

Antitumor Effects and Mechanism of n-butanol Fraction from Aril of *Torreya grandis* in H₂₂ Mice

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ABSTRACT

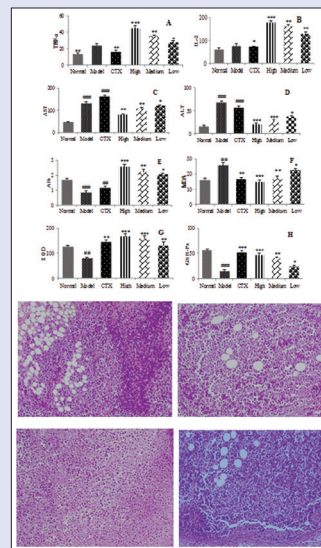
Background: To determine the antitumor effects and its mechanism of n-butanol fraction from aril of *Torreya grandis* (BFAT) on H₂₂ mice models of liver cancer. **Materials and Methods:** Sixty ICR male mice were used to establish H₂₂ mice models of liver cancer and then randomly divided into six groups, the normal control group, the model control group, the positive group (cyclophosphamide [CTX]), the BFAT-treated group (high, 4 g/kg, medium, 2 g/kg, and low, 1 g/kg). The animals were sacrificed 15 days after oral administration, and tumors were taken out for the tumor weights and antitumor rates, while thymus and spleen were taken for thymus index and spleen index. Blood in eyeball was collected for the determination of aspartate aminotransferase (AST), alanine aminotransferase (ALT), albumin (Alb), malondialdehyde (MDA), superoxide dismutase (SOD), glutathione peroxidase enzyme (GSH-Px), tumor necrosis factor- α (TNF- α), interleukin-2 (IL-2), transforming growth factor- β 1 (TGF- β 1), and IL-10 in serum. Sections of tumor tissue were prepared, and morphological changes in tumor tissue cells were observed using hematoxylin and eosin staining technique. **Results:** Compared with the model control group, the tumor inhibition rate of the high-dose administered group is 60.15%, which is quite closed to the effect of CTX. Moreover, the tumor weight is decreased, the indexes of spleen, thymus were increased significantly. Furthermore, the administration of BFAT significantly enhanced the activities of TNF- α , IL-2, SOD, and GSH-Px and reduced the levels of AST, ALT, MDA, Alb, TGF- β 1, and IL-10 ($P < 0.01$). **Conclusions:** The results demonstrated that n-butanol fraction from aril of *T. grandis* showed out antitumor activity without obviously liver damage through potentiating immunologic function and antioxidant activity of tumor-bearing mice and which may become one potential as anticancer drug alternatives or supplements.

Key words: Antitumor, aril of *Torreya grandis*, H₂₂ tumor strains, n-butanol fraction

SUMMARY

- High and medium groups could significant elevate the thymus and spleen indexes and the interleukin-2 and tumor necrosis factor- α level in serum of H₂₂ mice
- n-butanol fraction from aril of *Torreya grandis* (BFAT) could ameliorate the levels of aspartate aminotransferase, alanine aminotransferase and albumin to almost normal, and increase the concentrations of superoxide dismutase and glutathione peroxidase enzyme, decrease the malondialdehyde level in serum of mice significantly
- BFAT may indirectly play the role of antitumor activity through improving immunologic function

- BFAT had potent antitumor properties without obviously liver damage.



Abbreviations used: DDP: Cisplatin; CTX: Cyclophosphamide; AST: Aspartate aminotransferase; ALT: Alanine aminotransferase; Alb: Albumin; MDA: Malondialdehyde; SOD: Superoxide dismutase; GSH-Px: Glutathione peroxide enzyme; TNF- α : Tumor necrosis factor- α ; IL-2: Interleukin-2; TGF- β 1: Transforming growth factor- β 1; IL-10: Interleukin-10; HE: Hematoxylin and eosin; PBS: Phosphate-buffered saline; PFAT: Petroleum ether fraction from aril of *Torreya grandis*; EFAT: Ethyl acetate fraction from aril of *Torreya grandis*; BFAT: N-butanol fraction from aril of *Torreya grandis*.

