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Food Safety Evaluation of Papaya Fruits Resistant to Papaya Ring Spot Virus

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ABSTRACT

Genetically modified (GM) papaya plant resistant to infection by Papaya Ring Spot Virus (PRSV) was successfully generated by cloning the coat protein (CP) gene of the PRSV. However, the food safety of GM foods remains controversial. This study assesses the food safety of two GM papayas, 16-0-1 and 18-2-4, and compares them with that of non-GM papaya, Tainung-2 (TN-2), using genetic and animal toxicity assays. Pulps of fresh papaya fruits were lyophilized and grounded before use. Three genotoxicity assays were performed: the Ames test of *Salmonella typhimurium* tester strains (TA98, TA100, TA102, TA1535 and TA1537); the chromosomal aberration of Chinese hamster ovary (CHO-K1) cells (*in vitro*); and micronucleus assays for mice (*in vivo*). Experimental results demonstrated that both non-GM and GM papaya fruits had no genotoxicity. Acute oral toxicity and 28-day repeated feeding toxicity tests for these papayas were performed via the oral gavage method for rats. All GM papaya fruit had no acute toxicity at a maximal dose of 5 g/kg body weight. Furthermore, the results of the 28-day study at 1 g/kg body weight in rats revealed no adverse-related effect in the body weight, feed consumption, hematology, blood biochemical parameters, organ weights and pathology in both GMs and non-GM papaya groups. In conclusion, PRSV-resistant papaya fruit lines 16-0-1 and 18-2-4 are comparable to their parent non-GM (TN-2) counterparts and are equivalent in food safety.

Key words: genetically modified papaya, genotoxicity, animal toxicity study, Papaya Ring Spot Virus

INTRODUCTION

Papaya (*Carica papaya* L.), which is rich in nutrients and vitamins, is cultivated in subtropical and tropical regions[(1)]. However, papaya is susceptible to papaya ringspot virus (PRSV), which has caused considerable damage to papaya crops[(2)]. A genetically modified (GM) papaya that resists PRSV infection was recently developed[(3,4)]. Bau *et al*.[(5)] demonstrated that GM papaya lines 16-0-1 and 18-2-4 have high yields of papaya fruit and are highly resistant to the PRSV. In Taiwan, transgenic papaya lines carrying the coat protein (CP) gene of a severe Taiwanese strain of the PRSV YK, have been generated via agrobacterium-mediated transformation. These transgenic papaya lines have great potential for the control of the PRSV in large-scale commercial papaya production[(6)].

Papaya, an edible fruit, can be used in soups, salads and teas. However, the ingredients of papaya, such as papaya enzyme papain, benzyl glucosinolate (BG) and benzyl isothiocyanate (BITC) are known to pose adverse effects by using the different laboratory animal models[(7,8)]. In fact, the distribution and changes of levels are variable during fruit development and ripening[(9)]. Papain can induce interaction with warfarin which is an anti-coagulant[(10,11)]. Ingestion of high-purity papain powder can cause gastric ulcers, esophageal perforation and hypernatremia in the management of phytobezoars[(12)]. Notably, BG adversely affects the male rat's reproduction system by reducing fertility[(13,14)] and goitrogenic or anti-thyroid activity in the pregnant female rats[(14)]. Musk *et al*.[(15)] demonstrated that BITC induces both

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chromosomal aberrations and sister chromatid exchanges in Chinese Hamster Ovary (CHO) cells *in vitro*. An *in vivo* study revealed that BITC can promote urinary bladder carcinogenesis[16-18] and papaya seed extract reversibly reduces the contractile response of cauda epididymal tubules in male rats[19,20]. Administration of 200 mg/kg of BITC decreased rat body weight and feed consumption, increased serum cholesterol and decreased serum triglyceride concentrations. The renal function of rats was also injured[21].

There have been worldwide concerns about the safety of GM foods. The current safety assessment concept for GM foods is the "substantial equivalence", which is generally adapted by GM crops safety assessment guidelines[22]. The approval and labeling of GM food have been legislated in various countries. Therefore, the characterization of a particular GM food is crucial to ensuring regulatory compliance and monitoring unauthorized marketing of GM foods. For example, two GM papaya lines, 16-0-1 and 18-2-4, were developed that are resistant to PRSV infection. The polymerase chain reaction (PCR) patterns were used to characterize the DNA sequence of the transgene insert in these two selected transgenic papaya lines. The molecular characterizations of the PRSV in Taiwan were monitored in the previous study[23]. This study further determined the food safety of PRSV-resistant GM papaya lines 16-0-1 and 18-2-4 and its parent non-GM plant Tainung-2 (TN-2) using genetic and animal toxicity assays, according to the safety assessment guidelines developed by the Department of Health of Taiwan[24] based on Codex guidelines[25].

MATERIALS AND METHODS

I. *Preparation GM Papaya Fruit*

Plants from two GM papaya lines, 16-0-1 and 18-2-4, and a non-GM variety, TN-2, were grown in greenhouses at the National Plant Genetic Resources Center, Taiwan Agricultural Research Institute, Wufeng, Taiwan. Fresh papaya fruits pulps were harvested and lyophilized. The ratio of fresh to lyophilized papaya fruit was approximately 8 : 1. The lyophilized papaya fruits were grounded into powder and stored at -20°C before use. The transgene-specific and event-specific molecular markers for the characterization of these two GM papaya lines, which are resistant to the PRSV, and non-transgenic TN-2, were confirmed in a previous report[25].

II. *Chemicals and Reagents*

The positive control (PC) mutagens were 4-nitroquinoline-N-oxide (4-NQO), sodium azide, 9-aminoacridine (9-AA), 2-Aminoanthracene (2-AA), mitomycin C ethyl methanesulfonate, cyclophosphamide, acridine orange and colchicine. They were purchased from Sigma Co. (MO, USA). Histidine was obtained from Merck (Germany). Other chemicals used were of analytical grade.

III. *Ames Test*

The Ames test was performed as described previously[26,27]. Five test histidine- dependent *Salmonella typhimurium* strains (TA98, TA100, TA102, TA1535 and TA1537) were used. The TA98 and TA1537 strains were mainly used to detect frame shift mutation; the TA100 and TA1535 strains were used to detect base-pair substitution; and the TA102 strain was used to detect transitional mutation and oxidative stress[26]. The TA98 and TA100 strains (histidine needed a mutant) were purchased from the Bioresources Collection and Research Center (BCRC) (Hsinchu, Taiwan). The TA102, TA1535, and TA1537 strains were obtained from Discovery Partners International (DPI) (CA, USA).

