Antimutagenicity against Urethane of Mangosteen, Durian Products and Their Combinations in Somatic Mutation and Recombination Test

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ABSTRACT

Lyophilized durian meat, lyophilized mangosteen meat, durian chip, durian paste and the combinations (1:1, 1:2 and 2:1) of each durian product and mangosteen were determined for their effect on urethane induced somatic mutation and recombination in *Drosophila melanogaster*. The three-day old trans-heterozygous (*mwh flr*$^{+}$*/mwh TM3*) larvae were transferred to an experimental medium (substituted each sample for 25, 50, 75 or 100 % of corn flour) that had urethane (20 mM). We analyzed for the occurrence of mutant spots of the wings from the surviving flies and found that most samples enhanced the mutagenicity of urethane with different degree. The enhancement of urethane mutagenicity might involve in the phenomenon that the chemical compounds in the samples induced the activity of mixed function oxidases and saturation of enzymatic systems involved in the DNA repair pathways since the amount of each sample incorporated into the fly medium seemed to be very high. The results as such indicated that high consumption of durian and mangosteen should be with caution since it might enhance the mutagenicity of the compounds contaminated in our daily food. However, we surprisingly found that the combination of durian paste and mangosteen (2:1) had the highest antioxidant activity (determined with DPPH scavenging capacity and ferric reducing antioxidant power assays) as well as the content of phenolic compounds (determined with Folin-Ciocalteu reagent) while durian chip contained the least antioxidant and phenolic compounds.

Keywords: Durian, Mangosteen, SMART, antimutagenicity, antioxidant activity

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Introduction

Currently, a focus towards finding natural compounds in Thai fruits that may prevent or treat cancer is just the beginning. Thailand is an agricultural country. Thai people have a unique food culture owing to climate and geographical features of place and perhaps also to Thai religious and racial features. Many tropical plants have interesting biological activities with potential therapeutic applications. For example, Durian (*Durio zibethinus L*.) is named 'king of tropical fruit' refers to two facets: the highly nutritional meat and the big thorns on the skin, which apparently resembles the thorny thrones of the Asian kings of old [1]. Mangosteen (*Garcinia mangostana Linn*.) is named 'the queen of fruits' because many people agree that it is one of the best tasting fruit in the world. The pericarp of mangosteen has been used as traditional medicines for the treatment of abdominal pain, diarrhea, astringent, dysentery, infected wound, suppuration, chronic ulcer, leucorrhoea and gonorrhoea [2]. It has also shown anti-inflammatory [3], antitumor, antioxidant [4], anticancer [5,6], inhibition of prostaglandin E2 synthesis [7] and antibacterial activities.

Both mangosteen and durian have been cultivated and the consumption increases yearly. Most consumers may be experienced of warmth in their bodies after having durian fruit as dessert. The traditional method to counteract this effect is to have mangosteen that is considered to have quenching property. However, there is no study carried out to evaluate other health benefit of mangosteen and durian. We are interested to determine whether mangosteen, durian and their combinations can modify the mutagenicity of a genotoxin in somatic mutation and recombination test (SMART) using *Drosophila melanogaster*.

Materials and Methods

Chemicals: Urethane (URE) was purchased from Sigma Chemical (St. Louis, Mo, USA). 2, 4, 6-tripyridyl-s-triazine (TPTZ), ferric chloride hexahydrate, and ferrous sulfate heptahydrate were purchased from Sigma Chemical (St. Louis, Mo, USA). 2, 2- diphenyl-1-picrylhydrazl (DPPH), gallic acid and Folin-Ciocalteu reagent were purchased from Fluka Chemika (Buchs, Switzerland). Trolox was purchased from Aldrich Chemical (Milwaukee, WI, Germany). Other chemicals were of laboratory grade.

Sample Preparation: Durian (Chanee variety) and mangosteen were purchased from local markets at Salaya. The meat of each fruit was separated, lyophilized and ground to be fine powder. Durian chip was ground to be powder. Durian paste sample was sliced into small pieces and dried in a desiccator containing silica gel desiccant at room temperature. The combinations (1:1, 1:2 and 2:1) of durian or each durian product and mangosteen were prepared. Each prepared sample was stored in a refrigerator until use.

