

## Protective Effect of Polysaccharides Isolated from *Tremella fuciformis* against Radiation-induced Damage in Mice

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### Radioprotection/*Tremella fuciformis*/Hematopoietic system/Micronuclei/Chromosome aberration.

WTF-B, a type of water-soluble homogeneous polysaccharide, was isolated and purified from *Tremella Fuciformis*. To investigate the radioprotective effect of WTF-B, we employed a 30-day survival assay. Mice were treated with WTF-B once per day for three consecutive days before 8-Gy gamma irradiation. The treatment groups receiving 54 and 72 mg/kg body weight (b.w.) of WTF-B showed 50% survival post-irradiation. The hematological parameters of the peripheral blood indicated that WTF-B, when administered at doses of 72 mg/kg b.w., significantly restored hemoglobin, white blood cell counts and red blood cell counts by the 14th day and 18th day. In addition, spleen colony forming units (CFU-S), the number of nucleated cells in bone marrow (BMNC) and spleen index were used to investigate the radioprotective effect of WTF-B on the hematopoietic system. The treatment groups receiving WTF-B at 18, 54 and 72 mg/kg b.w. doses presented significantly higher BMNC compared to radiation-only group. The group administered 72 mg/kg b.w. WTF-B presented a significant change in CFU-S compared to the radiation-only group. We also completed micronucleus and chromosome aberration assays to explore genotoxicity. The results of those assays indicated that the number of micronuclei induced by 2-Gy irradiation in a group treated with 72 mg/kg b.w. WTF-B decreased from 30.30‰ to 11.32‰. The chromosomal aberration produced by 3-Gy irradiation in the group receiving 72 mg/kg b.w. WTF-B decreased from 56.01% to 28.13%. The results of the present study indicate a potential use for WTF-B as a radioprotector.

### INTRODUCTION

Wide varieties of people are exposed to ionizing radiation and are potentially at increased risk for adverse health effects. Radioprotective agents are of significant importance in medical, industrial, environmental, military and space applications. Radioprotectors might reduce the cancer risk in a population exposed to radiation directly or indirectly through industrial or military applications. Over the past 50 years, the possible radioprotective effects of many synthetic or natural agents have been investigated. Earlier studies were centered on thiols, aminothiols and their derivatives. Through these studies, the “gold standard”, the radioprotective drug amifostine, also known as Ethylol or WR2721 was discovered and applied in clinical therapy. Some of the other

agents also had the effects of protecting cells, membranes and biomolecules, such as DNA and proteins in vitro, and demonstrated promising results in laboratory studies, but were of limited utility for human use due to several factors including toxicity at radiation protective doses and availability.<sup>1)</sup> Plant extracts, such as those from *Emblica officinalis Gaertnd.*,<sup>2)</sup> *Phyllanthus Amarus*,<sup>3,4)</sup> *Amaranthus Paniculatus*,<sup>5)</sup> *Tinospora cordifolia*,<sup>6)</sup> *Myristica fragrans*<sup>7)</sup> and *Ganoderma lucidu*,<sup>8)</sup> have been reported to have radioprotective effects in animal model systems.

*Tremella fuciformis Berk* belongs to the Tremellaceae family of the class Heterobasidiomycetes. The fungus is known as a nutritious mushroom and an important medicine for people in China. Polysaccharides are one of the main bioactive components of *Tremella fuciformis Berk*. Great advances have been made in chemical and bioactive studies of *Tremella fuciformis* polysaccharides in the last twenty years.<sup>9–11)</sup> Experimental results indicate that the fungus has immunomodulatory,<sup>12–14)</sup> anti-cancer<sup>15)</sup> and anti-inflammatory activity.<sup>16)</sup> In our study, a homogeneous polysaccharide, WTF-B, was obtained and identified from crude extracts of *Tremella fuciformis Berk*. The chemical and physical characteristics of WTF-B were determined. In addition, its pro-

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doi:10.1269/jrr.11073

protective effects in mice exposed to radiation were explored in terms of whole body survival, protection of the hematopoietic system, genotoxicity and hematological parameters.

