Food taboo of taking pineapple and milk at a time

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Introduction

There are many taboos, rumors, myths, and misconceptions which are ingrained in the developing countries, most often due to underlying cultural, political, educational, economical and environmental factors that determine the complex human behaviors, including food consumption practices.1 There are many food taboos also in Bangladesh among them, related to pineapple fruits, have two food taboos: a) Eating pineapple during pregnancy results in miscarriage and b) Eating milk and pineapple together cause toxicity, even death. To clarify about the first one, only one experimental study had been done.2 However, to the best of our knowledge, no experimental study regarding this second food taboo was found.

Milk is regarded as a complete food in a human diet. It provides all the nutrients essential for the nourishment of the human body.3 Pineapple (Ananas comosus) fruit is a good source of various vitamins like A, B, C; minerals like calcium, phosphorus, iron, and enzymes.1 It also contains tannins, cardenolides, drienolides, cardiac glycocide and flavonoids (bromelain).3,4 The individual food is healthy and non-toxic. Why would it be toxic together? If so, what types of toxicity (hepatotoxicity, neurotoxicity, hemotoxicity, etc) it causes? Still, it remains mysterious. Therefore, the purpose of this study was to assess whether potential toxic interactions occur between the pineapple and milk following oral administration in rats by evaluating the clinical signs, hematological and biochemical parameters, gross and microscopic findings by comparing with the normal control and toxic standard group.

Abstract

The purpose of this study was to assess whether potential toxic interactions occur between the pineapple and milk following oral administration in rats by comparing clinical signs, hematological and biochemical parameters with the normal control and toxic standard groups. Pineapple and milk solutions were made by 1:1 (PMS1) and 2:1 (PMS2) ratio, administered 12 mL/kg body weight. Forty rats were equally divided into 4 groups treated for 3 days: a) normal control (only vehicle treated), b) toxic standard (CCl4 was suspended in corn oil, 20% v/v; treated 1.25 mL/kg), c) PMS1 and d) PMS2 groups. CCl4 administration altered the normal behavior, changes gross and microscopic morphology. Toxicity related hematological and serum biochemistry changed significantly (p<0.05) than the normal group. However, all these clinical and pathological changes were completely absent in PMS treated groups. These results suggest that taking pineapple with milk is not toxic and this food taboo is wrong.

Materials and Methods

Preparation of pineapple and milk mixture solution

Pineapple fruits (Jinwon Trading, Co. Korea) and pasteurized milk (Seoul milk®, Korea) were bought from the local market of Guri City, South Korea. Fruits were cut into small pieces and weighed. Then juice was made by electric blender without mixing water and suck by mesh. Two types of pineapple and milk solutions were made by mixing juice and milk by the ratio of 1:1 (PMS1) and 2:1 (PMS2).

Experimental animals

A total of 40 male Sprague-Dawley rats (375–448 g, Orient Bio, Korea) were used for this study. The rats were housed in an environment with a controlled temperature (23 ± 2°C) and humidity (50 ± 5%) with a 12 to 12 hours light-dark cycle. Food and water were available ad libitum before started the experiment. After 7 days acclimatization, rats were equally divided into four groups (n=10): a) Normal control group (treated with only vehicle 12 mL/kg body weight), b) Toxic standard group (carbon tetrachloride, CCl4), c) PMS1 group (treated with PMS 1:1, 12 mL/kg body weight) and d) PMS2 group (treated with PMS 2:1, 12 mL/kg body weight). The design of CCl4-induced
toxicity such as dosage and timing of CCl₄ were adapted and modified as described previously. The CCl₄ was suspended in corn oil (20% v/v) and administered orally (1.25 mL/kg body weight). The control animals received saline (12 mL/kg) orally at 12 hours intervals up to 72 hours.

**Lethality and behavioral observation**

Visual physical examination of the rat was performed carefully before starting the experiment to ensure a good state of health. The rats were closely observed for any indication of toxicity effect within the treatment period and finally at the 72th hours before sacrifice. Visual observations included checking mortality, rectal temperature, behavioral changes (weakness, aggressiveness, food or water refusal), diarrhea or loose feces, salivation, discharge from eyes and ears, noisy breathing and clonic convulsion were recorded at 0, 24, 48 and 72 hours.

**Sample collection**

After 72 hours of treatment, the rats were sacrificed by anesthesia and the blood (collected caudal from vena cava), stomach, liver, heart and kidney were collected for gross and histological analysis.

**Hematological parameters**

Whole blood was collected into the test tube containing the anticoagulant, ethylenediamine tetraacetic acid (EDTA). Hematological and biochemical changes were measured by a fully-automated hematology system (ADVIR 2120i, Siemens).

