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Full Length Research Paper

Effect of garlic's mode of administration on erythrocytes and plasma parameters in Wistar rat

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Garlic preparations are recognized as hypolipidemic, cardioprotective and antihypertensive agents. However, there are some discrepancies about the beneficial effects of garlic according to dosage and mode of administration. We aimed to determine the ability of high dosage garlic (5 g/kg *bw*) to modulate erythrocytes and plasma parameters when administered orally (p.o.) or via intraperitoneal (i.p.) route. With regard to erythrocytes parameters, p.o. garlic treatment was found to have beneficial effects as it increased hemoglobin and hematocrit levels. Garlic i.p. treatment showed detrimental activity as it decreased these parameters. Our results reveal that garlic administered by p.o. does not involve any significant variation on mean cell volume (MCV), mean cell hemoglobin (MCH) and mean cell hemoglobin concentration (MCHC). Nevertheless, garlic i.p. increased MCV but reduced the MCH. The MCHC remained invariable even in intraperitoneal way. Concerning plasma parameters, our data show that garlic did not induce any variation on glycaemia and plasma electrolytes whatever its mode of administration. High garlic dosage was found to be relatively safe when administered orally.

Key words: Garlic, erythrocytes, hemoglobin, hematocrit, glycaemia, plasmatic electrolytes, administration mode.

INTRODUCTION

It is well known that dietary factors play a key role in the prevention of metabolic disorders (Riccardi and Rivellese, 2000). Among all such agents, garlic (*Allium sativum* L.) has attracted the attention of modern medical science because of its widespread over the counter use. Garlic exhibits antihypertensive (Asdaq and Inamdar, 2011), hypolipidemic (Madkor et al., 2011) or antibacterial (Rahman et al., 2011) activities. However, several reported effects were deviating and conflicting, depending on experimental duration, garlic dosage and mode of administration (Banerjee and Maulik, 2002). Garlic is generally administered either orally or by intraperitoneal route. The latter, which avoids gastric barrier, was previously shown to be more effective than gastric gavage (Alnaqeeb et al., 1996). It was interesting to observe that i.p. treatment with garlic was more effective with regard to its hypocholesterolemic effect when compared with other modes of treatment (Heidarian et al., 2011).

From a recent study, it was established that garlic high dose oral treatment exhibited profound antianemic, antifatigue and lipid-lowering activity when compared with i.p. route of treatment (Hamlaoui-Gasmi et al., 2011a). This

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Abbreviations: **Hb**, Hemoglobin; **HCT**, hematocrit; **MCV**, mean cell volume; **MCH**, mean cell hemoglobin; **MCHC**, mean cell hemoglobin concentration; **bw**, body weight.

study compared the two modes of garlic administration on erythrocytes and plasma parameters, that is, p.o. and i.p. routes in chronic experiments of one month duration. The data obtained during the study showed that garlic oral treatment, possessed anti-anemia effect. The putative link between anti-anemia nature and effect of garlic on free iron overload was studied and the results were presented in a recent communication (Hamlaoui-Gasmi et al., 2011a).

MATERIALS AND METHODS

Preparation of garlic extract

Garlic was purchased from the local market, peeled and ground with an electric mincer. 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 10,000 g and 4°C). The supernatant obtained was aliquoted and stored at – 80°C until it was used.

Animals and treatment

Male Wistar rats (180–200 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 diet and 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 National Institute of Health (NIH) guidelines (NIH, 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 injected (i.p.) with 9% NaCl (control). Group IV was injected (i.p.) with garlic (5 g/kg *bw*). Animals were treated daily for 30 days and checked for weight gain or loss. At the end of treatment duration, blood samples were collected following standard procedure and processed for studies on erythrocytes and plasma parameters.

Blood processing

Whole blood was obtained by cardiac puncture and collected into heparinized tubes. Erythrocytes were isolated from plasma by centrifugation at 1000 g for 10 min at 4°C and homogenized using a hypotonic buffer Tris-HCl 10 mM pH 7.5, $MgCl_2$ 5 mM, NaCl 10 mM.