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INTRODUCTION

Liver cancer is still one of the most serious malignant tumor threats to human's health.^[1] In China, liver cancer has become the second leading cause of death in tumor patients with serious damage to their health and life.^[2] In recent years, the therapeutic schedule of liver cancer is the combination therapy of surgery, radiotherapy, and chemotherapy.^[3] However, Although these treatments have improved greatly, for over 90% of the liver cancer patients, all the aforementioned treatments have not reached the optimal efficiency.^[4] In addition, most of the antitumor drugs, such as cisplatin (DDP) and cyclophosphamide (CTX) used

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in chemotherapy are chemical synthesis medicine or natural product derivatives which are cytotoxic to normal cells when killing cancer cells, leading to multiple organ toxicity, and immunotoxicity.^[5] Therefore, it is crucial to clarify the mechanism of tumorigenesis and find out newly, low toxicity anticancer drugs, which are applied more widely. It is well known that plants are vast repository of natural compounds with wide range of biological activities such as antibacterial, anti-inflammation, and antifatigue, antitumor. Those rich medicinal values need to be developed and utilized comprehensively.^[6] Researches showed that most of the plants which possess taxol and flavonoids have a direct or indirect anticancer activity and are expected to be the ideal supplement or agents for antitumor because of its lower side effects.^[7,8]

Torreya grandis Fort Ex. Lindl. (Taxaceae) is an evergreen tree. There are six species in the world, and three of them distribute in China.^[9] *T. grandis* is one of the characteristic economic species mainly growing in Zhejiang and Anhui provinces. It is well-known for its edible seeds that taste-like nuts with a unique flavor and its bioactivities that are employed in traditional Chinese medicine.^[10] The seeds of *T. grandis* have been used to cure insect abdominal pain thanks to its many medicinal values in ancient China as record in shennong materia medica.^[11] In addition, the seeds are rich in fatty oils composed of unsaturated fatty acids as high as 80% and fatty acids mainly oleic, linoleic, and palm acids, and have found preventive effects on experimental arteriosclerosis in rats.^[12] As a rare snack, *T. grandis* has been widely used because of its abundant functional ingredients and nutritional values, but the succulent seed aril as one of the by-products that completely encloses the seeds and represents about 50% of the fresh weight of the seeds, is normally discarded which consequently resulted in great waste of resources and generated some environmental problems.^[13] All along, investigations of aril of *T. grandis* mainly focused on the isolation and constituents analysis of its essential oil and found that many volatile oil components show out mosquito repellent effects. Other reports indicated that flavonoids^[14] and taxol were found in the aril of *T. grandis* which possess antivirus, antitumor activities, but there are some disputes about the content of taxol.^[15,16] In the screening of cytotoxicity natural substances in our preliminary work, it was found that n-butanol fraction from aril of *Torreya grandis* (BFAT) showed out significant cytotoxic activity. Based on literature review, to supply more scientific evidence for the development of aril of *T. grandis* in the field of pharmaceutical, we started our recent work about observing the antitumor effect and its mechanism of BFAT on H₂₂ mice models of liver cancer.

MATERIALS AND METHODS

Instruments and reagents

RV8 Rotatory Evaporating Instrument (Germany IKA); Infinite M 200 Microplate Reader (Swiss, Tecan), AP280-2 Histocentre (Germany MICROM); HM335E Ultra-Thin Semiautomatic Microtome (Germany MICROM); ST5010 dyeing machine; (Germany Leica); DC300 photomicroscope (Germany Leica); KQ-250 B Ultrasonic Cleaner (Kunshan City Ultrasonic Instrument Corporation); and DGG-924A Electric Heat Constant Temperature Drying Oven (Shanghai Senxin Experimental Instrument Corporation, Ltd.).

Artemia salina prawn egg is purchased from Tianjin Fengnian Aquaculture Corp., Ltd.; 96-hole microporous plate and all the assay kits were obtained from Nanjing Jiancheng Bioengineering Institute (Nanjing, Jiangsu Province, China). All other chemicals and solvents used were of analytical reagent grade and were obtained from Huadong Medicine Co., Ltd. (Zhejiang, Hangzhou Province, China).

Collection and preparation of plant material

Fresh aril of *T. grandis* was obtained from Zhuji, Zhejiang Province. Plant material was authenticated by Professor Dr. Pin-zhang Tong, the Plant taxonomy expert of Forestry Bureau Zhuji, Zhejiang Province. About 6 kg of the fresh arils was immersed in cold 75% ethanol for 24 h and then extracted at room temperature. The above procedures were repeated three times. The combined ethanolic extract was concentrated in a rotary evaporator at reduced pressure at 45°C. A brownish-black-colored residue was obtained (yield 1.08 kg [18%, w/w]), which was dispersed into H₂O (5 L) with vigorous stirring and then partitioned sequentially with petroleum ether, ethyl acetate, and n-butanol. These parts were concentrated in a rotary evaporator to yield petroleum ether fraction (PF, 412 g), ethyl acetate fraction (EF, 326 g), and n-butanol fraction (BF, 255 g).

