exhibited profound antianemic, antifatigue, lipid and transaminases lowering activities as compared to i.p. route of treatment (Hamlaoui-Gasmi et al., 2011a). In the present work, we investigated the antioxidant effect of garlic when p.o. or i.p. administered on heart redox status by evaluating malondialdehyde (MDA), free iron, hydrogen peroxide, lactate dehydrogenase (LDH) and antioxidant enzyme activities as catalase (CAT), peroxidase (POD) and superoxide dismutase (SOD). Data are in favor of an efficient antioxidant effect of garlic when orally administered. Moreover, the putative link between pro-antioxidant effect of garlic and free iron overload is discussed.

*Corresponding author. E-mail: sonia_hamlaoui@yahoo.fr. Tel: (216) 98 96 81 13.

Abbreviations: CAT, Catalase; **SOD,** superoxide dismutase; **LDH,** lactate dehydrogenase; **MDA,** malondialdehyde.

#Both authors contributed equally to the work.

MATERIALS AND METHODS

Chemicals

2-Thiobarbituric acid (TBA); 2,6,-di-tert-butyl-4-hydroxy-toluene (BHT); trichloroacetic acid (TCA); hydrogen peroxide (H_2O_2); 2-methoxyphenol (guaiacol); bovine catalase and 4-(1-Hydroxy-2-

methylamino-ethyl)-benzene-1,2-diol (epinephrine) were obtained from Sigma-Aldrich Co (Germany).

Preparation of garlic extract

Garlic was purchased from local market, peeled and ground with an electric mixer. It was diluted in double distilled water at 2 g/ml on the basis of the weight of the starting material and centrifuged (Beckman J20, 15 min at 10 000 g and 4°C). Supernatant was aliquoted 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 of 22 ± 1°C and a 12 /12 h dark/light cycle. They were supplied with standard feed and tap water *ad libitum*. Procedures with laboratory animals and their care were in accordance with the NIH guidelines. 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 i.p. injected with 9‰ NaCl (control) and group IV was i.p. injected with garlic (5 g/kg bw). Animals were treated daily for 30 days and checked for weight gain or loss. The rats were anesthetized with 0.5 ml urethane (40 mg/ml) and sacrificed 24 h after the last treatment. Their hearts were collected and processed for biochemical determination of antioxidant status parameters.

Lipid peroxidation measurement

Lipid peroxidation was determined by MDA measurement according to the double heating method (Draper and Hadley, 1990). Briefly, aliquots from heart homogenates were 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 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 determination

Total soluble proteins were determined according to Biuret method (Ohnishi and Barr, 1978). Briefly, at acidic pH soluble proteins constituted with copper a colourful complex measurable at 546 nm.

Analysis of antioxidant enzyme activities

All spectrophotometric analyses were performed with a Beckman DU 640B spectrophotometer. 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 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 50 µL of enzyme 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. Peroxidase activity was expressed in 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; while competing with this reaction, SOD decreased the adenochrome formation. 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

Free iron was determined according to Leardi et al. (1998) using a commercially available kit from Biomaghreb (Tunisia). Briefly, at acidic pH 4.8 all Fe^{3+} is released from transferrine. Ascorbic acid reduced Fe^{3+} in Fe^{2+} which constituted with ferrozine a colourful complex measurable at 560 nm.

H_2O_2 determination

H_2O_2 was determined according to Chance et al. (1979) using a commercially available kit from Biomaghreb (Tunisia).

LDH determination

Lactate dehydrogenase activity was determined using a commercially available kit from Biomaghreb (Tunisia). Briefly, heart homogenate was added to reaction mixture containing NADH and Tris buffer pH 7.2. LDH activity was assayed by measuring the initial rate of NADH disappearance at 340 nm.

Statistical analysis

All data were expressed by mean values ± SEM. Statistical analysis was carried out using student's t-test and one way analysis of variance (ANOVA test). Statistical p value less than 0.05 was considered significant.

RESULTS

Effect of garlic (p.o. and i.p.) treatment on heart lipoperoxidation and LDH level

The results presented in Figure 1 showed the data of garlic dosage administered either by p.o. or i.p. route on heart lipoperoxidation (Figure 1A) and LDH (Figure 1B). When administered by p.o. route, garlic has no effect on heart MDA but decreases LDH level. However, i.p. garlic increased MDA (+ 30%) and LDH (+ 112%) levels in heart.