Hematological parameters

Erythrocytes counts, hemoglobin, haematocrit, mean cell volume (MCV), mean cell hemoglobin (MCH) and mean cell hemoglobin concentration (MCHC) were determined using Coulter counts apparatus (Nihon Kohden Celtac E automate).

Estimation of glucose and plasma electrolytes

Plasma samples were analyzed for estimation of glycemia and electrolytes (calcium, phosphate, sodium and potassium) using an auto blood analyzer (Coulter).

Statistical analyses

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

RESULTS

Differential effect of garlic (p.o. and i.p.) treatment on hemoglobin and hematocrit

The results presented in Figure 1 showed the effects inside of data of garlic dosage administered either by p.o. or i.p. on hemoglobin (Figure 1A) and hematocrit (Figure 1B). When administered by p.o. route, garlic increased hemoglobin (+14%) and hematocrit (+18.16%). However, intraperitoneal garlic induced a highly significant reduction in the hemoglobin reaching (-18.93%) and the hematocrit (-12.54%).

Erythrocytes parameters

The data shown in Table 1 dealt with the effect of garlic mode of administration on erythrocyte parameters. Oral garlic treatment does not involve any significant variation on MCV, MCH and MCHC. Nethertheless, i.p. garlic increased the MCV, reduced the MCH but the MCHC remains invariable even in intraperitoneal way.

Effect of garlic mode of administration on glycemia

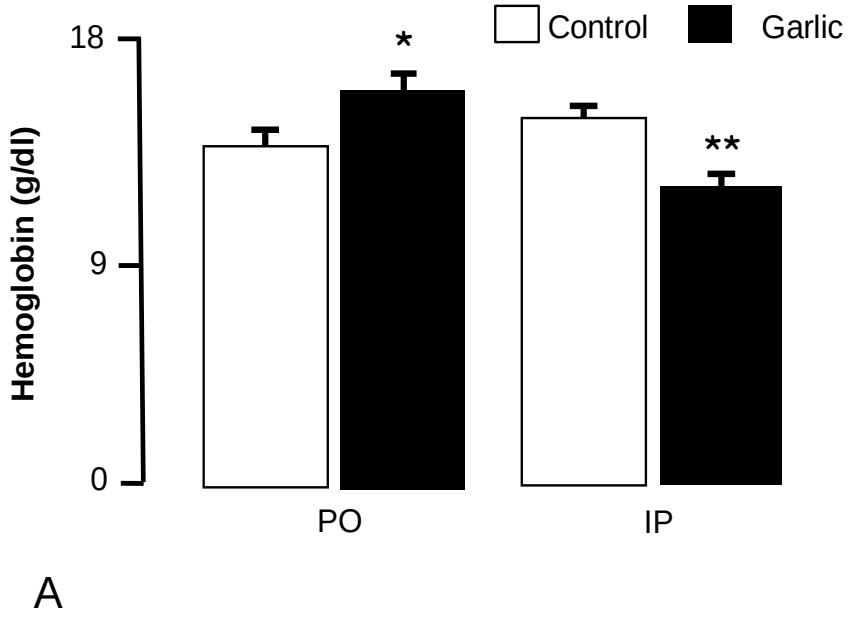
The effect of garlic mode of administration (p.o. and i.p.) on glycemia is presented in Figure 2 and the data showed that garlic did not induce any variation concerning the glycemia, however, it could be due to its mode of administration.

Effect of garlic mode of administration on plasma electrolytes

Table 2 shows that garlic did not induce any variation concerning the plasma electrolytes concentration whatever its mode of administration.