Blood samples for other biochemical analysis were collected into plain sample tubes. The serum was separated by centrifugation at 3,000 rpm for 10 min and stored at -20°C until analysis. Serum creatinine kinase, aspartate aminotransferase (AST), alanine aminotransferase (ALT), lactate dehydrogenase, total bilirubin, direct bilirubin, creatinine, blood urea nitrogen and uric acid levels were determined with a Hitachi 7180 instrument (Hitachi, Japan).

Differences between groups were evaluated by analysis of variance (ANOVA) with the Student’s t-test versus control group using Prism 5.03 (GraphPad Software Inc., USA).

**Results**

**Lethality and behavioral observation**

Mortality was not observed in all the experimental groups during the study period. After the administration of CCl₄, the rectal temperature was increased significantly (p<0.01) from 48 hours to end of the experiment and decreased the amount of feed and water intake (Table I). Restlessness, weakness and diarrhea also observed in toxic standard group. However, all of these clinical signs were absent in the normal control, PMS1 and PMS2 groups. Other clinical signs such as salivation, discharge from eyes and ears, noisy breathing, convulsion and tremor all were absent in all groups.

**Organ and body analysis**

There were no significant differences in the mean body weight of rats among groups. The relative changes of the weight of stomach, liver and kidney to the body weight increased significantly than the control group but the PMS administered groups had no change. No remarkable changes

<table>
<thead>
<tr>
<th>Table I</th>
<th>Observation of clinical signs and behavioral changes of normal and experimental rats</th>
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<tbody>
<tr>
<td>Time (hours)</td>
<td>Normal control</td>
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<tr>
<td>Rectal temperature (°C)</td>
<td></td>
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<tr>
<td>0</td>
<td>33.1 ± 0.3</td>
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<tr>
<td>24</td>
<td>33.0 ± 0.3</td>
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<tr>
<td>48</td>
<td>32.8 ± 0.2</td>
</tr>
<tr>
<td>72</td>
<td>33.0 ± 0.3</td>
</tr>
<tr>
<td>Restlessness</td>
<td>0</td>
</tr>
<tr>
<td>24</td>
<td>-</td>
</tr>
<tr>
<td>48</td>
<td>-</td>
</tr>
<tr>
<td>Weakness</td>
<td>0</td>
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<td>24</td>
<td>-</td>
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<td>48</td>
<td>-</td>
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<td>Diarrhea or loose feces</td>
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PMS1 group treated with mixed solution (1:1) 12 mL/kg body weight and PMS2 group treated with mixed solution (2:1) 12 mL/kg body weight. *p<0.05, Bonferroni post hoc test following one-way ANOVA versus the normal control group. **p<0.01; Bonferroni post hoc test following one-way ANOVA versus the carbon tetrachloride group. ‘+’ is presence of clinical sign and ‘-’ is absence of clinical signs; Data are mean ± SD.
were found in the weight of heart among all groups (Table II).

### Hematological parameters

There were significantly increased in leucocyte, lymphocyte, neutrophil, basophil and monocyte in the toxic standard group compared to the normal group. But PMS1 and PMS2 groups showed no statistically significant (p>0.05) difference in the hematological parameters than the normal control group (Table III).

### Serum biochemistrys

Cytosolic enzymes (ALT, AST, lactate dehydrogenase and creatinine kinase), direct bilirubin, total bilirubin, blood urea nitrogen, creatinine and uric acid were markedly increased (p<0.001) in the CCl₄ group than the normal control group (Figure 1). However, no alteration was observed in the pineapple and milk treated groups. As shown in Figure 2, serum inflammatory cytokines (TNF-α and IL-6) also significantly increased (p<0.001) in the CCl₄ group than the normal control group. But no changes were found in the PMS administered groups.

### Histological analysis

In the normal control rats, histological analysis revealed normal hepatic and renal cells (Figure 3). Conversely, CCl₄-induced toxicity rats exhibited extensive necrosis and loss of architecture of hepatocytes, slight hydropic degeneration, apoptotic nuclei, occasional bi-nucleation, cellular infiltration, hemorrhage and congestion. Histological analysis of PMS treated rats was like vehicle-treated control rats.

