Roles of Mangosteen, Durian Products and Their Combinations in Reduction of Mutagenesis and Mutagen Formation: Studies on Nitrite Treated 1-Aminopyrene

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ABSTRACT

Mangosteen, durian products and their different combinations were investigated for their mutagenicity, antimutagenicity and inhibition on mutagen formation by Ames test. All tested samples (dimethylsulfoxide extracts) showed no mutagenic potential towards Salmonella typhimurium strains TA98 and TA100 without metabolic activation after nitrination at pH 3.0-3.4. Each sample was further evaluated for its mutagenicity against the products from 4-h nitration of 1-aminopyrene (AP-nitrite model). It was demonstrated the possibility of the samples in reducing direct-acting mutagenicity of compounds occurred in the model on both strains. Each tested sample was also added into the model at the beginning of the reaction to observe its inhibitory effect on mutagen formation. The results revealed the potentials of the samples in inhibiting mutagen formation. In conclusion, the reduction of the direct-acting mutagenogenesis and the inhibition on mutagen formation of the DMSO extract of mangosteen, durian products and their combinations were detected in both S. typhimurium strains TA98 and TA100.

Keywords: Mangosteen, durian, combinations, antimutagenicity, Ames test

INTRODUCTION

Thailand is an agricultural country. Thai people have a unique food culture owing to climate and geographical features and perhaps also to Thai religions and racial features. A number of tropical plants have the interesting biological activities with potential therapeutic applications. Mangosteen (Garcinia mangostana Linn.) and durian (Durio zibethinus Linn.) are among these plants. The former has been named as “Queen of Fruits” due to its taste that is agreed to be one of the best tasting fruits of the world, while the latter has been named as “King of Tropical Fruits” referring to two facets: the highly nutritional flesh and thorn-covered husk that apparently resembles the thorny thrones of the Asian kings of old. In terms of medicinal use, mangosteen is recorded to be good for the treatment of abdominal pain, diarrhea, dysentery, infected wound, suppuration, chronic ulcer, leucorrhoea and gonorrhoea as well as having the astringent, anti-inflammatory, antitumor, antioxidant, anticancer, prostaglandin E2 synthesis inhibitory and antibacterial activities.

For the therapeutic benefits of durian, it was stated in Thai folk medicine to be good for antipyretic.

Both mangosteen and durian have been cultivated and consumed increasingly every year. Most consumers feel warm after durian consumption. To minimize this effect, Thai people are always taught to take mangosteen after consuming durian. Besides such counteract health effect,
there is no any additional study to show other health beneficial properties of these two fruits when consumed together. It was of interest to investigate the potentials of the extracts from mangosteen and durian as individual or combination against mutagens and mutagen formation through the process mimic the gastric causing mutagens by using Ames test.

**MATERIALS AND METHODS**

**Chemicals**

1- Aminopyrene (Aldrich, USA.), dimethylsulfoxide (DMSO, E. Merck, Germany) and sodium nitrite (BDH Chemicals, England) were the used chemicals. Oxoid nutrient broth no. 2 (Oxoid, England) was used bacterial medium. Other chemicals were of laboratory grade.

**Tested bacteria**

*Salmonella typhimurium* strains TA98 (hisD3052 mutation) and TA100 (hisG46 mutation) containing R - plasmid (pKM101), rfa and D uvrB mutation were used for the whole study.

**Sample preparation and extraction**

Mangosteen, durian and durian products including durian chip and paste were purchased from local markets at Salaya, Nakhon Pathom. The separated fruit was lyophilized and ground. Durian chip was ground and for durian paste, it was cut thinly, dried and ground. Each sample was stored in a desiccator until use. The combination of mangosteen and durian, or its products, was prepared as indicated in Table 1. Afterwards, each sample was mixed vigorously with DMSO at the ratio of 1:6 (w/v) in a 10 ml test tube fitted with a plastic stopper by a vortex mixer. The mixture was then shaken (180 rev/ min) at 37°C for 10 min. After centrifugation, the same procedure was repeated twice. The pooled supernatant was kept at 4°C under the dark condition until used for the study.