Heart antioxidant enzyme activities

The data outcome shown in Figure 2 dealt with the effect

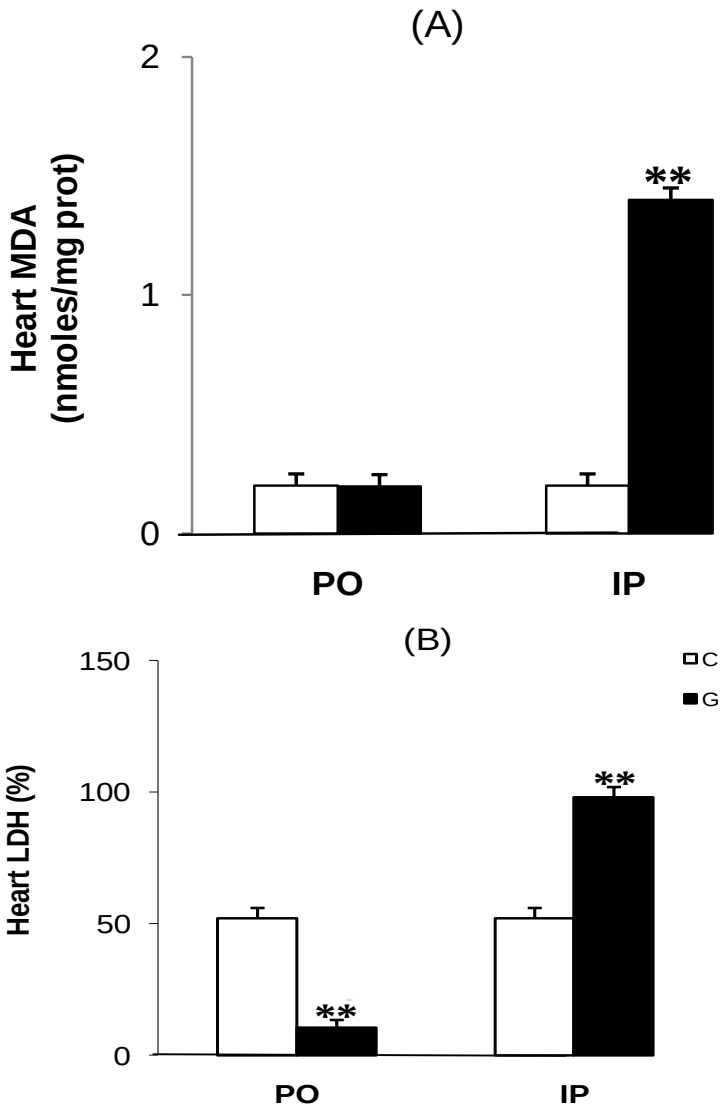

Figure 1. Effect of garlic way of administration on heart lipoperoxidation and LDH. NaCl 9‰ (C □) or garlic (G ■) was p.o. or i.p. administered to rats during 30 days and heart MDA (A) and LDH (B) determined. Results are expressed by mean ± SEM of 10 rats per group. Data are representative of 3 independent experiments; ** indicated $p < 0.01$.

of garlic mode of administration on heart antioxidant enzyme activities. Oral garlic treatment has no effect on heart CAT (Figure 2A) and SOD, (Figure 2B) but decreased POD (- 40%) (Figure 2C) activities. Garlic i.p. treatment increased CAT (- 40%) and SOD (- 34%) but has no effect on POD activities.

Effect of garlic mode of administration on heart free iron and hydrogen peroxide levels

We further analyzed heart free iron level and data showed that whatever the mode of administration, garlic significantly increases cardiac free iron level (Figure 3A). This increase is more important with i.p. (+140%) route than p.o (+25%). Cardiac hydrogen peroxide level was also investigated and the results are presented in Figure 3B. As expected, garlic p.o. treatment decreased H_2O_2 level (-22%) in heart, however, i.p. garlic treatment increased it (+160%).

