Mutagenicity of Four Salted Foods and Their Modulating Effects on the Mutagenicity of Urethane

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ABSTRACT

We determined the mutagenicity of boiled salted duck egg, pickled green mussel, fried salted Spanish mackerel and fried salted beef by transferring the three-day old trans-heterozygous (*mwh flr*$^{+}$*/mwh TM3*) larvae to an experimental medium (containing each sample 75% substituted for yeast). We evaluated the antimutagenicity of each sample by transferring 3-day old larvae to an experimental medium that had urethane (20 mM)) as the co-administration study while pre-feeding studies were also performed by mating the parental flies on the experimental medium to obtain 3-day old larvae that were subsequently raised on the regular medium containing urethane as the type 1 study or the experimental medium containing urethane as the type 2 study. The round wings of the surviving flies were analyzed for the occurrence of mutant spots. The results showed that none of the sample was mutagenic. Interestingly, boiled salted duck egg revealed its antimutagenicity in all studies while pickled green mussel exhibited its antimutagenicity only in the pre-feeding studies. Some digested proteins of pickled green mussel and the digested egg yolk protein occurred in the digestive tract of the larvae might eliminate the free radical generated via the activation of urethane. On the other hand, fried salted Spanish mackerel enhanced the mutagenicity of urethane in all studies while salted beef enhanced the mutagenicity of urethane only in the pre-feeding type 2. The enhancing effect was supposed to be due to the amount of sodium chloride in the sample could impair the repairing system during DNA damage.

Keywords: Mutagenicity, antimutagenicity, urethane, nitrite treated methylurea, salted foods, *Drosophila melanogaster*

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Introduction

Salt has a crucial property that made it important for the development of human society. Salt was used to preserve meat, fish and vegetables and to create delicacies which added variety to the diet. However, scientist suggested that dietary consumption of salt is a risk factor in mortality from gastric ulcer.[1-4] Higher risk of gastric cancer is found in people who consume salty food. There is an association between increased risk of gastric cancer and frequent intake of salted or meat.[5] Reducing consumption of salt or salted food and increasing intake of fruits and vegetables are thought to be beneficial for the prevention of gastric cancer.[6,7] In addition, various food produced in Asia were reported on their direct-acting mutagenicity after nitrite treatment. Kimchis, sun-dried fishes, sun-dried squid, soy sauces, fish sauces, bean pastes and shrimp past produced in Korea, Philippines and Thailand showed direct acting mutagenicity after nitrite treatment.[8] Dmitrieva *et al*.[9] concluded that high concentrations of sodium chloride disrupted DNA damage signaling that associated with failure of DNA damage-repair network and led to DNA damage accumulation. A case-control study in Thailand indicated that relatively high levels of *N*-nitrosodimethylamine (NDMA), *N*-nitrosopiperidine (NPIP) and *N*-nitrosopyrrolidine (NPYR) detected in fermented fish and NDMA detected at levels ranging from trace amounts to 66.5 mg/kg in several salted and dried fish was the etiology a high incidence of liver cancer in Thailand rather than other common risk factors such as hepatitis B infection, afatoxin intake and alcohol consumption.[10] Therefore, it was of interest to elucidate the mutagenicity and their modulating effects on mutagenicity of urethane of boiled salted duck egg, pickled green mussel, fried salted Spanish mackerel and fried salted beef.

Materials and Methods

Chemicals and Samples: Urethane (URE) was purchased from Sigma Chemical (St. Louis, Mo, USA). Five replicates of salted beef, boiled salted duck egg, pickled green mussel and salted Spanish mackerel were collected from local markets. Salted beef (5.0 g) and salted Spanish mackerel (5.0 g) were separately fried at 180º C for 5 min before study. Each sample was cut and chopped into small pieces and lyophilized. All dehydrated samples were homogenized and kept in a desiccator before further study.

