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Full Length Research Paper

Effect of the route of garlic treatment on modulation of liver and spleen redox status in rats

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Garlic is a widely used medicinal plant exhibiting beneficial health effects such as antidiabetic, antioxidant or hypolipidemic. However several controversies persist about the beneficial or toxic effects of garlic according to its mode of administration in rat. We analyzed the ability of high dosage garlic administered per orally (p.o.) or through intraperitoneal (i.p.) route to act on liver and spleen antioxidant status in rats. In these tissue p.o. garlic is antioxidant as it decreased malondialdehyde (MDA) and H_2O_2 and increased catalase (CAT), peroxidase (POD) and superoxide dismutase (SOD) activities. Intraperitoneal garlic is pro-oxidant as revealed by high malondialdehyde and H_2O_2 levels, a decrease in free iron deposition and in catalase, peroxidase and superoxide dismutase activities. In conclusion in the liver and the spleen high garlic dosage is more safe when orally administered. These effects are free iron mediated and organ specific.

Key words: Garlic, liver, spleen, antioxidant status, administration way, lipoperoxidation, free iron, hydrogen peroxide.

INTRODUCTION

Garlic (*Allium sativum L.*) is a medicinal plant which has been widely used as a nutritional supplement in acute as well as in long term experiments (Lawson and Bauer, 1998). Garlic was shown to exert a wide range of pharmacological such as antiviral, antibacterial (Taratinsev et al., 1992), antitumor (Schaffer et al., 1996), antiatherosclerotic (Campbell et al., 2001), antioxidant (Banerjee et al., 2002) and hypolipidemic activity (Yeh and Liu, 2001). 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). In a recent study, we could establish that garlic high dose oral treatment exhibited profound antianemic, antifatigue, lipid-lowering activity and transaminases lowering as compared to i.p. route of treatment (Hamlaoui-Gasmi et al., 2011). In the present work, we investigated the antioxidant effect of garlic when p.o. or i.p. administered on liver and spleen antioxidant status by evaluating MDA, free iron, hydrogen peroxide and antioxidant enzyme activities (catalase, peroxidase and superoxides dismutase). Data are in favour 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.

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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 (gaiacol); bovine catalase and 4-(1-Hydroxy-2-methylamino-ethyl)-benzene-1,2-dio l (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 kept in plastic cages and 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 chewed tap 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. 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 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 organs 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 liver or spleen 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, 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-1cm-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 minute 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 (0.4U/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 colorful 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).

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

Differential effect of garlic (p.o. and i.p.) treatment on liver and spleen lipoperoxidation

The results presented in Figure 1 show the data of garlic dosage administered either by p.o. or i.p. way on liver (Figure 1A) and spleen (Figure 1B) lipoperoxidation. When administered by p.o. route, garlic decreased liver (-20%) and spleen (-45%) MDA. However, the i.p. administered garlic increased MDA in liver (+57%) and on spleen (+51%).

Liver antioxidant enzyme activities

The data outcome shown in Figure 2 dealt with the effect of garlic mode of administration on liver antioxidant enzyme activities. Oral garlic treatment increased liver CAT (+81%), (Figure 2A), POD (+40%) (Figure 2B) and SOD (+61%) (Figure 2C) activities; in this latter case, Fe-SOD is strongly up-regulated (+88%) when compared to Cu/Zn or Mn isoform. Garlic i.p. treatment decreased