DISCUSSION

In a prior study we demonstrated that high dosage garlic exhibited dual effects in rat that is antioxidant or prooxidant depending on the mode of administration. Oral garlic treatment exerted antianemic and lipid-lowering

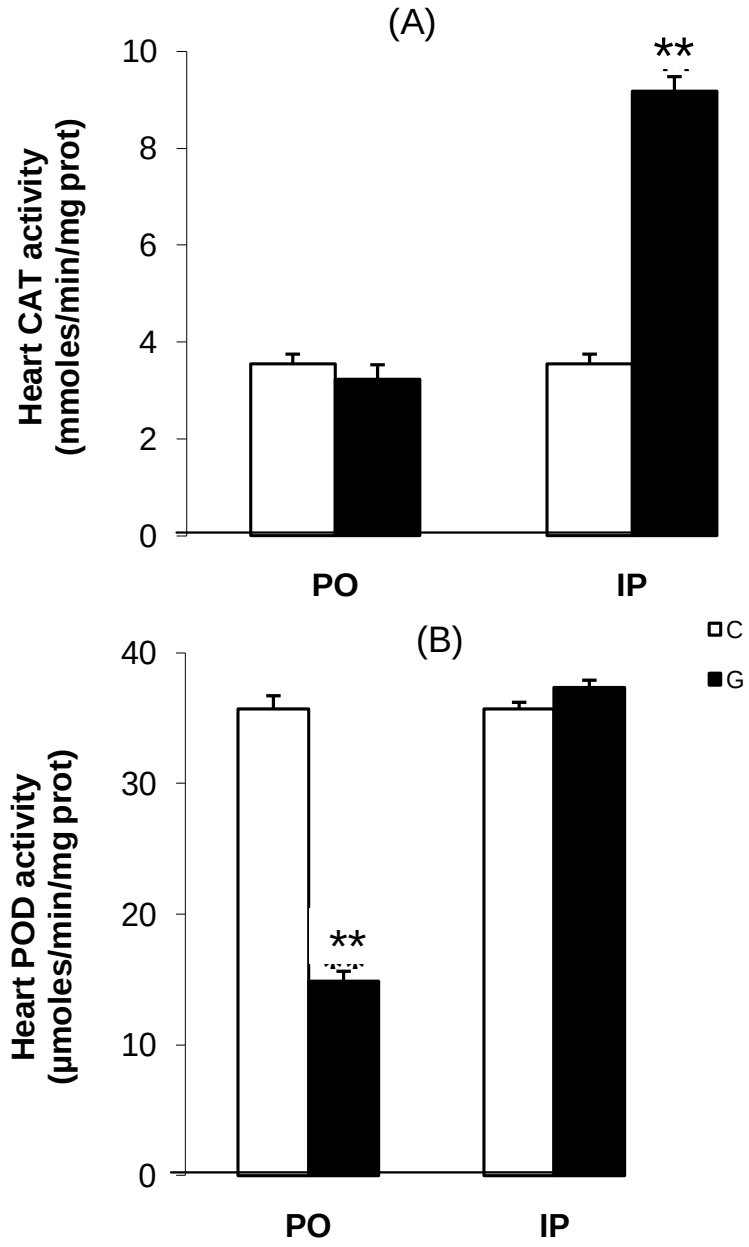

Figure 2. Effect of garlic way of administration on heart antioxidant status. NaCl 9‰ (C □) or garlic (G ■) were p.o. or i.p. administered to rats during 30 days and cardiac CAT (A), POD (B) and SOD (C) activities determined. Results are expressed by mean ± SEM of 10 rats per group. Data are representative of 3 independent experiments; ** indicated $p < 0.01$.

effect whereas garlic i.p. treatment, induced anemia and hepatotoxicity as assessed by elevation in plasma transaminases (Hamlaoui-Gasmi et al., 2011a). Moreover, i.p. garlic-induced toxic effects were shown to be mediated by increased erythrocyte free iron and H_2O_2, whereas p.o. garlic-induced beneficial effects were mediated by a decrease in both free iron and H_2O_2 (Hamlaoui-Gasmi et al., 2011b). The main conclusion drawn was that there is a harmful/prooxidant effect of i.p. garlic and a beneficial/ antioxidant effect of p.o. garlic (Hamlaoui-Gasmi et al., 2011b).

