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Full Length Research Paper

Acute and subchronic toxicity evaluation of the hydroethanolic extract of mangosteen pericarp

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In this study, we examined the effects of a hydroethanolic extract from the fruit pericarp of mangosteen that contained epicathechin based tannins as a major constituent upon acute and subchronic treatments to experimental animals. Intragastric administration of this extract to Swiss albino mice at a single dose of 2 and 5 g/kg body weight produced no toxicity signs during 14 days of observation. For the subchronic toxicity study, the mangosteen extract at 400, 600 and 1,200 mg/kg body weight was administered by oral gavage to male and female Wistar rats daily for 12 weeks. In all instances, consumption of the extract showed no effect on behavior, food and water intake, growth or health status of these animals. In both sexes, their hematology values monitored throughout the study period did not alter from those of the control. After the 12 weeks, no significant dose-related differences in blood biochemical parameters were detected among the female groups, but in all male groups, there were dose-variation increases in direct bilirubin compared with the control. However, either gross necropsy or histopathological examination of their livers as well as other internal organs did not reveal any abnormal appearances.

Key words: Acute toxicity, *Garcinia mangostana,* hydroethanolic extract, mangosteen, pericarp, subchronic toxicity.

INTRODUCTION

Garcinia mangostana (*G. mangostana*) Linn., commonly known as mangosteen, is a tropical fruit tree belonging to the family Clusiaceae (Guttiferae). A sweet and slightly sour flavor together with a pleasant aroma of its white, soft and juicy edible pulp make mangosteen one of the best tasting tropical fruits and is called “the queen of fruits”. The pericarp or fruit hull (rind) which is thick, hard, and a dark purple to red purple in color, has a tradition of use in Southeast Asia to cure a broad range of ailments (Morton, 1987). Extensive phytochemical studies have revealed that the mangosteen-fruit pericarp is rich in xanthones with many diverse structures (Pedraza-Chaverri et al., 2008).

Of these, mangostins have been mostly reported for their remarkable biological activities such as anti-oxidant (Williams et al., 1995), anti-HIV (Chen et al., 1996), anti-fungus (Gopalakrishnan et al., 1997), anti-allergy (Chairungsrilerd et al., 1998), anti-bacterial (Sakagami et al., 2005), anti-malarial (Mahabusarakam et al., 2006), anti-cancer (Nagakawa et al., 2007), and anti-inflammation (Tewtrakul et al., 2009). Other than xanthones, the pericarp of mangosteen also contains an abundance of epicathechin based tannins

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(Mahabusarakam et al., 1987; Yu et al., 2007). These polymeric flavonoids are astringents that can relieve irritation in the bowel and stop excess secretion from the intestinal lining (Salunkhe et al., 1990). This explains why a boiling-water extract of mangosteen fruit hull has been employed as a folk remedy for cholera, dysenteries, and diarrhea (Morton, 1987). Even though the long-term and/or a large intake of tannins may cause some deleterious effects (Salunkhe et al., 1990), consumption of a whole fruit (rind and pulp) in the form of beverages, teas, pills, powders, crushed extracts etc., has gained much popularity during the last decade due to its alleged health benefits. Although, earlier studies have demonstrated that mangostin extracted from the mangosteen fruit hull is not toxic (Sornprasit et al., 1987; Jujun et al., 2008), a systemic toxicological evaluation of its tannin-rich extract for human consumption remains to be established.

In this study, we have carried out both acute and subchronic toxicity assays to investigate the safety of a mangosteen pericarp extract containing epicathechin-based tannin as the principal polyphenolic constituent.

MATERIALS AND METHODS

Plant material and preparation of the extract

The mangosteen (*G. mangostana*) fruits were purchased from Chumporn Province, Thailand. The voucher specimen (SKP 0803071301) is kept at the herbarium of the Faculty of Pharmaceutical Sciences, Prince of Songkla University, Thailand. Their hulls were washed, cut into small pieces, dried at 50 - 60°C, and then ground to a powder. One kilogram of the mangosteen powder was successively extracted by maceration with 1:1 (v/v) of water: ethanol (3 L × 3 times). The extract was then filtered, concentrated to dryness under reduced pressure, and freeze-dried to obtain a hydroethanolic extract of the mangosteen pericarp. The percentage yield of the extract was 21.7% (w/w) and contained (-) epicathechin 11.38 mg/g according to HPLC analysis. Dried extract was stored at 4°C in sterilized sealed plastic containers and kept away from light until use. The same batch of material was used throughout this study.