## MATERIALS AND METHODS

### *Preparation polysaccharide of tremella fuciformis berk*

Fungal spores (150 g) were extracted three times with boiling water. The extract was concentrated in a rotary evaporator under reduced pressure, precipitated by ethanol at 4°C for 24 h, and then centrifuged (5000 *r*, 10 min). The precipitate was dissolved in water and then deproteinated with chloroform and *n*-butanol eight times. The resulting aqueous fraction was extensively dialyzed against running water for 3 d and then against distilled water for 1 d. The retentate was concentrated under reduced pressure to a small volume, and 4 volumes of ethanol were added stepwise with stirring at 4°C. The mixture was stored overnight at 4°C. The resulting precipitate obtained by centrifugation consisted of crude polysaccharide.

To purify the crude polysaccharide, sequential column filtration was used. First, the extract was applied to a DEAE-Sephadex A-25 column and eluted with 0.1 M NaCl. No carbohydrates were detected via a phenol-sulfuric acid color reaction in the fractions prior to linear gradient elution at 3.9 M and 0.1 M NaCl. The corresponding fractions, WTF-A and WTF-B were pooled, dialyzed, and lyophilized. WTF-B, with the highest activity, was further fractionated on a Sephadex G-200 column and eluted with 0.1 M NaCl, resulting in one fraction. This fraction was purified by rechromatography on the same exclusion column three times. The resulting homogeneous polysaccharide obtained was designated WTF-B (yield: 2.5 g).

The sugar composition of WTF-B was analyzed by gas chromatography (GC), paper chromatography (PC) an infrared spectrum (IR). The carbohydrate and protein contents were measured using the phenol-sulfuric acid method and Lowry method, respectively. The glucuronic acid content was measured using the sulfuric acid-carbazole method. Homogeneity and molecular weight measurements were made with high power liquid chromatography (HPLC).

### *Animals*

ICR mice (6–8 weeks old) with an average body weight of  $22 \pm 2$  g were obtained from the Animal Center of the Chinese Academy of Medical Sciences. They were maintained under controlled laboratory condition at a temperature of  $25 \pm 2$  °C with a controlled light cycle (14 hours of light and 10 hours of darkness). The mice were fed standard animal food pellets and tap water *ad libitum*. All animal experiments were conducted according to the guidelines of the institutional ethics committee.

### *Irradiation of animals*

Total body gamma irradiation (TBI) was accomplished at room temperature using a <sup>137</sup>Cs Gamma Tissue Irradiator at a dose rate of 0.78 Gy/min (Theratron 780E, Canada) during the experimental period. Each mouse was kept in a perforated plastic container. The mice being irradiated were placed on a rotating platform to ensure even dose delivery to all tissues.

### *Administration of WTF-B*

WTF-B was dissolved in normal saline for administration at the desired concentrations, and the dose was expressed in mg/kg b.w. Different doses of WTF-B were administered to mice through an intra-peritoneal (i.p.) route in a maximum volume of 0.2 ml. Control animals received 0.2 ml of normal saline.

### *Maximum tolerated dose (MTD)*

The acute toxicity of WTF-B was studied in terms of percent survival, change in behavior, alteration in neuromuscular coordination, and respiratory disorders for 14 days after the administration of single dose of WTF-B at 125, 250, 500, 1000 and 2000 mg/kg b.w. The maximum dose of WTF-B that yielded no toxic manifestations was considered the MTD.

### *Animal survival*

The effects of the administration of different concentrations of WTF-B and irradiation on survival were investigated. Mice were randomly divided into six groups of 12 animals. The control group and the radiation group were treated with saline intraperitoneally once a day for three consecutive days. The treated group included a 6 mg/kg WTF-B-treated subgroup, an 18 mg/kg WTF-B-treated subgroup, a 54 mg/kg WTF-B-treated subgroup and a 72 mg/kg WTF-B-treated subgroup. The animals received WTF-B or saline intraperitoneally once a day for three consecutive days at group aforementioned body-weight doses, and then on the 3<sup>rd</sup> day, they were irradiated with gamma rays at an 8-Gy dose 30 min after the administration of WTF-B.

Survival was observed daily up to 30<sup>th</sup> post-irradiation day, and data were expressed as % survival and average survival days.

### *Assay of hematological parameter in peripheral blood*

The mice groups in this assay were the same as those in the animal survival assay.

Blood was collected from the caudal vein into heparinized tubes one day before irradiation and the 8<sup>th</sup>, 14<sup>th</sup> or 18<sup>th</sup> post-irradiation day, and white blood cell (WBC) counts, red blood cell (RBC) counts and hemoglobin (Hb) were analyzed using a Coulter LH755 Hematology Analyzer.

