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Full Length Research Paper

Regulatory effects of *Tenebrio molitor* Linnaeus on immunological function in mice

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This paper describes the results of experiments to test the effect of the larvae of *Tenebrio molitor* Linnaeus on the immune systems of mice. Mice were given a decoction of *T. molitor* in water at doses of 1.87, 3.75 and 7.50 g/kg/d for four weeks, after which their immune function was studied. The results indicate that *T. molitor* observably enhanced the carbon expurgatory index and phagocytic index. The half of hemolysis values in serum of treated mice increased compared to the control group. Furthermore, serum nitric oxide (NO) content in all treatment groups was higher than that of the control group whereas acid phosphatase and alkaline phosphatase activity was only significantly higher in the high dose group relative to the control group. We conclude that *T. molitor* can enhance the immune function of mice and therefore, this insect has the potential of a health food supplement.

Key words: *Tenebrio molitor* Linnaeus, mice, immunoregulation, immunological function.

INTRODUCTION

Yellow mealworm beetles *Tenebrio molitor* Linnaeus (Tenebrionidae, Coleoptera) are considered scavengers and are typically found to be injurious to insects in warehouses for agricultural products. Most prefer to feed on decaying grain or milled cereals in damp, poor conditions, sometimes infesting cornmeal, flour, cake mixes, cereals, meat scraps, dead insects, bran and litter from chicken houses; they have even been found in sparrow's nests where they feed on the droppings (Cotton, 1963; Weaver et al., 1990; Ye et al., 2001).Traditionally, mealworm has a long history of use as food supplement. They are typically used as a food source for reptile, fish, and avian pets. They are also provided to wild birds in bird feeders, particularly during the nesting season, when birds are raising their young and appreciate a ready food supply. *T. molitor* larvae are generally regarded as a rich source of protein, vitamins, essential amino acids, minerals and essential fatty acids like linolenic acid. The contents of toxic heavy metals were lower than national standards (Bai and Cheng, 2003; Gerber, 1984; Xia, 1994). Experiments on mice fed with the filtrate of larval powder solution showed that the insect sample was safe to the mice, and it was effective as an antifatigue, delaying aging, decreasing the level of serum total cholesterol and micronuclear rate in polychromatic erythrocytes, and increasing the perilymphocyte transformative rate in tested mice or mice (Yang et al., 1999). The various healthful function of the insect sample is based on the integration of its effective nutrition components. As a result, they are known as "golden grubs" and make excellent fish bait and serve as food for animals in aquariums and zoological parks. The insect is worthy of exploration as the healthful food for human. Chang (1994) reported that yellow mealworm

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Abbreviations: SRBC, sheep red blood cells; **HC_{50},** half of hemolysis values.

larvae could become an ingredient for Chinese sauces, biscuits, etc. They can be purchased at many pet stores. The potential for annual mealworm production in China is estimated at more than 100 tons (Zhang et al., 2008).

The immune system is involved in the etiology as well as pathophysiologic mechanisms of various diseases and its function and efficiency is influenced by many exogenous and endogenous factors such as food, pharmaceuticals, physical and psychological stress, resulting in either immunosuppression or immunostimulation (Geetha et al., 2005). There is growing interest in identifying and characterizing insects with immunomodulatory activity. A number of insects used in Chinese traditional medicines and food systems for rejuvenation therapy have been demonstrated to modulate immune responses (Zhang and Xu, 1990). Unfortunately, our knowledge and understanding of the immunological function of mealworm is very limited. No systematic studies have been reported to prove scientifically the immunomodulatory activity of *T. molitor* larvae.

Here, we investigated the immunomodulatory activity of *T. molitor* larvae in normal mice. The objective of this work was to provide the scientific basis for the comprehensive utilization of the insect, improve the insect utilization efficiency, and evaluate the potential for future applications.