Bioactivity determination of the lethal-to-prawn

The cytotoxic activity of the fractions were tested by microtiter-plate which was modified from the prawn-larva-fatality biological determining method.^[17]

Weigh precisely 0.02 g of PF, EF, BF, and use dimethyl sulfoxide (DMSO) to dissolve them to a constant volume of 10 mL, respectively to get three sample solutions with a concentration of 2 mg/mL. Then, use DMSO to prepare three sample solutions with different concentration gradients (10, 100, and 1000 µg/mL) of the above PF, EF, and BF solutions to get nine sample solutions and take them separately with 25–30 prawn larva into the 96-hole microporous plate as the experimental groups. Only add DMSO into the control group and cultivate it for 24 h in the dark at 25°C and then calculate the number of dead prawn larva under the microscope in each trough. Finally, calculated the correction mortality rate of prawn larva according to the formula: correction mortality = $(t - c)/(1 - c) \times 100$ (t -death rate of sample group, c -death rate of blank control group). According to the average death rate under different concentrations of the three samples, we calculate their half-number-death concentration LC₅₀ by the SPSS method.

Animals experiment

A total of 60 male clean grade ICR mice, aged 3–4 week, and weighed (20 ± 2) g and 5 mice with H₂₂ ascites tumor were purchased from Zhejiang Academy of Medical Sciences Laboratory Animal Center (number of animal license: SCXK [Zhejiang] 2015-0033). The animals were acclimatized for a period of 1 week before the experiment and were allowed free access to the standard diet and sterile water and fed in a sterile and ventilated room under a controlled environmental condition (24°C ± 2°C, 55% ± 5% humidity, 12 h light/12 h dark cycle). Experiments were carried out in accordance with local guidelines for the care of laboratory animals of Zhejiang Agriculture and Forestry University and were approved by the Ethics Committee for research on laboratory animal use of the institution.

Model preparation and group treatments

Murine hepatocellular carcinoma H₂₂-H₂D₈ cells were maintained as ascites in ICR mice (6–8 weeks old) for 1 week. H₂₂-H₂D₈ cells collected from the peritoneal cavity of the tumor-bearing mice, were washed with aseptic phosphate-buffered saline and adjusted to a suspension with a concentration of 1 × 10⁶ cells/mL with sterile saline. The mice were implanted with 200 µL of cell suspension by subcutaneous injection to the fore right subaxillary. Seven days after the tumor inoculation, ten normal mice treated with only 0.9% normal saline (NS) served as the normal control (normal), and the tumor-bearing mice were randomly

divided into five groups (10 mice in each group): the model control group (model, 0.9% NS); the positive group (CTX, 20 mg/kg/day); the BFAT-treated group (high, 4 g/kg/day; medium, 2 g/kg/day; low, 1 g/kg/day). All treatments used oral administration and were administered once daily for 15 consecutive days, and meanwhile, observed the fur appearance, food intake, physical and mental activities of the mice during the period of treatment.

Indexes observation

After the last drug administration, diets were removed from the cages for 24 h before the mice were euthanized. Tumors were peeled off for weights of tumor, calculation of antitumor rate while thymus and spleen were taken for thymus index and spleen index. The blood in eyeball was collected and centrifuged at $1500 \times g$ for 10 min to produce the sera for the determination of aspartate aminotransferase (AST), alanine aminotransferase (ALT), albumin (Alb), malondialdehyde (MDA), superoxide dismutase (SOD), glutathione peroxidase enzyme (GSH-Px), tumor necrosis factor- α (TNF- α), interleukin-2 (IL-2), transforming growth factor- β 1 (TGF- β 1), and IL-10 followed the manufacturer's instructions. Sections of tumor tissue were also prepared, and the morphologic changes in tumor tissue cells were observed using hematoxylin and eosin dyeing technique. The inhibition ratio of tumor, thymus index, and spleen index was calculated using the following formulas: Inhibitory rate (%) = $(A - B)/A \times 100\%$ where A is the average tumor weight of the model control group while B is the average tumor weight of treated groups. Thymus index (mg/g) = weight of thymus (mg)/body weight (g). Spleen index (mg/g) = weight of spleen (mg)/body weight (g).

