

Full Length Research Paper

Grape seed extract mitigates high dosage garlic-induced toxic effects on erythrocyte and plasma parameters

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High dosage garlic exerts adverse health properties and grape seed extract (GSE) exhibit a variety of beneficial effects, even at high dosage. In the present study we evaluated the toxic effect of high dosage garlic treatment on blood parameters and the protective effect of GSE co-treatment. Rats were intraperitoneally injected with 5 g/kg body weight (bw) crude garlic extract during one month and co-treated or not (vehicle 10% ethanol) with GSE (500 mg/kg bw). Blood parameters were evaluated. Data confirmed that high dosage garlic induced anemia, increased MCV, reduced the MCHC, but has no effect on MCH. Garlic also has no effect on glycemia, calcemia, natremia and kaliemia. GSE co-treatment counteracted almost all garlic's effects. High dosage garlic induced a toxic effect into erythrocytes parameters and GSE has real contradictory property.

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

INTRODUCTION

Garlic is a commonly used spice in folk medicine, exhibiting antibacterial (Rahman et al., 2011), hypolipidemic (Madkor et al., 2011) or antihypertensive (Asdaq and Inamdar, 2011) activities. Garlic's beneficial health effects are mainly assumed to result from its antioxidant ability (Lu, 2011). However some controversies persist concerning garlic dosage or mode of administration. Garlic is generally administered either orally or by intraperitoneal route. This latter way of administration, which avoids the gastric barrier, was previously shown to be more effective

than gastric gavage (Alnaqeeb et al., 1996). However, in earlier studies, we that high dosage of garlic administered by intraperitoneal way could even be detrimental by inducing a pro-oxidant effect (Hamlaoui-Gasmi et al., 2011a), an increase in erythrocytes membrane fragility and ultimately anemia (Hamlaoui-Gasmi et al., 2011b).

Grape seed extract (GSE) is widely used as nutritional supplement affording tremendous preventing and healing effects (Suwannaphet et al., 2010). GSE contains polyphenols, such as resveratrol, which is at the basis of the "French Paradox". GSE effects are wide, ranging as cardioprotective (Charradi et al., 2011) or neuroprotective (Narita et al., 2011) mainly due to its antioxidant and anti-inflammatory properties (Jia et al., 2011). Moreover, GSE also exerts antineoplastic effects by inducing cytotoxicity toward some cancer cells (Kaur et al., 2011) by cell cycle arrest and apoptosis (Dinicola et al., 2010).

In the present work, we re-evaluated the toxic effect of high dosage garlic on blood parameters as hemoglobin, hematocrit, mean cell volume (MCV), mean cell

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Abbreviations: Hb, Hemoglobin; HCT, hematocrit; MCV, mean cell volume; MCH, mean cell hemoglobin; MCHC, mean cell hemoglobin concentration,

