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Anti-Inflammation and Anti-Infection Applicability of *Tremella flava* Chen Fermented Soymilk (TFS) in a BALB/c Mice Model

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Abstract. *Tremella flava* Chen, a novel yellow jelly and edible mushroom, was isolated in Taiwan. The objective of this study was to evaluate potential adverse effects, if any, of *T. flava*-fermented soymilk (TFS) and the influence of oral treatment of mice with the soy product on clinical isolate *Salmonella Typhimurium* NJ08.124 infections in BALB/c mice. After consecutive administration of TFS to mice for 28 days, most anti-inflammatory cytokines (IL-6 and IL-10) were increased significantly, while pro-inflammatory cytokines (IL-2 and IFN-γ) decreased significantly as the dosage of TFS increased. These results showed that TFS-treated mice had immuno-regulatory effects towards induced inflammation in splenocytes. In addition, the TFS also reduced the infection rate of *Salmonella* significantly, suggesting that TFS may have enhanced macrophage activity, reduced pathogen numbers, and, therefore, reduced fecal shedding in the T-S group compared to the infection group. These results demonstrated that TFS might be a new, natural alternative for use as an anti-infection and anti-inflammation agent in the future.

Keywords: anti-infection, anti-inflammation, *T. flava*, fermentation

1. Introduction

It has been found that the fermented solution, various extracts, or the polysaccharide from *Tremella* have significant bioactivity, such as enhancing the cellular and humoral immune functions by *T. mesenterica* polysaccharides [1]; it also has anti-tumor by *T. fuciformis* polysaccharides [2], hypoglycemic by *T. aurantia* polysaccharides [3], hypocholesterolemic by *T. fuciformis* Berk (TFB) dietary fiber [4], anti-inflammatory by *T. fuciformis* polysaccharides [5] and the hot water extract of *T. fuciformi* is neuroprotective [6]. However, *Tremella flava* Chen, a novel fungus isolated in Taiwan, was used to ferment the soymilk that was used as beverage in this study. The results of our preliminary study showed the antioxidative property of *T. flava* fermented soymilk (TFS), and, further, the TFS was determined to have anti-infection and anti-inflammation applicability in the food market.

2. Experimental Design

2.1. Microbes

Tremella flava is a novel, yellow and edible mushroom that belongs to the Tremellaceae family; it was isolated by Dr. Chee-Jen Chen in Taiwan. The genus *Tremella* belongs to the family of the Tremellaceae, which is a heterobasidiomycetous fungus.

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Salmonella typhimurium NJ08.124 was cultured in a nutrient broth at 37 ℃, and then the culture was prepared using approximately 1×10^7 CFU/mL with phosphate-buffered saline (PBS) with a pH of 7.4.

2.2. Soymilk fermentation

The soybeans were dipped in water and homogenized at 1:10 (w/v) ratio and filtered with a filtration fabric. The soymilk was sterilized further for 15 min at 121 ℃ [7]. One percent of the *T. flava* culture was added to the sterilized soymilk, which was then incubated at 25 ℃ and 100 rpm for 2 d. The fermented soymilk (TFS) was lyophilized for further use.

2.3. Animals

The BALB/c mice were obtained from the National Laboratory Animal Center, Taiwan, and they were identified uniquely with ear piercing and randomly assigned to cages in groups of 8. The mice were approximately six weeks old. The mice were divided into groups with different concentrations of TFS extract and control (C).

2.4. Experimental procedure

The mice were not fed for 16 hr before the administration of the TFS (1.5 g/kg) or sterilized water, and the samples were administered orally by gastric intubation via a cannula once daily for 11 consecutive days, through the day prior to the scheduled primary necropsy. The dosage volume for all groups was 0.2 mL/mouse. The anti-inflammation test included a cytokine immune assay and a non-specific antibody immune assay. The soymilk-infection group (T-S) and the infection group (S) were all challenged with *Salmonella typhimurium* NJ08.124 for 3 d after a week of TFS or sterilized water supplementation in the anti-infection test.