Statistical analysis

All data were presented as means \pm standard deviation. Analysis of variance was used to evaluate the significant difference among various groups. $P < 0.05$ was considered to be statistically significant.

RESULTS AND DISCUSSION

Cytotoxicity analysis and screening

The prawn-larva-fatality biological determining method is one of the most common and practical screening methods for sifting antitumor activity part of the plant which is used to measure cytotoxicity of the different extracts of AT.^[18] It is reported that it will show out a strong antitumor activity when the LC_{50} value of the plant crude extracts is $< 1000 \mu\text{g/mL}$, and the LC_{50} value of monomeric compound is less than $50 \mu\text{g/mL}$.^[19] As shown in Table 1, the LC_{50} value of Petroleum ether Fraction from Aril of *Torreya grandis* (PFAT) is more than $1000 \mu\text{g/mL}$ which means no significant cytotoxicity; however, the LC_{50} value of BFAT is $227.8 \mu\text{g/mL}$ showed out relatively strong cytotoxicity on prawn larva compared with Ethyl acetate Fraction from Aril of *Torreya grandis* (EFAT) which the LC_{50} value is $473.2 \mu\text{g/mL}$, so BFAT was selected to be the experiment sample due to its certain antitumor activity.

Effect of n-butanol fraction from aril of *Torreya grandis* on tumor growth *in vivo*

Significant reductions in the tumor weights were observed in the CTX and BFAT-treated mice compared with the model group. As shown in Figure 1, BFAT inhibited the growth of the transplanted tumor dose-dependently, with the inhibitory rates of 60.15%, 51.88%, and 22.56% at the respective dose of 4, 2, and 1 g/kg. Furthermore, mice in the high and medium BFAT-treated groups, as well as the normal control group, were vigorous with shiny fur and normal body weight increases ($P > 0.05$) which suggesting that BFAT was of low toxicity. As expected, CTX,

a conventional chemotherapeutic drug, showed the highest tumor inhibition rate of 70.68%. However, the toxicity in the CTX-treated group was also serious, manifested by anorexia listlessness and emaciation as well as had significant decrease in body mass compared with that of the model control group or the normal control group ($P < 0.05$).

Effect of n-butanol fraction from aril of *Torreya grandis* on immune organs in tumor-bearing mice

As we all know, the immune system plays an important role in preventing tumor development and restraining established tumors and the proliferation and metastasis of cancer occur more easily in a background of immunodeficiency.^[20,21] Hence, enhancing the host's immune functions in defending against tumors has become more and more popular.^[22] Thymus and spleen are the most important immune organs in human body, which can through participating in the differentiation and maturation of T and B immunologically active cells to promote the cellular and humoral immunity, hence, the indexes of immune organs are important indicators for immunologic function.^[23-25] As shown in Figure 2, compared to the normal control group, the relative thymus and spleen indexes of the tumor-bearing mice were significantly decreased ($P < 0.05$), suggesting that the thymus and spleen of the mice were probably damaged and diminished due to the tumor transplantation.^[26] Although oral administration of BFAT and CTX could obviously increase the thymus and spleen indexes as compared with the model control group ($P < 0.05$), the effects of the high and medium groups were more significant than the CTX group ($P < 0.01$). These results indicated that the immune organs were protected well from the grievous damage, and the innate immunity of the tumor-bearing mice was activated by BFAT to decrease the growth of the tumor which suggesting that the treatment of BFAT can apparently reduce the immunosuppressive reaction of H_{22} mice with liver cancer, accelerate the growth of immune organs, and strengthen the immunocompetence of the host.

Effects of n-butanol fraction from aril of *Torreya grandis* on serum cytokine levels in tumor-bearing mice

The cell-mediated immune response plays a very important role in antitumor. The cell is divided into two subgroups, Th1 cells secrete cytokines such as TNF- α , IL-2 which can regulate both cellular and humoral immune responses by affecting immune cell proliferation, differentiation, and functions.^[5] TNF- α causing apoptosis of tumor, resulting in tumor necrosis, and also can induce the expression of a number of other immunoregulatory and inflammatory mediators.^[27] IL-2 augments the cytotoxicities of T-cells and NK cells, stimulates the proliferation of T-cells and B-cells, all of which can participate in immunological antitumor mechanisms.^[28] Th2 cells produce IL-4, IL-10, these cytokines can inhibit the cellular immune response by suppressing Th1 cells.^[29] Tumor cells can produce large quantities of IL-10, which has the ability to reduce T cell proliferation,