The TN-2, 16-0-1 and 18-2-4 papaya fruit powders were dissolved in sterilized distilled water (DW). No bacterial toxicity of all tested *Salmonella* strains was found at up to 2 mg/plate of TN-2, 16-0-1 and 18-2-4 in the preliminary bacterial toxicity study (data not shown). The Ames test (plate incorporation method) was administered at doses of 0.125, 0.25, 0.5, 1 and 2 mg/plate of TN-2, 16-0-1 and 18-2-4. The test was performed with or without the S9-fraction from Aroclor 1254-induced rat livers (36.5 mg/mL) (Lot#1452, Moltox^{TM}, USA). The S9-fraction was prepared freshly by adjusting the S9-fraction to a final concentration of 10% with dilution buffer containing 4 μM of nicotinamide adenine dinucleotide phosphate (sodium salt), 5 μM of glucose-6-phosphate (mono sodium salt), 8 μM of $MgCl_2$, 33 μmole of KCl and 100 μM of sodium phosphate buffer (pH 7.4). Briefly, 0.1 mL of DW-soluble sample was first added to culture tubes containing 2 mL of top agar (containing 0.5% NaCl), 0.2 mL of 0.5 mM of histidine/biotin and 0.1 mL of tester strain suspension. The sample-loaded cultures were then plated. Metabolic activation experiments were conducted using the rat liver S9-fraction at a volume of 0.5 mL in activation mixtures. Plates were incubated at 37°C for 48 h. Control (DW) and PC experiments were also conducted. The PC mutagens incubation without S9-fraction treatment were 4-NQO (2.5 μg/plate), sodium azide (5 μg/plate), 9-AA (50 μg/plate) and mitomycin C (0.5 μg/plate), respectively. Otherwise, all tester bacterial strains incubation with S9-fraction in the PC groups used 2-AA (5 μg/plate). Test samples were assayed in triplicate at five concentrations and two independent experiments were performed for each bacterial strain.

IV. *Chromosome Aberration (CA) Assay*

To assess the ability of TN-2, and 16-0-1 and 18-2-4 papaya fruits to induce structural and numerical chromosome aberrations, an *in vitro* chromosome assay was applied to Chinese hamster ovary cell clone K1 (CHO-K1) according to methods described previously[15,28]. The CHO-K1 cells were obtained from the BCRC. Cells, 5-mL 1.5×10^5 in a 25 cm^2 flask, were seeded overnight before the treatment day. For cytotoxicity, 2 mg/mL of TN-2, 16-0-1 and 18-2-4 were dissolved in culture media and incubated with cells for 24 h. Cultures treated with 2 mg/mL of TN-2, 16-0-1 and

18-2-4, with or without the metabolic activation system were incubated with the S9-fraction for 3 h before treatment. The PC reagents, ethyl methanesulfonate (3.14 mM) and cyclophosphamide (20 μM), were used without or with S9-fraction incubation, respectively. After treatment for 18 - 21 h, the cells were harvested and then added to Colchicine (0.1 μg/mL) for 3 h before harvesting. After trypsinization, the cells were treated with hypotonic solution (0.5% KCl) for 5 - 7 min at 37°C pre-incubation, fixed with an acetic acid and methanol (1 : 3) solution, dropped onto slides, air-dried and stained with 10% Giemsa. For the analysis of chromosomal aberrations, at least 100 metaphases were scored for each group[28,29]. The number of cells with damaged chromosomes was calculated as aberration rate (%) = (number of cells with damaged chromosomes/total number of cells examined) × 100. Two independent treatments were conducted.

V. *Micronucleus Test*

Healthy 6-week-old male mice (ICR strain, body weight, 25 - 35 g), obtained from Biolasco Taiwan Co., Ltd. (I-Lan, Taiwan), were subjected to a general physical examination upon receipt and acclimatized for 1 week. The animals were housed in cages (5 per cage) and provided with food (Lab Diet 5001 Rodent diet; Purina Mills LLC, St. Louis, MO, USA) and water *ad libitum*. The stainless steel cages were kept at 21 ± 2°C with 50 - 70% humidity under a 12-h light/12-h dark cycle. This study was approved by the Institutional Animal Care and Use Committee (IACUC) of National Chung-Hsing University (IACUC: 96-34).

The micronucleus assay was conducted as previously described[30,31]. Mice were administered TN-2, 16-0-1 and 18-2-4 papaya fruit powder at a limited dose of 2 g/kg body weight by oral gavage. The administered volume was 10 mL/kg body weight. The PC group was intraperitoneously injected with 0.05 g/kg body weight of cyclophosphamide and DW as a control. Five mice were allocated randomly to each group. After dosing, the animals were examined for mortality and clinical signs. The animals were anesthetized using 2% isoflurane (Halocarbon Laboratories, USA) and 100 μL of orbital peripheral blood was withdrawn at 48 and 72 h. Slides were prepared for staining with 0.1% acridine orange. Reticulocytes (RETs) stained orange and micronuclei (MN) in RETs stained yellow-green on each slide were counted under a florescence microscope (BX50, Olympus, Münster, Germany). In total, 1000 RETs per animal were analyzed for the existence of MN. The ratio of RETs to normochromatic erythrocytes (NCEs) was determined based on 1000 NCEs. The MN-to-NCE ratio was recorded while counting 1000 RETs per animal and the MN-RETs/1000 RETs (‰) was calculated.

VI. *The Acute and 28-Day Repeated Feeding Toxicity Studies*

Toxicological studies for the non-GM and GM papaya fruits were performed according to the guidelines for food safety of genetically modified foods[23,32]. For the acute and 28-day repeated oral toxicity studies, the TN-2, 16-0-1 and 18-2-4 papaya fruit powder were prepared with distilled water (DW). The 5-week-old male and female Sprague Dawley albino rats were obtained from Biolasco Taiwan Co., Ltd. (I-Lan, Taiwan). The housing facility was maintained under appropriate environmental conditions, as mentioned. For the acute oral toxicity study, 5 male and 5 female rats were orally administered a single dose of TN-2, 16-0-1 and 18-2-4 papaya fruit powder at 5 g/kg body weight. The control animals were gavaged with DW. The administered volume was 10 mL/kg body weight. The animals were observed daily for signs of toxicity and behavioral changes after administration; body weight was determined weekly for 14 days. On day 15, the rats were anesthetized using 2% isoflurane and blood was withdrawn from the abdominal aorta. A complete necropsy was performed.

