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Short communications

Effects of garlic-derived lipid soluble organosulfur compounds on hematological parameters in mice

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ABSTRACT

The effects of garlic oil (GO) and 5 lipid soluble organosulfur compounds on hematological parameters were investigated. SPF male ICR mice were treated with 100 mg/kg body weight of GO, diallyl trisulfide (DATS), diallyl disulfide (DADS), diallyl sulfide (DAS), allyl methyl trisulfide (AMT), or allyl methyl disulfide (AMD) for 4 weeks. We found that 1 and 2 weeks of GO, DATS, and AMT treatment led to significant increase of the blood white blood cell (WBC) counts and a slight decrease of the red blood cell (RBC) and hemoglobin (HGB) levels, while platelet was not affected. The spleen weight and the spleen weight/body weight ratio in mice of GO, DATS, and AMT groups were significantly increased compared with control group mice. These results suggest that DATS and AMT may be the bioactive compounds responsible for the elevation of WBC, which might be related with the increased extramedullary hematopoiesis in the spleen.

1. Introduction

Garlic (*Allium sativum* L.) is believed to originate in central Asia and belongs to the Alliaceae family. The medicinal use of garlic dates back to thousands of years and at present, it remains to be one of the most popular herbal remedies worldwide (Amagase, Petesch, Matsuura, Kasuga, & Itakura, 2001; Guan, Zhao, Xie, & Zeng, 2018; Rana, Pal, Vaiphei, Sharma, & Ola, 2011; Zeng, Zhang, Zhao, & Xie, 2013). Experimental studies and clinical trials have provided clear evidences that garlic possesses multiple pharmacological effects including antioxidation, anti-inflammation, anticancer, immunomodulation, regulating serum lipids and blood pressures, and hepatoprotective effects against various kinds of chemicals-induced liver injuries (Amagase, 2006; Iciek, Kwiecien, & Wlodek, 2009; Rana et al., 2011). These biological activities likely arise from the volatile organosulfur compounds, which are also responsible for the pungent of garlic (Amagase et al., 2001; Iciek et al., 2009; Lanzotti, 2006). Garlic oil (GO), usually prepared by steam distillation, is one of commercially available garlic products which accounts for about 0.2–0.5% of garlic bulbs weight and has many beneficial effects(Banerjee, Mukherjee, & Maulik, 2003). Chemical analyses revealed diallyl trisulfide (DATS), diallyl disulfide (DADS), diallyl sulfide (DAS), methyl allyl trisulfide (AMT), and methyl allyl disulfide (AMD) are the 5 major organosulfur compounds in garlic and GO (O'Gara, Hill, & Maslin, 2000).

The effects of garlic on hematological parameters have been investigated in some pilot studies (HAMLAOUI-GASMI, Mokni, Aouani, Amri, & Marzouki, 2011; Iranloye, 2002; Oluwole, 2001; Zamani, Ghiasvand, Hadei, Babaahmadi-Rezaei, & Pishdadian, 2009). One study showed that garlic juice (extracted using distilled water) feeding for 30 d significantly increased the red blood cell (RBC) count, hemoglobin (HGB) concentration, packed cell volume (PCV), and the total white blood cell (WBC) count in male albino rats (Iranloye, 2002), while another study found that garlic tablet (100 mg/kg body weight) significantly increased the blood RBC count, the total WBC count, as well as the neutrophil and lymphocyte counts (Oluwole, 2001). However, the study by Zamani et al. showed that garlic powder could not significantly affect the levels of total WBC, RBC, and HGB in blood (Zamani et al., 2009). In another study, Hamlaoui-Gasmi et al. compared 2 mode of garlic administration (intraperitoneally vs. orally) on some hematological parameters, and found that garlic administration by intraperitoneal injection induced a significant decrease of RBC counts and affected osmotic fragility of RBC in male Wistar rats, while opposing results were obtained in rats orally treated with garlic (HAMLAOUI-GASMI et al., 2011). These results suggest that the effects of garlic may be related with the types, the doses, and the delivering methods. In our previous study, we found that GO significantly

Abbreviations: AMD, allyl methyl disulfide; AMT, allyl methyl trisulfide; DADS, diallyl disulfide; DAS, diallyl sulfide; DATS, diallyl trisulfide; EMH, extramedullary hematopoiesis; GO, garlic oil; GR, granulocyte; HCT, hematocrit; HGB, hemoglobin; LY, lymphocyte; MCH, mean corpuscular hemoglobin; MCV, mean corpuscular erythrocyte volume; MPV, mean platelet volume; MO, monocyte; PCT, plateletcrit; PCV, packed cell volume; PDW, platelet distribution width; PLT, platelet; RBC, red blood cell; RDW, red cell distribution width; SPF, specific pathogen free; WBC, white blood cell