Table 1: Bioactivity determination results of the lethal-to-prawn

Sample	Percent deaths (%) of different concentrations ($\mu\text{g/mL}$) at 24 h			LC_{50} ($\mu\text{g/mL}$)
	10	100	1000	
PFAT	10	18	33	> 1000
EFAT	13	22	58	473.2
BFAT	17	26	77	227.8

PFAT: Petroleum ether Fraction from Aril of *Torreya grandis*; EFAT: Ethyl acetate Fraction from Aril of *Torreya grandis*; BFAT: n-Butanol Fraction from Aril of *Torreya grandis*

inhibits antitumor factor and monocyte/macrophage phagocytosis, resulting in tumor cell growth and proliferation, apoptosis.^[30] TGF- β has been implicated as the most efficiency immunosuppressive factors which

can affect natural and acquired immune cell proliferation, activation, and differentiation. TGF- β can inhibit the cytotoxic T lymphocyte (CTL) differentiation and affect the produce of tumor-associated CTL *in vivo*.

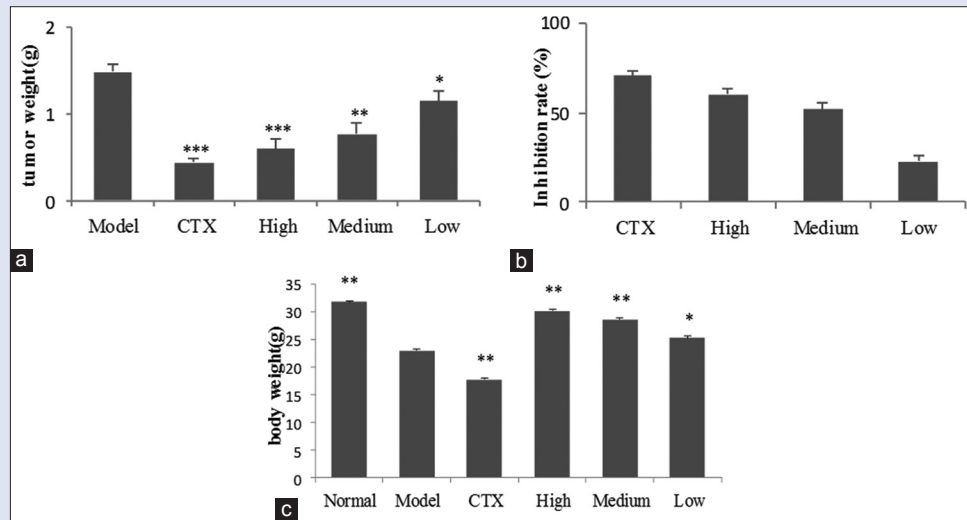


Figure 1: Tumor inhibition of n-butanol fraction from aril of *Torreya grandis* in H22 tumor-bearing mice. (a) tumor weight, (b) Inhibition rate, (c) body weight. Data were expressed as mean \pm standard deviation ($n = 10$). Compared with the model group, *** $P < 0.001$; ** $P < 0.01$; * $P < 0.05$

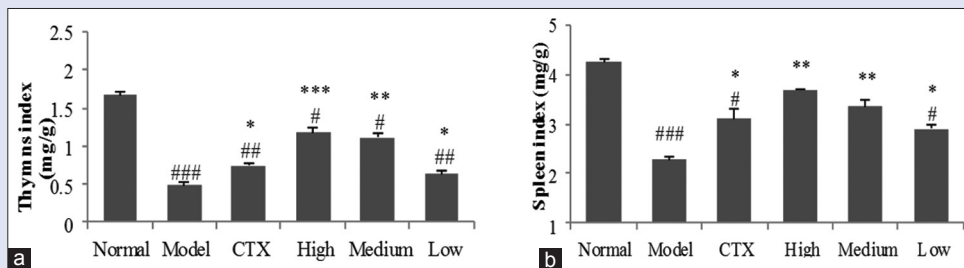


Figure 2: Effects of n-butanol fraction from aril of *Torreya grandis* on thymus and spleen indexes in H22 tumor-bearing mice. (a) Thymus index, (b) Spleen index. Data denoted were means \pm standard deviation ($n = 10$). Compared with the model group, *** $P < 0.001$; ** $P < 0.01$; * $P < 0.05$. Compared with the normal group, ### $P < 0.001$; # $P < 0.01$; # $P < 0.05$