For the 28-day repeated oral toxicity study, 10 male and 10 female rats were administered TN-2, 16-0-1, and 18-2-4 papaya fruit powder at 1 g/kg body weight daily via oral gavage. The control animals were gavaged with DW. The administered volume was 10 mL/kg body weight. The weekly body weight and daily feed consumption were recorded. At the end of day 28, all animals were fasted overnight and then anesthetized with 2% isoflurane in an inhalation chamber. Blood was completely withdrawn from the abdominal aorta into tubes (K3 EDTA syringes) (Vacutainer, NJ, USA). Routine hematological analysis, clinical chemistry analysis and a urinary examination were conducted on all animals. A thorough necropsy was performed on all animals and organs were weighed after dissection, and then examined grossly, removed, and fixed in 10% buffered formalin. The organs, including the brain, heart, thymus, liver, spleen, kidneys, adrenal glands, testes (males) and ovaries (females), were trimmed for histopathological examination. For semiquantitative grading, lesion severity was graded using the criteria developed by Shackelford *et al*.[33] Lesion severity was graded as follows: 1 = minimal (< 10%); 2 = slight (11 - 25%); 3 = moderate (26 - 50%); 4 = moderate/severe (51 - 75%); and 5 = severe/high (76 - 100%).

VII. *Statistical Analysis*

Data are expressed as mean ± standard deviation. Statistical differences are evaluated by Student's *t*-test. Differences are regarded as significant at $p < 0.05$.

RESULTS

I. *The Mutagenic Effect of GM Papaya Fruit in the Ames Test*

Five mutant *Salmonella* TA strains were tested for the papaya fruits. Tables 1 and 2 show Ames assay results for the tested papaya fruit. The TN-2, 16-0-1 and 18-2-4 groups (up to 2 mg/plate) did not have mutagenic responses either with or without the S9-fraction in the five strain testers. Conversely,

Table 1. Revertant colonies of TN-2, 16-0-1 and 18-2-4 papaya fruits in the absence of S9-fraction in *Salmonella* mutagenicity

Test article	Conc. (mg/plate)	Number of revertants (colony/plate)[c]				
		TA98	TA100	TA102	TA1535	TA1537
		–S9	–S9	–S9	–S9	–S9
C[a]		30.3 ± 1.7	170.3 ± 15.3	224.3 ± 5.3	13.3 ± 1.9	6.7 ± 1.7
PC[b]		265.3 ± 2.4*	1525.3 ± 130.3*	4881.0 ± 39.3*	746.3 ± 7.9*	180.3 ± 15.1*
TN-2	0.125	26.0 ± 5.7	166.3 ± 17.9	237.7 ± 4.0*	11.7 ± 0.5	10.3 ± 1.7
	0.25	28.0 ± 3.3	157.7 ± 9.6	227.3 ± 4.5	10.3 ± 4.0	9.3 ± 2.6
	0.5	27.7 ± 2.6	173.7 ± 12.0	241.0 ± 4.2	14.7 ± 2.6	11.7 ± 1.2
	1	33.3 ± 4.5	148.0 ± 19.1	240.7 ± 3.3	17.0 ± 3.3	7.7 ± 1.7
	2	29.0 ± 2.8	172.3 ± 18.2	237.3 ± 6.6	17.0 ± 0.0	8.0 ± 3.3
16-0-1	0.125	30.0 ± 2.8	152.0 ± 20.5	220.3 ± 4.0	14.7 ± 2.1	7.7 ± 0.5
	0.25	33.0 ± 1.6	140.3 ± 10.5	232.7 ± 3.8	11.3 ± 3.3	8.3 ± 0.9
	0.5	32.3 ± 0.5	156.0 ± 8.5	234.3 ± 4.2	10.7 ± 1.7	14.0 ± 1.4
	1	25.3 ± 2.5	151.3 ± 8.1	225.3 ± 2.9	11.7 ± 2.6	8.7 ± 0.9
	2	29.0 ± 4.2	158.7 ± 25.1	232.3 ± 2.5	19.7 ± 2.5	11.0 ± 1.6
18-2-4	0.125	33.3 ± 1.9	150.3 ± 11.7	214.7 ± 2.5	15.0 ± 0.0	10.3 ± 2.1
	0.25	45.7 ± 4.0	141.7 ± 23.7	221.3 ± 4.0	14.7 ± 2.9	11.0 ± 2.9
	0.5	26.3 ± 0.5	144.7 ± 24.2	221.3 ± 6.1	8.0 ± 0.8	11.3 ± 1.2
	1	23.3 ± 3.3	164.7 ± 4.5	226.0 ± 5.4	12.3 ± 2.5	8.7 ± 3.8
	2	26.3 ± 1.9	163.7 ± 14.6	219.3 ± 5.6	13.7 ± 1.7	10.7 ± 3.3

[a] C: control was distilled water; TN-2: Tainung No. 2; 16-0-1 and 18-2-4: GM papaya fruits.
[b] Positive reagents without S9-fraction reactions were 1 μg/plate 4-nitroquinoline- *N*-oxide (4-NQO) for TA98, 5 μg/plate sodium azide for TA100, TA1535, 0.5 μg/plate mitomycin C for TA102, and 50 μg/plate; 5 μg/plate sodium azide for TA1535, and 50 μg/plate 9-aminoacridine for TA1537.
[c] Data are presented as mean ± SD (n = 3).
* Significant difference of colonies more than two folds of control and treated groups at $p < 0.05$.

the reversed colonies for PCs were 8 - 57 times colonies than those for control samples, indicating that the TN-2, 16-0-1 and 18-2-4 papaya fruit did not cause mutagenic effects.

II. *The Effect of GM Papaya Fruit on Chromosome Aberration in CHO-K1 Cells*

The aberration rates of CHO-K1 cells administered 2 mg/mL TN-2, 16-0-1 and 18-2-4 papaya fruit powder without the S9-fraction treatment were in the range of 3.0 - 4.7%, while that for the PC increased to 24% (Table 3). Furthermore, the aberration rates of CHO-K1 cells that incubation of the S9-fraction with the TN-2, 16-0-1 and 18-2-4 groups were normal at 3.0 - 4.3%, compared with that of the PC of 23.7%, indicating that the TN-2, 16-0-1 and 18-2-4 papaya fruits had no detectable chromosomal aberration effect on CHO-K1 cells *in vitro*.

III. *Effect of GM Papaya Fruit on Mouse Micronuclei*

In micronucleus tests, mice were gavaged TN-2, 16-0-1 and 18-2-4 papaya fruit powder at 2 g/kg body weight. No clinical or body weight changes were noted in the tested mice (data not shown). The frequency of micronucleus ratios for all test animals was within the normal range of 1.2 - 2.6‰, except for that of PCs increased to 8.0 and 15.6‰ at 72 h and 48 h, respectively (Table 4). The experimental results revealed that TN-2, 16-0-1 and 18-2-4 papaya fruit did not induce an increase in micronucleus ratios *in vivo*.