Experimental animals

Swiss albino mice (*Mus musculus*) weighing 28 - 35 g and Wistar rats (*Rattus norvegicus*) weighing 150 – 210 g of both sexes were obtained from the Laboratory Animal Facility Unit, Faculty of Science, Prince of Songkla University, Hat Yai, Thailand. They were housed in identical wire-mesh-bottomed stainless-steel cages containing four (4) rats or ten (10) mice per cage and maintained in an air-conditioned room at 25 ± 2°C, 50 - 60% relative humidity and artificial illumination between 06:00 and 18:00 h. Commercial chow diets (C.P. Mice Feed®, Charoen Phokphand Group, Bangkok, Thailand) and drinking water were provided *ad libitum*. All procedures concerning animal treatments and experimentations in this study were reviewed and approved by the Institutional Committee for Ethical Use of Experimental Animals at Prince of Songkla University (Approval no. MOE 0521.11/320).

Acute toxicity study

The single-dose acute oral toxicity test in this study was performed in Swiss mice according to OECD 420 (OECD, 2001). Mice were randomly divided into one control and two treatment groups. Each group contained five (5) males and five (5) females. The mangosteen extract suspended in a co-solvent consisting of 4:4:1 (v/v/v) of propylene glycol: water: Tween 80 was orally given to mice in the test groups at a single dose of 2 and 5 g/kg body weight.

Each control animal, however, received the co-solvent instead of the extract suspension. Following administration, animals were observed closely during the first day, and occasionally, thereafter, for 14 days, for toxic signs and symptoms, and death. At the end of the period, all survivors were sacrificed to examine gross changes in their vital organs.

Subchronic toxicity study

Male and female Wistar rats were randomly divided into four groups of six. During the 12 weeks of the experimental period, the extract suspension prepared as above was administered orally to the animals in each treatment group daily at doses of 0, 400, 600 and 1,200 mg extract/kg body weight. The animals were observed daily for their clinical signs and any behavioral changes. They were weighed initially and then once a week until the end of the experiment. Food and water intakes were also measured daily.

Once every 4 weeks, heparinized blood was collected by ocular bed puncture for hematology and biochemical analyses, following overnight fasting. Packed red cell volume measurements and counts of total and differential leukocytes were performed. Plasma was also separated from the collected blood for assays of aspartate aminotransferase (AST), alanine aminotransferase (ALT), alkaline phosphatase, total and direct bilirubins, glucose, creatinine, urea nitrogen, uric acid, albumin, total protein, and Na^+, K^+, Cl^-, and HCO_3^- levels using a Lab Automation Model Synchron CX3 Delta (Beckman Coulter, Palo Alto, USA). Diagnostic kits (CPT Diagnostics, Barcelona, Spain) based on enzymatic methods were used for total cholesterol, HDL-cholesterol, LDL-cholesterol, and triglyceride assays in these plasma samples.

At the end of the study, the rats were anesthetized with ether for blood collection and then sacrificed by cervical dislocation. An autopsy was performed during which any macroscopic abnormalities were noted. The heart, liver, spleens and kidneys were weighed immediately after removal. Samples of these organs were fixed in 10% (v/v) neutral buffered formalin and kept in that solution for further histopathological examination.

Statistical analysis

All data are presented as means ± S.E.M. Statistical evaluations were performed by one-way analysis of variance (ANOVA) and a post hoc least-significant difference (LSD) test at the 95% confidence level using an SPSS program for Windows 11. Significance was judged at $p < 0.05$.

