

Antimutagenic and Antimicrobial Activities of γ -Aminobutyric Acid (Gaba) Tea Extract

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Abstract. This study evaluated the antimutagenic and antimicrobial activities of 50% ethanolic extract obtained from γ -aminobutyric acid (GABA) tea. The antimutagenic effects of the extract were determined using Ames test. *Vibrio parahaemolyticus*, *Staphylococcus aureus*, *Bacillus cereus*, *Salmonella typhimurium* and *Escherichia coli* were used to test the antimicrobial activity. The tea extract did not show toxicity and mutagenicity toward *S. typhimurium* TA98 and TA100 with or without S9 mix. The tea extract at 0.25–5 mg/plate exhibited a dose-dependent inhibition against the mutagenicity of 2-aminoanthracene, 4-nitro-*o*-phenylenediamine and sodium azide in *S. typhimurium* TA98 and TA100. Among food pathogens tested, *V. parahaemolyticus* was the most sensitive, followed by *S. aureus* and *B. cereus*. However, the growth of *S. typhimurium* and *E. coli* were not affected at 0-6 mg/mL. Based on the results obtained, the GABA tea extract exhibited good antimutagenic and antimicrobial activities.

Keywords: GABA tea; Antimutagenic activity; Antimicrobial activity.

1. Introduction

Tea is a beverage widely appreciated and consumed in vast quantities worldwide. The γ -aminobutyric acid (GABA) tea is a kind of special tea enriched with GABA. The amount of GABA was accumulated by the repeating treatments of alternative anaerobic and aerobic conditions. GABA could act effectively as a natural relaxant to induce relaxation and diminish anxiety, and its administration could concurrently enhance immunity under stress conditions [1]. Furthermore, GABA has a physiological role in many systems outside the central system, such as the regulation of cardiovascular functions, the inhibition of metastasis of cancer cells, and the modulation of renal function. Kanehira et al. [2] suggested that intake of GABA-containing beverages, especially those containing 50 mg of GABA, might help reduce both psychological and physical fatigue and improve task-solving ability.

Many bioactive actions of tea extracts, such as antimutagenic and antimicrobial activities were documented [3-5]. Green tea, oolong tea, pouching tea and black tea showed strong antimutagenic action against five indirect mutagens[3]. The antimutagenicity of the teas might be attributed to an interaction between tea extracts and S9 mix, which decreases the mutagenicity of mutagen metabolites [4]. Regarding antimicrobial activity of tea, tea leaf and green tea exerted the strongest antimicrobial activity followed by paochung and oolong teas whereas black tea showed the least antimicrobial activity [5].

Based on dried GABA tea extracts or leaves results obtained in our previous study [6], the optimal extraction conditions to obtain the highest extraction yield, highest contents of total phenols, various catechins, GABA and theanine are 50% ethanol (v/v) and 75-95 °C. Although the antimutagenic and antimicrobial activities of tea and tea-derived products have been reported, no information is available

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regarding the GABA tea extract on these activities. Therefore, the purpose of this study was to investigate the antimutagenic and antimicrobial activities of GABA tea extract.

2. Materials and Methods

2.1. Materials

The GABA tea was made from the leaves of *Camellia sinensis* L. (cultivar Shy-Jih-Chun) in the winter season and processed under the standard procedure as described in our previous study [6]. The 50% ethanolic extracts from GABA tea leaves were prepared according to the method of Lin et al. [6].

2.2. Determination of total phenols, various catechins, GABA and theanine

Total phenols, various catechins, GABA and theanine contents in extracts were determined according to the method of Lin et al. [6].

2.3. Ames test

Toxicity and mutagenicity were assayed by the standard Ames test (standard plate incorporation assay) [7-8]. Antimutagenicity assay was assayed according to the methods of Mortelmans and Zeiger [8], 2-aminoanthracene (2-AA), 4-nitro-*o*-phenylenediamine (4-NP) and sodium azide (NaN₃) were used.

2.4. Antimicrobial activity assay

The antimicrobial activity of tea extract was determined by counting bacterial CFU (colony forming units) according to the method of Kovalskaya and Hammond [9] with some modification. Test organisms included *V. parahaemolyticus*, *S. aureus*, *B. cereus*, *S. typhimurium* and *E. coli*.

2.5. Statistical analysis

All triplicate data were subjected to analysis of variance using the Statistical Analysis System software package (SAS Institute, Cary, NC, USA). Duncan's multiple range tests were used to determine the differences among the means at the level of $\alpha = 0.05$.