In this study, we investigated the effect of p.o. or i.p. garlic on rat heart. In this organ, when administered by p.o. route, garlic has no effect on MDA but decreases LDH and H_2O_2 levels. However, the i.p. administered garlic increased MDA, LDH and H_2O_2 levels. Our results,

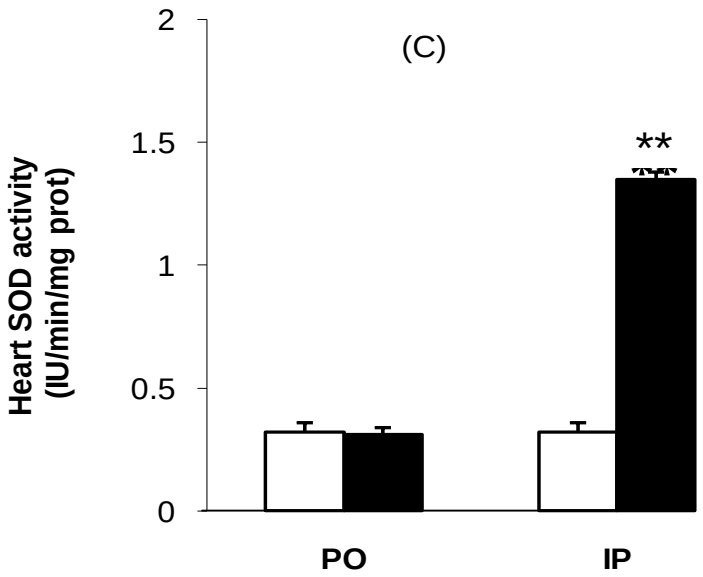

Figure 2. Effect of garlic way of administration on heart antioxidant status. NaCl 9‰ (C □) or garlic (G ■) were p.o. or i.p. administered to rats during 30 days and cardiac CAT (A), POD (B) and SOD (C) activities determined. Results are expressed by mean ± SEM of 10 rats per group. Data are representative of 3 independent experiments; ** indicated $p < 0.01$.

obtained by supplementation of garlic are consistent with the results reported in most studies on this subject as garlic dose-dependent reduction of basal lipid peroxidation obtained in the heart (Banerjee et al., 2001). Many other studies have shown that administration of garlic induced a significant decrease in cardiac (Saravanan and Prakash, 2004) and renal (Jabbari et al., 2005) MDA.

Elevated levels of thiobarbituric acid reactive species (TBARS) in heart can be an indicator of increased lipid peroxidation in cardiomyocyte membranes that suggests the participation of free-radical induced oxidative cell injury in mediating the toxicity of i.p. route. The formation of MDA in heart is a sign of lipid membrane degradation involving the deterioration of cellular integrity. As an affirmation, p.o. garlic induced antioxidant effect was further confirmed by its positive effects on catalase (CAT) and superoxide dismutase (SOD), while it has no effect on these antioxidant enzymes activities but unexpectedly decreases POD activity. However, i.p. garlic exerted a strong prooxidant effect also by having just an opposite result, while it has no effect on POD activity but unexpectedly increased CAT and SOD activities.

We further looked at heart free iron level and data showed that whatever the mode of administration, garlic significantly increases cardiac free iron level. This increase is more important with i.p. way than p.o. 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. Indeed in the case of heart, p.o. garlic only slightly increased free iron deposition (antioxidant role) whereas i.p. garlic increased it drastically (prooxidant role). Both iron deficiency and iron excess can lead to cellular dysfunction, hence maintaining normal iron homeostasis is therefore crucial (Andrews, 1999). Iron homeostasis is a highly complex and finely regulated network, involving several regulatory proteins. 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) where it exerted a pivotal role in the pathogenesis of iron overload (Papanikolaou et al., 2005) and high levels of hepcidin caused intracellular iron sequestration and decreased level in the plasma (Pigeon et al., 2001). It is tempting to speculate about i.p. garlic inducing up-regulation of hepcidin and drastic heart iron excess thus leading to increased oxidative stress. This apparent discrepancy is reminiscent of the paradoxal prooxidant effect of catalase (Heck et al., 2003) which should be interpreted in the light of oxidative stress-induced ROS activation of non receptor tyrosine kinases associated with CAT phosphorylation and activity stimulation (Borchi et al., 2010).