RESULTS

Acute toxicity study

All mice treated with the extract by intragastric gavage

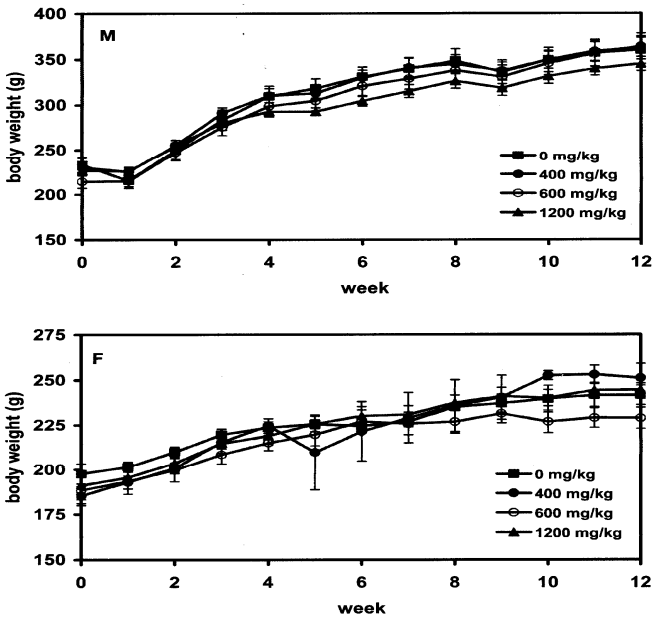

Figure 1. Average body weights of male (M) and female (F) rats fed daily for 12 weeks with the hydroethanolic extract of mangosteen fruit pericarp at various doses. Values are mean ± S.E.M.; n = 6.

survived until the end of the experimental period. They did not exhibit any signs of toxicity during the 14 days of observation. There were no obvious differences between the treated and control animals. The gross examinations of their internal organs also found no pathological abnormalities.

Subchronic toxicity study

Ingestion of mangosteen extract at any tested dose did not cause mortality or toxic symptoms in rats. The average daily food and water intake among the groups were similar throughout this study. There was no unusual behavior or physical appearance among these animals. In all cases, their feces were dry and dark. Measurements of the body weight over the whole experimental period found no significant differences between the treated and control groups of the same sex. They all gained weight as the experiment progressed. On average, the male rats grew faster than the females (Figure 1).

As shown in Table 1, feeding of the extract up to the highest dose of 1,200 mg/kg/day for 12 weeks did not produce any significant dose-related effects on blood chemistry parameters in female rats. In all male groups that received the extract, however, their direct bilirubin levels were significantly increased from the control at the end of treatment, whereas, their plasma protein concentrations became slightly lower (Table 1). Some alterations in blood lipids among our treated male groups were also detected. All of them had lower LDL-cholesterol levels than the control group, with significant differences being found in the low-dose (400 mg/kg/day) and middle-dose (600 mg/kg/day) groups. Their blood triglycerides were also elevated but only those of the low-dose (400 mg/kg/day) and high-dose (1,200 mg/kg/day) groups were statistically different from the control values.

To determine if the mangosteen extract could affect blood cells and the bone marrow activity of rats, hematological examinations were performed. As presented in Table 2, there were no significant differences in any of the parameters examined in either the control or treated groups of both sexes. In addition, normal blood smears were observed in all of the animals.

At autopsy, macroscopic examinations of vital organs including heart, liver, kidney and spleen in our experimental rats did not show any abnormality in their gross appearances and weights as a result of the consumption of the extract (Table 3). In addition, we did not detect any damage in their gastrointestinal tracts, the potential and direct target for toxic effects of ingested foods. The results from gross examination were also confirmed by histopathological assessment. The extract did not produce any significant histological changes in the organ tissues of any of the animals.

DISCUSSION

The mangosteen-fruit hull extract contains an abundance of condensed tannins which are epicatechin-based as commonly found in teas (Balentine et al., 1998). According to the OECD guidelines for the testing of chemicals No. 420 (OECD, 2001), the results of our acute toxicity study indicate that this tannin-rich extract at any given dose is non-toxic to mice. In a previous study by Jujun and others, the ethanol extract from a similar fruit rind containing 11.45% (w/w) mangostin also produced no toxic effects in rats after a single oral administration up to a dose of 5 g/kg body weight (Jujun et al., 2008). Acute hepatotoxic effects of mangostin have also been examined in mice after a single intraperitoneal injection. This compound at 200 mg/kg dosage increased the two liver enzymes, SGOT and SGPT, but the effects were relatively mild as compared with paracetamol (Sornprasit et al., 1987). During the 0 - 8 weeks of our subacute test, we occasionally observed different blood chemistry results among groups of both sexes. Since these differences were minor and not-dose related, they could have been the results of biological variation among rats rather than the treatment effects. In addition, all values of parameter obtained throughout that period were still within the reference intervals for Wistar rats (Boehm et al., 2007).