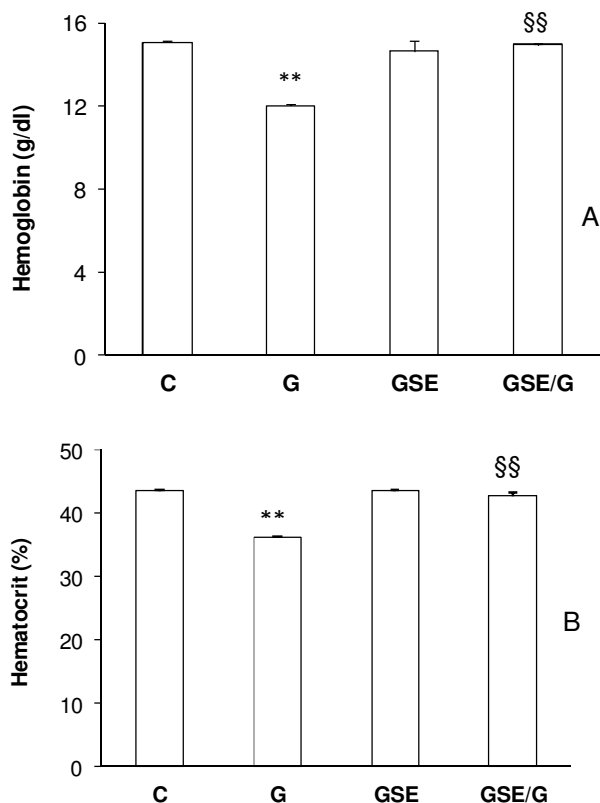


Figure 1. Effect of GSE on garlic-induced changes in hemoglobin and hematocrit. 10% ethanol (C), garlic (G), grape seed extract (GSE) or garlic plus GSE (GSE/G) were administered to rats during one month and haemoglobin (A) and hematocrit (B) were determined. Results are expressed by mean \pm SEM ($n = 10$) and assays were done in triplicate. **Indicated $P < 0.01$ versus C. §§Indicated $P < 0.01$ versus G.

haemoglobin (MCH) and mean cell hemoglobin concentration (MCHC). We also studied the effect of high dosage garlic on glycemia, calcemia, kaliemia and natremia as well as the putative protection offered by GSE pretreatment. Data reinforced the toxic effect of high dosage garlic as well as the potential protective one of GSE.

MATERIALS AND METHODS

Preparation of garlic and grape seed extract

Garlic was purchased from local market, peeled and grounded with an electric mincer. It was diluted in double distilled water at 2 g/ml on the basis of the weight of the starting material and was centrifuged (Beckman J20, 15 min at 10 000 g and 4°C). Supernatant was aliquoted and stored at -80°C until use.

Grape seed and skin extract (GSE) was processed from a grape cultivar (Alicante Bouschet) of *Vitis vinifera* from Northern Tunisia. Polyphenolic powder mixture containing grape seed (50%) and skin (50%) was dissolved in 10% ethanol, centrifuged 15 min at 10 000 g to eliminate insoluble material, and supernatant-containing soluble polyphenols was used.

Animals and treatment

40 male Wistar rats (200 to 240 g) from Pasteur Institute of Tunis were used for these experiments in accordance with the local ethic committee of Tunis University for use and care of animals in conformity with the National Institutes of Health (NIH) recommendations (NIH, 1985). They were provided with food and water *ad libitum* and maintained in animal house at controlled temperature ($22 \pm 2^{\circ}\text{C}$) with a 12 h light-dark cycle. Rats were divided into four groups of ten animals each. Group I received ethanol 10% (control), group II aqueous extract of garlic (5 g/kg bw), group III GSE (500 mg/kg bw) and group IV garlic plus GSE. Animals were daily intraperitoneally injected during one month. 24 h after the last injection, rats were anesthetized with 0.5 ml urethane (40 mg/ml) and sacrificed, their blood were collected and processed for erythrocytes and plasma parameters using automates.

Blood processing

Whole blood was obtained by cardiac puncture and collected into heparinized tubes. Erythrocytes were isolated from plasma as previously described (Hamlaoui-Gasmi et al., 2011a).

Hematological parameters

Erythrocytes counts, hemoglobin, haematocrit, MCV, MCH and 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 analyser (Coulter).

Statistical analysis

All data were expressed by mean values \pm 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 and GSE on hemoglobin and hematocrit

We reported in Figure 1, the effect of garlic and GSE either alone or in combination on hemoglobin content (Figure 1A) and hematocrit (Figure 1B). As expected, high dosage garlic decreased the hemoglobin content and the hematocrit, whereas GSE, which had no significant effect *per se*, counteracted all garlic-induced deleterious effect on erythrocyte parameters.