IV. *Acute Toxicity of GM Papaya Fruit in Rats*

To assess the safety of TN-2, 16-0-1 and 18-2-4 papaya fruit powder, the acute oral test was conducted first. During the 2-week post-dosing observation period, all test rats appeared healthy and normal. No abnormal signs or death were observed. Thus, the TN-2, and 16-0-1 and 18-2-4 papaya fruit at 5 g/kg body weight had no acute toxic effect in rats (data not shown).

V. *28-Day Repeated Oral Toxicity of GM Papaya Fruit to Rats*

(I) *Clinical Observations, Body Weight and Feed Consumption*

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Table 2. Revertant colony of TN-2, 16-0-1 and 18-2-4 papaya fruits in the presence of S9-fraction in *Salmonella* mutagenicity

Test article	Conc. (mg/plate)	Number of revertants (colony/plate)[c]				
		TA98	TA100	TA102	TA1535	TA1537
		+S9	+S9	+S9	+S9	+S9
C[a]		29.0 ± 0.8	167.7 ± 6.5	336.0 ± 9.9	9.0 ± 0.8	8.7 ± 1.7
PC[b]		391.3 ± 25.6*	3448.0 ± 240.4*	1035.4 ± 33.5*	179.3 ± 2.9*	113.0 ± 12.8*
TN-2	0.125	30.3 ± 4.9	146.7 ± 5.8	335.3 ± 8.7	8.3 ± 1.2	9.0 ± 3.7
	0.25	31.3 ± 8.3	148.3 ± 15.2	335.0 ± 14.4	8.7 ± 1.7	8.0 ± 5.3
	0.5	23.3 ± 1.2	169.7 ± 10.0	346.0 ± 24.1	8.0 ± 0.8	5.3 ± 0.5
	1	27.0 ± 3.7	174.3 ± 16.1	332.3 ± 12.8	8.3 ± 1.2	9.7 ± 3.4
	2	26.3 ± 0.9	174.0 ± 20.0	353.7 ± 17.2	8.7 ± 0.9	6.3 ± 1.9
16-0-1	0.125	32.0 ± 5.0	143.7 ± 13.9	320.3 ± 8.5	10.0 ± 0.8	8.7 ± 2.9
	0.25	30.3 ± 2.6	159.0 ± 6.5	341.3 ± 26.7	10.3 ± 0.5	9.0 ± 0.8
	0.5	31.0 ± 1.4	163.0 ± 11.3	352.7 ± 34.3	9.0 ± 0.8	7.7 ± 0.9
	1	34.7 ± 4.0	140.0 ± 8.7	315.3 ± 34.3	10.3 ± 0.5	6.0 ± 1.4
	2	22.3 ± 1.7	174.3 ± 2.1	356.3 ± 14.1	7.7 ± 0.9	7.3 ± 1.7
18-2-4	0.125	33.3 ± 0.9	151.3 ± 19.2	329.7 ± 11.6	10.7 ± 0.5	10.0 ± 3.7
	0.25	33.7 ± 5.2	143.3 ± 19.4	335.7 ± 19.1	10.3 ± 0.5	8.0 ± 0.8
	0.5	26.3 ± 2.9	125.3 ± 16.5	332.3 ± 20.1	11.7 ± 2.6	8.0 ± 1.6
	1	32.3 ± 1.2	158.7 ± 11.2	339.3 ± 7.9	10.3 ± 1.2	6.0 ± 2.2
	2	26.7 ± 3.9	161.7 ± 10.4	322.0 ± 10.8	9.0 ± 0.0	8.0 ± 1.4

[a] C: control was distilled water, DW; TN-2: Tainung No. 2; 16-0-1 and 18-2-4: GM papaya fruits.
[b] Positive reagents with S9-fraction was 50 μg/plate 2-aminoanthracene for all *Salmonella* strains.
[c] Data are presented as mean ± SD (n = 3).
* Significant difference of colonies more than two folds of control and treated groups at $p < 0.05$.

Table 3. Frequency of chromosomal aberration of TN-2, 16-0-1 and 18-2-4 papaya fruits treated with or without S9-fraction in cultured CHO-K1 cells

Group[a]	Concentration	Frequency of chromosomal aberration (%)[b]	
		–S9	+S9
C	0	4.7 ± 0.5	3.7 ± 0.5
EMS	3.14 mM	24.0 ± 4.3*	ND
CP	20 μM	ND	23.7 ± 2.5*
TN-2	2 mg/mL	3.0 ± 0.0*	3.7 ± 0.9
16-0-1	2 mg/mL	3.7 ± 0.9	4.3 ± 1.2
18-2-4	2 mg/mL	3.7 ± 1.2	3.0 ± 0.8

[a] C: control was distilled water, DW; EMS: Ethyl methanesulfonate; CP: cyclophosphamide; TN-2: Tainung No. 2; 16-0-1 and 18-2-4: GM papaya fruits; ND: Not done.
[b] A total number of 100 metaphases of chromosomes of CHO cells were counted in each treatment. The number of cells with damaged chromosomes was calculated as aberration rate (%) = (number of cells with damaged chromosomes/100) × 100.
* Significant difference between the control and treated groups at $p < 0.05$.

Table 4. Micronuclei assay of TN-2, 16-0-1 and 18-2-4 papaya fruits in the peripheral red blood cells of mice

Sampling intervals/Group[a]	Dose (g/kg)	RETs/1000 NCEs (‰)[b]	MN-RETs/ 1000 RETs (‰)[b]
48 h			
C	0	36.2 ± 6.1	1.4 ± 0.9
CP	0.05	19.8 ± 3.3*	15.6 ± 5.7*
TN-2	2	37.6 ± 5.5	1.2 ± 1.3
16-0-1	2	39.0 ± 5.7	1.4 ± 0.5
18-2-4	2	39.0 ± 2.4	1.8 ± 0.8
72 h			
C	0	34.8 ± 4.3	1.4 ± 0.5
CP	0.05	20.2 ± 2.3*	8.0 ± 3.1*
TN-2	2	32.8 ± 3.0	1.6 ± 0.9
16-0-1	2	37.4 ± 4.9	2.6 ± 1.5
18-2-4	2	36.6 ± 8.0	1.2 ± 1.3

[a] C: control was distilled water, DW; TN-2: Tainung No. 2; 16-0-1 and 18-2-4: GM papaya fruits; RETs: reticulocytes ; NCEs: normochromatic erythrocytes; MN-RETs: micronucleated reticulocytes; CP: cyclophosphamide (intraperitoneal injection) .
[b] Data are expressed as the mean ± SD (n = 5).
* Significant difference between the control and treated groups at $p < 0.05$.