**Formation of mutagen**

1-Aminopyrene was interacted with sodium nitrite to provide a direct-acting mutagen [the 1-aminopyrene-nitrite (AP-nitrite) model]. The method was briefly described as following. Ten μl (if tested with TA98) or 40 μl (if tested with TA100) of 1-aminopyrene (0.0375 mg/ml) was added into a tube, followed by 710-740 μl of 0.2 N hydrochloric acid (sufficient to acidify the reaction mixture to pH 3.0 - 3.4) and 250 μl of 2M sodium nitrite with thorough mixing. The final volume was adjusted to 1,000 μl with appropriate solvent. Each reaction tube was incubated at 37°C in a shaking water bath for 4 h and the reaction was stopped by placing the tube in an ice bath for 1 min. To decompose the residue nitrite, 250 μl of 2M ammonium sulfamate was added and allowed to stand for 10 min in an ice bath. Each mixture was assayed for its mutagenicity.

**Mutagenicity and antimutagenicity test**

The pre-incubation method of Ames test suggested by Yahagi et al in the absence of metabolic activation was performed. Before the antimutagenicity test, the DMSO extract of each sample was determined for its mutagenicity. In antimutagenicity test, the 4-h incubated mixture of AP-nitrite model was mixed with 200 μl of each DMSO extract (total volume 1,450 μl). One - hundred μl aliquot of the final mixture was determined for the mutagenicity as described above.

<table>
<thead>
<tr>
<th>Table 1</th>
<th>Preparation of the extracts from mangosteen and durian</th>
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<tr>
<td>Durian (g)</td>
<td>Mangosteen (g)</td>
</tr>
<tr>
<td>0.0</td>
<td>1.0</td>
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<tr>
<td>0.5</td>
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Anti-mutagen formation test

The tested sample was each added to AP-nitrite model during 4-h incubation to see their potentials in inhibiting mutagen formation during the incubation time. An aliquot (200 µl) of each DMSO extract sample was added at the beginning to the reaction mixture of the AP-nitrite model described above. The final volume of the mixture was 1,450 µl. DMSO (200 µl) was added in place of the sample to serve as the negative control. The 4-h incubation mixture (100 µl) was determined for its mutagenicity.

Table 2 shows the amount of the composition of the reaction sample in antimutagenicity and effect of DMSO extract during mutagen formation in the AP-nitrite model. The same amount of the composition was used for each in comparison of the results. The reduction of mutagen formation in the model by each sample was interpreted when the percent inhibition of his + revertants per plate of the sample in antimutagenicity test was higher than that shown by the sample during mutagen formation.

Data evaluation

The results were reported as mean of histidine (His +) revertants per plate. The mutagenicity of each sample was declared when the number of histidine (His +) revertants per plate was twice higher than of spontaneous revertants and with a concentration-response manner. The percent inhibition of samples towards the mutagens was calculated as following:

Percent inhibition (average) = (A-B)/(A-C) x100

Where A is a number of histidine revertants per plate induced by nitrite treated 1-aminopyrine. B is a number of histidine revertants per plate induced by nitrite treated 1-aminopyrine in the presence of the sample and C is a number of spontaneous histidine revertants per plate (with DMSO in place of the sample). The antimutagenic potential of the sample was interpreted or considered to be strong when the inhibition was higher than 60%, moderate, weak and none when the inhibition were 40-60%, 20-40% and less than 20%, respectively.

RESULTS

The mutagenicity test operated by the pre-incubation method of Ames Salmonella assays revealed that the DMSO extracts neither from mangosteen nor durian was mutagenic (data not shown). Besides, they were not mutagenic either when treated with nitrite (data not shown). For antimutagenic potential, most DMSO extracts showed no antimutagenicity towards the products from AP-nitrite model tested on S. typhimurium strains TA98 and TA100 (Figures 1 and 2). However, results revealed that the combination extracts between mangosteen and durian paste (2:1) had weak antimutagenicity tested on TA98.