3. Results and Discussion

3.1. Extraction Yields and Bioactive Components

Using the same batch of GABA tea powder in previous study [6] as test materials, the ethanolic extract was prepared according to the optimal extraction conditions (50% aqueous ethanol and 75 °C). The extraction yield and the total phenol content of GABA tea extract were $32.21 \pm 0.68\%$, and 492.08 ± 3.16 mg/g extract, respectively. The contents of GC, EGC, EC, EGCG, GCG, ECG, GABA, and theanine of GABA tea extract were 2.57 ± 0.27 , 40.98 ± 2.01 , 18.82 ± 0.41 , 87.64 ± 3.41 , 1.57 ± 0.13 , 16.78 ± 0.53 , 5.74 ± 0.19 , and 6.29 ± 0.27 mg/g extract, respectively. Compared with the previous study [6], those contents were comparable.

3.2. Toxicity, Mutagenicity and Antimutagenicity

Many phenolic compounds were found to show antimicrobial activity. A high percentage of total phenols and various catechins were present in the 50% ethanolic extracts from GABA tea in this study. Therefore, a toxicity test with various concentrations preceding the antimutagenicity studies was performed. The results show that the various concentrations (0.25, 0.5, 1.0, 2.5 and 5.0 mg/plate) of GABA tea extract added to Ames indicator bacteria did not influence their viability. Result from this test (Table 1A) show no toxicity in the tested *S. typhimurium* strains TA98 and TA100 at concentrations of ≤ 5.0 mg/plate, with or without S9 metabolic system [7]. Therefore, the highest concentration of tea extract for the mutagenicity/antimutagenicity assay was arbitrarily selected as 5 mg/plate. Regarding mutagenesis, we found that in the presence of different concentrations of 50% ethanolic extracts, the mutation frequencies for the tested *S. typhimurium* strains TA98 and TA100 did not change significantly when compared to spontaneous mutation frequencies (Table 1B). Indeed, none of the tested concentrations induced a significant increase in the revertant number of TA98 and TA100 strains with or without S9 mix. It appears that the DNA

is not a relevant target for 50% ethanolic extracts and they do not produce DNA lesions. Thus, the GABA tea extract appears to be non-genotoxic to the tested TA98 and TA100 strains.

In this study, 2-AA was used as indirect mutagen requiring metabolic activation, and 4-NP and NaN₃ were used as direct acting mutagens. The ethanolic extract markedly and dose-dependently decreased the mutagenicity of 2-AA in the Ames test with the *S. typhimurium* TA98 and TA100 strains (Fig. 1). The ethanolic extract in the range of 0.25 to 5.0 mg/plate showed an inhibition of 53.03 to 100% on 2-AA toward TA98 (Fig. 1A), and showed an inhibition of 33.82-100% on 2-AA toward TA100 (Fig. 1B). The inhibitory effect of the ethanolic extract on the mutagenicity of 4-NP and NaN₃ toward *S. typhimurium* TA98 and TA100 increased with increasing dose of extracts (Fig. 1). The ethanolic extract exhibited an inhibition of 8.31-44.18% on 4-NP toward TA98 (Fig. 1A). For TA100, it showed an inhibition of 5.58-35.28% on NaN₃ toward TA100 (Fig. 1B). In the case of TA98, a strong and moderate inhibition [10] was shown by extract at 5.0 and 2.5 mg/plate, respectively, and it showed a weak inhibition at 0.25-1.0 mg/plate. In the case of TA100, a moderate inhibition was shown by extract at 5.0 mg/plate, and it showed a weak inhibition at 0.25-2.5 mg/plate. The ethanolic extract tended to inhibit the mutagenicity of indirect-acting mutagen that required metabolic activation of S9 mix more markedly than that of direct-acting mutagen. Yen and Chen [3] indicated that compounds with antioxidant activity could inhibit mutation and cancer due to the fact that they could scavenge a free radical or induce an antioxidant enzyme. Furthermore, the flavanol structures in catechins provide a nucleophilic characteristic to react with an electrophilic mutagen to form flavanol-mutagen adducts which may prevent the occurrence of mutagenicity [11]. The ethanolic extract exhibited good scavenging effect on free radicals, reducing power and metal-binding ability in previous study [6]. Thus, it appears that these relevant bioactive components and properties of the ethanolic extract might contribute to antimutagenic potency against different types of chemical mutagen.