Table 1. Mean blood chemistry values in rats treated with the mangosteen pericarp extract for 12 weeks.

Parameter	Dose administered (mg/kg of body weight/day)							
	0		400		600		1,200	
	Male	Female	Male	Female	Male	Female	Male	Female
AST (IU/L)	96.38±7.23	74.50 ± 12.50	92.50 ± 8.74	97.00 ± 35.00	88.86 ± 3.49	93.60 ± 15.25	97.43 ± 12.43	100.63 ± 11.62
ALT (IU/L)	54.38 ± 3.33	58.00 ± 0.00	52.83 ± 2.80	43.50 ± 3.50	50.43 ± 4.29	42.00 ± 3.26	50.14 ± 2.27	44.50 ± 4.19
ALP (IU/L)	63.88 ± 2.92	34.50 ± 3.50	59.50 ± 2.26	44.50 ± 11.50	56.71 ± 6.71	32.20 ± 4.36	64.71 ± 5.77	31.13 ± 1.98
Direct bilirubin (mg/dl)	0.01 ± 0.01	0.05 ± 0.05	0.03 ± 0.02*	0.10 ± 0.00#	0.07 ± 0.02*	0.08 ± 0.02#	0.10 ± 0.01*	0.04 ± 0.02
Total bilirubin (mg/dl)	0.10 ± 0.00	0.10 ± 0.00	0.12 ± 0.02	0.10 ± 0.00	0.10 ± 0.00	0.10 ± 0.00	0.11 ± 0.00	0.11 ± 0.01
Total protein (g/dl)	6.28 ± 0.12	5.43 ± 0.08	5.73 ± 0.08*	5.63 ± 0.50	5.76 ± 0.05*	6.25 ± 0.17	5.87 ± 0.14*	6.21 ± 0.21
Albumin (g/dl)	3.05 ± 0.12	3.37 ± 0.02	3.10 ± 0.12	3.53 ± 0.17	3.19 ± 0.09	3.92 ± 0.18	3.26 ± 0.49	3.60 ± 0.17
Cholesterol (mg/dl)	50.16 ± 2.45	46.67 ± 8.24	47.51 ± 1.97	50.98 ± 1.22	42.65 ± 2.73*	50.54 ± 11.20	48.58 ± 2.99	49.46 ± 2.98
TG (mg/dl)	57.39 ± 3.01	62.90 ± 2.36	72.76 ± 3.48*	58.25 ± 6.86	66.55 ± 3.58	63.01 ± 4.10	69.37 ± 5.35*	56.83 ± 3.78
HDL-C (mg/dl)	25.49 ± 2.52	24.44 ± 2.22	26.33 ± 2.59	31.65 ± 1.00	22.59 ± 2.27	27.87 ± 3.46	25.87 ± 1.70	26.26 ± 1.46
LDL-C (mg/dl)	13.19 ± 1.15	9.65 ± 5.54	6.63 ± 3.61*	7.68 ± 0.85	6.75 ± 1.91*	10.07 ± 4.52	8.83 ± 1.62	11.84 ± 2.68
Glucose (mg/dl)	114.29 ± 8.96	106.67 ± 12.02	112.00 ± 8.60	110.00 ± 5.77	121.67 ± 11.38	117.50 ± 4.78	108.00 ± 6.63	105.00 ± 10.88
BUN (mg/dl)	19.25 ± 1.28	22.50 ± 1.50	16.50 ± 1.02	16.50 ± 1.50	15.71 ± 1.06*	21.60 ± 0.87	16.71 ± 0.97	18.63 ± 1.53
Creatinine (mg/dl)	0.70 ± 0.03	0.80 ± 0.10	0.55 ± 0.09*	0.70 ± 0.00	0.67 ± 0.04	0.80 ± 0.00	0.63 ± 0.03	0.70 ± 0.03#
Uric acid (mg/dl)	1.01 ± 0.21	0.95 ± 0.15	1.02 ± 0.15	1.05 ± 0.25	0.93 ± 0.10	1.22 ± 0.28	0.84 ± 0.16	1.24 ± 0.18

AST, aspartate aminotransferase; ALT, alanine aminotransferase; ALP, alkaline phosphatase; BUN, blood urea nitrogen; TG, triglycerides.
*$p < 0.05$ vs. male control group (0 mg/kg/day), #$p < 0.05$ vs. female control group (0 mg/kg/day). Values are mean ± S.E.M., n = 6.