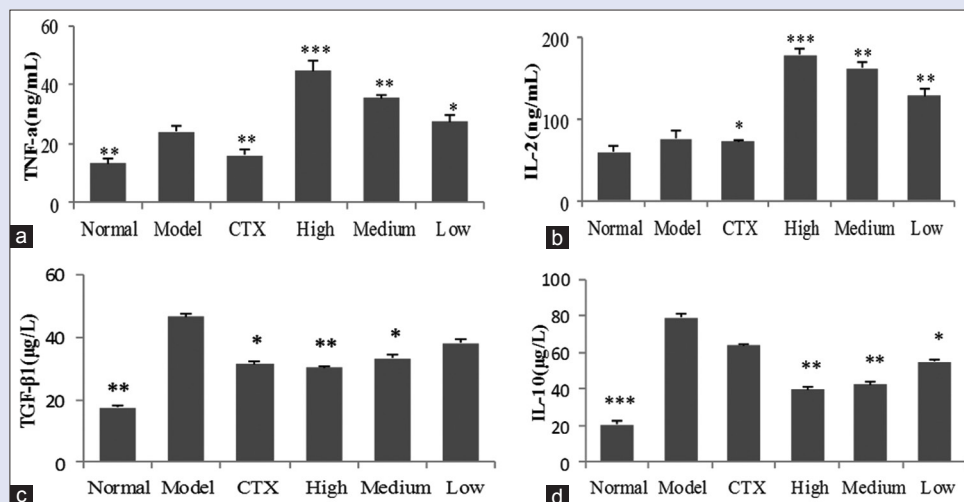


Figure 3: Effect of n-butanol fraction from aril of *Torreya grandis* on serum tumor necrosis factor- α (a), interleukin-2 (b), transforming growth factor- β 1 (c), and interleukin-10 (d) in H₂₂-bearing mice. Data denoted were means \pm standard deviation ($n = 10$). Compared with the model group, *** $P < 0.001$; ** $P < 0.01$; * $P < 0.05$

There are three subtypes of TGF- β , TGF- β 1 is the major subtypes. TGF- β 1 is highly expressed in various cancers such as thyroid carcinoma,^[31] breast cancer,^[32] bladder carcinoma,^[33] liver cancer.^[34] Aberrant TGF- β 1 expression is active in aggressive tumor progression and induce poor prognosis. In this study, we assessed the effect of BFAT on the production of serum cytokines IL-2, TNF- α , TGF- β 1, and IL-10 in H₂₂-bearing mice by ELISA. As expected in Figure 3a-d, the productions of TNF- α and IL-2 were significantly decreased in CTX-treated mice as compared with model control group ($P < 0.05$). However, in the BFAT-treated mice, the productions of TNF- α and IL-2 were restored in a dose-independent manner, especially in the High and Medium BFAT-treated groups which were significantly enhanced when compared with the model control group ($P < 0.01$). Meanwhile, the levels of TGF- β 1 and IL-10 in the high BFAT-treated group were significantly decreased when compared with the model control group ($P < 0.01$). The result indicated that BFAT influences immuno-regulating property by increasing the secretion of IL-2 and TNF- α and decreasing the levels of TGF- β 1 and IL-10 in serum of H₂₂-bearing mice, which may be involved in its antitumor activity.

Effects of n-butanol fraction from aril of *Torreya grandis* on aspartate aminotransferase, alanine aminotransferase, and albumin levels in tumor-bearing mice

Liver dysfunction is the most important diagnostic basis of liver cancer. The AST, ALT, and Alb activities as biochemical markers for hepatic damage were determined in the plasma of H₂₂-bearing mice as showed in Figure 4a-c. The mice in CTX and model group exhibited a significant increase ($P < 0.01$) in the levels of AST, ALT, and meanwhile the level of Alb

decreased obviously ($P < 0.01$) as compared to those of the normal control group. When the tumor-bearing mice were given BFAT administration, especially in the high and medium groups, the levels of AST, ALT, and Alb were significantly ameliorated to almost normal ($P < 0.01$). Which revealed that the CTX could induce hepatic damage (or fail to protect the liver) though it possess a strong tumor-inhibiting effect, on the contrary, the effect of BFAT is quite satisfactory.