Experimental Design The mutagenicity of each sample of each sample was assayed as described by Graf *et al*.[11] Virgin females of Oregon wing flare strain (*ORR/ORR*; *flr*^{3}*/TM3, Ser)* and males of multiple wing hair strain *(mwh/mwh)* were mated on regular medium which had propionic acid (0.01 ml) as a preservative to produce *trans*-heterozygous larvae of improved high bioactivation cross (IHB). Both strains were obtained from the Institute of Toxicology (Swiss Federal Institute of Technology, and the University of Zurich). Each salted food was substituted for yeast to the final 25%, 50% or 75% of the protein source of the regular medium and was named *experimental medium*. It was used for mutagenicity evaluation of each sample. URE (20 mM) was substituted for deionized water in the regular medium and was used as a *positive control medium*. Adding the highest concentration of each sample that provided more than 50% surviving adult flies of each salted food into the positive control medium, the *experimental medium containing URE* was prepared. This medium was used for both co-administration and pre-feeding studies for the investigation on the modulating effect of each food on urethane induced mutagenicity. Induction frequencies of wing spots of each salted food were compared with that of the deionized water negative control group. The estimation of spot frequencies and confidence limits of the estimated mutation frequency were performed with significant level of $\alpha = \beta = 0.05$. A multiple-decision procedure was used to decide whether a sample was positive, weak positive, inconclusive or negative mutagen as described by Frei and Wurgler.[12]

The modulation activity of each sample on the mutagenicity of urethane was calculated as follows: percentage of modulation = (a-b)/a x 100. Where “a” is the number of total spots per wing induced by URE, “b” is the number of total spots per wing induced with URE administered with each salted food. It is proposed that percents of modulation between 0–20%, 20–40%, 40–60% and higher than 60% indicate negligible, weak,

moderate and strong modulation, respectively. When "b" is higher than "a" the result is classified as enhancement; on the other hand, when "b" is lower than "a" the result is classified as inhibition.

Results

A substitution 75 percentage of each sample for yeast was the highest concentration that provided more than 50% of surviving adult flies. This percentage of sample was also used for co-administration and pre-feeding study. The results showed that none of the sample was mutagenic (Table 1). However, feeding of salted foods to newborn larvae affected size and number of newborn larvae as well as of the surviving flies. The size of adult fly derived from the larva fed on salted foods was smaller than that fed on regular medium. The co-administration and pre-feeding study were showed in Table 2 and Table 3, respectively. Two samples namely, boiled salted duck egg and pickled green mussel decreased the mutagenicity of urethane. Pickled green mussel revealed its antimutagenicity only in the pre-feeding studies. Contrastingly, fried salted Spanish mackerel enhanced the mutagenicity of urethane in all studies. Fried salted beef also enhanced the mutagenicity of urethane but only in the co-administration study.

Discussion

The effect of salted foods on size and number of newborn larvae as well as of surviving flies was not surprised since Kangsadalampai and Somany.[13] previously reported this effect too. The mutagenicity evaluation demonstrated that none of the sample was mutagenic in both trials. Mereto and Ghia[14] indicated that pretreatment of rat with NaCl, which by itself did not produce any genotoxic response, markedly increased the frequency of nuclear anomalies (micronuclei, pyknosis, and karyorrhexis) in the forestomach mucosa in Sprague-Dawley male rats induced by *N*-methyl-*N*'-nitro-*N*-nitrosoguanidine (MNNG).

The discovery that boiled salted duck egg and pickle green mussel decreased the frequency of induced wing spots in adult flies in the co-administration and pre-feeding studies suggested that some components of such salty foods performed such activity. It was proposed that *N*-hydroxyurethane, a urethane metabolite[15,16] was hydrolyzed by esterase to generate hydroxylamine and exerted its mutagenic effect in multiple organs via generating $O_2^{\bullet -}$ and $NO^{\bullet}$ to cause oxidation and depurination of DNA.[17] It was possible that antioxidant activity of the proteinase-hydrolyzed egg yolk protein in the digestive tract might reduce $O_2^{\bullet -}$ and/or $NO^{\bullet}$ in urethane metabolism. Since there was not any change in protein pattern during the fermentation of salted egg[18] and Sakanaka and Tachibana[19] demonstrated that hydrolysates of egg yolk proteins by proteinase were a good source of natural antioxidants. Carotenoids are notable as the pigments responsible for the typical yellow-orange colors of the egg yolks of birds.[20] Karadas *et al*.[21] preformed carotenoids content in the yolk egg of hatched chicks were between 11.2-79.2 µg/g. Surai and Speake[22] showed that the carotenoids content of the yolk of the newly laid fertile eggs was 13.3 µg/g. It was suggested that carotenoids in the egg yolk might take a role as important factor of antimutagenicity of boiled salted duck egg. Polyakov *et al*.[23] showed the scavenging ability of the carotenoids towards peroxyl free radical (•OOH) correlates with their redox properties. It was documented that *N*-hydroxyurethane, a urethane metabolite[15,16] was hydrolyzed by esterase to generate hydroxylamine and exerted its mutagenic effect in multiple organs via generating $O_2^{\bullet -}$ and $NO^{\bullet}$ to cause oxidation and depurination of DNA.[17] Therefore, it was possible that carotenoids remained in the boiled salted duck egg might reduced free radical of activation of urethane.