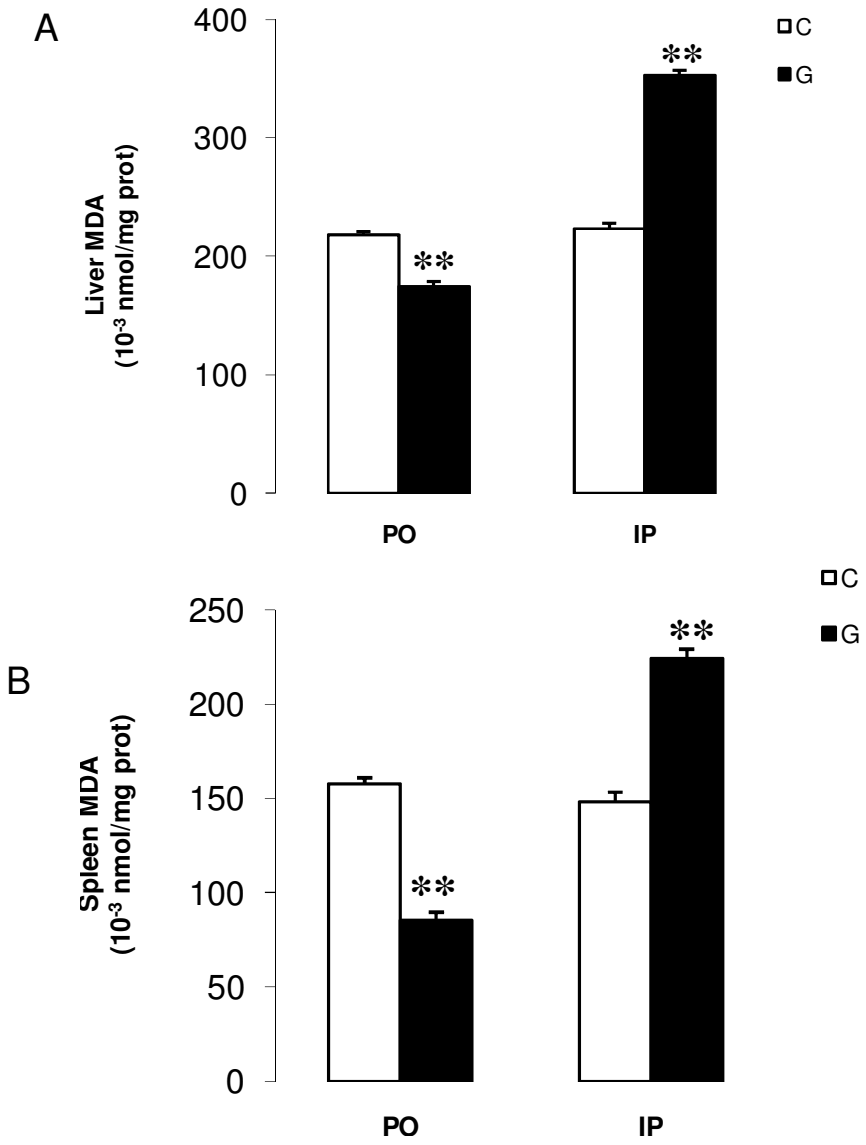

Figure 1. Effect of garlic way of administration on liver and spleen lipoperoxidation. NaCl 9‰ (C□) or garlic (G■) were p.o. or i.p. administered to rats during 30 days and liver (A) and spleen (B) MDA determined. Results are expressed by mean ± SEM of 10 rats per group. Data are representative of 3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

CAT (-40%), POD (-34%) and SOD (-37%); moreover in this latter case, the two isoforms that is Cu/Zn and Mn-SOD were decreased respectively by -63 and -37%.

Spleen antioxidant enzyme activities

The effect of garlic mode of administration (p.o. and i.p.) on spleen antioxidant enzyme activities is present in Figure 3. Oral garlic treatment increased all three enzyme activities CAT (+76%) (Figure 3A), POD (+88%) (Figure 3B) and SOD (+67%) (Figure 3C) activities. However garlic i.p treatment slightly increased CAT (+35%), decreased POD (-22%) and SOD (-40%) especially the Fe isoform.

Effect of garlic mode of administration on liver and spleen free iron levels

We further looked at tissues free iron level (Figure 4) and data showed that whatever the mode of administration, garlic significantly decreased hepatic (Figure 4A) and spleen (Figure 4B) free iron levels.