### *Studies of radioprotection on hematopoietic system*

Animals were randomly divided into five groups of 5 animals. The control group and the radiation group were treated with intraperitoneal saline once a day for three consecutive days. The treated group included a 6 mg/kg WTF-B-treated subgroup, an 18 mg/kg WTF-B-treated subgroup, a 54 mg/kg WTF-B-treated subgroup and a 72 mg/kg WTF-B-treated subgroup. The mice received WTF-B or saline administered intraperitoneally once a day for three consecutive days at the aforementioned group body-weight doses, and then on the 3<sup>rd</sup> day, they were irradiated with gamma rays at an 8-Gy dose 30 min after administration of WTF-B. Mice were sacrificed by cervical dislocation on the 9<sup>th</sup> post-irradiation day in all groups. Their spleens and bones were collected. The spleen index, endogenous spleen clone forming unit count and bone marrow nucleated cells were investigated to estimate the radioprotective effects of WTF-B on the hematopoietic system.

### *Endogenous spleen colony forming unit (CFU-S)*

Spleens were removed from mice the 9<sup>th</sup> day post-irradiation and fixed in Bouin's solution (trinitrophenol and methanal) for 24 h. Macroscopic colonies (CFU-S) visible to the naked eye were scored in each spleen.<sup>17)</sup>

### *Bone marrow nucleated cells (BMNC)*

Mouse femoral bones were collected, and bone marrow was flushed out with 3% acetic acid. The number of bone marrow nucleated cells was counted using a light microscope.

### *Micronucleus (MN) assay*

The frequency of micronucleated polychromatic erythrocytes in femoral bone marrow was evaluated according to the procedure described by Schmid<sup>18,19)</sup> with some modifications.<sup>20,21)</sup>

Fifteen mice were divided into the following groups of 5 animals per group:

Group I (Control), animals were administered 0.9% NaCl intraperitoneally (n = 5); Group II (Irradiated group), animals were given 0.2 ml 0.9% NaCl intraperitoneally before 2-Gy gamma irradiation (n = 5) and Group III (Treated group): animals were treated with 0.2 ml WTF-B (72 mg/kg b.w.) before 2-Gy gamma irradiation (n = 5).

The treated group started receiving WTF-B three days prior to radiation administration. All animals were exposed to a single dose of radiation (2 Gy) on the 4<sup>th</sup> day. Then, 24 hr after irradiation, the animals were sacrificed. Bone marrow from the femur was flushed out, vortexed and centrifuged. The pellet was resuspended in a few drops of fetal calf serum. Smears were made on pre-cleaned, pre-coded, dry slides, air dried and fixed in absolute methanol for 30 min and stained with Giemsa. The slides were observed under a light microscope to seek micronuclei in polychromatic

erythrocytes (PCE). A total of 2000 erythrocytes were scored in each animal. The number of micronuclei in PCEs was expressed as a percentage value to depict the MN frequency.

### *Chromosome aberration assay*

Fifteen mice were divided in following groups of 5 animals per group:

Group I (Control), animals treated with 0.2 ml normal saline intraperitoneally (n = 5); Group II (Irradiated group), 3-Gy irradiated animals treated with 0.2 ml normal saline intraperitoneally (n = 5) and Group III (treated group), 3-Gy irradiated animals treated with 0.2 ml WTF-B (72 mg/kg b.w.) intraperitoneally (n = 5).

The treated group started receiving WTF-B three days prior to radiation administration. All animals were exposed to a single dose of radiation (3 Gy) on the 4<sup>th</sup> day. Next, 24 hr after irradiation, the animals were sacrificed. In addition, 90 min before sacrifice all animals were given a single dose of colchicine (1 mg/kg b.w., in saline) intraperitoneally (i.p.). Bone marrow cells from the animal femurs were flushed into PBS containing 2% FCS. The cell button was separated after centrifugation and 5 ml of 0.075 M KCL (maintained at 37°C) was added to each tube and incubated for 30 min. The tubes were centrifuged again and 5 ml of an ice cold methanol-acetic acid mixture (3:1) was added and centrifuged again at 1000 rpm for 5 min. Centrifugation and fixation (in the cold) were repeated two times. The cell pellet was dropped into a chilled clean glass slide and blown off. The slides were air dried and kept in the dark for 5 days for maturation. The slides were stained with Giemsa solution for 5 min and washed with water, air dried and observed under oil immersion with a light microscope. A minimum of 100 metaphase spreads were scored for aberrations.<sup>22)</sup>