The antimutagenicity of pickled green mussel against urethane might be due to the same phenomenon as of boiled salted duck egg. During fermentation, microbial proteases digests food proteins to produce some bioactive peptides[24] that may be antimutagenic against urethane since Rajapakse *et al*.[25] demonstrated that fermented marine blue mussel (*Mytilus edulis*) derived peptide was highly effective for radical scavenging. Pickled green mussel might produce some peptides that reduce the radicals were

Table 1 Mutagenicity of each salted food in Drosophila melanogaster obtained by introducing 100 trans-heterozygous (mwh+/+flr^3) larvae of improved high bioactivation cross to experimental medium

Trial	Treatment	Percent of sample substituted for yeast	Spots per wing*(40 wings)			
			Small single (m=2)	Large single (m=5)	Twin (m=5)	Total (m=2)
1	Boiled salted duck egg	75%	0.150(6)i	0.025(1)i	0	0.175(7)i
		50%	0.200(8)i	0.05(2)i	0	0.250(10)i
		25%	0.05(2)-	0.05(2)i	0	0.100(4)-
	Pickled green mussel	75%	0.400(16)i	0.050(2)i	0	0.450(18)i
		50%	0.100(4)-	0.150(6)i	0.050(2)i	0.300(12)i
		25%	0.025(1)-	0.025(1)i	0	0.050(2)-
	Fried Salted Mackerel	75%	0.250(10)-	0.025(1)i	0	0.275(11)-
		50%	0.400(16)i	0.025(1)i	0	0.425(17)i
		25%	0.125(5)-	0.025(1)i	0.050(2)i	0.200(8)-
	Fried Salted Beef	75%	0.200(8)-	0.025(1)i	0.025(1)i	0.250(10)-
		50%	0.150(6)-	0.075(3)i	0	0.225(9)-
		25%	0.100(4)-	0	0	0.100(4)-
2	Boiled salted duck egg	75%	0.350(14)i	0	0	0.350(14)i
		50%	0.250(10)i	0	0	0.250(10)i
		25%	0.250(10)i	0	0	0.250(10)i
	Pickled green mussel	75%	0.125(5)-	0	0	0.125(5)-
		50%	0.275(11)i	0	0.025(1)i	0.300(12)i
		25%	0.325(13)i	0	0.050(2)i	0.375(15)i
	Fried Salted Mackerel	75%	0.150(6)-	0	0	0.150(6)-
		50%	0.150(6)-	0.025(1)i	0.025(1)i	0.200(8)-
		25%	0.175(7)-	0	0.025(1)i	0.200(8)-
	Fried Salted Beef	75%	0.050(2)-	0.025(1)i	0	0.075(3)-
		50%	0.075(3)-	0.050(2)i	0.025(1)i	0.150(6)-
		25%	0.125(5)-	0	0.025(1)i	0.150(6)-

Number of induced spots per wing of the positive control (urethane) was 10.29 $\pm$ 3.27 and that of negative control (distilled water) was 0.31 $\pm$ 0.08

* Statistical diagnoses using estimation of spot frequencies and confidence limits according to Frei and Wurgler[12] for comparison with negative control:

+ = positive; - = negative; i = inconclusive; Probability level: $\alpha = \beta = 0.05$. One-sided statistical tests

Table 2 Modulating effects of each salted food on urethane induced somatic mutation and recombination in *Drosophila melanogaster* in the co-administration study.