Rats were orally fed with 1 g/kg body weight of non-GM (TN-2) or GMs of 16-0-1 or 18-2-4 papaya fruits powders for 28 days. Overall, body weight and weight gain were comparable for the TN-2, 16-0-1, 18-2-4 and control groups (Figure 1). Feed consumption by the TN-2, 16-0-1 and 18-2-4 groups was similar to that of the control group. Although the TN-2 and 18-2-4 groups exhibited less feed consumption than the control group ($p < 0.05$), this was not consistent throughout the study. The feed efficiency of male rats in the TN-2 and 18-2-4 groups was higher than that of the control group (Table 5). No treatment-related effect on feed consumption and feed efficacy was noted for the TN-2, 16-0-1 and 18-2-4 groups.

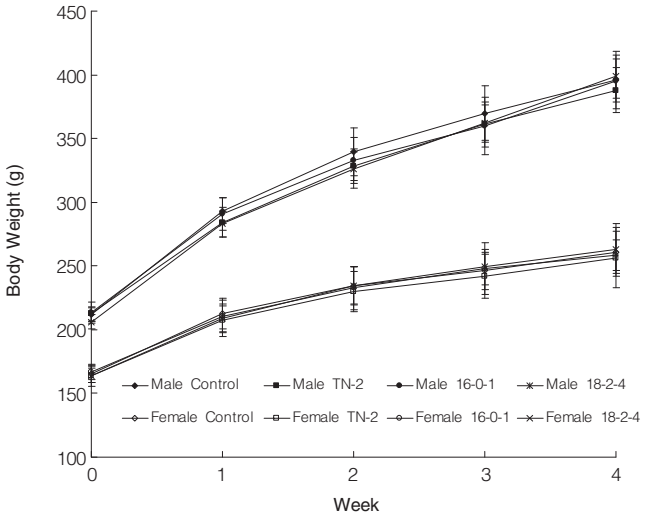

Figure 1. Body weight changes of rats after oral administration with papaya fruit for 28 days. No significant difference was observed in the body weights of male and female rats after daily gavage with either genetically modified papaya fruits (plant lines 16-0-1 and 18-2-4) (1 g/kg body weight) when compared with the non-GM line of Tainung-2 (TN-2) or control group.

(II) *Hematology, Serum Biochemistry and Urine Chemistry*

Table 6 lists measured hematology parameters. The TN-2, 16-0-1 and 18-2-4 groups had significantly lower red blood cell count (RBC), hematocrit (HCT), platelet counts and higher hemoglobin (Hb), mean corpuscular hemoglobin (MCH) and mean corpuscular hemoglobin concentration (MCHC) values than that of the control group ($p < 0.05$). However, these differences were within background data[34] and were not considered treatment-related. No difference in Prothrombin time (PT), activated partial thromboplastin time (APTT) and fibrinogen levels for blood coagulation was observed among the TN-2, 16-0-1, 18-2-4 and control groups.

The serum biochemistry results for male and female rats were listed in Tables 7 and 8. The 18-2-4 male rats had lower alanine aminotransferase (ALT), aspartate aminotransferase (AST), blood urea nitrogen (BUN), creatinine kinase (CK) and lactate dehydrogenase (LDH) than those of the control group; otherwise, K^+ and Na^+ levels in male rats were higher, and amylase, LDH, triglyceride (TG) and inorganic phosphate (P^{3-}) in female rats were lower in the 16-0-1 and 18-2-4 groups when compared with those of the TN-2 group ($p < 0.05$). Most of these parameters were slightly lower or higher than those of the control group. The slight decrease

Table 5. Weekly feed consumption and feed efficiency of rats treated with TN-2, 16-0-1 and 18-2-4 papaya fruits for 28 days

Group[a]/Week	Feed consumption (g/day)[b]			
	C	TN-2	16-0-1	18-2-4
Male				
1	29.0 ± 2.6	25.1 ± 1.5*	26.4 ± 2.2	25.2 ± 2.4
2	31.2 ± 3.1	25.6 ± 2.4*	28.0 ± 4.3	25.2 ± 2.5*
3	31.6 ± 2.7	26.0 ± 2.9*	28.2 ± 4.9	26.8 ± 3.0*
4	27.8 ± 2.9	25.8 ± 2.1	27.0 ± 3.3	27.9 ± 3.2
Feed efficiency (%)[c]	22.1 ± 2.8	24.3 ± 1.6*	24.0 ± 3.1	26.2 ± 2.3*
Female				
1	20.5 ± 1.3	19.7 ± 1.8	20.2 ± 1.5	19.8 ± 2.0
2	22.4 ± 3.2	22.9 ± 3.0	22.1 ± 4.5	23.1 ± 2.7
3	22.1 ± 2.9	23.7 ± 3.4	22.5 ± 2.6	24.1 ± 2.7
4	20.8 ± 2.4	21.1 ± 1.4	20.9 ± 4.8	23.6 ± 1.6*
Feed efficiency (%)	15.8 ± 1.9	15.2 ± 1.5	15.2 ± 2.5	15.7 ± 2.6

[a] C: control was distilled water, DW; TN-2: Tainung No. 2; 16-0-1 and 18-2-4: GM papaya fruits.
[b] Feed consumption (g/day) = [Total feed intake (g)/test period (day)].
[c] Feed efficiency (%) = [Daily body weight gain (g)/daily feed intake (g)] × 100.
Data are expressed as the mean ± SD (n = 10).
* Significant difference between the control and treated groups at $p < 0.05$.

Table 6. Hematological and coagulate parameters of rats treated with TN-2, 16-0-1 and 18-2-4 papaya fruits for 28 days