In terms of anti-mutagen formation resulting from AP-nitrite model, the test on TA98 revealed the extracts from mangosteen, durian, durian chip and the combination of mangosteen and durian or durian chip (1:1) had no inhibitory activity on TA98 (Figure 3). On the contrary, the test on TA100 showed that all DMSO extracts could prevent the formation of mutagenic species from AP-nitrite model. The DMSO extracts from both mangosteen and durian had the moderate anti-mutagen formation (Figure 4).
**Figure 1** Antimutagenicity of DMSO extract of mangosteen, durian products and their combination expressed as percent inhibition of number of revertants of *S. typhimurium* strain TA98 induced by sodium nitrite treated 1-aminopyrene (average of 2 trials)

**Figure 2** Antimutagenicity of DMSO extract of mangosteen, durian products and the combination expressed as percent inhibition of number of revertants of *S. typhimurium* strain TA100 induced by sodium nitrite treated 1-aminopyrene (average of 2 trials)
Figure 3  Effect of DMSO extract of mangosteen, durian products and the combination in inhibition mutagen formation during the reaction between sodium nitrite and 1-aminopyrene on *S. typhimurium* TA 98 (% inhibition average of 2 trials)

Figure 4  Effect of DMSO extract of mangosteen, durian products and the combination in inhibition mutagen formation during the reaction between sodium nitrite and 1-aminopyrene on *S. typhimurium* TA 100 (% inhibition average of 2 trials)
Figure 5  Difference of percent inhibition of mutagenicity on *S. typhimurium* TA98

Figure 6  Difference of percent inhibition of mutagenicity on *S. typhimurium* TA100
Figure 5 presents the different percentage of mutagenicity inhibition of samples on S. typhimurium TA98 from two experimental designs. It revealed that most samples had the inhibitory effect on mutagen formation. The difference in percentage of inhibition on mutagenicity of samples on TA100 from two experimental designs also revealed that most samples had the inhibitory effect on mutagen formation resulting from AP-nitrite model (Figure 6).

DISCUSSION

All DMSO extracts of mangosteen, durian products and their combinations tend to be safe for consumption because neither of them showed the mutagenicity towards the tested bacterial stains, S. typhimurium TA98 and TA100. Either were the nitrosated products of these samples. This indicates consumers would not be in a risk to consume these two kinds of fruit simultaneously with any nitrite containing dishes. It might be that mangosteen and durian contain some compounds that are likely to interfere or inhibit nitrosation of some nitrosable compounds as dietary amines or amides.

Since nitrite ion and nitrous acid (the product of the reaction between nitrite ion with proton of the acidic medium) are not active, but they can form very reactive nitrous anhydride (N₂O₃) under moderately acidic condition. Under strongly acidic condition, for example acidic pH in human stomach, nitrous acid is converted to more powerful nitrosating agents such as nitrous acidum ion (H₂ONO⁻) or nitrosonium ion (NO⁺) that can be inhibited by antioxidants such as ascorbic acid (vitamin C), tocopherols (vitamin E), phenolic compounds and other phytochemicals.18 Jitwiriyatham19 reported that mangosteen, durian with its products (chip and paste) and their combinations which were the same samples as in the present study contained antioxidants and phenolic compounds. This might be the reason why they could protect themselves from being converted to be mutagenic by nitrite. Therefore, these samples might possess antimutagenicity and protect against mutagen formation in acid condition simulated gastric digestion.