3.3. Antimicrobial Activity

Common food pathogens in Taiwan are *V. parahaemolyticus*, *S. aureus*, *B. cereus*, *S. typhimurium* and *E. coli*. Therefore, the inhibition of the tea extract on the growth of various food pathogens was assayed. Among the organisms tested (Table 2), *V. parahaemolyticus* was most susceptible to the inhibition of the GABA tea extract at added concentration (1 mg/mL), followed by *S. aureus* (3 mg/mL) and *B. cereus* (4 mg/mL) whereas the growth of *S. typhimurium* and *E. coli* were not affected by the tested concentration (0-6 mg/mL) of GABA tea extract. The results show that the growth of *V. parahaemolyticus*, *S. aureus*, and *B. cereus* were inhibited by GABA tea extract, but not those of *S. typhimurium* and *E. coli*. Besides, *S. aureus* and *B. cereus* are gram-(+) bacteria, and *V. parahaemolyticus*, *S. typhimurium* and *E. coli* are gram-(-) bacteria. Based on the antimicrobial activity results obtained, the growth of gram-(+) microbes was easily inhibited by GABA tea extract; and however, gram-(-) microbes were not affected by GABA tea extract except for *V. parahaemolyticus*. General speaking, the structures of flavonoids possess antifungal, antiviral, and antibacterial activity [12]. The structure of gallic catechins and gallates are the main chemical moieties responsible for the antimicrobial activity of tea extracts [13]. In this study, the ethanolic extract from GABA tea contains a large amount of total polyphenols, especially catechins. Therefore, the antimicrobial activity of tea extract may be closely related to its content of catechins.

4. Conclusion

The 50% ethanolic extract from GABA tea did not show toxicity and mutagenicity toward *S. typhimurium* test strains TA98 and TA100 with the concentrations that enabled antimutagenic activity. Besides, the ethanolic extract exist strong antimutagenicity when TA98 and TA100 were severed as experimental microorganisms in the presence of metabolic activator. In addition, the growth of *V. parahaemolyticus*, *S. aureus*, and *B. cereus* was inhibited by the addition of tea extract. The GABA tea not only provides a healthy drink, but also its extract can be developed as antimutagenic and antimicrobial agents.

5. Acknowledgement

The authors would like to thank National Science Council of Taiwan for financially supporting this research under Grant No. NSC-97-2221-E-241-001 and NSC-98-2221-E-241-010.

6. References

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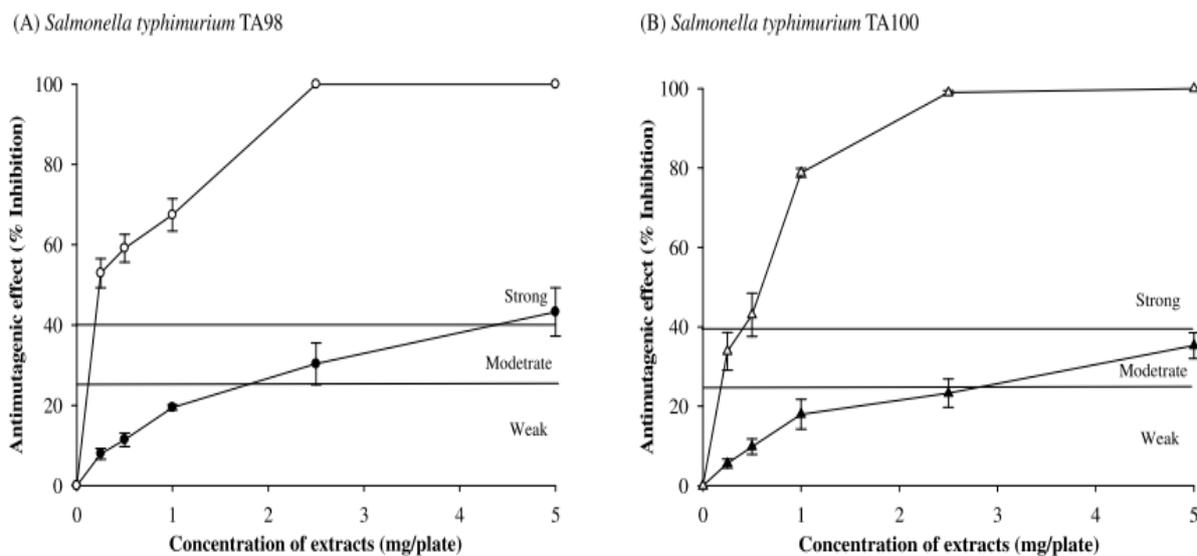


Figure 1. Inhibition of ethanolic extract from GABA tea against the mutagenicity of mutagens to *Salmonella typhimurium* TA98 and TA100. (A) *S. typhimurium* TA98, -●- direct mutagen, 4-nitro-*o*-phenylenediamine without S9 mix; -○- indirect mutagen, 2-aminoanthracene with S9 mix. (B) *S. typhimurium* TA100, -▲- direct mutagen, sodium azide without S9 mix; -△- indirect mutagen, 2-aminoanthracene with S9 mix. Each value is expressed as mean \pm standard deviation ($n = 3$).