Group[a]/Items	C	TN-2	16-0-1	18-2-4
Male				
RBC (10^6/μL)	9.2 ± 1.2	8.1 ± 1.3	7.2 ± 0.9*	7.9 ± 0.4*
HGB (g/dL)	13.4 ± 3.6	12.5 ± 4.2	14.1 ± 2.1	15.8 ± 1.2#
HCT (%)	54.4 ± 6.3	49.2 ± 7.9	44.1 ± 6.0*	48.4 ± 3.1*
MCV (fL)	59.6 ± 2.2	60.8 ± 2.5	60.9 ± 3.6	61.4 ± 2.4
MCH (pg)	15.0 ± 4.6	15.6 ± 5.5	19.4 ± 1.6#,*	20.1 ± 1.2#,*
MCHC (g/dL)	25.1 ± 7.5	25.6 ± 8.7	31.9 ± 2.5#,*	32.7 ± 2.0#,*
PLT (10^3/μL)	1054.5 ± 327.3	907.0 ± 456.5	796.9 ± 272.1	623.3 ± 244.4*
PT (s)	12.1 ± 0.7	11.4 ± 0.4	13.6 ± 1.6	12.7 ± 1.0
APTT (s)	25.5 ± 1.7	26.2 ± 2.0	25.6 ± 0.8	23.9 ± 1.8
Fbg (mg/dL)	240.8 ± 29.6	217.3 ± 16.8	248.6 ± 22.9	216.1 ± 11.4
Female				
RBC (10^6/μL)	7.4 ± 3.8	6.4 ± 0.3	7.4 ± 0.6#	6.9 ± 0.7
HGB (g/dL)	14.8 ± 1.2	14.0 ± 0.6	14.7 ± 0.9#	14.5 ± 1.0
HCT (%)	45.2 ± 23.9	39.4 ± 2.4	41.7 ± 3.6	40.5 ± 3.3
MCV (fL)	61.2 ± 2.7	61.5 ± 1.3	56.6 ± 1.5#,*	58.6 ± 2.5#,*
MCH (pg)	28.8 ± 26.4	21.9 ± 1.2	20.0 ± 0.8#	21.0 ± 1.0
MCHC (g/dL)	47.5 ± 44.6	35.6 ± 2.1	35.4 ± 1.4	35.8 ± 0.9
PLT (10^3/μL)	898.5 ± 468.0	1014.2 ± 207.2	915.9 ± 216.4	808.8 ± 244.5
PT (s)	12.8 ± 2.5	10.1 ± 0.1	10.4 ± 0.1	10.3 ± 0.1
APTT (s)	31.7 ± 3.8	25.8 ± 1.5	27.0 ± 1.5	24.7 ± 1.7
Fbg (mg/dL)	174.1 ± 7.0	183.7 ± 5.0	192.3 ± 8.3	201.5 ± 27.6

[a] C: control was distilled water, DW; TN-2: Tainung No. 2; 16-0-1 and 18-2-4: GM papaya fruits; RBC, red blood cell; HGB, hemoglobin; HCT, hematocrit; MCV, mean corpuscular volume; MCH, mean corpuscular hemoglobin; MCHC, mean corpuscular hemoglobin concentration; PLT, platelets. Data are expressed as the mean ± SD (n = 10).

* Significant difference between the control and treated groups at $p < 0.05$.

Significant difference between the non-GM (TN-2) and GM papaya-treated groups at $p < 0.05$.

or increase in serum biochemistry did not indicate clinical cell injury, and these differences were within background data ranges[34] and were not considered treatment effects. Additionally, no difference in urinary chemistry parameters or urinary sediment existed among the TN-2, 16-0-1, 18-2-4 and control groups (data not shown).

(III) *Organ Weight and Pathology Examination*

Table 9 lists the organ weight for the TN-2, 16-0-1, 18-2-4 and control groups. No difference in organ weight was observed among the TN-2, 16-0-1 and 18-2-4 rats fed with papaya fruit powder and control rats, except that the liver weight of the 16-0-1- and 18-2-4-treated male rats and that of the 16-0-1-treated female rats decreased slightly ($p < 0.05$). Ovary weight in the 18-2-4-treated female rats increased when compared with that of the TN-2-treated rats ($p < 0.05$). Microscopically, affected livers and ovaries from the 16-0-1- and 18-2-4-treated rats had no significant lesions. Non-specific lesions, including minimal to small renal cysts and minimal renal tubular regeneration of the kidney, or congenital hydrocephalus of the brain, were distributed randomly in the TN-2, 16-0-1, 18-2-4 and control groups (Table 10). Experimental results for tissues from the TN-2-, 16-0-1 and 18-2-4-treated rats indicate no repeated feeding toxicity, suggesting that substantial equivalence between the non-GM- and GM-papaya fruits in rats.

DISCUSSION

In this study, the food safety of GM 16-0-1 and 18-2-4 papaya fruit was evaluated for genotoxicity using three assays. Results indicate no genotoxicity for these two GM 16-0-1 and 18-2-4 papaya lines and the non-GM TN-2. Currently, to assess the safety of GM foods, the European Food Safety Authority (EFSA)[35] recommended using an appropriate *in silico* or *in vitro* test method to improve specificity or serve as a substitute for animal testing, including *in vitro* genetic toxicology tests and screening for point

Table 7. Serum biochemistry of male rats treated with TN-2, 16-0-1 and 18-2-4 papaya fruits for 28 days

Group[a]/Items	C	TN-2	16-0-1	18-2-4
Albumin (g/dL)	3.4 ± 0.9	3.6 ± 0.3	3.6 ± 0.4	2.9 ± 1.1
ALP (U/L)	89.9 ± 39.5	133.7 ± 34.9*	144.4 ± 40.0*	99.7 ± 56.5
Amylase (U/L)	770.4 ± 238.1	522.2 ± 203.2*	342.0 ± 64.9*	547.1 ± 278.8
ALT (U/L)	24.6 ± 10.7	24.0 ± 7.6	28.8 ± 6.7	15.2 ± 10.9#
AST (U/L)	120.6 ± 36.2	116.9 ± 22.7	132.2 ± 31.4	84.3 ± 43.0#
BUN (mg/dL)	13.8 ± 3.5	18.0 ± 2.0*	16.2 ± 2.9	13.3 ± 4.6#
Creatinine (mg/dL)	0.4 ± 0.1	0.5 ± 0.1	0.5 ± 0.1	0.4 ± 0.1
CK (U/L)	391.3 ± 151.0	332.9 ± 132.3	207.9 ± 64.9#	224.9 ± 269.4
Glucose (mg/dL)	71.3 ± 25.2	98.9 ± 29.1*	81.5 ± 16.7	110.5 ± 44.0*
LDH (U/L)	1791.4 ± 754.3	1512.0 ± 373.3	1479.9 ± 303.7	833.0 ± 511.0#
TC (mg/dL)	74.4 ± 23.5	60.7 ± 11.6	67.6 ± 12.1	62.3 ± 32.8
TG (mg/dL)	60.7 ± 27.3	49.3 ± 20.6	32.0 ± 17.6*	43.2 ± 24.5
TP (g/dL)	4.5 ± 1.3	4.8 ± 0.4	5.0 ± 0.6	4.1 ± 1.5
Ca^{2+} (mg/dL)	7.7 ± 2.0	8.3 ± 0.6	7.7 ± 0.7	6.6 ± 2.5
Cl^- (mEq/dL)	107.8 ± 14.5	105.1 ± 10.8	122.0 ± 12.5#	134.9 ± 25.0#
K^+ (mEq/dL)	5.2 ± 0.6	4.6 ± 0.6*	5.3 ± 0.7#	7.1 ± 1.7#,*
Mg^{2+} (mEq/dL)	2.3 ± 0.6	1.9 ± 0.4	2.3 ± 0.9	1.8 ± 1.1
Na^+ (mEq/dL)	150.4 ± 19.0	144.4 ± 15.8	169.2 ± 18.5#	164.0 ± 18.2#
P^{3-}(mg/dL)	7.5 ± 2.3	6.7 ± 0.4	6.5 ± 1.1	6.4 ± 2.2