This investigation was the first attempt to elucidate the antimutagenicity of mangosteen, durian and its products, and the mixture of each mangosteen and durian with its products. Due to the suspicion of the presence of the antimutagenic compounds from their ability to inhibit the mutagenicity of the AP-nitrite produced mutagens, they were likely to be able to inhibit the enzymatic activation of direct mutagens by the existing flavonoids that have been known to be the potent antimutagenic phytochemicals. Kuo et al.20 found that a flavonoid, apigenin could inhibit nitropyrene reductase. Nitroreductase and O-acetyltransferase in Salmonella cells are activating enzymes of many direct mutagens and are speculated to be the target on the inhibition study. Besides such mechanism of antimutagenicity, some compounds may modify the permeability of mutagen across bacterial membranes. Edenharder and Tang21 reported 1-nitropyrene was effectively antagonized by potent antimutagenic flavonoids. However, carotenoids in the samples might have some other unknown inhibitory mechanism in addition to the above. Durian is a good source of carotenoids with β-carotene in a large portion.22

More interestingly, the studied DMSO extracts extended their mutagenicinhibition towards nitrite treated 1-aminopyrene to their inhibition towards formation of mutagenic species during such treatment. Most samples inhibited mutagen formation better than inhibited mutation. The inhibition on formation of direct-acting mutagens by DMSO extracts might be due to the antioxidants and phenolic compounds detected in this investigation. It was reported that ascorbic acid was found in both mangosteen and durian.23 These compounds seem to be the promising inhibitors of the chemical
reaction involving with nitrite in fermented meats and some food components during stomach digestion. Mirvish concluded that ascorbic acid was the most suitable inhibitor because of its activity at acidic pH and lack of toxicity. Polyphenolic compounds, the constituents of the extract, are believed to be anti-nitrosating agents. Mirvish suggested that polyphenols could inhibit the formation of nitrosoprine during in vivo gastric nitrosation.

In conclusion, mangosteen and durian are safe for consumption in consideration of mutagenicity. Most importantly, they are good for body health in terms of their ability to inhibit mutagenesis and mutagen formation under nitrosating condition in the stomach if they have to be consumed with nitrite related foods.

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บทบาทของมังคุด ผลิตภัณฑ์ทุเรียน และผลรวมกันของสองผลิตภัณฑ์ในการลดการก่อสายพันธุ์ และการเกิดสารก่อมกลางสายพันธุ์ โดยศึกษาในปฏิกิริยาในเคราะห์บนสิ่งมีชีวิต

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บทคัดย่ำ

ตัวอย่างมังคุด ทุเรียน ผลิตภัณฑ์ทุเรียน และผลรวมกันของมังคุดและผลิตภัณฑ์ทุเรียนในอัตราส่วนต่างกัน ถูกนำมาวิเคราะห์ความสามารถในการก่อสายพันธุ์ การต้านการก่อสายพันธุ์ และผลในการยับยั้งการเกิดสารก่อมกลางสายพันธุ์โดยใช้วิธีเอมส์เทสต์ จากการศึกษาพบว่าทุกตัวอย่าง (สกัดด้วย DMSO) ไม่แสดงศักยภาพในการก่อสายพันธุ์ เมื่อนำมาทดสอบด้วย Salmonella typhimurium สายพันธุ์ TA98 และ TA100 ซึ่งพบว่าไม่มีเม็ดเชื้อที่มีมันหมายความเป็นพิษหลังจากทำปฏิกิริยาในดีอีซีที่ pH 3.0-3.4 และเมื่อนำตัวอย่างมาทำการศึกษาด้านการก่อสายพันธุ์ โดยทำปฏิกิริยาแบบทดสอบจากปฏิกิริยาของ 1-aminopyreneสามารถแสดงให้เห็นถึงความสามารถในการลดการก่อสายพันธุ์ในแบบจำลองที่มีตัวอย่างที่เกิดจากการปฏิกิริยาในช่วงเริ่มต้นของปฏิกิริยา เพื่อค้นหาการยับยั้งการเกิดสารก่อมกลางสายพันธุ์ ผลการทดลองพบว่าตัวอย่างทั้งสองมีศักยภาพในการยับยั้งการเกิดสารก่อมกลางสายพันธุ์ แต่ได้รับการนามัตว์การต้านการก่อสายพันธุ์ ผลิตภัณฑ์ทุเรียนที่สกัดด้วย DMSO สามารถลดการก่อสายพันธุ์และยับยั้งการเกิดสารก่อมกลางสายพันธุ์ได้ใน S. typhimurium ทั้งสายพันธุ์ TA98 และ TA100

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