Increased *in vitro* anticancer activity in HepG2 human hepatoma cells and *in vivo* hepatitis protective effect of bamboo salt

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Bamboo salt as a functional food was originated in Korea. Bamboo salt was evaluated for the anticancer effect in human cancer cells and determined preventive effects on hepatitis in SD rats. The solar salt was filled in the internodes of young bamboo, and sealed using the natural red clay, then baked at $1000°C \sim 1500°C$[1,2] using pine as fuel. *In vitro* anticancer effect of the bamboo salt was evaluated in HepG2 human hepatoma cells using MTT assay (Table 1). At 1% of salts treated HepG2 cells, showed growth inhibitory effects of bamboo salt ($9 \times$, 9 times baked) was 65%, which was higher than that of bamboo salt ($1 \times$), solar salt and purified salt (Hanju Corporation, Ulsan, Korea).

Table 1. Inhibitory effect of aqueous solutions from different kinds of salt products on the growth of Hep G2 cells in 3-(4,5-dimethyl-thiazol)-2, 5-diphenyl tetrazolium (MTT) assay

Treatment	OD_{540} (% inhibited compared with control)	
	0.5	1
Control		0.581 ± 0.004^a
Purified salt	0.523 ± 0.008^b (10)	0.436 ± 0.010^e (25)
Solar salt	0.500 ± 0.011^c (14)	0.413 ± 0.007^{ef} (29)
Bamboo salt ($1 \times$)	0.459 ± 0.013^d (21)	0.349 ± 0.007^g (40)
Bamboo salt ($9 \times$)	0.395 ± 0.009^f (32)	0.203 ± 0.012^h (65)

Table 2. Plasma AST, ALT and LDH levels

	Plasma levels (IU/L) BS: Bamboo salt		
	AST	ALT	LDH
Normal	169.5 ± 12.3^f	45.7 ± 5.6^e	1173.5 ± 77.2^f
Control	1693.8 ± 74.2^a	1438.2 ± 82.2^a	6742.6 ± 45.6^a
Purified salt	1553.1 ± 34.5^b	998.4 ± 65.2^b	5875.2 ± 44.2^b
Solar salt	1285.3 ± 45.2^c	804.9 ± 34.5^c	5432.9 ± 32.7^c
BS ($1 \times$)	1077.0 ± 25.7^d	790.2 ± 27.6^{cd}	4776.4 ± 55.2^d
BS ($9 \times$)	1038.1 ± 14.2^e	634.4 ± 22.5^d	3877.8 ± 37.6^e

[a–h]Means with different letters on the bars are significantly different ($p = 0.05$) by Duncan's multiple range test ($n = 3$).

To determine the inhibitory mechanisms of apoptosis and anti-inflammatory effect of bamboo salt samples in HepG2 cells the expressions of Bax, Bcl-2 and iNOS, COX-2 genes were determined by RT-PCR. The bamboo salt ($9 \times$) induced a stronger anticancer effect than the other salts (Fig. 1). We also evaluated whether bamboo salt exhibits hepatoprotective effects in SD-rats. Bamboo salt effectively prevented CCl_4 induced hepatitis in the rats ($n = 36$, 7-week-old, 400 mg/kg bw of salt dissolved in water for 2 weeks). Expressions of inflammation related genes in the livers of the rats were tested. Bamboo salt ($9 \times$) significantly decreased mRNA expressions of COX-2, iNOS, TNF-α and IL-1β (Fig 2). Bamboo salt ($9 \times$) showed the lowest levels of plasma AST, ALT and LDH levels (Table 2). Accordingly, bamboo salt ($9 \times$) can be expected to contribute to prevention of hepatitis, bamboo salt ($1 \times$) also revealed better hepatitis preventive effects than solar salt and purified salt. On the whole, bamboo salt showed much better *in vitro* anti-cancer activity than solar salt and purified salt in HepG2 cells and the hepatitis induced in SD rats was effectively prevented by bamboo salt.
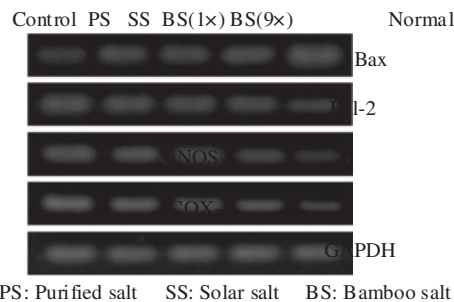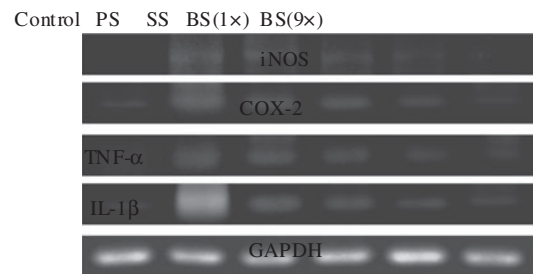

PS: Purified salt   SS: Solar salt   BS: Bamboo salt

Fig. 1. Gene expression (cells).

Fig. 2. Gene expression (liver).

1. Shin HY, Na HJ *et al*. (2004) *Food Ind Nutr* **9**, 36–45.
2. Chun H, Ying Z *et al*. (2000) *J Agr Food Chem* **48**, 3170–3176.