

# Comparison of Immunomodulatory Effects Among Three Tissue Extracts Of Tartary Buckwheat In Mice

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**Abstract:** Buckwheat such as common “sweet” buckwheat (*Fagopyrum esculentum* Moench.) and tartary buckwheat (*Fagopyrum tataricum*) contains numerical nutritional compounds, which makes it having several benefits for human health. Although, tartary buckwheat has been shown to have positive effects on lowering blood cholesterol level, whether it has benefits to improve immune system hasn't been reported. To investigate the effects of tartary buckwheat on immune system regulation, I conducted a series of pharmacological tests using three different tissue extracts in mice. Results demonstrated that unmaturing seeds and leaves extracts significantly improved both humoral and nonspecific immune functions in normal adult mice, while little immunomodulatory effect was observed with mature seeds extracts.

**Keywords:** Tartary buckwheat, *Fagopyrum tataricum*, Immunomodulation.

## 1. Introduction

Buckwheat is one of the most versatile crops with a traditional cultivation range stretched from eastern Europe to Japan. The origin of buckwheat is thought to be eastern Tibetan plateau, bordering the Chinese province of Yunnan [1-3]. Due to its strong ability to adapt to various environmental conditions, common “sweet” buckwheat (*Fagopyrum esculentum* Moench.) is grown in many countries such as China, Japan, Korea, Nepal and Bhutan and widely cultivated in Canada and USA [4]. However, tartary buckwheat (*Fagopyrum tataricum*) has been only found in mountain regions of China [5-6].

Buckwheat has been well known to have beneficial effects on human health. Various tissues of buckwheat contain multiple nutritional compounds such as high level of proteins in grains and a variety of vitamin and amino acids in seeds [7]. In addition, tartary buckwheat has been reported to contain numerous compounds such as general flavone, vitamin E and glucose, which are important in health protection including lowering blood lipid and sugar levels [8-9]. Therefore, tartary buckwheat is considered to have more medical and healthcare values as compared to sweet buckwheat.

Here, I evaluated and compared the effects of tissue extracts from mature seeds, unmaturing seeds and leaves of tartary buckwheat on regulating immune system in normal adult mice. The paper is organized with sections as follows. Section 2 presented experimental design and details about determination of immune system profiles in mice. The analysis and comparison of effects among three tissue extracts were demonstrated in section 3. Concluding remarks were revealed in the last section.

## 2. Materials and Methods

### 2.1. Ethics Statement

All experiments and procedures involving live animals were conducted in compliance with protocols approved by the Wenzhou Medical School Institutional Animal Care and Use Committee. All efforts were made to minimize suffering.

## 2.2. Animals and Diets

Female Kunming mice (specific pathogen-free, 8 weeks, 40-50 g) were purchased from Experimental Animal Center of Wenzhou Medical School. All mice were single caged in a room with a 12-h light-dark cycle with free access to food and water.

## 2.3. Tartar Tissue Extracts

Total aqueous extracts from mature seeds, unmaturing seeds and leaves of tartary buckwheat were produced from Tianci Green Biotech Industries, Beijing, China. The concentration of tartary tissue extracts is 20 µg/ml. The chemical analysis report provided by the manufactures showed no presence of endotoxin.

## 2.4. Evaluation of immune system profiles

Solutions of three different tissue extracts from tartar buckwheat were orally administered to mice daily at a dose of 0.1 ml/10 mg body weight (equals 200 mg/kg bw) consecutively for 30 days. Control animals were given double distilled water in the same way as extracts-treated mice. Experimental groups were as follows: I. Control, II. Mature seeds extracts, III. Unmaturing seeds extracts, IV. Leaves extracts. Each cohort had 10 mice. The immune system profiles were measured 24-h after the last dose as follows.

*Delayed-Type Hypersensitivity (DTH) test:* Mice from both experimental and control cohorts were injected with 2% sheep red blood cells (SRBCs) in 0.2 ml 0.9% normal saline (NS) intraperitoneally (IP). After 4 days of initial injection, right hind feet of mice were subcutaneously administered with 20% SRBCs. Thickness of the footpad was measured with a digital calliper (Fisherbrand Digital Callipers, Traceable, Fisher Scientific) at 24-h and 48-h after the antigen injection [10].