### *Statistical analysis*

Statistical analysis was performed using SPSS 12.0 for Windows. Data obtained were expressed as the mean  $\pm$  SD. A Student's t-test was used to make statistical comparison between the groups. The significance levels were set at  $p < 0.05$ ,  $p < 0.01$  and  $p < 0.001$ . The significance between survival curves was analyzed by Kaplan-Meier survival analysis and a log-rank test. The significant differences between MN frequency and chromosomal aberration were evaluated by using one-way analysis of variance. A difference was considered to be statistically significant when  $p < 0.05$ .

## RESULTS

### *Structural features of WTF-B*

The alditol acetate composition of WTF-B as determined by PC and GC indicated that WTF-B consisted of glucose and mannose in an 8:2 molar ratio. The strong absorption in the infrared spectrum (IR) of WTF-B at 1747.2 cm<sup>-1</sup> and PC

analysis confirmed that WTF-B contained glucuronic acid. WTF-B was determined to contain 32.88% glucuronic acid by the sulfuric acid-carbazole method.

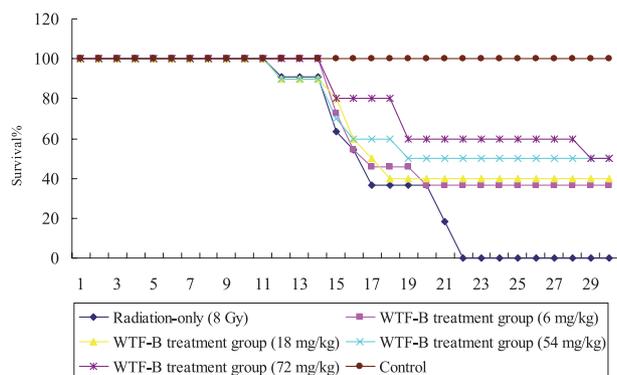
The polysaccharide was eluted out as a single symmetric peak on Ultrahydrogel™ 2000, Ultrahydrogel™ 500 and Ultrahydrogel™ Linear serial columns, corresponding to a molecular weight of 68000 daltons, which suggested WTF-B was homogeneous.

#### Maximum tolerated dose (MTD)

The acute toxicity of WTF-B was studied in terms of percent survival, change in behavior, alteration in neuromuscular coordination, and respiratory disorders for 14 days post-administration of a single dose of different concentrations of WTF-B. As the maximum dissolubility of WTF-B is approximately 2000 mg/kg, single doses of WTF-B up to 2000 mg/kg b.w. were studied. Even at 2000 mg/kg, the doses were well-tolerated by the mice, and no deaths were noted.

#### Survival studies

Overall, 45.5% of irradiated animals that were not administered WTF-B died by the 16<sup>th</sup> post-irradiation day, and the other non-treated animals died by the 17<sup>th</sup> to 21<sup>st</sup> post-irradiation day (Fig. 1). The administration of WTF-B at 54 and 72 mg/kg b.w. doses before 8-Gy whole body gamma-irradiation resulted in 50% 30-day survival. The average number of survival days of animals receiving WTF-B at a 72 mg/kg b.w. dose was higher than those receiving 54 mg/kg



**Fig. 1.** The radioprotective effect of WTF-B was studied with a 30-day survival curve. The mice were administered different doses of WTF-B once per day for three consecutive days, and then on the 3<sup>rd</sup> day they were exposed to 8-Gy whole body gamma irradiation 30 min after WTF-B administration and monitored for survival until the 30<sup>th</sup> post-irradiation day. The data are presented as the mean  $\pm$  SD of three independent experiments carried out with a minimum of twelve animals/group each time. The significance of the differences between the survival curves was analyzed by Kaplan-Meier survival analysis along with a log-rank test. The difference in survival between the radiation-only and treatment groups at 18, 54 and 72 mg/kg b.w. of WTF-B was statistically significant ( $P < 0.05$ ,  $P < 0.01$ ,  $P < 0.001$ , respectively).

b.w. dose. The administration of WTF-B at 18 mg/kg b.w. before 8-Gy whole body gamma-irradiation resulted in 40% 30-day survival. The significance between the survival curves was analyzed by Kaplan-Meier survival analysis and a log-rank test. The difference in survival between the radiation-only group and the 18, 54, 72 mg/kg b.w. treatment groups was statistically significant ( $P < 0.05$ ,  $P < 0.01$ ,  $P < 0.001$ , respectively).