Experimental Design Virgin females of Oregon wing flare strain (*ORR/ORR*; *flr*^{3}*/TM3, Ser)* were mated with males of multiple wing hair strain *(mwh/mwh)* on regular medium 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) and maintained on the regular medium modified from the formula of Roberts *et al*. [8] which had propionic acid (0.01 ml) as a preservative.

Firstly, the highest concentration of each sample that provided more than 50% survival of adult flies was determined. It was also explored whether each sample was mutagenic in the somatic mutation and recombination test (SMART). The *experimental media* containing 25, 50, 75 and 100 % of each sample or each combination of durian and mangosteen substitute for corn flour were prepared and were used for mutagenicity testing of each sample. URE solution (20 mM) was substituted for deionized water in the regular medium and was used as a *positive control medium*. An *experimental medium containing URE* was prepared by substituting the highest concentration of each sample that gives more than 50% survival rate for corn flour in the positive control medium. Equal amount of each medium was transferred into a 15 ml test tube. This medium was used for antimutagenicity study. The mutagenicity of each sample (in the experimental medium) was assayed as described by Graf *et al*. [9] and the antimutagenicity of each sample was assayed using the experimental medium containing URE. The larvae were maintained on medium at 25±1°C until pupation. The surviving adult flies bearing the marker trans-heterozygous (*mwh+/+flr*^{3}) indicated with round wings were

collected. Subsequently, the wings were removed, mounted and scored under a compound microscope for recording of the wing spot.

Induction frequencies of wing spots of sample treated groups 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[10].

Antimutagenicity was estimated using percentage of inhibition of total spots per wing calculated as follows: percentage of inhibition = (a-b)/a × 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 sample. It was proposed that percent of inhibition between 0–20%, 20–40%, 40–60% and higher than 60% would indicate negligible, weak, moderate and strong antimutagenicity, respectively.

One gram of sample (lyophilized durian meat, lyophilized mangosteen meat, durian chip or durian paste) was extracted twice with 50 ml of 80% methanol at room temperature for 2 h; then the mixture was filtered through cotton mesh and Whatman filter paper No. 1. The combination of each durian product and mangosteen (1.5 g) was also extracted in the same manner with 75 ml of solvent. Each extract was assayed for its antioxidant activity and total phenolic content. DPPH assay for free radical scavenging activity [11] and ferric reducing antioxidant power [12] of each methanol extract were performed. The total phenolic content [13] of methanol extract from each sample was determined.

Results

The highest concentration of each sample that provided more than 50% survival was determined for its mutagenicity and antimutagenicity. The number of total spots/wing obtained from the larvae fed each sample were between 0.10 to 1.20 in trial 1 and 0.20 to 0.94 in trial 2 while that of the negative control were 0.47 to 0.78 in trial 1 and 0.38 to 1.92 in trial 2 and of positive control were 10.20 to 14.60 in trial 1 and 7.10 to 19.67 in trial 2. This indicates that none of the sample was mutagenic.

The data shown in Table 1 demonstrate that all samples potentiately increased the number of total spots induced by urethane. The percentages of enhancement are within the range of 9.00% of durian meat:Mangosteen (2:1) to 77.41% of durian chip:Mangosteen (2:1).

Table 2 shows the antioxidant activity of each sample. The percentage of DPPH radical scavenging activity of each sample is between 12.58 and 48.48%. The combination of durian paste and mangosteen (2:1) has the highest scavenging activity. In addition, the FRAP values (μM ferrous tripyridyltriazine) of all samples are between 58.00 and 494.33. The durian paste:mangosteen (1:2) has the highest reducing power. The total phenolic content expressed as mg gallic acid equivalent per liter of each sample extract is in the range of negligible to 101.833. Durian chip contains the least amount of phenolic compound while the combination of durian paste and mangosteen (2:1) has the highest value.

Discussion

The results confirmed that such samples are safe for most consumers. None of samples was mutagenic since they did not significantly induce the frequencies of mutant spots at any testing concentrations to be higher than that of the negative control medium. The average size and survival rates of adult flies obtained from larvae fed on medium containing each sample with various percents did not show any difference compared with that of the control group (fed on regular medium).

