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Full Length Research Paper

Garlic-mode treatment effects on rat brain redox status

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Garlic (*Allium sativum*) has played an important medicinal role in human history. We analyzed the ability of high dosage garlic administered per orally (PO) or via intraperitoneal (IP) route to act on brain antioxidant status in rats. In this organ, PO garlic is antioxidant as it decreased malondialdehyde (MDA), hydrogen peroxide (H_2O_2) and lactate dehydrogenase (LDH) levels. As a confirmation, oral garlic increases antioxidant enzyme activity as catalase (CAT), peroxidase (POD) and superoxide dismutase (SOD). IP garlic is pro-oxidant as revealed by high MDA, H_2O_2 and LDH levels. However, IP garlic decreases CAT and SOD activities but unexpectedly it has no effect on POD activity. Garlic also increases brain free iron level whatever its mode of administration however this increase is more important with IP way than PO. In conclusion, in the brain, high garlic dosage is safer when orally administered; these effects are free iron mediated and organ specific.

Key words: Garlic, brain, redox status, administration route, lipoperoxidation, free iron, hydrogen peroxide, antioxidant enzymes.

INTRODUCTION

Brain diseases constitute a major cause of morbidity and mortality all over the world. Compelling evidences support the central role of oxidative stress in the pathophysiology of neurodegenerative diseases as Alzheimer and Parkinson. Increased reactive oxygen species (ROS) lead to a neurodegenerative signalling cascade triggered by oxidation of vital cellular components which induced cellular damage and cell death (Chauhan, 2006). Cells possess effective mechanisms to control ROS among which antioxidant enzymes as CAT, POD and SOD. As a result, they convert ROS to less harmful species (Pari et al., 2007). A promising approach in the prophylaxis and treatment of neurodegenerative diseases is the use of antioxidants. These latter are able to scavenge ROS and to up-regulate endogenous antioxidant defences. Garlic has been shown to have a number of medicinal properties including antithrombotic (Steiner and Li, 2001), antibiotic (Bakri and Douglas, 2005), hypoglycemic (Liu et al., 2006), hypotensive (Brankovic et al., 2011) and hypolipidemic (Hamlaoui-Gasmi et al., 2011a) activities. Various garlic preparations and components have also been shown to have antioxidant activity (Hasani-Ranjbar et al., 2009). 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 PO or by IP route. This latter way of administration which avoids the gastric barrier was previously shown to be more effective than gastric gavage especially concerning the hypocholesterolemic effect of garlic (Syed et al., 2009). In a recent study, we have established that garlic high dose oral treatment exhibited profound antianemic, antifatigue, lipid-lowering activity and transaminases lowering as

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Abbreviations: IP, Intraperitoneal; **PO,** per orally; **MDA,** malondialdehyde; **LDH,** lactate dehydrogenase; **CAT,** catalase; **POD,** peroxidase; **SOD,** superoxide dismutase; **ROS,** reactive oxygen species; **TBA,** 2-thiobarbituric acid; **BHT,** 2,6,-di-tert-butyl-4-hydroxy-tolu ene; **TCA,** trichloroacetic acid; **UV**, ultraviolet; **NADH,** nicotinamide adenine dinucleotide hydride.

compared to IP route of treatment (Hamlaoui-Gasmi et al., 2011a). We also showed that garlic high dose oral treatment exhibited profound antioxidant activity in red blood cells, plasma, liver and spleen (Hamlaoui-Gasmi et al., 2011b, c) as compared to IP route of treatment. In this present work, we investigate the effect of garlic when PO or IP administered on brain statut redox by evaluating MDA, free iron, hydrogen peroxide, lactate dehydrogenase and antioxidant enzyme activities as CAT, POD and SOD. Data are in favour of an efficient antioxidant effect of garlic when orally administered.

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-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 10000 g and 4ºC). Supernatant was aliquoted and stored at 80ºC 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 ± 1ºC 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 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 brains 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 brain 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 0.5N HCl, 120 mM TBA in 26 mM Tris and then heated at 80ºC 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. 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. POD activity was measured at 25ºC 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. POD 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).

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.

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).