Garlic-induced iron excess or deficiency seems to be organ specific. For instance we previously showed that in erythrocytes iron deficiency (p.o. garlic treatment) was antioxidant (Hamlaoui-Gasmi et al., 2011b). We also previously showed that in the liver, slight iron deficiency is associated with antioxidant effect (p.o. garlic

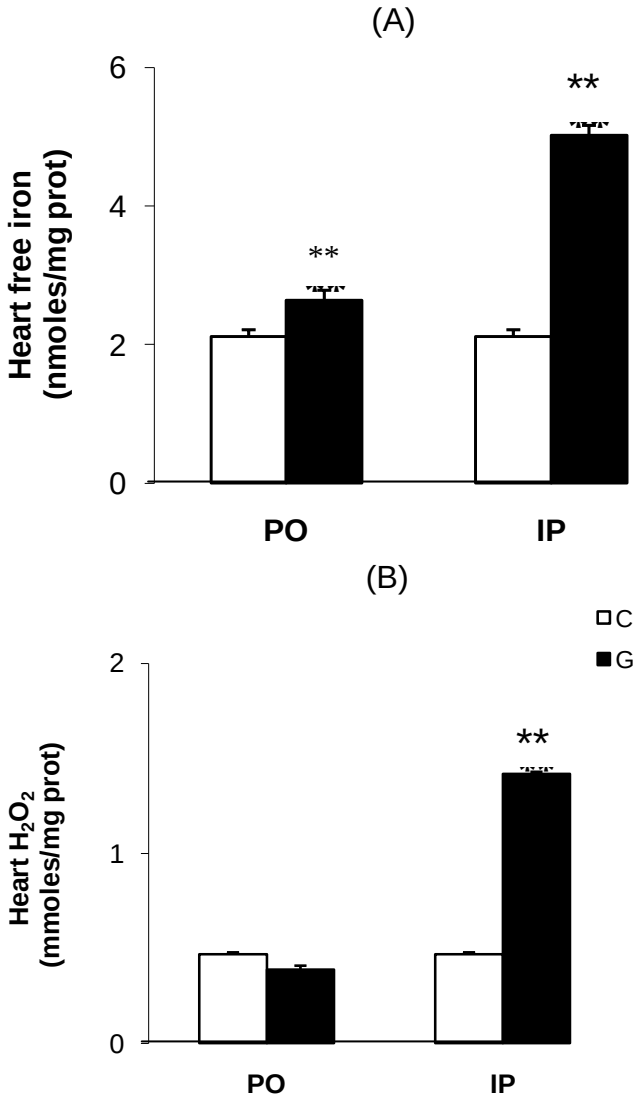

Figure 3. Effect of garlic route of administration on heart free iron and H_2O_2 levels. NaCl 9‰ (C □) or garlic (G ■) were p.o. or i.p. administered to rats during 30 days and heart free iron (Figure 3A) and H_2O_2 (Figure 3B) levels determined. Results are expressed by mean ± SEM of 10 rats per group. Data are representative of 3 independent experiments; ** indicated $p < 0.01$.

treatment), although, high iron deficiency is rather associated with prooxidant effect (i.p. garlic) (Hamlaoui-Gasmi et al., 2011c). In conclusion, the prooxidative or antioxidant effect of high dosage garlic is linked to route of administration and to the extent it modulates (excess or deficiency) labile iron pool, the threshold of which is organ specific.

ACKNOWLEDGEMENT

This work was financially supported by the Tunisian Ministry of Research.

REFERENCES

Aebi H (1984). Catalase *in vitro*. Methods Enzymol. 105: 121-126.
Alnaqeeb MA, Thomson M, Bordia T, Ali M (1996). Histopathological effects of garlic on liver and lung of rats. Toxicol. Lett. 85: 157-164.
Andrews NC (1999). Disorders of iron metabolism. N. Engl. J. Med. 341: 1986-1995.
Banerjee SK, Maulik SK (2002). Effect of garlic on cardiovascular disorders: a review. Nutr. J. 1: p. 4.
Banerjee SK, Maulik M, Gupta SK, Manchanda SC, Dinda AK, Maulik SK (2001). Effect of chronic garlic intake on endogenous antioxidants and ischemic-reperfusion injury in isolated rat heart. Ind. J. Pharm. 33: p. 298
Borchi E, Bargelli V, Stillitano F, Giordano C, Sebastiani M, Nassi PA, d'Amati G, Cerbai E, Nediani C (2010). Enhanced ROS production