#### Hematological studies in the peripheral blood Hemoglobin (Hb)

Changes in the Hb amount in different treatment groups were observed at different post-irradiation intervals (Fig. 2-a). The Hb level of the radiation group decreased continuously up to the 14<sup>th</sup> post-irradiated day but recovered thereafter. The treatment groups receiving WTF-B prior to radiation did not exhibit any change in Hb levels in comparison to the radiation-only group up to the 8<sup>th</sup> day, but WTF-B treatment groups maintained higher Hb levels than the radiation-only group up to the 14<sup>th</sup> day. Then a recovery became evident, and the Hb levels in the WTF-B treatment groups receiving 54 and 72 mg/kg b.w. doses prior to radiation achieved nearly normal levels by the 18<sup>th</sup> day. The Hb level in the WTF-B treatment group receiving 72 mg/kg b.w. doses remained higher levels than the radiation-only group up to the 14<sup>th</sup> day and the 18<sup>th</sup> day. The difference was statistically significant ( $P < 0.05$ ).

#### White blood cell (WBC) counts

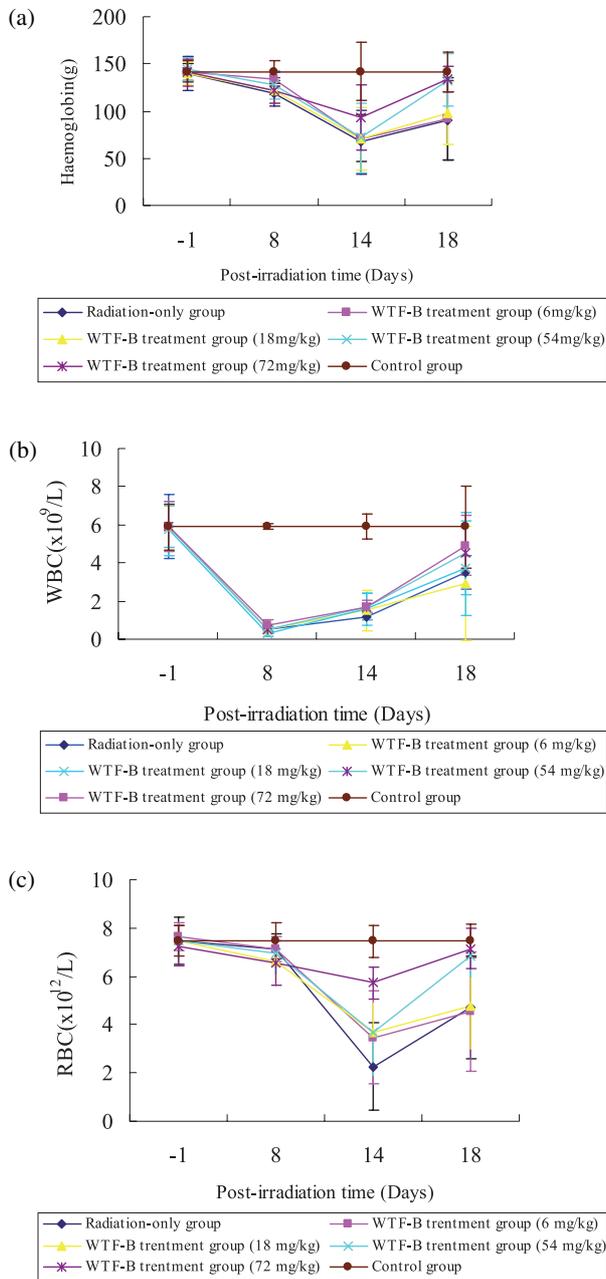
The WBC counts decreased sharply up to the 8<sup>th</sup> day in the radiation-only group, but recovered slowly and until reaching  $3.5 \times 10^9/L$  (Fig. 2-b). The treatment groups receiving WTF-B prior to radiation, there was no change in WBC count in comparison to the radiation-only group up to the 8<sup>th</sup> day. However, the recovery became evident thereafter, and the WBC value in the treatment groups receiving 72 mg/kg b.w. doses of WTF-B increased markedly compared to the radiation-only group up to the 14<sup>th</sup> day and the 18<sup>th</sup> day. The difference was statistically significant ( $P < 0.05$ ).