LDH determination

Lactate dehydrogenase activity was determined using a commercially available kit from Biomaghreb (Tunisia). Briethly, brain homogenate was added to reaction mixture containing nicotinamide adenine dinucleotide hydride (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.

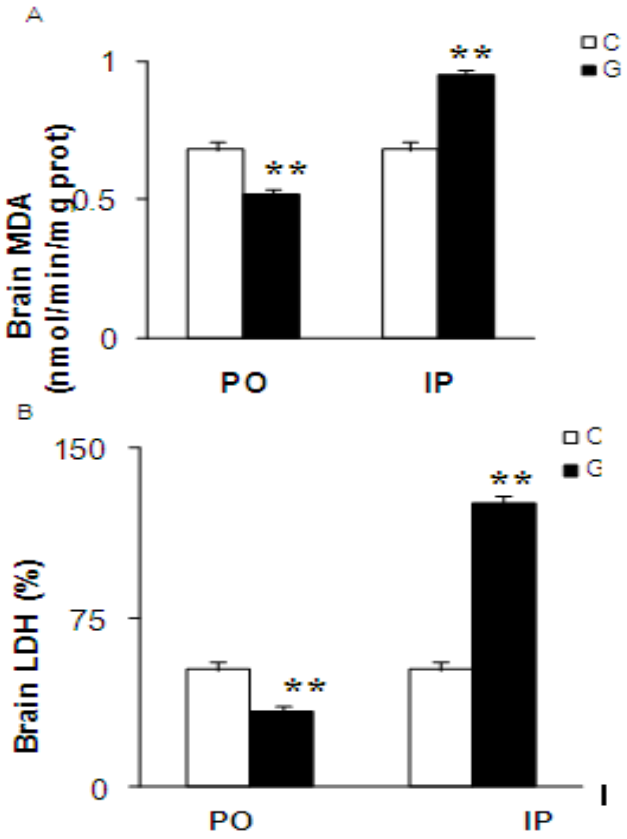

Figure 1. Effect of garlic way of administration on brain lipoperoxidation and LDH. NaCl 9‰ (C □) or garlic (G ■) were p.o. or i.p. administered to rats during 30 days and brain MDA (Figure 1A) and LDH (Figure 1B) determined. Results are expressed by mean ± SEM of 10 rats per group. Data are representative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

RESULTS

Effect of garlic (PO and IP) treatment on brain lipoperoxidation and LDH level

The results presented in Figure 1 show the data of garlic dosage administered either by PO or IP way on brain lipoperoxidation (Figure 1A) and LDH (Figure 1B). When administered by PO route, garlic decreases brain MDA (- 24%) and LDH (- 36%) levels. However, IP garlic increased MDA (+ 40%) and LDH (+ 112%) levels in brain.

Brain antioxidant enzyme activities

The data outcome shown in Figure 2 dealt with the effect of garlic mode of administration on brain antioxidant enzyme activities. Oral garlic treatment increases brain CAT (Figure 2A) (+ 175%), POD (Figure 2B) (+ 640%) and SOD (Figure 2C) (+ 150%) activities. Garlic IP treatment decreases CAT (- 45%) and SOD (- 38%) but has no effect on POD activity.

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

We further looked at brain free iron level and data showed that whatever the mode of administration, garlic significantly increases brain free iron level (Figure 3A). This increase is more important with IP than PO way. Hydrogen peroxide level was also investigated and the results are presented in Figure 3B. Garlic PO treatment decreases H_2O_2 level (- 45%) in brain, however, IP garlic treatment increased it (+ 150%).