By NADPH oxidase is correlated to changes in antioxidant enzyme activity in human heart failure. Biochem. Biophysica Acta. 1802: 331-338.
Campbell JH, Efendy JL, Smith NJ, Campbell GR (2001). Molecular basis by which garlic suppresses atherosclerosis. J. Nutr. 131: 1006-1009.
Chance B, Maehly AC (1955). Assay of catalases and peroxidases. Methods Enzymol. 2: 764-817.
Chance B, Sies H, Boveris A (1979). Hydroperoxide metabolism in mammalian. Ann. Rev. Physiol. 59: 527-604.
Draper HH, Hadley M (1990). A review of recent studies on the metabolism of exogenous and endogenous malondialdehyde. Xenobiotica, 20: 901-907.
Hamlaoui-Gasmi S, Mokni M, Aouani E, Amri M, Marzouki L (2011a). Modulation of hematological parameters by garlic based on route of administration in rat. J. Food Biochem. 35. 442-453.
Hamlaoui-Gasmi S, Mokni M, Aouani E, Amri M, Marzouki L (2011b). Effect of the route of garlic treatment on modulation of erythrocytes and plasma redox status in rats J. Med. Plants Res. (In Press).
Hamlaoui-Gasmi S, Mokni M, Aouani E, Amri M, Marzouki L (2011c). Effect of the route of garlic treatment on modulation of liver and spleen redox status in rats J. Med. Plants Res. (In Press).
Heck DE, Vetrano AM, Mariano TM, Laskin JD (2003). UVB light stimulates production of reactive oxygen species unexpected role of catalase. J. Biol. Chem. 278: 2243-2246.
Jabbari A, Argani H, Ghorbanihaghjo A, Mahdavi R (2005). Comparison between swallowing and chewing of garlic on levels of serum lipids, cyclosporine, creatinine and lipid peroxidation in renal transplant recipients. Lipids Health Dis. 4: 11-14.
Kulaksiz H, Fein E, Redecker P, Stremmel W, Adler G, Cetin Y (2008). Pancreatic -cells express hepcidin and iron uptake regulatory peptide. J. Endocrinol. 197: 241-249.
Leardi A, Caraglia M, Selleri C, Pepe S, Pizzi C, Notaro R, Fabbrocini A, De Lorenzo S, Musicò M, Abbruzzese A, Bianco AR, Tagliaferri P (1998). Desferioxamine increases iron depletion and apoptosis induced by ara-C of human myeloid leukaemic cells. Br. J. Haematol. 102: 746-752.
Merle U, Fein E, Gehrke SG, Stremmel W, Kulaksiz H (2007). The iron regulatory peptide hepcidin is expressed in the heart and regulated by hypoxia and inflammation. Endocrinology, 148: 2663-2668.
Misra HP, Fridovich I (1972). The role of superoxide anion in autoxidation of epinephrine and a simple assay for SOD. J. Biol. Chem. 247: 3170-3175.
Ohnishi ST, Barr JK (1978). A simple method of quantitating protein using the biuret and phenol reagent. Anal. Biochem. 86: 193-200.
Papanikolaou G, Tzilianos M, Christakis JL, Bogdanos D, Tsimirika K, McFarlane J, Goldberg YP, Sakellaropoulos N, Ganz T, Henneth E (2005). Hepcidin in iron overload disorders. Blood, 105: 4103-4105.
Park CH, Valore EV, Waring AJ, Ganz T (2001). Hepcidin, a urinary antimicrobial peptide synthesized in the liver. J. Biol. Chem. 276: 7806-7810.
Pigeon C, Ilyin G, Courseland B, Leroyer P, Turlin B, Brissot P, Loreal O (2001). A new mouse liver-specific gene, encoding a protein homologous to human antimicrobial peptide hepcidin, is overexpressed during iron overload. J. Biol. Chem. 276: 7811-7819.
Saravanan G, Prakash J (2004). Effect of garlic (*Allium sativum*) on lipid peroxidation in experimental myocardial infarction in rats. J. Ethnopharmacol. 94: 1155-158.
Schaffer EM, Liu JZ, Green J, Dangler CA, Milner, J.A (1996). Garlic and associated allyl sulphur components inhibit N-methyl-N-nitrosurea induced rat mammary carcinogenesis. Cancer Lett. 102: 199-204.
Taratinsev AV, Vrizheshch PV, Schegolev AA et al. (1992). Ajoene antagonizes integrine-dependent processes in HIV-infected T lymphoblast. AIDS, 6: 1215-17.
Wang Q, Du F, Qian ZM, Ge XH, Zhu L, Yung WH, Yang L, Ke Y (2008). Lipopolysaccharide induces a significant increase in expression of iron regulatory hormone hepcidin in the cortex and substantia nigra in rat brain. Endocrinology, 49: 3920-3925.