3. Results and Discussion

3.1. Anti-inflammation

Table 1 shows the different cytokine secretions of spontaneous splenocytes. At 0.5g/kg, a significant increase of IL-10 and a decrease in IFN-γ secretions were observed in the male mice. At 1.0 g/kg, a significant increase of IL-4, IL-10, and TNF-α were observed in the male mice; a significant decrease of IL-2 and IFN-γ were noted in both male and female mice. At 2.0 g/kg, the results showed a significant increase of IL-4, IL-6, IL-10, and TNF-α as well as a significant decrease of IL-2 and IFN-γ in both the male and female mice. The results showed that all fermented soymilk samples had a concentration-dependent increase in IFN-γ and IL-2 and a concentration-dependent decrease in IL-4, IL-6, IL-10, and TNF-α. No significant differences in NO secretions were noted in the male and female mice. .

Table 1: Effects of TFS administration for 28 consecutive days on cytokine and NO secretions by spontaneous splenocytes of BALB/c mice**

		Dose (g/kg)*			
		0	**0.5**	**1**	**2**
IL-2 (pg/mL)	**M**	31.99 ±0.67^{a}	31.38±0.49^{a}	20.81±0.21^{b}	20.88±0.29^{b}
	F	29.52±0.28^{a}	29.10±0.42^{a}	21.91±0.37^{b}	20.37±0.22^{c}
IL-4 (pg/mL)	**M**	1.94±0.67^{b}	1.08±0.35^{b}	2.04±0.51^{a}	2.86±0.33^{a}
	F	1.69±0.28^{b}	1.73±0.16^{b}	1.97±0.38^{b}	2.97±0.41^{a}
IL-6 (pg/mL)	**M**	9.81±2.44b^{c}	7.78±0.94^{c}	11.34±1.42^{b}	15.59±0.59^{a}
	F	4.14±2.72b^{c}	4.41±0.37^{c}	6.97±1.47^{b}	20.43±1.73^{a}
IL-10 (pg/mL)	**M**	8.39±2.58^{c}	19.44±8.58^{b}	20.93±1.13^{b}	41.25±2.08^{a}
	F	5.40±4.29^{b}	14.36±5.72^{b}	16.16±1.33^{b}	32.93±8.39^{a}
TNF-α (pg/mL)	**M**	9.10±4.92^{c}	9.59±5.76^{c}	19.39±13.88^{b}	40.47±2.49^{a}
	F	14.37±6.52^{b}	6.53±3.65^{b}	13.27±5.44^{b}	32.97±6.82^{a}
IFN-γ (pg/mL)	**M**	28.41±0.27^{a}	27.14±0.23^{b}	26.89±0.35^{b}	25.93±0.32^{c}
	F	28.08±0.19^{a}	28.10±0.26^{a}	24.34±0.21^{b}	23.58±0.40^{c}
NO (μM)	**M**	1.79±0.69^{a}	1.96±0.37^{a}	2.24±0.27^{a}	2.77±0.53^{a}
	F	3.01±0.70^{a}	3.02±0.90^{a}	3.74±1.44^{a}	3.82±0.38^{a}

* Data are expressed as mean ± SD (n=4); M = male, F = female. Data bearing different superscript letters within a row are significantly different ($P < 0.05$), as determined by Duncan's multiple range test.

** Splenocyte concentration used was 1×10^7 cells/mL.

Table 2 shows the different cytokine secretions of LPS-stimulated splenocytes. After LPS treatment, IL-10 increased significantly in both the male and female mice, while IFN-γ decreased significantly in the male

mice only at 0.5 g/kg. At 1.0 g/kg, significant increases of IL-6 and IL-10 were noted for both the male and female mice, but TNF-α was significantly higher only in the males. Significant decreases of IL-2 and IFN-γ were noted in both the male and female mice. At 2.0 g/kg, IL-4, IL-6, IL-10, and TNF-α were found to be significantly greater in both the male and female mice, while IL-2 and IFN-γ were decreased significantly. No significant differences of NO secretion were found for either the male or female mice. The above data indicate that TFS-treated mice had immuno-regulatory effects towards induced inflammation.