Trial	Sample	Mutagen	No. of wings	Spots per wing*				% Modulation (rank**)	
				Small single (m=2)	Large single (m=5)	Twin (m=5)	Total (m=2)	Inhibition	enhancement
1	-	-	40	0.325(13)	0	0.025(1)	0.350(14)		
	-	Urethane	28	3.679(103)+	2.964(83)+	0.464(13)+	7.107(199)+		
	Boiled salted duck egg	Urethane	36	3.417(123)+	1.722(62)+	0.417(15)+	5.556(200)+	21.83(w)	
	-	-	40	0.325(13)	0	0.025(1)	0.350(14)		
	-	Urethane	28	3.679(103)+	2.964(83)+	0.464(13)+	7.107(199)+		
	Pickled green mussel	Urethane	32	4.313(138)+	1.875(60)+	0.250(8)+	6.438(206)+	9.42(n)	
	-	-	40	0.275(11)	0.050(2)	0	0.325(13)		
	-	Urethane	40	6.525(261)+	4.575(183)+	1.850(74)+	12.950(518)+		
	Fried Salted Mackerel	Urethane	36	16.389(590+	3.750(135)+	1.750(63)+	21.889(788)+		69.03(s)
	-	-	40	0.275(11)	0.050(2)	0	0.325(13)		
	-	Urethane	40	6.525(261)+	4.575(183)+	1.850(74)+	12.950(518)+		
	Fried Salted Beef	Urethane	40	9.400(376)+	3.025(121)+	1.175(47)+	13.600(544)+		5.02(n)
2	-	-	40	0.300(12)	0	0.025(1)	0.325(13)		
		Urethane	40	6.875(275)+	4.850(194)+	0.875(35)+	12.600(504)+		
	Boiled salted duck egg	Urethane	40	6.100(244)+	2.100(84)	0.425(17)+	8.625(345)+	31.55(w)	
	-	-	40	0.225(9)	0.025(1)	0	0.250(10)		
	-	Urethane	40	5.800 (232)+	4.025(161)+	0.850(34)+	10.675(427)+		
	Pickled green mussel	Urethane	36	5.083(183)+	2.972(107)+	0.917(33)+	8.972(323)+	15.95(n)	
	-	-	40	0.175(7)	0.025(1)	0.050(2)	0.250(10)		
	-	Urethane	35	8.800(308)+	5.200(182)+	0.600(21)+	14.600(511)+		
	Fried Salted Mackerel	Urethane	30	15.067(452)+	5.233(157)+	2.300(69)+	22.600(678)+		54.80(m)
	-	-	40	0.300(12)	0	0.025(1)	0.325(13)		
	-	Urethane	40	6.875(275)+	4.850(194)+	0.875(35)+	12.600(504)+		
	Fried Salted Beef	Urethane	40	8.625(345)+	3.800(152)+	0.775 (31)+	13.200(528)+		4.76(n)

*Statistical diagnoses using estimation of spot frequencies and confidence limits according to Frei and Wurgler[32] for comparison with negative control:

+ = positive; - = negative; i = inconclusive; Probability level: $\alpha = \beta = 0.05$. One-sided statistical tests.

** w = weak antimutagenicity, m = moderate antimutagenicity, s = strong antimutagenicity, n = negligible antimutagenicity

Table 3 Modulating effects of each salted food on urethane (20 mM) induced somatic mutation and recombination in *Drosophila melanogaster* in the pre-feeding type 1 and 2 studies