### Discussion

The food taboo is that eating pineapple and milk together induce toxicity but what types of toxicity (hepatotoxicity, neurotoxicity, hemotoxicity, etc) it causes, still not known. So, a specific standard group was not able to make. However, CCl₄ is well known toxic chemical. So, CCl₄ was used as a toxic standard in this experiment. After 4 times administration of CCl₄ acute toxicity were occurred which were represented by the alteration of normal behavioral, significant increased of rectal temperature and at the end of experiment significant elevation of hematological (leucocyte, lymphocytes, monocytes, basophils and neutrophils) parameters, serum cytosolic enzymes (ALT, AST, lactate dehydrogenase and creatinine kinase), direct bilirubin, total bilirubin, blood urea nitrogen, creatinine and uric acid and serum inflammatory cytokines (TNF-α and IL-6) were found. These are consistent with other reports of CCl₄-induced acute toxicity. Interestingly, no clinical signs, pathological or biochemical changes related to toxicity were observed in pineapple and milk (different doses) at a time administered group. These indicated that this mixture is non-toxic.

This is common food taboo in Bangladesh often reported in the Bangladeshi newspaper. However, many favorite foods and recipe are made mixing milk and pineapple in many countries. Moreover, pineapple and its constituents exert anti-oxidative, anti-inflammatory, anti-cancer, hepatoprotective and neuroprotective effects.
Any types of toxicity cause injury to the cells. As consequences of injury, cytosolic enzymes are leak out to the bloodstream and increased their normal level.

Like this study, other studies have reported that CCl4 intoxication elevated serum ALT, AST, lactate dehydrogenase, creatinine kinase, direct bilirubin, and total bilirubin levels following acute liver injury. Additionally, increases in serum creatinine, blood urea nitrogen and uric acid levels are all indicative of kidney toxicity. Both acute and chronic CCl4 administration can alter the kidney function and has been shown to reduce renal function by promoting interstitial edema and nephritis, which were absent in PMS administered groups.

Inflammation is a complex biological response to injury as a result of different stimuli such as pathogens, damaged cells, or irritants. Moreover, TNF-α and IL-6 are considered as

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**Figure 1:** Effect of pineapple and milk mixed solution on serum biochemical changes

NC, normal control group treated with only vehicle; TS group, toxic standard group, CCl4 treated group, 12 mg/kg body weight; PMS1 group, treated with mixed solution (1:1) 12 mL/kg body weight and PMS2 group, treated with mixed solution (2:1) 12 mL/kg body weight. a: p<0.01, b: p<0.001, Bonferroni post hoc test following one-way ANOVA versus the NC group. ALT alanine aminotransferase, AST aspartate aminotransferase, CK creatinine kinase, LDH lactate dehydrogenase, CRE creatinine, BUN blood urea nitrogen and UA uric acid.

**Figure 2:** Effect of pineapple and milk mixed solution on serum inflammatory cytokines

NC, normal control group treated with only vehicle; TS group, toxic standard group, CCl4 treated group, 12 mg/kg body weight; PMS1 group, treated with mixed solution (1:1) 12 mL/kg body weight and PMS2 group, treated with mixed solution (2:1) 12 mL/kg body weight. a: p<0.001, Bonferroni post hoc test following one-way ANOVA versus the NC group. TNF-α tumor necrotic factor-alpha, IL-6 interleukin-6.
indications of hepatotoxicity experimental model of liver injuries. Inflammatory cytokines and inflammation related blood cells were also not found in the PMS-treated groups which were extremely elevated in the toxic standard group when compared with normal.

It might be due to the absence of tissue injury in PMS-treated groups which further confirmed by histopathological examination. Microscopically, it was found that necrosis of tissue and infiltration of inflammatory in hepatic and renal tissues in the CCl₄ group, which was absent in the normal control and PMS groups and showed normal architectures of cells and tissues. The relative change in liver and kidney weight to the body weight were increased markedly in the toxic standard group, might be attributed to increased infiltration of neutrophils, edematous cellular space and increased protein content due to tissue injuries or necrosis evidenced by histopathology. These postmortem findings are absent in PMS-treated group which furthermore confirmed this mixture is not toxic. Logically, altered behavioral changes and the high rectal temperature were found in the CCl₄ group while in PMS-treated groups it was like normal and indicating that this foods combination is safe.

Conclusion
Evaluating the toxicity related clinical signs, hematological and biochemical parameters, gross and microscopic findings propose that taking pineapple and milk at a time is non-toxic. So, this food taboo in Bangladesh is wrong.

Ethical Issue
All experimental protocols employed herein were approved by the committee on the care of laboratory animal resources, Knotus Pvt, Co., Ltd, Korea, (Certificate number: IACUC 16-KE-104) and were conducted in accordance with the Guide for the Care and Use of Laboratory Animals published by the US National Institute of Health (NIH Publication No. 85-23, revised 1996).

Conflict of Interest
Author has no conflict of interest.

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