DISCUSSION

We have previously 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 IP treatment, induced anemia and hepatotoxicity as assessed by elevation in plasma transaminases (Hamlaoui-Gasmi et al., 2011a). Interestingly, IP garlic-induced toxic effects were shown to be mediated by increased erythrocyte MDA, free iron and H_2O_2 whereas PO garlic-induced beneficial effects were mediated by a decrease in these parameters. We also showed that garlic high dose oral treatment exhibited profound antioxidant activity in red blood cells, plasma, liver and spleen (Hamlaoui-Gasmi et al., 2011b, c) as compared to IP route of treatment. The main conclusion drawn was that the harmful/prooxidant effect of IP garlic and the beneficial/antioxidant effect of PO garlic. This study clearly investigates the effect of PO or IP garlic on brain where oral garlic treatment decreased MDA, H_2O_2 and LDH. When administered by IP way garlic significantly increases brain MDA, H_2O_2 and LDH levels. These data which fully corroborated our recent work (Hamlaoui-Gasmi et al., 2011b) add some new information on the relationship between garlic modes of administration. Elevated levels of MDA in brain can be an indicator of increased lipid peroxidation in cerebral cell membranes that suggests the participation of free-radical induced oxidative cell injury in mediating the toxicity of IP route. The formation of MDA in brain is a sign of lipid membrane degradation that involving the deterioration of cellular integrity. In addition, our results confirm the works of Sofiyan et al. (2006) which shown a significant decrease in cerebral MDA level after administration of Age (Aged Garlic Extract). Our study clearly shows a reduction of H_2O_2 in the brain when garlic is administered by PO way. This data can be explained by the garlic activity as a scavenger of free radicals such as H_2O_2, O_2^- and $OH^\bullet$ (Pedraza-Chaverri et al., 2007). As a confirmation, PO garlic stimulated in brain all three

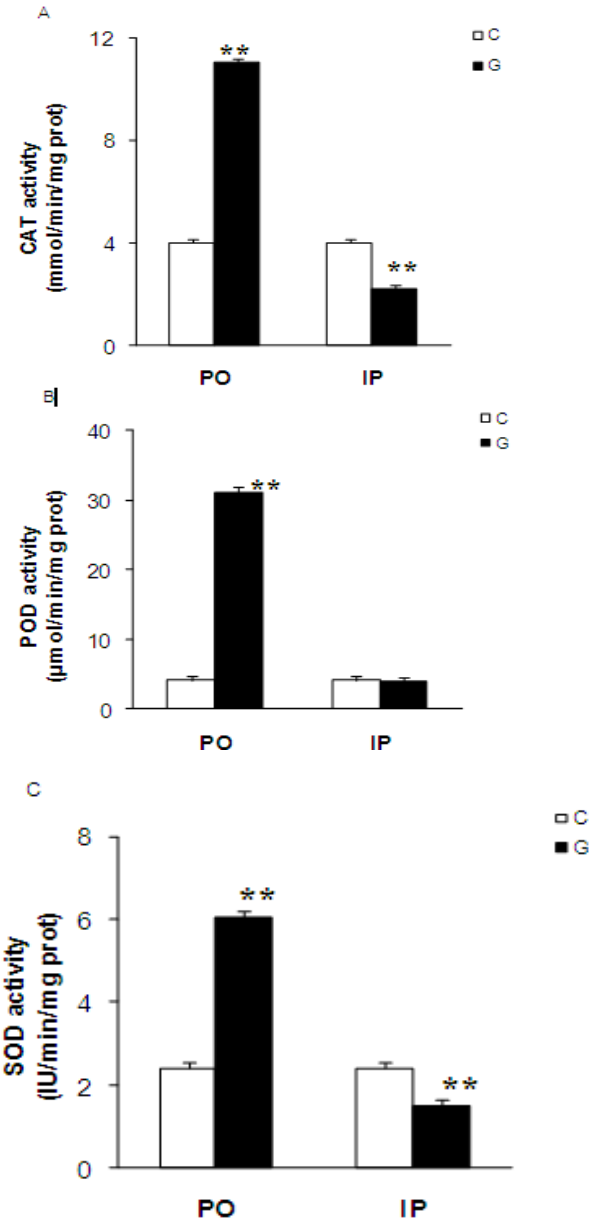

Figure 2. Effect of garlic way of administration on brain antioxidant status, NaCl 9‰ (C□) or garlic (G ■) were p.o. or i.p. administered to rats during 30 days and brain CAT (Figure 2A), POD (Figure 2B) and SOD (Figure 2C) activities determined. Results are expressed by mean ± SEM of 10 rats per group.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