DISCUSSION

In the present study, we used garlic in subchronic experiments of one month duration at high dosage and



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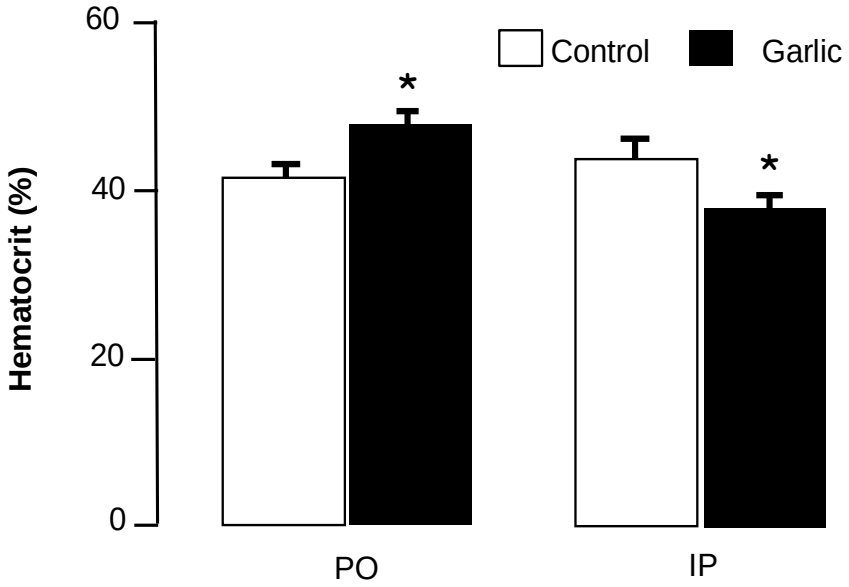

B

Figure 1. Effect of garlic (p.o. and i.p.) treatment on hemoglobin (A) and haematocrit (B).

Table 1. Effect of garlic mode of administration on erythrocyte parameters.

Parameter	Control (p.o.)	Garlic (p.o.)	Control (i.p.)	Garlic (i.p.)
MCV (μm^3)	52.85±0.7	52.5±0.5	52.32±0.2	54.63±0.7**
MCH (g/l)	33.6±0.3	34. ±0.1	34.78±0.4	33.32±0.4
MCHC (pg)	18.15±0.3	17.52±0.2	19.18±0.1	18.20±0.3

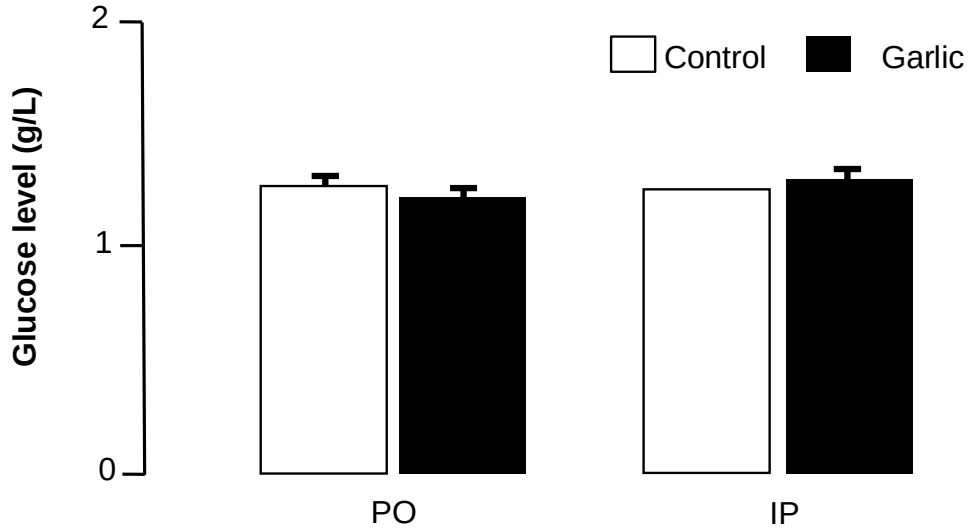

Figure 2. Effect of garlic mode of administration (p.o. and i.p.) on glycemia.