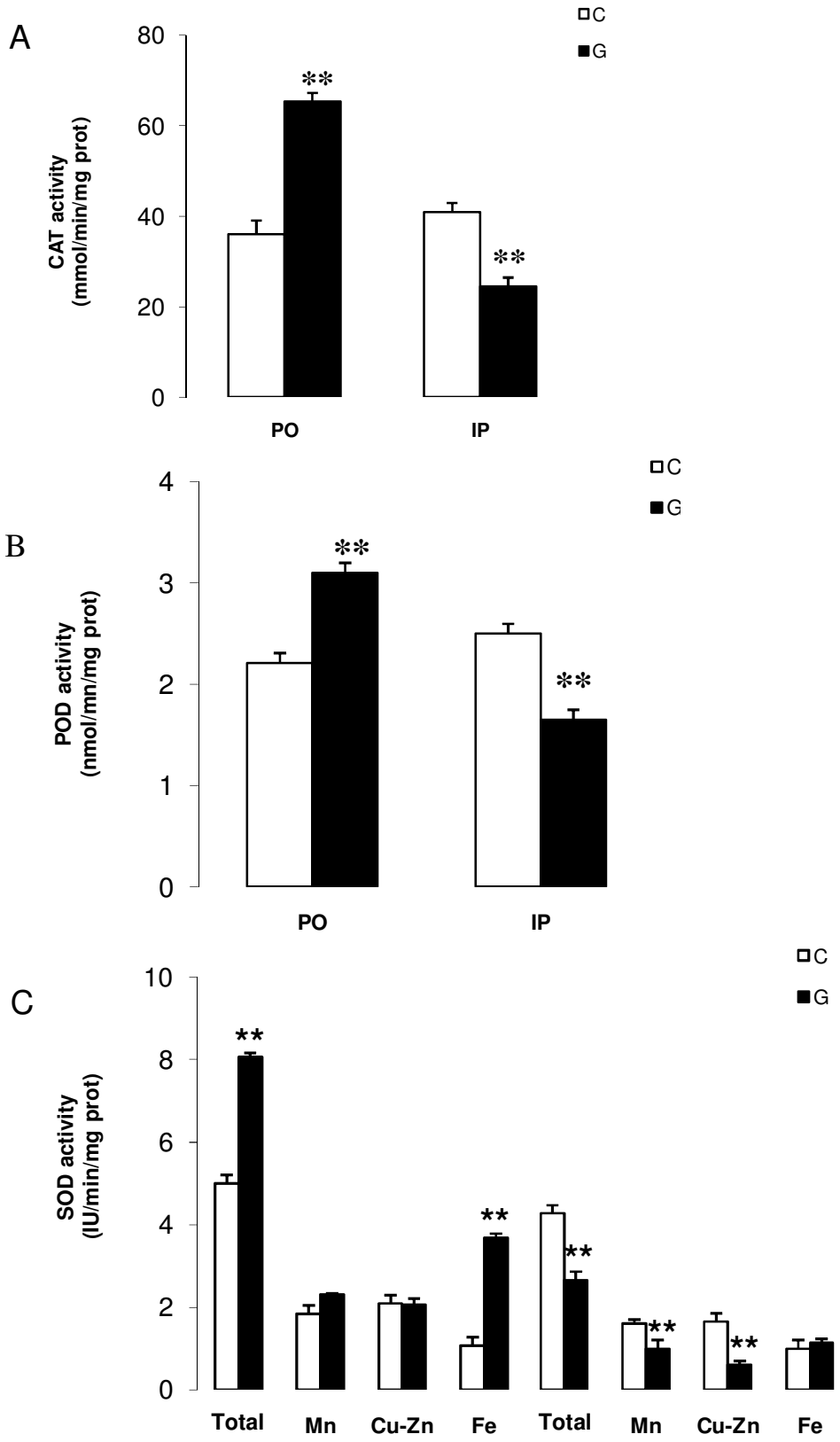

Figure 2. Effect of garlic way of administration on liver antioxidant status. NaCl 9‰ (C□) or garlic (G■) were p.o. or i.p. administered to rats during 30 days and hepatic CAT (A), POD (B) and SOD (C) activities determined. Results are expressed by mean ± SEM of 10 rats per group. Data are representative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