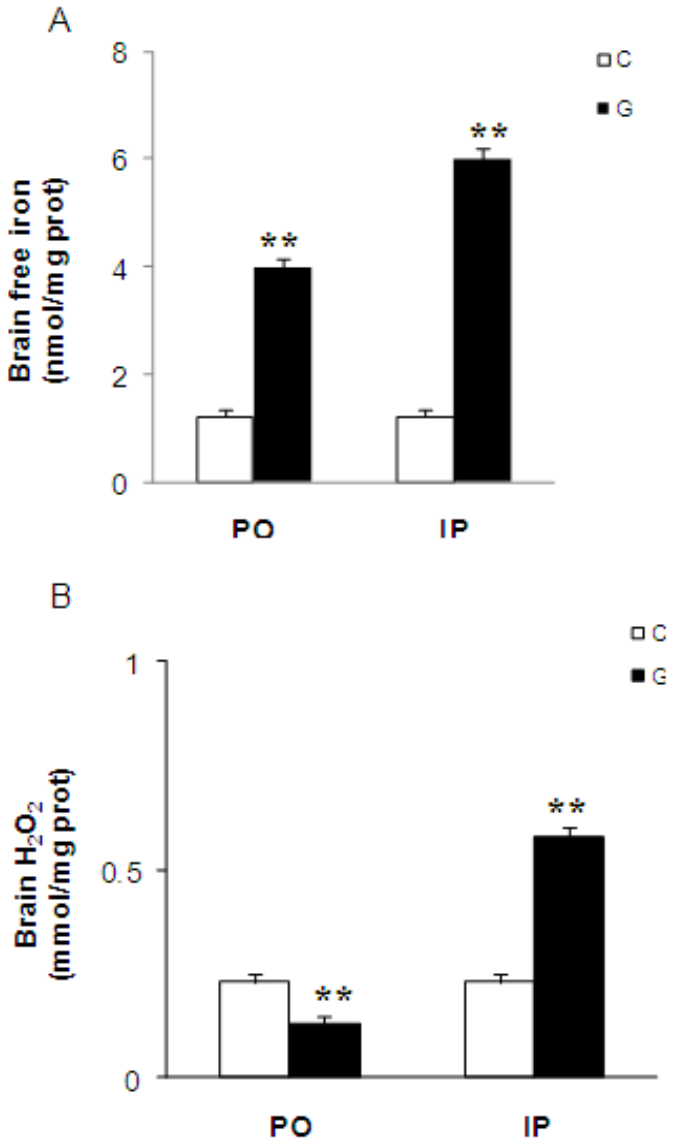

Figure 3. Effect of garlic way of administration on brain free iron and H_2O_2 levels, NaCl 9‰ (C□) or garlic (G ■ were p.o. or i.p, Administered to rats during 30 days and brain free iron (Figure 3A) and H_2O_2 (Figure 3B) levels determined. Results are expressed by mean ± SEM of 10 rats per group.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

antioxidant enzymes as CAT, POD and SOD whereas IP garlic had just an opposite effect. Our data are consistent with previous results showing that in the brain garlic has antioxidant effects by increasing levels of antioxidant enzymes such as CAT, SOD and GPx (Rahman, 2003; Rajasree et al., 2009). The same results were confirmed by Sofiyan et al. (2006) who noted an increase in antioxidant enzymes in brain on rats treated with garlic.

Indeed, in the case of brain, whatever the mode of administration, garlic significantly increases free iron level. 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 IP garlic inducing up-regulation of hepcidin and drastic brain iron excess thus leading to increased oxidative stress. Interestingly, in the case of brain, PO garlic slightly increased free iron deposition (antioxidant role) whereas IP garlic increased it drastically (prooxidant role). It is noteworthy that similar data were found in plasma compartment that is, a positive correlation between PO garlic-induced slight increase in free iron and antioxidant effect and between IP garlic-induced high increase in free iron and prooxidant effect (Hamlaoui-Gasmi et al., 2011b). Garlic-induced iron excess or deficiency seems organ specific. For instance, we previously showed that in erythrocytes, iron deficiency (PO garlic treatment) was antioxidant. In the liver and spleen, 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., 2011c).

In conclusion, we showed that garlic could modulate oxidative stress in rats depending on its route of administration as it behaves either as prooxidant PO or as antioxidant (IP). Differential intestinal absorption of garlic might explain these results and the underlying mechanism seems also to involve disturbances in iron homeostasis and likely iron shuttling proteins.

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