Table 2: Effects of TFS administration for 28 consecutive days on cytokine and NO secretions by LPS-stimulated splenocytes of BALB/c mice**

		Dose (g/kg)*			
		0	**0.5**	**1**	**2**
IL-2 (pg/mL)	**M**	30.24 ±0.26^{a}	29.88 ±0.72^{a}	26.12 ±0.94^{b}	26.86 ±0.52^{b}
	F	29.41 ±0.51^{a}	29.28 ±0.60^{ab}	27.91 ±0.74^{b}	25.12 ±0.82^{c}
IL-4 (pg/mL)	**M**	1.73 ±0.81^{b}	1.59 ±0.32^{b}	1.94 ±0.29^{b}	2.75 ±0.36^{a}
	F	1.63 ±0.22^{b}	1.61 ±0.33^{b}	1.88 ±0.29^{ab}	2.45 ±0.47^{a}
IL-6 (pg/mL)	**M**	60.06 ±13.61^{b}	83.30 ±11.94^{b}	115.18 ±18.71^{a}	142.61 ±11.13^{a}
	F	53.71 ±11.16^{b}	68.71 ±13.45^{b}	98.43 ±11.92^{a}	129.09 ±19.13^{a}
IL-10 (pg/mL)	**M**	270.07 ±12.31^{c}	300.54 ±11.27^{b}	318.52 ±10.79^{b}	446.92 ±7.86^{a}
	F	223.17 ±4.22^{c}	268.41 ±9.56 ^{b}	281.73 ±6.42^{b}	382.99 ±8.77^{a}
TNF-α(pg/mL)	**M**	93.30 ±15.75^{c}	95.51 ±15.89^{c}	130.80 ±16.53^{b}	171.37 ±15.48^{a}
	F	99.92 ±12.60^{b}	72.22 ±11.87^{b}	91.34 ±10.36^{b}	111.31 ±7.71^{a}
IFN-γ (pg/mL)	**M**	48.82 ±1.93^{a}	37.16 ±0.58^{b}	23.17 ±0.29^{c}	23.20 ±2.84^{c}
	F	40.29 ±3.85^{a}	34.32 ±2.84^{a}	18.02 ±8.53^{b}	20.12 ±0.49^{b}
NO (μM)	**M**	1.79 ±0.69^{a}	1.97 ±0.52^{a}	2.25 ±0.27^{a}	2.77 ±0.54^{a}
	F	3.02 ±0.90^{a}	3.02 ±0.70^{a}	3.75 ±1.45^{a}	4.27 ±0.53^{a}

*Data are expressed as mean ± SD (n=4); M = male, F = female. Data bearing different superscript letters within a row are significantly different ($P < 0.05$), as determined by Duncan's multiple range test.

**LPS and splenocyte concentration was 5 μg/mL and 1×10^{7} cells/mL, respectively

3.2. Anti-infection

In this study, *Salmonella typhimurium* NJ08.124 was disseminated in feces and internal organs, and no *Salmonella* spp. was isolated before infection in either the T-S group or the S group. The results showed a significant decrease of *Salmonella* in the T-S group on days 1 and 2 post-infection compared to S group (Fig. 1), however no significant difference was noted between T-S and S groups on day 3 post-infection. Among visceral organs, *Salmonella* was significantly decreased in the livers of T-S group compared to the S group (Fig. 2), however, no significant differences were noted in the spleens of the two groups. Some pathogens cause intestinal infection via invasion of intestinal epithelial cells and further infect internal organs, such as the liver, spleen, and blood [8].