#### Red blood cell (RBC) counts

The radiation-only group had low RBC counts in comparison to pre-irradiation treatment groups receiving WTF-B by the 14<sup>th</sup> post-treatment day (Fig. 2-c). The lower RBC counts were elevated by WTF-B administration with 54 and 72 mg/kg b.w. doses by the 18<sup>th</sup> day. RBC counts in the WTF-B treatment group receiving a 72 mg/kg b.w. dose were significantly elevated compared to the radiation-only group up to the 14<sup>th</sup> day and the 18<sup>th</sup> day. The difference was statistically significant ( $P < 0.05$ ).

#### Radioprotection of the hematopoietic system

The effects of radiation on the hematopoietic system and its modulation by pre-irradiation administration of various



**Fig. 2.** The effects of WTF-B treatment pre-irradiation on mice hematological parameters were studied. Blood was collected from the caudal vein into heparinized tubes at one day before irradiation and the 8<sup>th</sup>, 14<sup>th</sup> or 18<sup>th</sup> post-irradiation day. The data are represented as the mean value of three independent sets of experiments with 12 animals of each group: (2-a) Hb content, (2-b) WBC counts and (2-c) RBC counts. A Student's t-test was used to make statistical comparison between the groups. The Hb level, WBC counts and RBC counts in the treatment groups receiving WTF-B at doses of 72-mg/kg b.w. doses of WTF-B were significantly elevated compared with the non-treated radiation-only group up to the 14<sup>th</sup> day and the 18<sup>th</sup> day. The differences were statistically significant ( $P < 0.05$ ).

**Table 1.** Effects of WTF-B treatment pre-irradiation on the hematopoietic system in radiation-damaged mice. Mice were sacrificed by cervical dislocation on the 9<sup>th</sup> day post-irradiation in all groups. Spleens and bones were collected. Spleen index, CFU-S and bone marrow nucleated cells were investigated to estimate radioprotection of the hematopoietic system. The data are represented as the mean value of three independent sets of experiments each having 5 animals per group. A Student's t-test was used to make statistical comparisons between the groups.

Group	$(\bar{x} \pm SD, n = 15)$		
	BMNC $\times 10^6$	Spleen index (mg.g <sup>-1</sup> )	CFU-S
Control	2.67 $\pm$ 0.21	4.65 $\pm$ 1.27	0
Radiation-only (8 Gy)	1.31 $\pm$ 0.18 $\triangle\triangle\triangle$	0.99 $\pm$ 0.21 $\triangle\triangle\triangle$	1.29 $\pm$ 1.14 $\triangle\triangle\triangle$
WTF-B treatment (18 mg/kg)	1.53 $\pm$ 0.33*	1.08 $\pm$ 0.19	1.31 $\pm$ 0.96
WTF-B treatment (54 mg/kg)	1.73 $\pm$ 0.49**	1.12 $\pm$ 0.20	1.38 $\pm$ 0.02
WTF-B treatment (72 mg/kg)	2.01 $\pm$ 0.38***	1.13 $\pm$ 0.19	2.80 $\pm$ 0.32***

\*  $P < 0.05$  \*\*  $P < 0.01$  \*\*\*  $P < 0.001$  against radiation group

$\triangle\triangle\triangle P < 0.001$  against control group

Spleen index = weight of spleen (mg)/ body weight of animal (g)

doses of WTF-B are listed in Table 1.

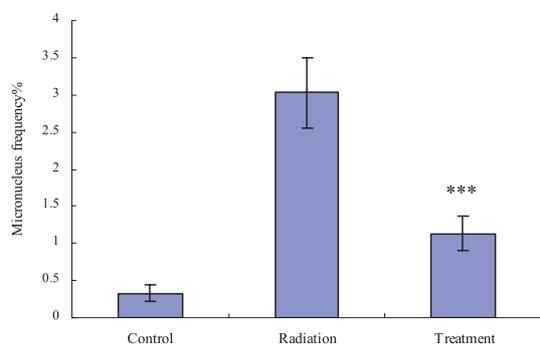
The number of nucleated cells in bone marrow, spleen colony-forming units and spleen index in the radiation-only group decreased markedly compared with the control group in mice. Pre-irradiation treatment groups with receiving 18, 54 and 72 mg/kg b.w. doses of WTF-B had significantly higher BMNC counts in comparison to the radiation-only group. In addition, only pre-irradiation treatment with WTF-B administration at 72 mg/kg b.w. rendered a significant change in CFU-S compared with the radiation-only group. The spleen index of the treatment groups receiving WTF-B increased compared to the radiation-only group, but there was no statistically significant difference.