MATERIALS AND METHODS

Insect

The yellow mealworm were subsequently cultured in the insect rearing room at the Anhui Agricultural University, on a diet of wheat bran, whole wheat flour, and brewer's yeast (50:45:5 w/w) at 26±1°C and 55± 5% relative humidity and a photoperiodic regime of 12 h light and 12 h darkness. Mealworm larvae (7 to 10th instars) that were used were obtained from the laboratory stocks. The larvae, which were removed from culture and held in clean Petri dish without food for 24 h, were burnt to death by boiling water and dried to constant weight at 80°C. Lastly, the powders of dried *T. molitor* larvae were pulverized.

30 g powdered larvae samples were weighed and extracted with distilled water by stirring at room temperature for 2 h. The resultant suspension was filtered through a two-fold layer of muslin. The filtrate was concentrated to 100 ml with rotary evaporator, and the liquid was 0.3 g/ml (equivalent to raw material) and diluted before use. The extract was prepared just before the experiments.

Animals

Normal KunMing mice were purchased from the laboratory animal center of Anhui Institute of Medical Science, and the animals were kept in air controlled rooms. The experiments were conducted on female KunMing mice weighing 20±2 g maintained at 25±2°C with normal mouse chow and water *ad libitum*. The animals were housed, five per cage, and maintained on 12 h day and night cycle. The animals were divided into four groups comprising ten mice each: Group I, control animals (distilled water control); Group II: animals fed with larvae powder extract (low-dosage group 1.87 g · kg^{-1} · d^{-1}); Group III: animals fed with larvae powder extract (moderate-dosage group 3.75 g · kg^{-1} · d^{-1}); Group IV, animals fed with larvae powder extract (high-dosage group 7.50 g · kg^{-1} · d^{-1}).

Mice were orally administered a dose of 0.025 ml · g^{-1} body weight of larvae powder extract once daily for four consecutive weeks. The control mice were given distilled water only by the same method. After four weeks of oral administration, the immunomodulatory effect of the larvae of yellow mealworm was obtained from each experimental group by the previously described method.

Carbon clearance test

To test the function of the macrophages in treated and control animals, experimental mouse was injected with Indian ink which was used as granular foreign body. The Indian ink was phagocytized and cleared by mononuclear macrophage after it went into circulation. After four consecutive weeks of oral administration, each mouse was injected with Indian ink (0.1 ml/10 g body weight) through mouse tail vein. In the second (t_1) and tenth minute (t_2) after injection, 20 µl of blood was drawn from the orbital venous plexus of mice' eye sockets with suction tube which was moistened with heparin solution and diluted in 2 ml 0.1% sodium carbonate solution. Mice were sacrificed and the body, thymus, and spleen were collected and weighed.

The optical density (OD) values of blood solution were assayed with a 721 spectrophotometer at a wavelength of 600 nm, and the *K* (representing the capability of carbon granule clearance from mouse blood) and α (representing the phagocytosis activity of macrophage) values were calculated according to the formula:

$$K = \frac{\lg OD \text{ at } 2 \text{ min} - \lg OD \text{ at } 10 \text{ min}}{t_2 - t_1}$$

$$\alpha = \frac{\text{Body weight}}{\text{Spleen weight} + \text{Thymus weight}} \times \sqrt[3]{K}$$

Serum hemolysin assay

Sheep blood was put into a sterile flask containing crystal ball and shaken to remove the fiber. The solution was rinsed three times with saline before centrifugation at 2000 rpm for 10 min. The supernatant was abandoned and the sheep red blood cells (SRBC) were prepared.

After 24 days oral administration, 0.2 ml of 20% (v/v) SRBC was intraperitoneally injected into each mouse. Five days later, blood was drawn from the orbital venous plexus of mice' eye sockets with suction tube and serum was isolated from normal mice' blood as well. Serum samples were diluted with normal saline to 1/400. The value of absorbance at the 50% hemolytic dose (HC_{50}) was determined by colorimetric method with a 721 spectrophotometer at a wavelength of 540 nm:

$$HC_{50} = \frac{\text{Value of samples absorbance}}{\text{Value of absorbance at SRBC 50\% hemolytic dose}} \times \text{Dilution of serum}$$

Acid phosphatase and alkaline phosphatase assay

After four consecutive weeks of oral administration, blood was drawn from the orbital venous plexus of mice' eye sockets with suction tube. The centrifuge tubes remained relatively static for 10 min and were incubated at 4°C in the refrigerator overnight, fixed with the blood from which serum precipitated. According to the method of Reagent Kit (Nanjing jiancheng Bioengineering Institute),

Table 1. Effect of *T. molitor* on the carbon particles clearance of mice (n=10, $\overline{X}$ ±SD).