*Plasma Haemagglutination Antibody titer test:* Mice were immunized by injecting 2% SRBCs in 0.2 ml NS intraperitoneally. After 5 days of initial injection, blood was sampled by retro-orbital collection technique and collected into heparin-coated capillary tubes and centrifuged at 1,000 x g for 15 min to yield plasma. Animals were killed by transcardial perfusion using saline after anesthetization with 98 mg/ml sodium pentobarbital containing 12.5 mg/ml phenytoin (a 1:4 dilution of Euthasol; Delmarva Laboratories, Midlothian, VA). Spleen and thymus were collected and stored at -80°C. Serial two-fold dilutions of plasma were performed with 0.2 mL of NS and a volume of 0.2 mL of serum was added to 10 test tubes. Plasma was not treated with DTT. The SRBCs were washed three times in NS and a 3% RBC suspension was prepared for the test. In addition to 50 µL of the group A or B 3% SRBCs suspension, 100 µL of diluted serum was pipetted into 10 test tubes. Tubes were incubated at RT for 30 min followed by incubation for 30 min at 37°C using monospecific anti-IgG (Lorne Laboratories Ltd., Reading, UK). Titer was determined as the highest dilution showing 1+ agglutination [11-12].

*Plaque Forming Cell (PFC) Assay:* Spleen cell suspensions were prepared by gently tamping the spleen through a 60-mesh stainless steel screen, and collecting the cells in balanced salt solution (BSS). The spleen cells were washed and made up to 15 ml with BSS. SRBCs were washed twice and made up to a 10% concentration. Complement (Gibco) is diluted 1/20 with BSS. Spleen cell suspensions were centrifuged and supernatant was discarded. Pellet was washed twice in fresh BSS. Mixture of spleen cells (50 µl), SRBCs (70 µl) and complement solution (500 µl) in a test tube was incubated at 37°C for 1 min. All the contents transferred to slide chambers were sealed and incubated at 37°C for 1-h. The number of PFC was counted by both macro- and microscopic examination [13-15].

*Macrophage Phagocytosis Assay:* Mice were immunized by IP injecting 1% chicken red blood cells (CRBCs) suspensions in 1 ml NS. Animals were sacrificed by cervical dislocation and the abdominal cavity was washed with 2 ml NS. Incubated the washing solution (0.5 ml) at 37°C on the slide for 30 min. Giemsa staining was performed after the slide was fixed with methanol. The slides were observed under light microscope using oil immersion. At least 200 cells were counted. The phagocytic activity was expressed as phagocytic index (PI) and phagocytic capacity (PC) using the following equations.

$$PI = A \times B$$

where A is the percentage of CRBC-ingesting phagocytes and B is the number of CRBC-ingested per phagocytes. PC is the mean percentage of cells that engulfed CRBCs.

*Statistical analysis:* Data were statistically analyzed using Student's t test to determine significant differences in the data of various groups. P values less than 0.5 were considered significant. The values were expressed as means  $\pm$  S.D.

### 3. Results

I firstly investigated the effects of tartary buckwheat extracts on ratio of lymphoid organs to body weight. None of the tartary buckwheat extracts showed toxicity or mortality in extracts-treated mice. No significant effects on body weight were found for treated groups (data not shown). The ratio of spleen or thymus to whole body weight did not significantly change upon treatment with either tissue extract (TABLE I).

TABLE I: Effects of Tartary buckwheat extracts on ratio of lymphoid organs to body weight

Group	Spleen/BW	Thymus/BW
I. Control	0.41 $\pm$ 0.04	0.16 $\pm$ 0.03
II. Mature seeds	0.39 $\pm$ 0.03	0.18 $\pm$ 0.06
III. Unmatured seeds	0.40 $\pm$ 0.08	0.15 $\pm$ 0.02
IV. Leaves	0.42 $\pm$ 0.06	0.14 $\pm$ 0.03

Values are means  $\pm$  S.D. of 10 mice per group.

DTH assay was performed to evaluate the cell mediated immunity, which is a T-cell mediated defense mechanism against microbes in phagocytes. No significant effects were observed with each tartary buckwheat extract as compared to control group at both 24-h and 48-h after injection of antigen (TABLE II).

TABLE II: Effects of Tartary buckwheat extracts on cell mediated immunity parameters.

Group	Foot pad thickness (mm)	
	24-h	48-h
I. Control	0.41 $\pm$ 0.04	0.16 $\pm$ 0.03
II. Mature seeds	0.39 $\pm$ 0.03	0.18 $\pm$ 0.06
III. Unmatured seeds	0.40 $\pm$ 0.08	0.15 $\pm$ 0.02
IV. Leaves	0.42 $\pm$ 0.06	0.14 $\pm$ 0.03

Values are means  $\pm$  S.D. of 10 mice per group.

Effects on humoral immunity in response to tartary buckwheat extracts were analyzed using hemagglutination titer and PFC assays. Hemagglutination titer assay revealed that all three extracts displayed a dramatic increase in antibody titer ( $P < 0.01$  for mature seeds,  $P < 0.001$  for unmaturred seeds and leaves). Results from PFC assay indicated that responses to both unmaturred seeds and leaves were significantly elevated ( $P < 0.01$ ), while mature seeds extracts did not have statistically significant effects as compared to control mice (Fig. 1).