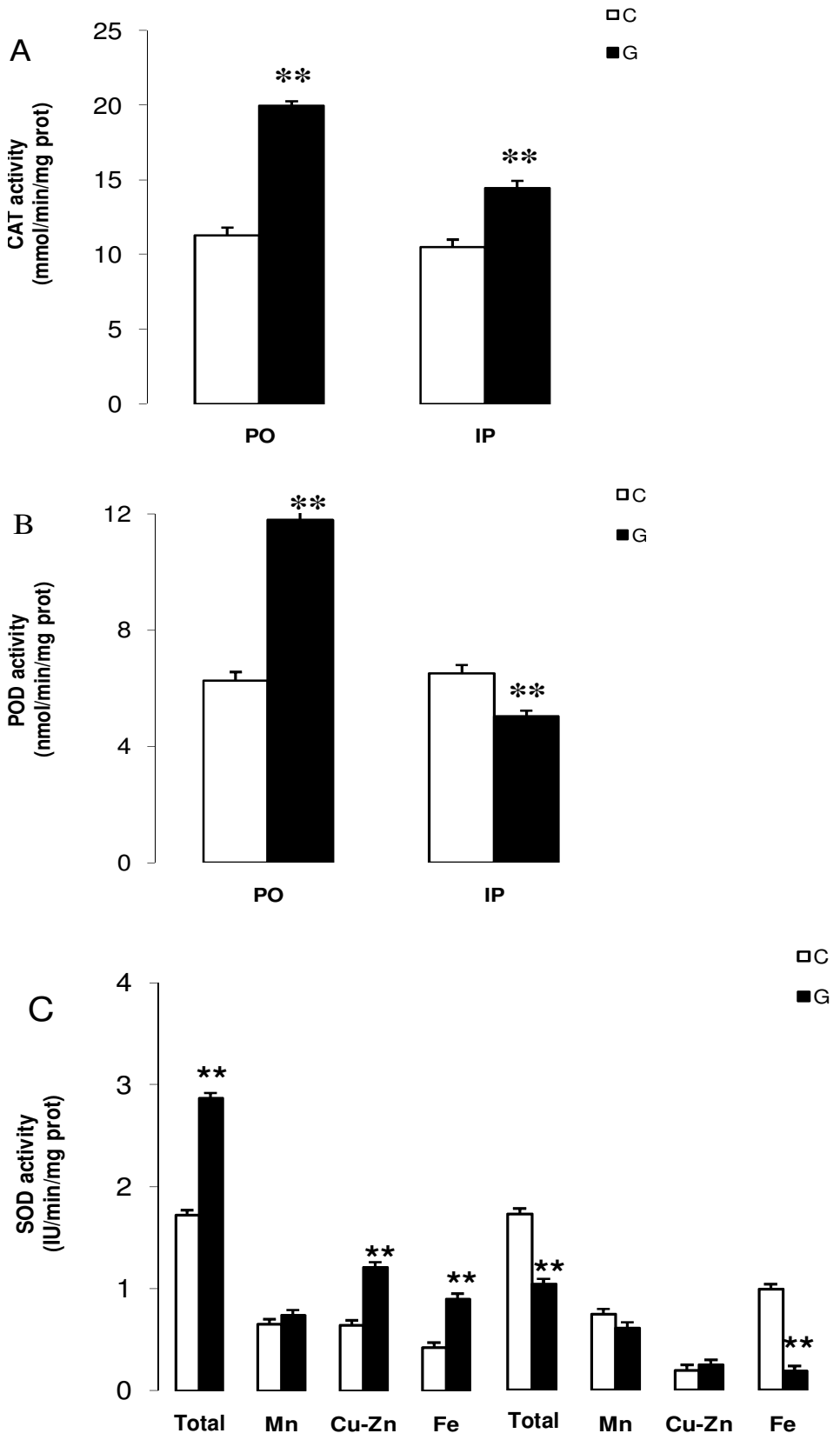

Figure 3. Effect of garlic way of administration on spleen antioxidant status. NaCl 9‰ (C□) or garlic (G■) were p.o. or i.p. administered to rats during 30 days and splenic CAT (A), POD (B) and SOD (C) activities determined. Results are expressed by mean ± SEM of 10 rats per group. Data are representative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