[a] C: control was distilled water, DW; TN-2: Tainung No. 2; 16-0-1 and 18-2-4: GM papaya fruits; ALP: alkaline phosphatase; ALT: alanine aminotransferase; AST: aspartate aminotransferase; ALT: alanine aminotransferase; BUN: blood urea nitrogen; CK: creatine kinase; LDH: lactate dehydrogenase; TC: total cholesterol; TG: triglyceride; TP: total protein. Data are expressed as the mean ± SD (n =10).

* Significant difference between the control and treated groups at $p < 0.05$.

\# Significant difference between the non-GM (TN-2) and GM papaya-treated groups at $p < 0.05$.

mutation, chromosomal mutation and DNA damage or repair. Thus, numerous GM foods, such as sweet peppers and tomatoes, which are resistant to the cucumber mosaic virus and Cry1C protein of *Bacillus thuringiensis* (Bt) in rice, have been subjected to genetic toxicology tests[3,36]. Ames test results show that for both non-GM and GM papaya fruit treated plates, either with or without the S9-fraction, the numbers of colonies are within their respective background levels and no mutagenicity was indicated.

Although some substances present no mutagenic effect, animal experiments or epidemiological investigations have proven that they are carcinogenic[37]. Therefore, test substances cannot be evaluated using the *Salmonella* reverse mutation test as the sole judge of genotoxicity. The evaluation of micronucleus induction is the primary *in vivo* test in a battery of genotoxicity tests and is also recommended by regulatory agencies worldwide as part of product safety assessments[30]. Chromosome aberration tests can be utilized to determine morphological abnormalities of chromosomes to predict the potential genotoxicity and carcinogenicity of chemical substances[28]. The CA test cell line should induce the growth of the background, as well as in terms of karyotype, number, chromosomal diversity and spontaneous mutation frequency, to lie in a stable state[30]. The cell cycle of CHO-K1 cells is 12 - 14 h, such that one can easily observe a subject chromosome; thus, the CHO-K1 cell line is typically used in the CA test[38]. Notably, BITC, a papaya ingredient, can induce CHO-K1 cell chromosomal aberration, sister chromatid exchange and DNA breakage[15]. However, chromosomal aberration in the non-GM TN-2 and the two lines of GM papaya fruit, 16-0-1 and 18-2-4, was less than 5%, and no significant difference existed when compared with that of the control group. Hence, GM papaya had no chromosomal aberration effects.

Micronuclei, also hematologically known as Howell-Jolly bodies, are induced by oxidative injury to and precipitation of hemoglobin. The micronucleus assay is primarily used to evaluate the ability of test agents to induce structural and/or numerical chromosomal damage. The polychromatic erythrocytes (PCEs)-to-NCE ratio between test-agent-treated animals and vehicle-treated control animals is a toxicity index. Experimental results in this study show that all tested groups included the non-GM and GM papaya fruit did not increase micronucleus incidence in mice, compared with that of the negative control group. Additionally, the number of immature reticulocytes in the PC group decreased significantly, while the number of micronuclei increased significantly.

Table 8. Serum biochemistry of female rats treated with TN-2, 16-0-1 and 18-2-4 papaya fruits for 28 days

Group[a]/Items	C	TN-2	16-0-1	18-2-4
Albumin (g/dL)	3.6 ± 0.7	3.3 ± 0.5	3.2 ± 0.6	3.9 ± 0.3[#]
ALP (U/L)	72.7 ± 23.6	93.6 ± 36.7	60.1 ± 25.7[#]	86.2 ± 40.8
Amylase (U/L)	529.6 ± 100.4	474.9 ± 120.2[*]	359.2 ± 102.2[#]	508.7 ± 79.2
ALT (U/L)	22.2 ± 7.8	21.2 ± 3.7	16.8 ± 5.8	27.2 ± 9.0
AST (U/L)	133.5 ± 36.2	115.6 ± 26.1	96.5 ± 32.5[*]	146.8 ± 48.0
BUN (mg/dL)	13.8 ± 2.9	15.7 ± 3.2	15.5 ± 3.8	19.8 ± 2.7[#]
Creatinine (mg/dL)	0.6 ± 0.1	0.5 ± 0.1	0.5 ± 0.1	0.6 ± 0.1[#]
CK (U/L)	482.2 ± 149.3	266.6 ± 149.3[*]	151.9 ± 71.4[#]	522.9 ± 292.1[#]
Glucose (mg/dL)	66.3 ± 22.0	81.6 ± 22.4	66.3 ± 8.1	71.7 ± 14.8
LDH (U/L)	2130.0 ± 681.9	1746.1 ± 468.8	1267.0 ± 546.0[#,*]	2050.8 ± 781.4
TC (mg/dL)	85.5 ± 20.1	80.4 ± 22.2	70.8 ± 23.6[#]	67.2 ± 11.2[*]
TG (mg/dL)	52.4 ± 23.8	45.6 ± 14.3	34.4 ± 8.8	29.8 ± 7.0[#,*]
TP (g/dL)	4.7 ± 1.1	4.1 ± 0.7	4.3 ± 1.0	5.4 ± 0.7[#]
Ca^{2+} (mg/dL)	7.7 ± 1.6	6.4 ± 0.8[*]	5.9 ± 1.4[*]	8.2 ± 1.4[#]
Cl^- (mEq/dL)	132.8 ± 18.5	139.3 ± 11.7	146.0 ± 11.4	136.7 ± 30.3
K^+ (mEq/dL)	6.6 ± 0.9	6.1 ± 0.6	6.5 ± 0.5	6.7 ± 1.3
Mg^{2+} (mEq/dL)	1.0 ± 0.7	0.7 ± 0.5	0.5 ± 0.5[#,*]	1.2 ± 0.7[#]
Na^+ (mEq/dL)	172.4 ± 23.7	186.0 ± 6.9	194.4 ± 5.5[#,*]	150.5 ± 29.0[#]
P^{3-}(mg/dL)	8.6 ± 2.5	5.5 ± 1.3[*]	5.6 ± 1.1[*]	8.3 ± 1.9[#]

[a] C: control was distilled water, DW; TN-2: Tainung No. 2; 16-0-1 and 18-2-4: GM papaya fruits; ALP: alkaline phosphatase; ALT: alanine aminotransferase; AST: aspartate aminotransferase; BUN: blood urea nitrogen; CK: creatine kinase; LDH: lactate dehydrogenase; TC: total cholesterol; TG: triglyceride; TP: total protein. Data are expressed as the mean ± SD (n =10).