Table 1: Toxicity and mutagenicity of ethanolic extract from GABA tea toward *Salmonella typhimurium* TA98 and TA100 with and without S9 mix

(A) Toxicity				
Extract (mg/plate)	Survival (%) ^a			
	TA98		TA100	
	-S9	+S9	-S9	+S9
0.0	100 A ^b	100A	100D	100BC
0.25	87.62 ± 6.73B	100.60 ± 2.55A	97.91 ± 6.14D	113.65 ± 6.02A
0.5	93.29 ± 2.75AB	100.00 ± 1.13A	129.11 ± 6.89B	96.51 ± 9.57C
1.0	92.32 ± 7.23AB	96.11 ± 1.47AB	149.42 ± 10.21A	106.60 ± 6.23ABC
2.5	93.81 ± 6.49AB	89.54 ± 5.20B	117.07 ± 11.07BC	107.19 ± 4.21AB
5.0	91.13 ± 6.18AB	93.43 ± 6.99AB	112.35 ± CD 2.27	102.82 ± 2.32BC

(B) Mutagenicity				
Extract (mg/plate)	His ⁺ revertants/plate (Mutagenicity ratio) ^c			
	TA98		TA100	
	-S9	+S9	-S9	+S9
0.0	26 ± 4 (1.00)A ^b	30 ± 3 (1.00)A	164 ± 8 (1.00)A	151 ± 9 (1.00)A
0.25	24 ± 5 (0.92)A	34 ± 9 (1.14)A	160 ± 4 (0.98)A	149 ± 3 (0.99)A
0.5	27 ± 4 (1.04)A	33 ± 3 (1.11)A	163 ± 16 (0.99)A	148 ± 8 (0.98)A
1.0	26 ± 2 (1.00)A	34 ± 6 (1.13)A	164 ± 7 (1.00)A	153 ± 3 (1.02)A
2.5	28 ± 3 (1.08)A	32 ± 6 (1.06)A	158 ± 10 (0.96)A	152 ± 8 (1.01)A
5.0	25 ± 3 (0.96)A	29 ± 12 (0.97)A	162 ± 13 (0.99)A	153 ± 5 (1.02)A

^a Survival (%) = (number of colonies of extract per plate/number of colonies of control per plate) × 100%.

^b Each value is expressed as mean ± standard deviation (*n* = 3). Means with different capital letter within a column are significantly different (*P* < 0.05).

^c Mutagenicity ratio = induced revertants per plate/spontaneous revertants (control) per plate.

Table 2: Antimicrobial activity of ethanolic from GABA tea on the growth of various food pathogens

Bacterial strain	Concentration (mg/ml)	Bacterial count (log CFU/ml)			Inhibition (%) ^b	
		0 h	24 h	48 h	24 h	48 h
<i>Vibrio parahaemolyticus</i>	0	5.04 ± 0.02 ^a	8.74 ± 0.05	8.69 ± 0.02		
	1		–	–	100	100
	2		–	–	100	100
	3		–	–	100	100
	4		–	–	100	100
	5		–	–	100	100
<i>Staphylococcus aureus</i>	0	5.89 ± 0.5	9.13 ± 0.05	9.23 ± 0.06		
	1		6.84 ± 0.07	5.93 ± 0.02	25.08	35.75
	2		4.00 ± 0.05	4.20 ± 0.03	56.19	54.50
	3		–	–	100	100
	4		–	–	100	100
	5		–	–	100	100
<i>Bacillus cereus</i>	0	4.90 ± 0.05	9.96 ± 0.08	10.02 ± 0.11		
	1		6.45 ± 0.04	6.62 ± 0.04	35.24	33.93
	2		3.32 ± 0.04	3.40 ± 0.03	66.67	66.07
	3		1.24 ± 0.03	1.29 ± 0.05	87.55	87.13
	4		–	–	100	100
	5		–	–	100	100
<i>Salmonella typhimurium</i>	0	6.18 ± 0.03	9.46 ± 0.04	9.96 ± 0.03		
	1		9.45 ± 0.04	9.47 ± 0.08	0	0
	2		10.03 ± 0.08	10.08 ± 0.06	0	0
	3		9.97 ± 0.05	10.00 ± 0.03	0	0
	4		9.58 ± 0.07	9.61 ± 0.03	0	0
	5		9.69 ± 0.10	9.71 ± 0.09	0	0
<i>E. coli</i>	0	6.33 ± 0.03	9.45 ± 0.04	9.59 ± 0.07		
	1		9.47 ± 0.06	9.52 ± 0.05	0	0
	2		9.59 ± 0.06	9.60 ± 0.03	0	0
	3		9.57 ± 0.06	9.56 ± 0.04	0	0
	4		9.67 ± 0.03	9.64 ± 0.03	0	0
	5		9.56 ± 0.04	9.48 ± 0.05	0	0
	6		9.55 ± 0.05	9.63 ± 0.04	0	0

^a Each value is expressed as mean ± standard deviation (*n* = 3). –: not detected.