Group	Dosage (g · kg^{-1} · d^{-1})	Expurgatory index (*K*)	Phagocytic index (α)
Negative control	0	0.009±0.005	5.02±1.32
Low-dosage group	1.87	0.012±0.008	5.09±1.09
Moderate-dosage group	3.75	0.019±0.011*	5.19±1.21
High-dosage group	7.50	0.027±0.009*	5.95±1.02*

Values in the same column followed by one asterisk means significant difference at 0.05 level compared to the control group.

Table 2. Effect of *T. molitor* on the hemolysin of mice (n=10, $\overline{X}$ ±SD).

Group	Dosage (g · kg^{-1} · d^{-1})	Half of hemolysin values (HC_{50})
Negative control	0	105.23±7.26
Low-dosage group	1.87	108.11±6.13
Moderate-dosage group	3.75	110.94±9.65
High-dosage group	7.50	119.84±9.06*

Values in the same column followed by one asterisk means significant difference at 0.05 level compared to the control group.

the activity of acid phosphatase and alkaline phosphatase was measured.

Serum nitric oxide (NO) assay

After four consecutive weeks of oral administration, blood was drawn from the orbital venous plexus of mice' eye sockets with suction tube. The centrifuge tubes remained relatively static for 10 min and were incubated at 4°C in the refrigerator overnight, fixed with the blood from which serum precipitated. According to the method of Reagent Kit (Nanjing jiancheng Bioengineering Institute), the content of NO was measured.

Statistical analysis

The results are reported as mean ± standard deviations. Statistical evaluation of the data was done using Student's *t*-test. A probability value of < 0.05 was considered significant.

RESULTS

Effect of *T. molitor* on the carbon particles clearance of mice

Effect of *T. molitor* administration on the carbon particles clearance of mice is given in Table 1. *T. molitor* extract administration was found to enhance the expurgatory index (*K*) and phagocytic index (α). It was dose-dependent to potentiate the capability of carbon granule clearance and the phagocytosis activity of macrophage in mice. Expurgatory index was significantly increased from 0.009 in negative control to 0.019 and 0.027, respectively in the group treated with 3.75 g and 7.50 g · kg^{-1} body weight *T. molitor*. The phagocytic index was significantly increased from 5.02 in negative control to 5.95, in the group treated with 7.50 g · kg^{-1} body weight *T. molitor*.

Effect of *T. molitor* on the hemolysin of mice

The effect of *T. molitor* on humoral immune response in mice was tested. As shown in Table 2, difference in HC_{50} was significantly increased from 105.23 in negative control to 119.84, in group treated with 7.50 g · kg^{-1} body weight *T. molitor*. No significant differences were found in dosage of 1.87 g and 3.75 g · kg^{-1} body weight compared to negative control. However, it was dose-dependent to potentiate the humoral immune response in mice.

Effect of *T. molitor* on the acid phosphatase and alkaline phosphatase activity of mice

Effect of *T. molitor* administration on the acid phosphatase and alkaline phosphatase activity of mice is given in Table 3. *T. molitor* extract administration was found to enhance the acid phosphatase and alkaline phosphatase activity. It was dose-dependent to potentiate the activity of the acid phosphatase and alkaline phosphatase in mice. The acid phosphatase activity was significantly increased from 4.81 in the negative control to 7.09 in the group treated with 7.50 g · kg^{-1} body weight *T. molitor*. The alkaline phosphatase activity was significantly increased from 8.01 in the negative control to 11.93 in the group treated with 7.50 g · kg^{-1} body weight *T. molitor*. However, no significant differences were found

Table 3. Effect of *T. molitor* on the acid phosphatase and alkaline phosphatase activity of mice (n=10, $\overline{X}$ ±SD).