#### Micronucleus assay

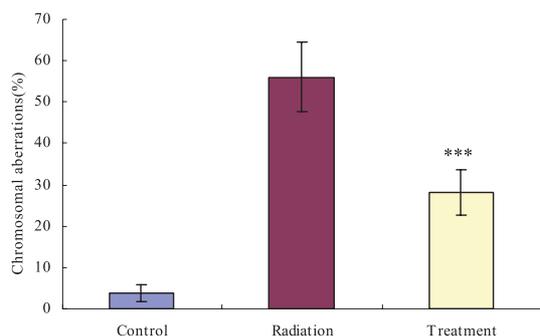
The effects of irradiation on micronucleus (MN) induction and its modification by treatment with WTF-B at 72 mg/kg b.w. were depicted in Fig. 3. The MN frequency in the control group was 3.31%  $\pm$  1.02. Exposure to 2.0-Gy gamma irradiation resulted in a significant increase in the MN frequency (30.30%  $\pm$  4.75) in comparison to the control group. The group treated with a 72 mg/kg b.w. dose of WTF-B before irradiation presented a significantly ( $p < 0.001$ ) reduced frequency of radiation-induced MN (11.32%  $\pm$  2.25) in comparison to the radiation-only group.

#### Chromosomal aberrations

The 3-Gy irradiation produced significant chromosomal



**Fig. 3.** The effect of WTF-B on 2.0-Gy gamma irradiation induced MN frequency in mice. Smears of bone marrow cells were fixed with methanol and stained with Giemsa. At least 2000 cells were scored from each animal. The data are presented as the mean  $\pm$  SD of three independent experiments carried out with a minimum of five animals/group each time. Statistical significance was determined using a one-way ANOVA followed by a Dunnett's test. \*\*\*  $P < 0.001$  compared to the radiation-only group.



**Fig. 4.** The effect of WTF-B on 3-Gy gamma irradiation induced chromosomal aberrations in mouse chromosomes. Bone marrow cells from femurs were flushed out, centrifuged, fixed and stained with Giemsa. A minimum of 100 metaphase spreads was scored for aberrations. The data are presented as the mean  $\pm$  SD of three independent experiments carried out with a minimum of five animals/group each time. Statistical significance was performed using a one-way ANOVA followed by a Dunnett's test. \*\*\*  $P < 0.001$  when compared to the radiation-only group.

aberrations in mouse chromosomes as observed from numerical aberrations (Fig. 4). The percentage of aberrations in the radiation-only group was  $56.01\% \pm 8.37$ , while that of the normal control group was  $3.75\% \pm 1.93$ . WTF-B treatment at 72 mg/kg b.w. doses significantly ( $P < 0.001$ ) decreased the radiation-induced aberrations in chromosomes compared to the radiation group, with the percentage of aberration being  $28.13\% \pm 5.46$ .

## DISCUSSION

In the present study, significant radioprotection was achieved when WTF-B was administered at doses of 18, 54,

72 mg/kg b.w. i.p. for three consecutive days before irradiation. The study revealed that pre-irradiation administration of WTF-B at doses of 54 and 72 mg/kg b.w. resulted in 50% 30-day survival in mice exposed to 8-Gy whole body lethal gamma irradiation, but irradiated mice without WTF-B treatment suffered 100% mortality within 12–21 days. The average survival days of animals receiving WTF-B at a dose of 72 mg/kg b.w. was higher than those receiving 54 mg/kg b.w. WTF-B. Most of the synthetic and herbal radioprotective agents render their maximum radioprotective effect at a dose approaching the MTD.<sup>23–26</sup> A single dose of WTF-B up to 2000 mg/kg b.w. was tolerated well by mice. However, WTF-B provides significant radioprotection at 54 and 72 mg/kg b.w. doses. Therefore, it might have potential as an agent for human application due to its low toxicity and no side effects.