Trial	Sample	Mutagen	type	No. of wings	Spots per wing*				% Modulation (rank**)	
					Small single (m=2)	Large single (m=5)	Twin (m=5)	Total (m=2)	Inhibition	enhancement
1	-	-		40	0.175(7)	0	0.025(1)	0.200(8)		
	-	urethane		40	3.350(134)+	2.075(83)+	0.500(20)+	5.925(237)+		
	Boiled salted duck egg	urethane	1	40	2.575(103)+	1.475(59)+	0.325(13)+	4.375(175)+	26.16(w)	
	-	-		40	0.175(7)	0	0.025(1)	0.200(8)		
	-	urethane		40	3.350(134)+	2.075(83)+	0.500(20)+	5.925(237)+		
	Boiled salted duck egg	urethane	2	28	1.000(28)+	1.429(40)+	0.071(2)i	2.500(70)+	57.81(m)	
	-	-		40	0.400(16)	0.025(1)	0.025(1)	0.450(18)		
	-	urethane		40	7.325(293)+	4.525(181)+	0.525(21)+	12.375(495)+		
	Pickled green mussel	urethane	1	40	4.900(196)+	3.700(148)+	0.500(20)+	9.100(364)+	26.46(w)	
	-	-		40	0.400(16)	0.025(1)	0.025(1)	0.450(18)		
	-	urethane		24	7.325(293)+	4.525(181)+	0.525(21)+	12.375(495)+		
	Pickled green mussel	urethane	2	37	5.541(205)+	2.297(85)+	0.405(15)+	8.243(305)+	33.39(w)	
	-	-		40	0.400 (16)	0.025(1)	0.025(1)	0.450(18)		
	-	urethane		40	7.325(293)+	4.525(181)+	0.525(21)+	12.375(495)+		
	Fried Salted Mackerel	urethane	1	40	6.625(265)+	3.775(151)+	0.700(28)+	11.100(444)+	10.30(n)	
	-	-		40	0.400 (16)	0.025(1)	0.025(1)	0.450(18)		
	-	urethane		40	7.325(293)+	4.525(181)+	0.525(21)+	12.375(495)+		
	Fried Salted Mackerel	urethane	2	40	12.125(485)+	3.475(139)+	1.600(64)+	17.200(688)+		38.99(w)
	-	-		40	0.400 (16)	0.025(1)	0.025(1)	0.450(18)		
	-	urethane		40	7.325(293)+	4.525(181)+	0.525(21)+	12.375(495)+		
	Fried Salted Beef	urethane	1	40	7.625(305)+	4.150(166)+	1.150(46)+	12.925(517)+		4.44(n)
	-	-		40	0.400 (16)	0.025(1)	0.025(1)	0.450(18)		
	-	urethane		40	7.325(293)+	4.525(181)+	0.525(21)+	12.375(495)+		
	Fried Salted Beef	urethane	2	34	10.441(355)+	4.412(150)+	2.147(73)+	17.000(578)+		37.37(w)

Table 3 Modulating effects of each salted food on urethane (20 mM) induced somatic mutation and recombination in *Drosophila melanogaster* n the pre-feeding type 1 and 2 studies (continued)

Trial	Sample	Mutagen	type	No. of wings	Spots per wing*				% Modulation (rank**)	
					Small single (m=2)	Large single (m=5)	Twin (m=5)	Total (m=2)	Inhibition	enhancement
2	-	-		40	0.175(7)	0.025(1)	0.050(2)	0.250(10)		
	-	urethane		35	8.800(308)+	5.200(182)+	0.600(21)+	14.600(511)+		
	Boiled salted duck egg	urethane	1	40	6.825(273)+	6.300(252)+	0.900(36)+	14.025(561)+	3.94(n)	
	-	-		40	0.400(16)	0.025(1)	0.025(1)	0.450(18)		
	-	urethane		40	7.325(293)+	4.525(181)+	0.525(21)+	12.375(495)+		
	Boiled salted duck egg	urethane	2	40	3.250(130)+	1.800(72)+	0.300(12)+	5.350(214)+	56.77(m)	
	-	-		40	0.075(3)	0	0	0.075(3)		
	-	urethane		28	9.286(260)+	5.143(144)+	1.214(34)+	15.643(438)+		
	Pickled green mussel	urethane	1	30	5.333(160)+	3.400(102)+	0.633(19)+	9.367(281)+	40.12(m)	
	-	-		40	0.075(3)	0	0	0.075(3)		
	-	urethane		28	9.286(260)+	5.143(144)+	1.214(34)+	15.643(438)+		
	Pickled green mussel	urethane	2	10	2.700(27)+	1.400(14)+	0.500(5)i	4.600(46)+	50.89(m)	
	-	-		40	0.300(12)	0	0.025(1)	0.325(13)		
	-	urethane		40	6.875(275)+	4.850(194)+	0.875(35)+	12.600(504)+		
	Fried Salted Mackerel	urethane	1	40	7.700(308)+	3.175(127)+	0.775(31)+	11.650(466)+	7.54(n)	
	-	-		40	0.300(12)	0	0.025(1)	0.325(13)		
	-	urethane		40	6.875(275)+	4.850(194)+	0.875(35)+	12.600(504)+		
	Fried Salted Mackerel	urethane	2	40	9.950(398)+	5.900(236)+	1.475(59)+	17.325(693)+		37.50(w)
	-	-		40	0.300(12)	0	0.025(1)	0.325(13)		
	-	urethane		40	6.875(275)+	4.850(194)+	0.875(35)+	12.600(504)+		
	Fried Salted Beef	urethane	1	40	6.800(272)+	4.625(185)+	0.900(36)+	12.325(493)+	2.16(n)	
	-	-		40	0.300(12)	0	0.025(1)	0.325(13)		
	-	urethane		40	6.875(275)+	4.850(194)+	0.875(35)+	12.600(504)+		
	Fried Salted Beef	urethane	2	40	10.975(439)+	5.100(204)+	1.200(48)+	17.275(691)+		37.10(w)

* Statistical diagnoses using estimation of spot frequencies and confidence limits according to Frei and Wurgler[12] for comparison with negative control:

+ = positive; - = negative; i = inconclusive; Probability level: $\alpha = \beta = 0.05$. One-sided statistical tests.