Effects of n-butanol fraction from aril of *Torreya grandis* on malondialdehyde, superoxide dismutase, and glutathione peroxidase enzyme levels in tumor-bearing mice

Many reports indicated that the imbalance of oxidation-reduction system exists in almost all cancer cells.^[35] The imbalance of oxidation-reduction system is the essence of oxidative stress, which mainly cause lipid peroxidation and the excessive of free radicals in the host.^[36] MDA is the most important by-product, and a reliable sign of lipid peroxidation and the amount of MDA can indirectly reflect the extent of oxidative damage in the body; SOD, and GSH-Px are considered to be two important enzymatic antioxidants which can reflect the antioxidant's ability of the body.^[37] As show in Figure 4d-f, MDA serum level in the BFAT groups, especially in High and Medium groups were statistically decreased ($P < 0.01$), simultaneously, the concentrations of SOD and GSH-Px were significantly increased ($P < 0.01$) compared with the model group. Thus, these results suggested that the administration of 4 g/kg and 2 g/kg BFAT can improve the antioxidant activity in the serum of H₂₂ tumor-bearing mice, which may delaying the aging of normal cells and slowing down the growth of tumor cells.

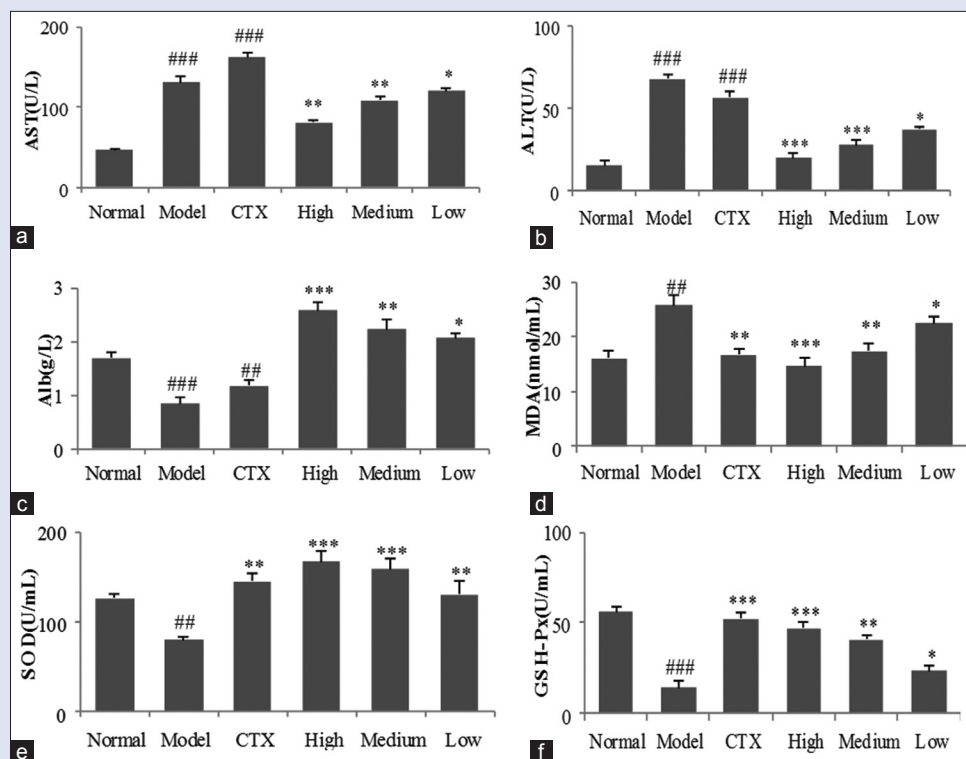


Figure 4: Effect of n-butanol fraction from aril of *Torreya grandis* on serum aspartate aminotransferase (AST: a), alanine aminotransferase (ALT: b), albumin (Alb: c), malondialdehyde (MDA: d), superoxide dismutase (SOD: e), and glutathione peroxidase enzyme (GSH-Px: f) in H₂₂-bearing mice. Data denoted were means \pm standard deviation ($n = 10$). Compared with the model group, *** $P < 0.001$; ** $P < 0.01$; * $P < 0.05$. Compared with the normal group, ### $P < 0.001$; ## $P < 0.01$