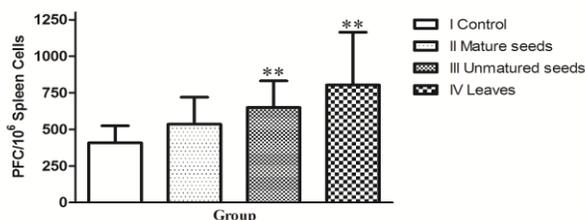


Fig. 1: Effect of tartary buckwheat extracts on humoral immunity via plaque forming cell assay. Data are means  $\pm$  S.D. of ten animals; \*\* $p < 0.01$  when compared with the control animals (significantly different).

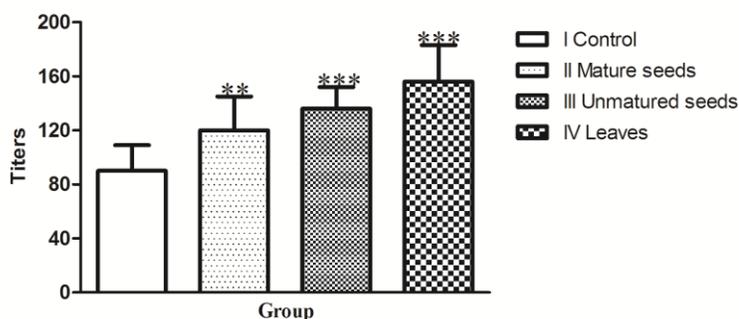


Fig. 2: Effect of tartary buckwheat extracts on humoral immunity via hemagglutination titer assay. Data are means  $\pm$  S.D. of ten animals; \*\* $p < 0.01$  when compared with the control animals (significantly different). \*\*\* $p < 0.001$  when compared with the control animals (significantly different).

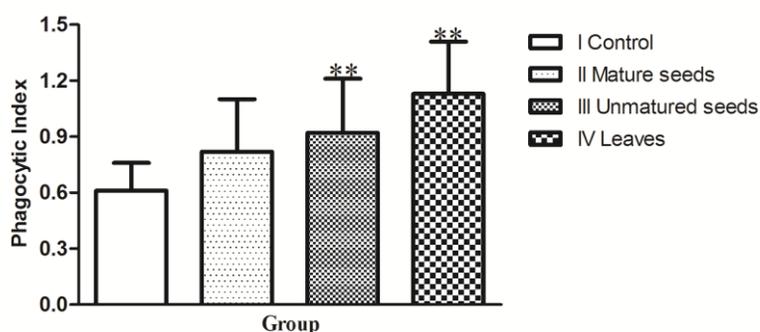


Fig. 3: Effect of tartary buckwheat extracts on nonspecific immunity evaluated by phagocytic index (PI). Data are means  $\pm$  S.D. of ten animals; \*\* $p < 0.01$  when compared with the control animals (significantly different).

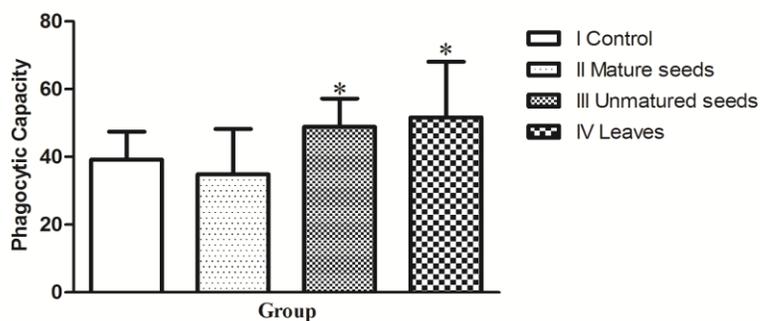


Fig. 4: Effect of tartary buckwheat extracts on nonspecific immunity evaluated by phagocytic capacity (PC). Data are means  $\pm$  S.D. of ten animals; \* $p < 0.05$  when compared with the control animals (significantly different).

To assess nonspecific immune response to tartary buckwheat, phagocytic activities of macrophages in mice abdominal cavity were determined and shown in Figure 3 and 4. Both unmaturred seeds and leaves showed significant increase in PI ( $P < 0.01$ ) and PC ( $P < 0.5$ ) when the results were compared with control animals. However, no effect in PC and only slight increase in PC were observed with mature seeds extracts.

## 4. Concluding Remarks

In this study, I analyzed and compared the effects of three tissue extracts from tartary buckwheat on immune system regulation of mice. Results demonstrated that both unmaturred seeds and leaves extracts showed significant positive immunomodulatory effects with regard to humoral and nonspecific immune responses as compared to the control group. In addition, leaves extracts had better immunoregulatory activity than unmaturred seeds. However, the mature seeds extracts showed little to no effect to improve immune system profiles. This study firstly provides the evidence that tartary buckwheat has positive immunomodulatory effects in mice and further reveals its significant value and potential to be developed as alternative medicine and protective food.

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