Table 2. Effect of garlic mode of administration on plasma electrolytes

Parameter (mM/L)	**Control (p.o.)**	**Garlic (p.o.)**	**Control (i.p.)**	**Garlic (i.p.)**
Calcium	2.29± 0.08	2.44 ± 0.1	2.18 ± 0.01	2.20 ± 0.02
Sodium	132.54 ± 2	129.86 ± 2	130.9 ± 2.7	134.6 ± 0.6
Potassium	6.20 ± 1.10	5.90 ± 1.00	6.39 ± 0.30	6.31 ± 0.14

compared i.p. versus p.o. mode of administration in order to bring some clues to several discrepancies about the effectiveness of garlic beneficial health effects (Agarwal, 1996). We used garlic at 5 g/kg dosage previously shown to exert cholesterol and glucose lowering activities (Mokni et al., 2006). In a previous study, we showed that when orally administered, garlic exerted positive growth effects evaluated by weight gain and increased the erythrocytes number (Hamlaoui-Gasmi et al., 2011a). Garlic-induced increase in erythrocytes count might be linked either to an increase in erythropoiesis (Morihara et al., 2007) or to the ability of garlic in decreasing membrane rigidity inherent to its cholesterol lowering effect (Hamlaoui-Gasmi et al., 2011a).

Overall in this work, we found that p.o. garlic exhibited much more beneficial effects than the i.p. mode of administration. When orally administered garlic exerted positive growth effects evaluated by increased hemoglobin and hematocrit. The data reveal that garlic administered by p.o. did not result in any significant variation of MCV, MHV or MCHC. These data which fully corroborated previous works further strengthened its putative use as an antifatigue agent (Morihara et al., 2007). This last effect might have been provoked by garlic increased erythrocytes deformability due to its hypolipidemic effect (Hamlaoui-Gasmi et al., 2011a). In this respect, osmotic fragility is a determinant of the deformability property which is essential for their function; and survival against the destruction by the spleen (Kempaiah and Srinivasan, 2005).

Conversely, i.p. garlic induced weight loss, slightly decreased erythrocytes number as well as their deformability (Hamlaoui-Gasmi et al., 2011a). Thus, garlic mode of administration appeared essential. Intraperitoneal garlic administration exerted negative effects evaluated by decreased hemoglobin and hematocrit. Moreover, the results obtained in this study reveal that garlic administered by i.p. increased the MCV but reduced the MHV and has no effect on the MCHC.

Garlic is commonly allowed like a hypoglycemiant agent whatever the mode of administration. We showed that garlic does not exert any effect on glycemia and on plasmatic electrolytes whatever the route of administration. In this order, Rosen et al. (2001) mentioned the absence of hypoglycemiant effect of garlic after intragastric way. In the same way it was shown (Liu et al., 2006) that a daily treatment of diabetics' rats by p.o. garlic oil or by DADS did not affect the glycemia. Contradictory results showed (Chang and Johnson, 1980) that p.o. garlic powder induced a reduction in blood glucose. Moreover in diabetics rat treatment using garlic, improved all the changes of these parameters on a level comparable with

that reached by treatments with insulin or the glibenclamide (Sheela and Augusti, 1992). Also, administration of raw garlic to fructose fed rats (diabetic) significantly reduced serum glucose, insulin as well as insulin resistance when compared with fructose fed rats after 8 weeks of treatment (Padiya et al., 2011).

Garlic p.o. exhibited beneficial effects with no toxicity even at high dosage used in the present study. In this respect, p.o. garlic has been shown to exert antioxidant properties in plasma and erythrocytes of elderly subjects (Avc et al., 2008). Although, preliminary our data opened the way to putative use of high garlic concentration either as antioxidant (when p.o. administered) or as prooxidant (by i.p. way). In previous studies, we established that garlic high dose oral treatment exhibited profound antianemic, antifatigue, lipid-lowering activity and transaminases lowering when compared with i.p. 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 and plasma (Hamlaoui-Gasmi et al., 2011b), liver and spleen (Hamlaoui-Gasmi et al., 20011c) and brain (Hamlaoui-Gasmi et al., 2011d) when compared with i.p. route of treatment, which could even be detrimental by inducing a prooxidant effect and ultimately toxicity in these organs.

From the above discussion, we conclude that the antianemic effects of p.o. garlic are original, interesting and could have promising therapeutic applications.

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