Group	Dosage ($g \cdot kg^{-1} \cdot d^{-1}$)	Acid phosphatase activity (U/100 ml)	Alkaline phosphatase activity (King unit /100 ml)
Negative control	0	4.81±0.94	8.01±1.64
Low-dosage group	1.87	5.04±1.21	8.29±2.18
Moderate-dosage group	3.75	5.59±1.04	8.37±2.06
High-dosage group	7.50	7.09±1.38*	11.93±1.98*

Values in the same column followed by one asterisk means significant difference at 0.05 level compared to the control group.

Table 4. Effect of *T. molitor* on the serum NO content of mice (n=10, $\overline{X}$ ±SD).

Group	Dosage ($g \cdot kg^{-1} \cdot d^{-1}$)	NO content (μmol/L)
Negative control	0	8.94±1.49
Low-dosage group	1.87	8.62±1.98
Moderate-dosage group	3.75	14.65±2.31*
High-dosage group	7.50	16.69±2.88*

Values in the same column followed by one asterisk means significant difference at 0.05 level compared to the control group.

in dosage of 1.87 g and 3.75 $g \cdot kg^{-1}$ body weight compared to the negative control.

Effect of *T. molitor* on the serum NO content of mice

The effect of *T. molitor* on the serum NO content in mice was tested. As shown in Table 4, difference in ear thickness was significantly increased from 8.94 in the negative control to 14.65 and 16.69, in the group treated with 3.75 and 7.50 $g \cdot kg^{-1}$ body weight *T. molitor*, respectively. However, no significant differences were found in dosage of 1.87 $g \cdot kg^{-1}$ body weight compared to the negative control.

DISCUSSION

Immunoregulation is a complex balance between regulatory and effector cell and any imbalance in the immunological mechanism can lead to pathogenesis (Davis and Kuttan, 2000). Immunity has been shown to be suppressed in cancer. Chemotherapy and radiation therapy, useful in cancer treatment, were found to deteriorate the immunity. The yellow mealworm, as an important food supplement, contains abundant active components such as proteins, amino acids, unsaturated fatty acids, flavones and alkaloids, etc. In fact, essential nutrients and an array of phytonutrients have been shown to affect almost every aspect of the immune system (Geetha et al., 2005). Our laboratory has reported earlier that an extract from the yellow mealworm could stimulate the immunity in normal mouse. In this study, the immunomodulatory activity of *T. molitor*, an important food supplement was explored.

It is well known that macrophages are important cells in the immune response, especially in the anti-infection immunity. Administration of *T. molitor* was found to increase the carbon expurgatory index and phagocytic index significantly, indicating that the extract could stimulate the nonspecific phagocytic function. The humoral immune response is the aspect of immunity that is mediated by secreted antibodies produced in the cells of the B lymphocyte lineage. Humoral immunity is so named because it involves substances found in the humours, or body fluids. Through the determination of hemolysin in serum content, we can know the immune response and its intensity (Zheng, 1995; Jin, 2001; Si, 1999). The extract was found to enhance half of hemolysin values indicating that the extract could stimulate humoral immune response.

Macrophages are cells produced by the differentiation of monocytes in tissues. Macrophages function in both non-specific defense (innate immunity) as well as help initiate specific defense mechanisms (adaptive immunity) of vertebrate animals. Their role is to phagocytose cellular debris and pathogens, either as stationary or as mobile cells. They also stimulate lymphocytes and other immune cells to respond to pathogens. Phosphatase is an important biological detoxification enzyme systems and its activity reflects the activated degree of macrophages. NO is the important medium and the initial factor that play important cell functions, involved in a series of immune regulation effects (He and Sun, 1992; Ding et al., 2005). *T. molitor* extract administration was found to enhance the serum NO content, acid

phosphatase and alkaline phosphatase activity, indicating that *T. molitor* can effectively protect the biological immune system of mice, and enhance non-specific immune function.

Conclusion

In conclusion, *T. molito* enhanced immune indexes compared with the negative control. The results indicate that *T. molito* had a potency to potentiate the immune responses in mice. At present, we do not know which compounds are responsible for the immunostimulatory activity produced by this extract. Further studies using isolated compounds are in progress.

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