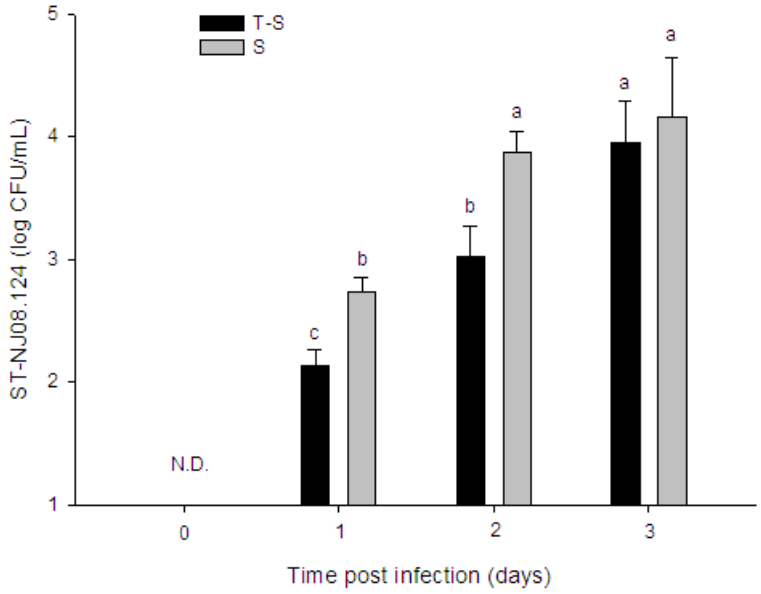

Fig. 1: *Salmonella typhimurium* NJ08.124 counts in feces of BALB/c mice during infection period (post infection) using TFS.T-S: Soymilk-infection group, S: Infection group.

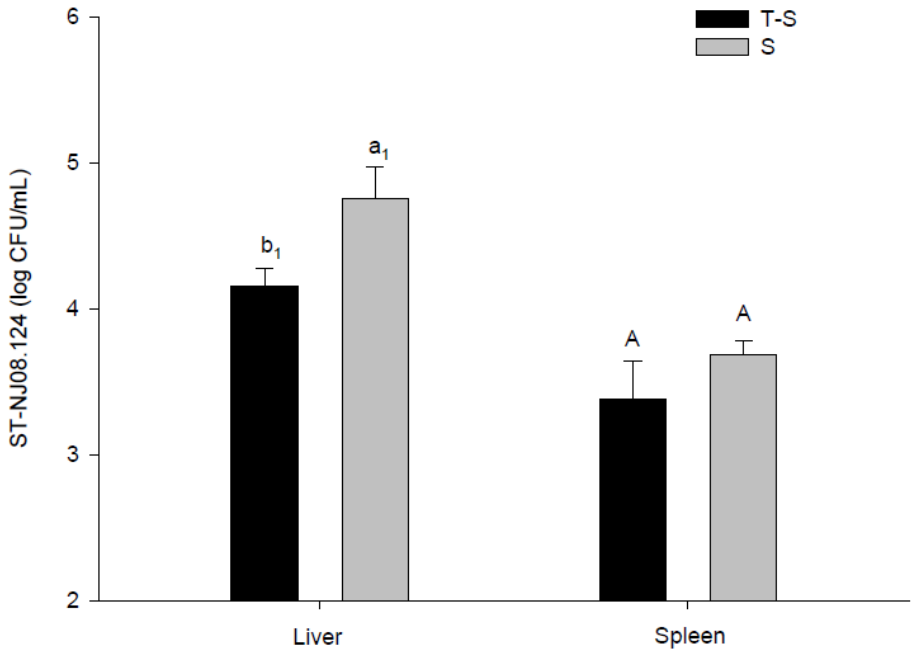

Fig. 2: *Salmonella typhimurium* NJ08.124 counts in livers and spleens of BALB/c mice 3 days post infection using TFS. T-S: Soymilk-infection group, S: Infection group.

After *Salmonella* infection, pathogenesis is characterized by three phases, i.e., 1) colonization of the intestines; 2) invasion of enterocytes, and 3) bacterial dissemination to lymph nodes and organs [9]. The results of our study were consistent with previous studies that showed the organs that were affected by *Salmonella* infection [10]. In this study, the infection rate of Salmonella was significantly reduced in the mice treated with TFS, indicating that TFS may have enhanced macrophage activity and reduced the number of pathogens, thereby reducing fecal shedding in the T-S group compared to the infection group.

4. Conclusion

The above data indicated that TFS-treated mice had immuno-regulatory effects, enhanced macrophage activity and reduced the number of pathogens. These results demonstrated that TFS might be a new, natural alternative for use as an anti-infection and anti-inflammation agent in the future.

5. Acknowledgements

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6. References

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