**w = weak antimutagenicity, m = moderate antimutagenicity, s = strong antimutagenicity, n = negligible antimutagenicity

generated by the activation of urethane. It is suspected that pickled green mussel of this study contaminated with some heavy metals since the Gulf of Thailand are now being polluted with many toxic substances including heavy metals from untreated municipal and industrial waste water.[26] It is known that most heavy metal stimulate the *in vivo* synthesis of metallothionein. Studying a defense system of bivalve mollusk *Mercenaria mercenaria* showed that it produced metallothionein and GSH for against cadmium toxicity that GSH was provided the initial defense against cadmium toxicity prior to MT induction.[27] It might be possible that such contaminated heavy metal in the shellfish stimulate the production of metallothionein (MT) in the *Drophilla melanogaster* and reduce the radical provided from the activation of urethane. Min *et al*[28] revealed that metallothionein reduced the formation of DNA adduct namely, 8-hydroxyl-2´-deoxyguanosine by hydroxyl radical generated from the Fe-NAT/H_2O_2 system. De Luca-Abbott *et al*[29] discovered that the antioxidant enzymatic systems namely, glutathione-S-transferase, catalase and glutathione peroxidase of mussel harvested from polluted area contained higher activity than that from the nonpolluted area. Such phase 2 detoxification system would be of benefit to the testing *Drophilla melanogaster* in terms of conjugation the reactive intermediate produced from phase 1 activation of urethane.

Hosono *et al*[30] demonstrated that cultured milk with *Lactobacillus bulgaricus* showed its antimutagenicity on mutagenicity of 2-(2-furyl)-3-(5-nitro-2-furyl) acrylamide and 4-nitroquinoline-*N*-oxide using streptomycin-dependent strains of *Salmonella* in an in *vitro* assay system. Since vinegar is always added in the fermentation of green mussel in order to let lactic acid bacteria to be the dominant organism. Lactic acid bacterium is a probiotic and its fermentation products are claimed to provide antimutagenicity and anticarcinogenic properties. Lankaputhra and Shah[31] showed that *Lactobaciluus acidophilus* showed its antimutagenicity on mutagenicity of eight mutagen namely, MNNG, 2-nitroflourene, 4-nitro-*O*-phenylene-diamine, 4-nitroquinoline-*N*-oxide, Aflatoxin-B, 2-amino-3-methyl-3H-imidazo- quinoline, 2-amino-1-methyl-6-phenyl-imidazo(4,5-b) pyridine and 2-amino-3-methyl-9H-pyrido (3,3-6) indole using Ames salmonella test. Zhang *et al*[32] showed that lyophilized lactic bacteria cell can inhibit the mutagenic effect of heterocyclic amines.

The enhancing effect of fried salted Spanish mackerel and fried salted beef showed on the mutagenicity of urethane was supposed to be due to the amount of sodium chloride in the sample that could impair the repairing system during DNA damage.[9] Vences-Mejía *et al*[33] investigated the effect of high salt on male Wistar rat. They reported that feeding diets containing different NaCl concentrations (0.6% control group, 6%, 12%, 18% and 24%) on rat for 12 weeks caused histological changes as well as modulation the activity of cytochrome P-450 in gastric mucosa. Chronic gastritis, regenerative hyperplasia and focal metaplasia were noted in animals receiving the 12%, 18% and 24% NaCl diets. In the same groups, induction of CYP1A1 and CYP3A2 was produced, mainly in areas of metaplasia. The expression of xenobiotic metabolizing enzymes in the gastric mucosa might contribute to chemical activation in the stomach, metabolizing both exogenous and endogenous compounds implicated in the development of gastric cancer. In addition, Yu *et al*[34] suggested that consumption of Cantonese-style salted fish during all time periods was significantly associated with nasopharyngeal carcinoma in Hong Kong Chinese population.

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