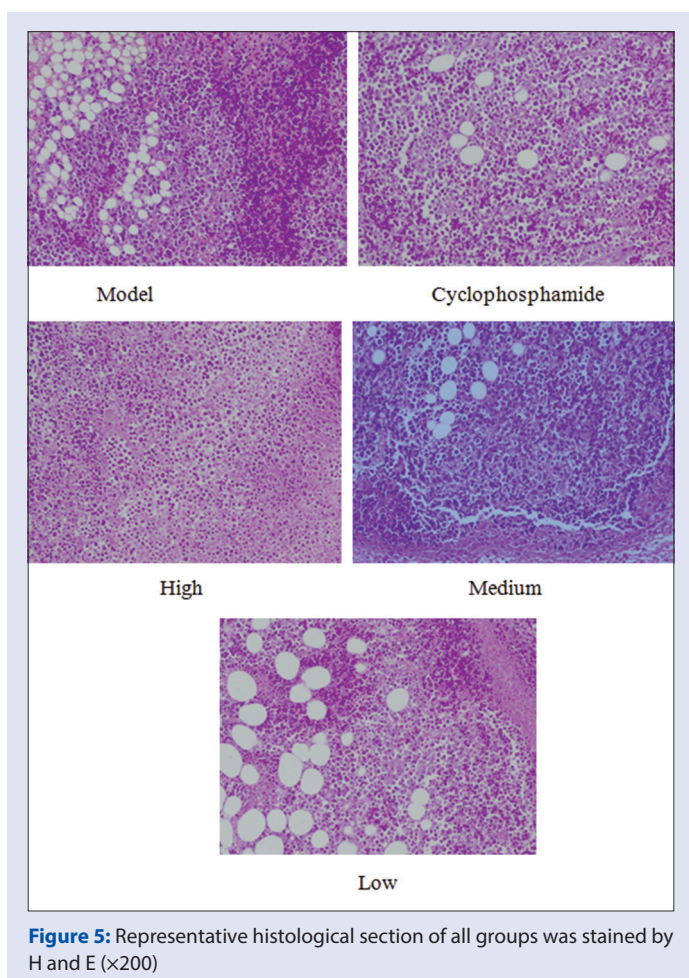


Figure 5: Representative histological section of all groups was stained by H and E (×200)

The pathological observation of tumor tissue

Cell pathological change in the tumor tissue can be showed directly by the sections of tumor tissue. As shown in Figure 5, there are many tumor cells with large vacuoles in the model group compared with the model group, the number of tumor cells of the medium and low BFAT groups decreased significantly, but still existed a little small bubbles. On the contrary, almost no normal tumor cells were found in the CTX group and the high group which further proved that BFAT possess significant antitumor activity *in vivo*.

CONCLUSION

Liver cancer represents the ninth most common cancer in women with 228,000 cases/year and fifth most common cancer in men with 554,000 new cases/year according to the 2014 World Cancer Report and its high fatality makes this type of cancer to be one of the major health burdens.^[38] While the pathogenesis of liver cancer still remains unclear and it is clinically believed to be related to many factors rather than one single factor.^[39] By far, the clinical treatments for liver cancer have been surgery combined with radiotherapy and chemotherapy, by which patients can attain satisfactory efficacy. However, the traditional treatments carry unsatisfactory efficacy due to the serious side effects of many antitumor drugs. Therefore, it is urgent to find new anticancer drugs applied more widely with low toxicity.

In the present study, the mouse liver H₂₂ cancer allograft model was created to investigate the antitumor and mechanism activities of BFAT *in vivo*. The significant reduction of tumor weights and increase of tumor

inhibition rate were observed in H₂₂ tumor-bearing mice following BFAT treatment at the dose of 4, 2, and 1 g/kg in a dose-dependent manner. Moreover, the high and medium groups could significant elevate the thymus and spleen indexes and the levels of IL-2 and TNF- α , decrease the IL-10 and TGF- β 1 levels in serum. The results indicated that BFAT may indirectly play the role of antitumor activity through improving immunologic function. At the same time, BFAT could ameliorate the levels of AST, ALT, and Alb to almost normal, and increase the concentrations of SOD and GSH-Px, decrease the MDA level in serum significantly which indicating that it could improve the antioxidant activity *in vivo* of H₂₂ tumor-bearing mice and has no toxicity to body weight and the liver. Therefore, all the data indicate that BFAT had potent antitumor properties without obviously liver damage, which would be explored as a potential adjuvant against cancer used in the health food and pharmaceutical therapy. It is also recommended that further study should be carried out to elucidate the antitumor and possible immune mechanism of BFAT.

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Nil.

Conflicts of interest

There are no conflicts of interest.

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