Radiation survival is a result of several factors, such as the prevention of damage through the inhibition of free free-radical generation; efficient scavenging of free radicals;<sup>27,28</sup> repair of DNA,<sup>29–31</sup> membrane and other damaged target molecules<sup>32</sup> and the replenishment of severely damaged or dead cells. Rapidly dividing tissues such as cells of the hematopoietic system are more prone to radiation-induced damage. The recruitment of cells to substitute for damaged cells could add to survival. Our present study indicated that WTF-B pretreatment elevated the number of radiation-induced endogenous spleen colonies and increased the number of nucleated cells in bone marrow when compared to a radiation-only no-treatment group. The increase in the CFU-S count in the spleen indicates a role for WTF-B in protecting stem cells.

The interaction of ionizing radiation with mammalian cells induces several types of molecular damage to cellular macromolecules and especially to DNA, where it causes single-strand breaks, double-strand breaks, bases damage, cross-linking between DNA and protein and a combination of all of these.<sup>33</sup> Of these lesion types, DNA double-strand breaks can be converted into chromosome aberrations. Micronuclei and chromosomal aberrations are the known cytogenetic end points generally used for evaluating genotoxicity. Micronuclei are formed in the cell due to chromosome damage by external factors such as radiation.<sup>34</sup> They are generally found in the cytoplasm outside the main nucleus of a cell. The WTF-B pretreatment mice were effective in preventing the formation of different types of aberrations in the chromosome as well as the induction of micronuclei. The present study revealed that WTF-B effectively prevented the genotoxic effects of radiation on mouse chromosomes.

Early experiments reported that polysaccharides from *Tremella fuciformis* Berk were divided into acidic and neutral polysaccharides.<sup>9–11</sup> Although sugar composition and relative molecular ratios are varied by published report, the reported effects are very similar and include anti-tumor

effects and immunomodulation. Acidic polysaccharides from *Tremella fuciformis* Berk induce human monocytes to produce interleukin-1 (IL-1), IL-6 and tumor necrosis factor (TNF). In addition, they induce production of IL-2 by mouse splenocytes according to Gao *et al.*<sup>13)</sup> and Ma *et al.*<sup>14)</sup> Pre-clinical and clinical studies demonstrated that a large number of cytokines could serve to accelerate bone marrow restoration after exposure to radiation.<sup>35)</sup> IL-1 plays an important role in the regulation of normal hematopoiesis by directly stimulating the most primitive stem cells and indirectly, by raising the production of other hematopoietic factors such as G-CSF, M-CSF, GM-CSF, and IL-6.<sup>36)</sup> In addition, an anti-IL-1R antibody reduces the survival of untreated irradiated mice, suggesting that natural levels of IL-1 contribute to radioresistance in mice. The kinetics of the radioprotective effect of IL-1 suggests this effect may be attributed to the cycling of progenitor cells and to the increased radioresistance of the cell in the late S-phase of the cell cycle.<sup>37)</sup> IL-1 has also been shown to have a role in stimulating bone marrow to overcome the myelosuppressive effect of irradiation and is able to protect bone marrow progenitor cells from irradiation injury *in vitro*.<sup>38)</sup> IL-6 has been shown to be an essential contributor to natural resistance to lethal irradiation and to IL-1- and TNF- $\alpha$ -induced recovery from lethal irradiation.<sup>39)</sup> Therefore, it is suggested that the remarkable radioprotection of WTF-B when is administrated before irradiation is mediated by the simultaneous secretion of proinflammatory cytokines IL-1, IL-6 and TNF. Due to these endogenous cytokines stimulating bone marrow, WTF-B manifested several other effects such as the protection of the hematopoiesis system, stimulation of stem cell proliferation and protection against radiation induced genotoxicity.

As stimulation of the secretion cytokines by WTF-B is the basis of radioprotection, some time is needed for production of the cytokines so they can mediate their effects in mice. Therefore, in our experiment, animals were administered WTF-B once per day for three consecutive days.

Our results demonstrated that the polysaccharide from *Tremella fuciformis* possesses the desirable properties of an ideal radioprotector. Further studies are required to reveal the full potential of this polysaccharide in clinical radiotherapy.

## ACKNOWLEDGEMENTS

This work was supported by the Ministry of Education of the People's Republic of China [Grant Number: 200800231054] and the Tianjin Municipal Science and Technology Commission [Grant Number: 10JCYBTC14500].

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*Received on April 22, 2011*

*Revision received on December 21, 2011*

*Accepted on December 22, 2011*