Erythrocytes parameters

The data outcome shown in Figure 2 dealt with the effect of garlic and GSE either alone or in combination on

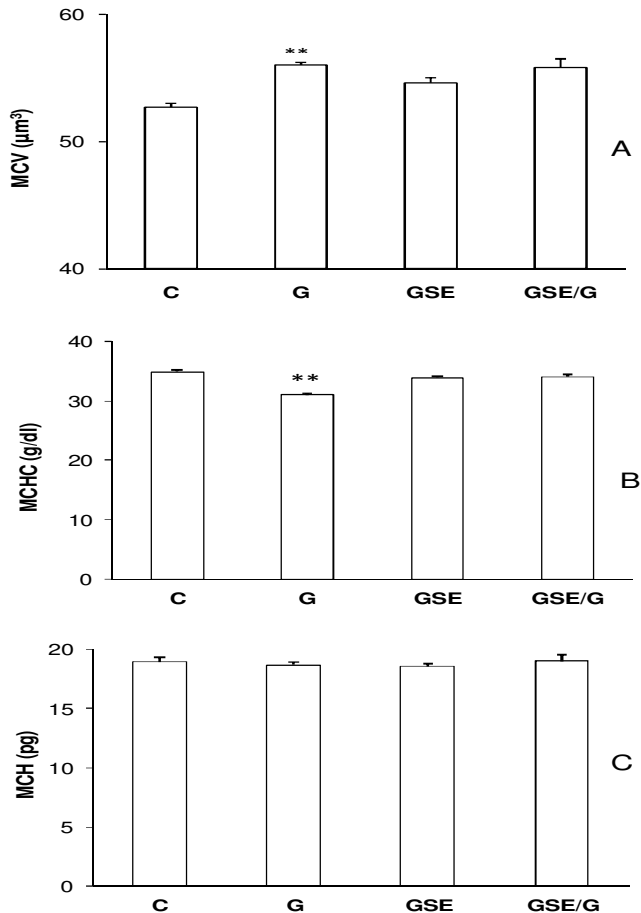


Figure 2. Effect of GSE on garlic-induced MCV, MCHC and MCH. Rats were daily administered with 10% ethanol (C), garlic (G), grape seed extract (GSE) or garlic plus GSE (GSE/G) for one month and MCV (Figure 1A), MCHC (Figure 1B) and MCH were determined. Results are expressed as means \pm SEM (n = 10) and assays were done in triplicate. **P<0.01 compared to control (C).

erythrocyte parameters (MCV, MCHC and MCH). High dosage garlic increased MCV, reduced the MCHC, but has no effect on MCH. Nevertheless, GSE which had no significant effect *per se* counteracted all garlic-induced deleterious effect on MCV, MCHC and MCH.

Effect of garlic and GSE on glycemia

The effect of garlic and GSE either alone or in combination on glycemia is presented in Table 1 and data show that garlic does not induce any variation concerning the glycemia. Moreover, GSE had no significant effect alone or in co-treatment with garlic.

Effect of garlic and GSE on plasma electrolytes

Table 2 shows that garlic does not induce any variation

concerning the plasma electrolytes concentration, no matter the mode of administration.

DISCUSSION

The main relevant result of the present study is that high dosage garlic administered by intraperitoneal injection to healthy rats induced anaemia and that garlic-induced deleterious effects are abolished by GSE treatment. We first confirmed the toxic role played by high dosage garlic. Indeed, i.p. garlic induced weight loss, slightly decreased erythrocytes number as well as their deformability (Hamlaoui-Gasmi et al., 2011b). In this study, intraperitoneal garlic administration exerted negative effects evaluated by decreased hemoglobin and hematocrit. Moreover, the results of this study revealed that garlic increased the MCV but reduced the MCHC and has no effect on the MCH.

Furthermore our data showed that co-treatment with GSE during one month reduced garlic-induced anaemia and oxidative stress (data not shown) within erythrocytes. First GSE was used at a dosage previously shown to be devoid of any toxicity. Our data corroborate with results already mentioned in literature dealing with antioxidant and anti-inflammatory effects of GSE that have been observed either *in vitro* or *in vivo* and in various experimental settings (Nassiri-Asl and Hosseinzadeh, 2009). Whether these protective properties of GSE are mediated by resveratrol as recently shown for human erythrocytes (Pandey and Rizvi, 2009) or result from a synergistic effect of various grape-containing polyphenols (Liu, 2004), is still a matter of debate.