The investigation indicated that high consumption of durian and mangosteen should be with caution since it might enhance the mutagenicity of the mutagenic species contaminated in our daily food. The presence of each sample in the medium increased the number of spots per wing induced by URE which had been found in some fermented food and beverages.[14] URE is metabolically activated by cytochrome P-450

Table 1 Modulation effect of each sample on urethane (20 mM) induced somatic mutation and recombination in *Drosophila melanogaster* derived from trans-heterozygous (*mwh*+/+*flr*^{3}) larvae of improved high bioactivation cross

Trial	Treatment	Ratio of durian products and mangosteen	No. of wing	Spots per wing (No. of spot) statistic diagnoses*				% Enhancement (rate**)
				Small single m=2	Large single m=5	Twin single m=5	Total m=2	
I	Negative control	-	40	0.58 (23)	0.03 (1)	0	0.60 (24)	
	Positive control	-	40	7.87 (315)+	3.33 (133)+	0.75 (30)+	11.95 (478)+	
	Durian meat	-	40	12.15 (486)+	6.13 (245)+	1.33 (53)+	19.60 (784)+	64.02 (s)
	Durian chip	-	39	10.90 (425)+	5.21 (203)+	1.49 (58)+	17.59 (686)+	47.19 (m)
	Durian paste	-	40	10.65 (426)+	6.35 (254)+	1.30 (52)+	18.30 (732)+	53.14 (m)
	Mangosteen	-	40	11.28 (451)+	5.80 (232)+	1.40 (56)+	18.48 (739)+	54.60 (m)
	Durian meat:Mangosteen	1:1	39	10.08 (393)+	4.33 (169)+	0.82 (32)+	15.23 (594)+	27.45 (w)
	Durian meat:Mangosteen	1:2	40	10.28 (411)+	5.23 (209)+	0.75 (30)+	16.25 (650)+	35.98 (w)
	Durian meat:Mangosteen	2:1	40	10.40 (416)+	5.28 (211)+	1.35 (54)+	17.03 (681)+	42.47 (m)
	Durian chip:Mangosteen	1:1	40	11.18 (447)+	4.95 (198)+	1.15 (46)+	17.28 (691)+	44.56 (m)
	Durian chip:Mangosteen	1:2	40	10.05 (402)+	3.70 (148)+	0.80 (32)+	14.55 (582)+	21.76 (w)
	Durian chip:Mangosteen	2:1	40	13.80 (552)+	5.98 (239)+	1.43 (57)+	21.20 (848)+	77.41 (s)
	Durian paste:Mangosteen	1:1	39	12.46 (486)+	4.82 (188)+	1.03 (40)+	18.31 (714)+	53.20 (m)
	Durian paste:Mangosteen	1:2	40	10.15 (406)+	4.80 (192)+	1.05 (42)+	16.00 (640)+	33.89 (w)
	Durian paste:Mangosteen	2:1	40	10.13 (405)+	4.58 (183)+	1.20 (48)+	15.90 (636)+	33.05 (w)
II	Negative control	-	40	0.48 (19)+	0	0	0.48 (19)+	
	Positive control	-	33	7.58 (250)+	4.61 (152)+	0.97 (32)+	13.15 (434)+	
	Durian meat	-	39	10.79 (421)+	5.18 (202)+	0.72 (28)+	16.69 (651)+	26.92 (w)
	Durian chip	-	33	10.00 (330)+	5.21 (172)+	0.70 (23)+	15.91 (525)+	20.97 (w)
	Durian paste	-	40	10.15 (406)+	4.18 (167)+	0.53 (21)+	14.85 (594)+	12.91 (n)
	Mangosteen	-	38	10.58 (402)+	4.87 (185)+	0.71 (27)+	16.16 (614)+	22.86 (w)

Table 1 Modulation effect of each sample on urethane (20 mM) induced somatic mutation and recombination in *Drosophila melanogaster* derived from trans-heterozygous (*mwh+/+flr*^{3}) larvae of improved high bioactivation cross (continued).