Table 2. Mean hematological values in rats treated with the mangosteen pericarp extract for 12 weeks.

Parameter	Dose administered (mg/kg of body weight/day)							
	0		400		600		1,200	
	Male	Female	Male	Female	Male	Female	Male	Female
Hct (%)	40.25 ± 1.30	36.50 ± 0.50	40.17 ± 1.11	38.00 ± 0.00	38.57 ± 1.25	36.40 ± 2.20	39.86 ± 0.70	34.88 ± 1.53
WBC (10^6/μL)	11.25 ± 1.32	7.50 ± 2.50	7.17 ± 1.62	8.00 ± 6.00	11.71 ± 2.55	8.40 ± 1.86	7.14 ± 1.18	9.63 ± 2.16
Neutrophil (%)	11.25 ± 1.32	7.50 ± 2.50	7.17 ± 1.62	8.00 ± 6.00	11.71 ± 2.55	8.40 ± 1.86	7.14 ± 1.18	9.63 ± 2.16
Lymphocyte (%)	78.50 ± 1.28	83.00 ± 1.00	82.00 ± 2.02	85.00 ± 4.00	74.43 ± 2.16	81.00 ± 2.77	79.71 ± 1.25	77.75 ± 2.85
Monocyte (%)	10.25 ± 1.11	9.50 ± 1.50	10.83 ±1.08	7.00 ± 2.00	13.86 ± 1.30*	10.60 ± 1.72	13.14 ± 0.74	12.63 ± 1.08

Hct, packed red blood cell volume; WBC, total white blood cell count.
*$p < 0.05$ vs. male control group (0 mg/kg/day). Values are mean ± S.E.M., n = 6.

Table 3. Mean organ weights in rats treated with the mangosteen pericarp extract for 12 weeks.

Organ weight (g)	Dose administered (mg/kg of body weight/day)							
	0		400		600		1,200	
	Male	Female	Male	Female	Male	Female	Male	Female
Liver	8.21 ± 0.32	6.35 ± 0.48	8.92 ± 0.51	5.68 ± 0.09	8.50 ± 0.37	5.59 ± 0.31	7.89 ± 0.42	5.76 ± 0.19
Kidney	1.73 ± 0.06	1.34 ± 0.08	1.77 ± 0.11	1.46 ± 0.06	1.77 ± 0.10	1.28 ± 0.02	1.70 ± 0.04	1.35 ± 0.05
Spleen	0.65 ± 0.04	0.59 ± 0.04	0.63 ± 0.02	0.50 ± 0.02	0.64 ± 0.07	0.50 ± 0.04	0.59 ± 0.05	0.51 ± 0.03
Heart	1.13 ± 0.06	1.02 ± 0.18	1.13 ± 0.07	0.90 ± 0.08	1.07 ± 0.08	0.07 ± 0.03	1.05 ± 0.03	0.87 ± 0.04

Values are mean ± S.E.M., n = 6.