Our data give some new insight into garlic as well as GSE mode of action. Garlic simultaneously increased free iron and H₂O₂ within erythrocytes, likely leading to the highly toxic hydroxyl radical formation via the Fenton reaction and ultimately to erythrocyte death and anaemia (Hamlaoui-Gasmi et al., 2011a, b). Erythrocytes are highly susceptible to oxidative damage, because of the high polyunsaturated fatty acid content of their membranes and the high cellular concentration of oxygen. Increased erythrocyte MDA level is known to cause a decrease in the fluidity of the membrane lipid bilayer (Bryszewska et al., 1995) and high MDA level is generally correlated to pathological conditions or stress, including aging (Rizvi and Maurya, 2007).

Importantly, consumption of a resveratrol rich diet as GSE is protective against human erythrocytes *in vitro* (Mikstacka et al., 2010). A possible mechanism by which GSE exerts its beneficial effect on erythrocytes is its ability to chelate free iron and scavenge H₂O₂ as recently demonstrated (Charradi et al., 2011).

Garlic is commonly allowed like a hypoglycemic agent what ever the mode of administration. In this study, we showed that garlic and GSE do not exert any effect on glycemia and on plasmatic electrolytes. The observation is in conformity with the observation of Rosen et al. (2001)

Table 1. Effect of garlic and GSE on glycemia.

	Control	Garlic	GSE	GSE/G
Glycemia (g/L)	1.32 ± 0.02	1.38 ± 0.04	1.3 ± 0.03	1.2 ± 0.05

Rats were daily administered with 10% ethanol (C), garlic (G), grape seed extract (GSE) or garlic plus GSE (GSE/G) for one month and glycemia determined. Results are expressed by mean ± SEM (n = 10) and assays were done in triplicate.

Table 2. Effect of GSE on garlic-induced calcemia, kalemia and natremia.

	Control	Garlic	GSE	GSE/G
Calcemia (mmol/L)	2.18 ± 0.01	2.20 ± 0.02	2.08 ± 0.01	2.1 ± 0.01
Natremia (mmol/L)	130.9 ± 2.7	134.6 ± 0.6	128.7 ± 2	130 ± 2
Kaliemia (mmol/L)	6.39 ± 0.30	6.31 ± 0.14	6 ± 0.50	6.3 ± 0.50

Rats were daily administered with 10% ethanol (C), garlic (G), grape seed extract (GSE) or garlic plus GSE (GSE/G) for one month and calcemia, kalemia and natremia determined. Results are expressed as means ± SEM (n=10) and assays done in triplicate.

who have also mentioned the absence of hypoglycemic effect of garlic after intragastric administration. In the same way, it was shown (Liu et al., 2006) that a daily treatment of diabetics rats by garlic oil or by diallyl disulfide (DADS) does not affect the glycemia. Contradictory results (Chang et al., 1980) showed that p.o. garlic powder induced a reduction in blood glucose. Moreover, in diabetics rat treatment by garlic improves all the changes of these parameters on a level comparable with that reached by treatments with insulin or the glibenclamide (Sheela and Augusti, 1992). Moreover, 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).

We also previously showed that garlic high dose oral treatment, exhibited profound antioxidant activity in red blood cells and plasma (Hamlou-Gasmi et al., 2011a), liver and spleen (Hamlou-Gasmi et al., 2011c) and brain (Hamlou-Gasmi et al., 2011d) as compared to i.p. route of treatment which could even be detrimental by inducing a pro-oxidant effect and ultimately toxicity in these organs.

In conclusion, we showed that high dosage garlic induced anemia and that GSE exerted antianemia effect.

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