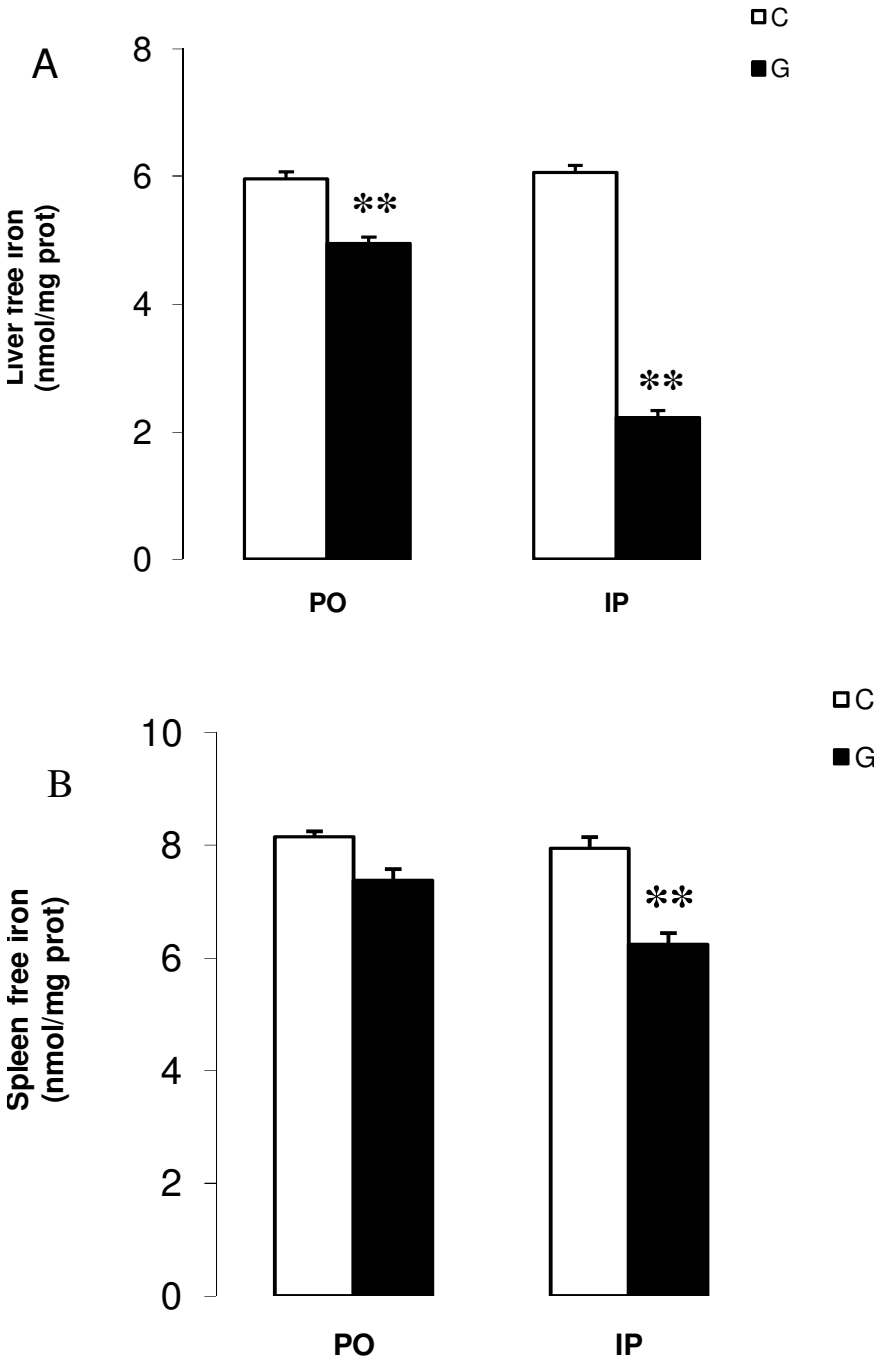

Figure 4. Effect of garlic way of administration on liver and spleen free iron level. NaCl 9‰ (C□) or garlic (G■) were p.o. or i.p. administered to rats during 30 days and hepatic (A) and splenic (B) free iron levels determined. Results are expressed by mean ± SEM of 10 rats per group. Data are representative of 3 independent experiments. ** indicated $p < 0.01$.

Effect of garlic mode of administration on liver and spleen hydrogen peroxide levels

In the present study, the effect of garlic treatment by using two modes of administration (p.o. and i.p.) on liver and spleen hydrogen peroxide level were investigated and the results are presented in Figure 5. As expected, garlic p.o. treatment decreased H_2O_2 level in liver (Figure 5A) (-45%) and spleen (Figure 5B) (-28%), however, i.p. garlic treatment increased the levels to 95 and 24%