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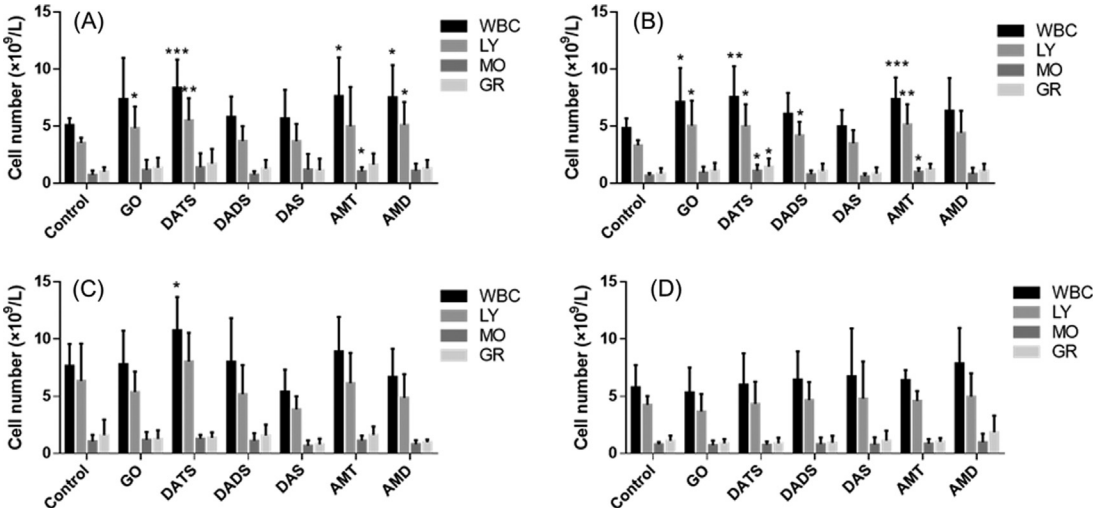

Fig. 1. Effects of garlic oil (GO) and the 5 major lipid soluble organosulfur compounds on total WBC count, lymphocyte (LY) count, monocyte (MO) count, and granulocyte (GR) count in mice. **A–D** represented the results of week 1, week 2, week 3, and week 4, respectively. $^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$, compared with the control group.

inhibited the decrease of the peripheral blood WBC levels induced by cyclophosphamide and radiation (Zeng et al., 2013). However, the effects of GO and its major organosulfur compounds on hematological parameters in normal animals remains unclear. The current study was designed to investigate the influence of GO and 5 major components in GO on the hematological parameters in mice.

2. Materials and methods

2.1. Materials

GO was purchased from Xuchang Yuanhua Biotechnology Co., Ltd. (Xuchang, China). DADS and DAS were purchased from Sigma-Aldrich CO (St. Louis, MO). DATS and AMDS were provided by Chengdu Micxy Chemical CO. (Chengdu, China). AMT were bought from Shenzhen Reagent Biotechnology CO. (Shenzhen, China).

2.2. Animals and treatments

Specific pathogen free (SPF) male ICR mice, 25–30 g, were provided by Jinan Pengyue experimental animal CO. LTD (Jinan, China). The animals were kept under standard laboratory conditions (12-h light/12-h dark cycle, 22 °C), and fed with mouse's pellets and tap water ad libitum. After acclimation to the environment for 5 d, the animals were randomized into 7 groups with 15 in each group. Mice in group I (control group) were treated with vehicle, while mice in group II-VII received 100 mg/kg body weight of GO, DATS, DADS, DAS, AMT or AMD, respectively daily, for consecutive 4 weeks. The dose of various garlic products (100 mg/kg body weight) were selected based on our preliminary experiments, which could not induce significant reduction of food intake and the slowing of body weight gain. Garlic products were administered to mice (0.2 ml/kg body weight, diluted in corn oil) by gavage between 8 am and 10 am, while the animals in control group were treated with same volume of corn oil. The mice were sacrificed at the end of the week 4, and the spleens and livers were rapidly dissected and weighed. The research was conducted in accordance with the National Institutes of Health guide for the care and use of Laboratory animals (NIH Publications No. 8023, revised 1978) and were approved by the Ethics Committee of Shandong University Institute of Preventive Medicine.

2.3. Hematological examination

At the end of week 1, week 2, week 3 and week 4, about 0.2 ml blood was drawn from each mouse via canthus intraocular, and collected into EDTA-2K-treated tubes. The total WBC count, lymphocyte (LY) count, monocyte (MO) count, granulocyte (GR) count, RBC count, HGB level, hematocrit (HCT), mean corpuscular erythrocyte volume (MCV), mean corpuscular hemoglobin (MCH), red cell distribution width (RDW), platelet (PLT) count, plateletcrit (PCT), mean platelet volume (MPV), and platelet distribution width (PDW) were detected by using CA-800 automatic blood cell counter.

2.4. Statistical analysis

All data were expressed as mean ± SD. All data were analyzed using one-way analysis of variance (ANOVA), followed by LSD's post hoc tests. Difference between groups was considered significantly at $P < 0.05$.The statistical software used for this analysis was SPSS (version 20).

3. Results

3.1. GO, DATS, and AMT treatment led to significant increase of blood WBC counts

As shown in Fig. 1, at the end of week 1, the blood WBC counts in DATS, AMT, and AMD-treated mice were all significantly higher than that of control mice ($P < 0.05$). At the end of week 2, blood WBC counts in GO, DATS and AMT–treated mice were all significantly increased compared with that in control group mice ($P < 0.05$). However, only DATS-treated mice showed elevated total WBC count at the end of week 3, and no significant difference was observed among these groups at the end of week 4. The changes of LY counts were similar to that of the total WBC. In regard with the changes of MO and GR levels, 1 and 2 weeks of AMT treatment resulted in the increase of MO counts, while 2 weeks of DATS treatment led to the elevation of both MO and GR levels.

3.2. Effects of GO and 5 major organosulfur compounds on blood RGB and PLT levels

GO and the 5 organosulfur compounds treatment also led to a slight decrease of the RBC counts and levels of HGB and HCT during the