Direct bilirubin is the approximate sum of conjugated bilirubin and biliprotein. An increased level of this bilirubin type in plasma generally results from functional or mechanical impairment in biliary excretion and is found in most cases of acute hepatitis and cholestasis (Dufour, 2006). In the present study, such an increase was evident in male rats after receiving the extract for 12 weeks. Their plasma direct bilirubin at the end was significantly elevated in the absence of abnormal total bilirubin levels. Although, these results are likely to be treatment-associated due to their dose-variable manner, they did not appear at any other time-points. The reason for such findings remains unknown. Since the extract did not contain only tannins, it is difficult to conclude that these polymeric compounds would be mainly responsible for the bilirubin stimulating effects observed among our male rats. For female animals, significant differences of plasma direct bilirubin levels were found only in the 400 and 600 mg/kg/day treated groups, and these were due mostly to their baseline levels (0.04 ± 0.02 mg/dl) being higher than that of the normal control (0.01 ± 0.01 mg/dl), rather than the results of the treatment. Most plasma proteins are synthesized and degraded in the liver. A measurable decreased amount of these proteins in the blood circulation thus, reflect either an impaired hepatocellular production and/or an increased catabolism that may occur in various physiological or pathological processes (Killingsworth, 1979; Johnson, 1999). At the 12 week, all male groups fed with the extract had lower levels of proteins except albumin in their plasma. Due to their protein-precipitating properties, dietary tannins have been reported to cause liver damage and disturbance of liver protein metabolism (Salunkhe et al., 1990; Marzo et al., 2002). The decrease of plasma proteins among our male rats, however, were unlikely to be caused by tannins in the extract since they were not dose dependent. These inhibitory effects were also slight and thus seem to have no clinical implication. Despite changes in both these biochemical factors, their livers did not reveal any abnormalities after both gross and histopathological examinations (Figure 2). Normal hematological values were also detected in all blood samples during the entire period and no hemolyzed plasma was obtained. Throughout this study, the total WBC numbers in our rat blood were within the normal range and lymphocytes were the major WBC populations as reported in the literature (Matsuda et al., 2000). Also at the final week, the LDL-cholesterol levels in all treatment groups of male rats decreased about two fold, but were found to be significantly different from the control value only in those receiving 400 and 600 mg/kg/day doses. These decreases are probably transient and need to be further investigated.

The effect of mangosteen fruit extract on the plasma electrolytes has not been determined previously (Jujun et al., 2008), although, a case of acidosis associated with the use of mangosteen juice has been reported recently (Wong and Klemer, 2008). In the present study, we found no accumulation of blood electrolytes in any treated animals throughout the 12 weeks of investigation. All measured values of Na^+, K^+, Cl^-, and HCO_3^- were within the same ranges of 150 - 155, 6 - 9, 105 - 112, and 17 - 22 mmole/L, respectively. These results together with their normal plasma urea, uric acid, and creatinine levels indicate that continuous intake of the mangosteen extract did not cause any deficiency in renal excretion.

In conclusion, we have investigated both acute and subchronic toxicities of the tannin-rich extract

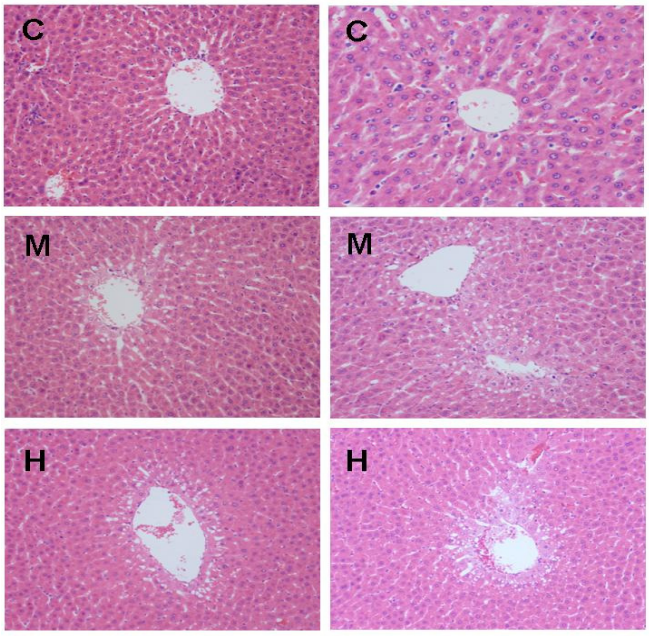

Figure 2. The histological morphology of male (left panel) and female (right panel) rat livers from the control (C), middle-dose (800 mg/kg/day) (M), and high-dose (1,600 mg/kg/day) (H) groups shown by hematoxylin and eosin staining at a magnification of 40×. No significant damage was detected in any treatment group.

from mangosteen fruit pericarp. With respect to our results, the extract at the doses tested did not produce any significant undesirable effects in the experimental animals. Their livers and kidneys which are the most sensitive organs to toxic factors were apparently normal and showed no signs of dysfunction. The toxicological data obtained from the present study are of significance in relation to increasing the consumption of the mangosteen rind crude extract for health benefits and remedial purposes worldwide. An additional study to evaluate chronic toxicity is needed to determine the long-term safety of this fruit extract.

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