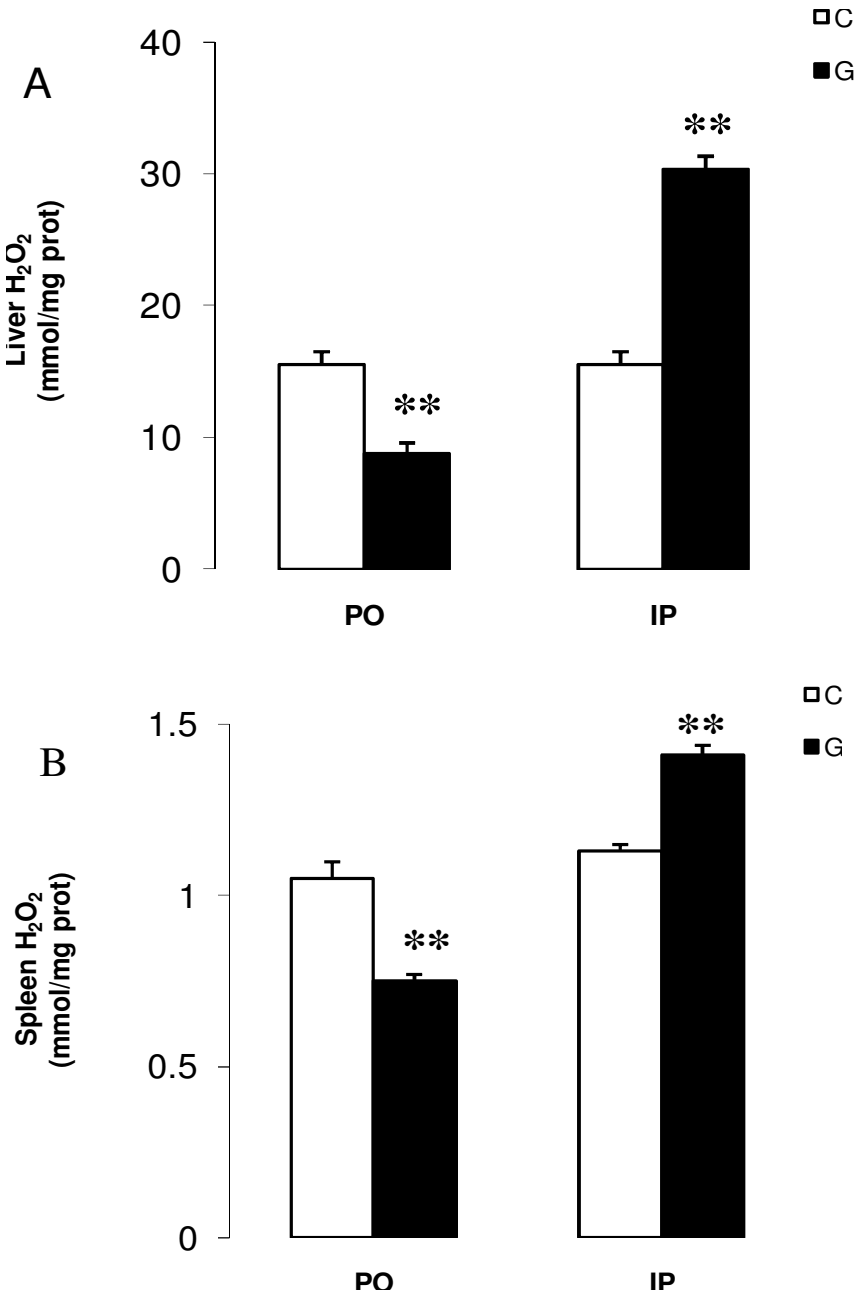

Figure 5. Effect of garlic way of administration on hepatic and splenic hydrogen peroxide level. NaCl 9‰ (C□) or garlic (G■) were p.o. or i.p. administered to rats during 30 days and liver (A) and spleen (B) H_2O_2 level activities determined. Results are expressed by mean ± SEM of 10 rats per group. Data are representative of 3 independent experiments. ** indicated $p < 0.01$.

respectively.

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 effect whereas garlic i.p. treatment, induced anemia and hepatotoxicity as assessed by elevation in plasma transaminases (Hamlaoui-Gasmi et al., 2011). 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. The

main conclusion drawn was that the harmful/prooxidant effect of i.p. garlic and the beneficial/antioxidant effect of p.o. garlic (Hamlaoui-Gasmi et al., 2011). In the present study, we investigated the effect of p.o. or i.p. garlic on two other organs that is the liver and the spleen, which play a pivotal role in iron metabolism. In the liver, oral garlic treatment decreased MDA an H_2O_2 and slightly free iron whereas i.p. garlic which increased MDA and H_2O_2 attenuated free iron more strikingly than by p.o. route. These data which fully corroborated our recent work (Hamlaoui-Gasmi et al., 2011) add some new information on the relationship between garlic mode of administration and its effect on either iron deficiency or iron excess. Indeed in the case of liver, p.o. garlic only slightly decreased free iron deposition (antioxidant role) whereas i.p. garlic decreased it drastically (prooxidant role). As a confirmation p.o. garlic stimulated all three antioxidant