Trial	Treatment	Ratio of durian products and mangosteen	No. of wing	Spots per wing (No. of spot) statistic diagnoses*				% Enhancement (rate**)
				Small single m=2	Large single m=5	Twin single m=5	Total m=2	
II	Negative control	-	40	0.58 (23)+	0.03 (1)+	0	0.60 (24)+	
	Positive control	-	40	7.88 (315)+	3.33 (133)+	0.75 (30)+	11.95 (478)+	
	Durian meat:Mangosteen	1:1	40	8.38 (335)+	4.50 (180)+	0.78 (31)+	13.65 (546)+	14.23 (n)
	Durian meat:Mangosteen	1:2	38	11.79 (448)+	4.68 (178)+	1.47 (56)+	17.95 (682)+	50.19 (m)
	Durian meat:Mangosteen	2:1	40	8.15 (326)+	3.98 (159)+	0.90 (36)+	13.03 (521)+	9.00 (n)
	Durian chip:Mangosteen	1:1	40	11.33 (453)+	4.40 (176)+	1.25 (50)+	16.98 (679)+	42.05 (m)
	Durian chip:Mangosteen	1:2	40	8.98 (359)+	4.60 (184)+	0.83 (33)+	14.40 (576)+	20.50 (w)
	Durian chip:Mangosteen	2:1	40	11.63 (465)+	4.60 (184)+	1.00 (40)+	17.23 (689)+	44.14 (m)
	Negative control	-	40	0.58 (23)+	0.03 (1)+	0	0.60 (24)+	
	Positive control	-	40	7.88 (315)+	3.33 (133)+	0.75 (30)+	11.95 (478)+	
	Durian paste:Mangosteen	1:1	40	10.48 (419)+	4.63 (185)+	0.80 (32)+	15.90 (636)+	33.05 (w)
	Durian paste:Mangosteen	1:2	40	9.83 (393)+	4.98 (199)+	0.73 (29)+	15.53 (621)+	29.92 (w)
	Durian paste:Mangosteen	2:1	40	9.00 (360)+	3.85 (154)+	0.78 (31)+	13.63 (545)+	14.02 (n)

* Statistical diagnoses using estimation of spot frequencies and confidence limits according to Frei and Wurgler (1988) for comparison with negative control: + = positive; - = negative; i = inconclusive; Probability level: $\alpha = \beta = 0.05$. One-sided statistical tests.

** (n) = negligible, (w) = weak, (m) = moderate, (s) = strong

Table 2 Antioxidant activity and total phenolic content of methanolic extracts of each sample.

Sample	Ratio of durian products and mangosteen	% DPPH Scavenging activity*	FRAP values†	Total phenolic content GAE (mg/l) ‡
Durian meat	-	24	137.00	32.83
Durian chip	-	13	58.00	0
Durian paste	-	38	291.33	56.50
Mangosteen	-	35	233.67	51.33
Durian meat:Mangosteen	1:1	36	190.33	41.00
Durian meat:Mangosteen	1:2	38	185.33	38.83
Durian meat:Mangosteen	2:1	35	261.67	51.83
Durian chip:Mangosteen	1:1	34	291.33	34.17
Durian chip:Mangosteen	1:2	30	244.00	20.17
Durian chip:Mangosteen	2:1	35	351.00	45.17
Durian paste:Mangosteen	1:1	43	368.67	48.00
Durian paste:Mangosteen	1:2	48	494.33	71.50
Durian paste:Mangosteen	2:1	48	431.67	101.83

*150 μM DPPH.in 80% methanol had been used for this investigation

† FRAP values = μM ferrous tripyridyltriazine form after the addition of sample

‡ GAE = gallic acid equivalent (mg gallic acid/l).

enzyme system[15] to be vinyl carbamate epoxide which is the carcinogenic active metabolite[16-18] that is detoxified with glutathione-S-transferase (GST) conjugation.[19] The mutagenicity of URE differently enhanced by the samples might be due to the natural components of the samples that induced the catalytic activities of cytochrome P-450 enzyme system (phase 1) or inhibited GST activity as well as decreased the amount of glutathione of phase 2 detoxifying system in *Drosophila melanogaster*. Therefore, there is a need to identify and/or quantify such compounds that pose such activities.

Fruits have long been regarded as having considerable health benefits, due to their main antioxidant compounds, of which phenolics are the most abundant.[20-22] A large screening study of the antioxidant capacity of methanolic extracts of fruits reported that these fruits contain different quantities of antioxidant compounds and have different levels of antioxidant capacity.[23]