enzymes as CAT, POD and SOD whereas i.p. garlic had just an opposite effect. Both iron deficiency and iron excess can lead to cellular dysfunction, 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 liver iron deficiency thus leading to increased oxidative stress.

In the spleen, p.o. garlic exerted antioxidant effect whereas i.p. garlic was pro-oxidant as assessed by MDA and H_2O_2 levels. Oral garlic-induced antioxidant effect was further confirmed by its positive effects on antioxidant enzymes CAT, POD and SOD. However, i.p. garlic which exerted strong prooxidant effect also attenuated POD and SOD (especially the Cu/Zn and Mn isoform) but unexpectedly increased CAT activity, although to a lesser extent than by p.o. way. 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). Oral garlic slightly decreased spleen iron deposition and behaved as antioxidant, whereas i.p. garlic which affected free iron more drastically is prooxidant. It is noteworthy that similar data were found in plasma compartment that is a positive correlation between p.o. garlic-induced slight increase in free and antioxidant effect and between i.p. garlic-induced high increase in free iron and prooxidant effect. Garlic-induced iron excess or deficiency seems organ specific. For instance we previously showed that in erythrocytes iron deficiency (p.o. garlic treatment) was antioxidant. In the liver and spleen, slight iron deficiency is associated with antioxidant effect (p.o. garlic treatment) although high iron deficiency is rather associated with prooxidant effect (i.p. garlic). 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.

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