The results showed that all tested sample extracts had antioxidative characteristics, including the abilities of radical-scavenging. The ripe Mon Thong durian had high content of bioactive compounds, namely, total polyphenols, flavonoids including a flavonol named quercetin and anthocyanins.[24] These compounds possessed high antioxidant capacity. Therefore, flavonoids of durian should present their antimutagenic activity mainly due to their ability to scavenge free radicals[25] that generated during the metabolism of urethane. It was documented that *N*-hydroxyurethane, a urethane metabolite,[26,27] 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.[28] However, many biological and pharmacological activities attributed to quercetin that may be beneficial to human health have been closely linked with the potential generation of reactive pro-oxidant intermediates.[29,30] Pro-oxidant effects of flavonoids that cause damage to the genetic material were reported.[31] Sahu and Gray [32] explained the quercetin genotoxicity as a result of the production of reactive oxygen species by redox cycling. Quercetin and other phenolic compounds give rise to the superoxide anion by auto-oxidation, which in turn may lead to the formation of H_2O_2[33] and subsequently to DNA damage,[34] base-pair substitutions and frame-shift mutations in the Ames test,[35] induction of chromosomal

aberrations and sister chromatid exchanges in Chinese Hamster Ovary (CHO) cells.[36] This might explain why durian and its products could enhance the mutagenicity of urethane.

Although the samples in this study had been lyophilized but it seemed to expose to oxygen after it was incorporated into the experimental media. Chin[37] identified 39 volatile compounds of durian including 22 esters, 9 sulphur-containing alkanes, 3 thioacetals, 2 thioesters, 2 thiolanes and 1 alcohol. Ethanethiol, propanethiol, ethyl propanoate, methyl 2-methylbutanoate, propyl propanoate, ethyl 2-methylbutanoate, propyl 2-methylbutanoate, diethyl disulfide, ethyl propyl disulfide and diethyl trisulfide were reported as the major volatile constituents that possessed the distinct strong onion-like odour, while esters were the predominant volatiles that corresponded to the fruity odour.[38-40] Rowe[41] indicated that thiols, especially primary compounds, were susceptible to oxidation even without the presence of oxygen. Thiol (SH)-containing compounds were reported to be mutagenic in the Ames *Salmonella typhimurium* assay.[42-45] It is well known that the oxidation of phenolic compounds can generate reactive oxygen species, partially responsible for the observed mutagenicity of many chemicals.[46, 47] Franke *et al*. [48] analyzed orange juices and found that they had both antioxidant and mutagenic potential that caused by polyphenol oxidase-generated quinones that were already present in the fruits prior to juice production as suggested by Patrineli *et al*. [46,47] These quinones can be converted to semiquinone radicals by loss of one electron, and can directly interact with DNA or facilitate redox cycling. Thus, reactive oxygen species would be generated in the presence of molecular oxygen, leading to oxidative stress and to DNA damage.[46,47,49]

Zadernowski *et al*.[50] identified the composition of phenolic acids in various parts of mangosteen fruit (*Garcinia mangostana*). They found that the major phenolic acids in the aril were *p*-hydroxybenzoic and protocatechuic acids. In addition, the aril contained 28.0 ± 3.0 mg per kg dry weight of other acids (such as benzoic, cinnamic, mandelic and piperonylic), respectively. Protocatechuic acids in mangosteen may be of interest since Krajka-Kuźniak *et al*.[51] observed in rat liver and found that the activity of pentoxyresorufin *O*-depentylase (PROD)-CYP2B in liver was increased by 21–27% after i.p. treatment with protocatechuic acid. On the other hand, the phase 2 enzyme namely, NAD(P)H:quinone oxidoreductase (NQO1) activity was also increased after protocatechuic acid treatment. NQO1 is generally assumed to possess the important protective properties, both by detoxifying some carcinogenic compounds as well as by preventing the generation of oxygen radicals. However, this enzyme may provide not only a cellular detoxifying system, but also, with some substrates, an activating mechanism. Therefore, the finding that NQO1 can affect the mutagenicity of urethane is warrant for further investigtation. Moreover, protocatechuic acid itself may be metabolized via the tyrosinase bioactivating pathway to reactive quinine intermediates.[52] To a great extent, this effect depends on the used species, tissue, dose and route of administration to the animals.

Both mangosteen and durian have been cultivated and the consumption increases yearly. Most consumers may be experienced of warmth in their bodies after having durian fruit as dessert. The traditional method to counteract this effect is to have mangosteen that is considered to have quenching property. The absence of mutagenicity of each sample containing oxidised polyphenolic compounds during mutagenicity test might possibly be due to lower concentration to express their mutagenicity or they were detoxified by the system of the fly; however, its notorious effect was expressed as potentiating effect during co-administered with urethane. Therefore, the modulating effect of both fruits in terms of enhancing the mutagenicity of urethane is still of interest to pursue clearer mechanism.

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