[*] Significant difference between the control and treated groups at $p < 0.05$.

[#] Significant difference between the non-GM (TN-2) and GM papaya-treated groups at $p < 0.05$.

To conduct a thorough safety assessment of GM foods, several *in vivo* experiments, such as the oral acute and 28-day repeated dose toxicities for rodents, were carefully designed and carried out[24,32]. In the acute oral toxicity test, no test animal died or experienced adverse effects when administered non-GM and GM papaya fruit powder at doses up to 5 g/kg body weight (data not shown). Furthermore, no abnormality or adverse observations were also noted in the 28-day repeated oral study. The TN-2- treated male and 18-2-4-treated male and female rats randomly decreased their feed consumption when compared with that of the control group. However, 18-2-4-treated rats exhibited no body weight change, indicating that these two lines of GM papayas had no harmful effects on test animal growth.

Although a statistically significant decrease in some hematological and clinical biochemical parameters existed in rats treated with non-GM and GM papaya, these differences were within the normal physiological ranges for rats[31] and were not considered adverse or related to the test substance treatment. Although total cholesterol (TC) and triglyceride (TG) levels in papaya-treated rats were lower than those in the control group. This is likely because papaya, which is rich in carotenoids that have anti-oxidant capacity, can hinder the formation of low-density cholesterol and attenuate atherosclerosis and cardiovascular disease[1,39]. Furthermore, papaya is rich in soluble fiber and pectin[40,41], a water-soluble dietary fiber, which can combine with bile acid in the liver (enterohepatic circulation), thereby reducing blood lipid levels[40,41], Moreover, pectin helps to reduce low-density lipoprotein levels[42]. No difference in organ weight existed among the TN-2, 16-0-1, 18-2-4 and control groups, except that liver weight decreased slightly in 16-0-1- and 18-2-4-treated male rats and 16-0-1-treated female rats. No histopathological changes were observed in the liver. No difference in clinical biochemistry parameters, such as AST and ALT, existed; indicating that 16-0-1 and 18-2-4 of GM papayas had no adverse effect on liver function and was considered to be spontaneous or non-treatment related.

Experimental results demonstrate that the two lines of PRSV-resistant papaya fruit, 16-0-1 and 18-2-4, had no genotoxicity or adverse effects in rodents, are comparable to their non-GM counterparts, and can be recognized as equivalent in terms of food safety.

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Table 9. Relative organ weight of rats treated with TN-2, 16-0-1 and 18-2-4 papaya fruits for 28 days

Group[a]/Items	C	TN-2	16-0-1	18-2-4
Male				
Brain	0.52 ± 0.04	0.53 ± 0.05	0.51 ± 0.09	0.52 ± 0.03
Heart	0.32 ± 0.04	0.31 ± 0.04	0.34 ± 0.08	0.31 ± 0.02
Thymus	0.12 ± 0.02	0.11 ± 0.02	0.12 ± 0.03	0.12 ± 0.03
Liver	2.78 ± 0.18	2.49 ± 0.24*	2.50 ± 0.20*	2.55 ± 0.17*
Kidney	0.65 ± 0.17	0.69 ± 0.08	0.69 ± 0.06	0.69 ± 0.04
Adrenal	0.013 ± 0.002	0.015 ± 0.002	0.015 ± 0.004	0.015 ± 0.003
Spleen	0.17 ± 0.02	0.16 ± 0.03	0.17 ± 0.03	0.17 ± 0.02
Testis	0.81 ± 0.09	0.79 ± 0.09	0.77 ± 0.07	0.80 ± 0.14
Female				
Brain	0.75 ± 0.06	0.74 ± 0.06	0.75 ± 0.05	0.74 ± 0.06
Heart	0.33 ± 0.03	0.33 ± 0.03	0.33 ± 0.04	0.32 ± 0.02
Thymus	0.17 ± 0.04	0.18 ± 0.03	0.15 ± 0.03*	0.16 ± 0.03
Liver	2.79 ± 0.40	2.67 ± 0.21	2.45 ± 0.12#,*	2.51 ± 0.20
Kidney	0.71 ± 0.06	0.72 ± 0.08	0.69 ± 0.06	0.68 ± 0.05
Adrenal	0.023 ± 0.002	0.023 ± 0.005	0.024 ± 0.003	0.050 ± 0.081
Spleen	0.20 ± 0.03	0.21 ± 0.02	0.18 ± 0.03*	0.22 ± 0.10
Ovary	0.031 ± 0.007	0.028 ± 0.006	0.031 ± 0.004	0.034 ± 0.004#

[a] C: control was distilled water, DW; TN-2: Tainung No. 2; 16-0-1 and 18-2-4: GM papaya fruits. Relative organ weight (%) = [organ weight (g)/ final body weight (g)] × 100. Data are expressed as the mean ± SD (n = 10).
* Significant difference between the control and treated groups at $p < 0.05$.
# Significant difference between the non-GM (TN-2) and GM papaya-treated groups $p < 0.05$.

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Table 10. Summary of pathological changes of rats after treatment with TN-2, 16-0-1 and 18-2-4 papaya fruits for 28 days

Sex	Organ	Histopathological findings[b]	Dose (1 g/kg)[a]			
			C	TN-2	16-0-1	18-2-4
Examined			10	10	10	10
Male	Adrenal		-	-	-	-
	Brain		-	-	-	-
	Heart		-	-	-	-
	Kidney					
		Cyst, focal, minimal to slight	-	1/10	1/10	-
		Regeneration, tubule, focal, minimal	1/10	-	1/10	1/10
	Liver		-	-	-	-
	Spleen		-	-	-	-
	Testes		-	-	-	-
	Thymus		-	-	-	-
Female	Adrenal		-	-	-	-
	Brain					
		Congenital hydrocephalus, locally extensive, moderate	-	-	1/10	-
	Heart		-	-	-	-
	Kidney					
		Cyst, focal, minimal	-	-	1/10	-
		Regeneration, tubule, focal, minimal	-	-	-	1/10
	Liver		-	-	-	-
	Ovary		-	-	-	-
	Spleen		-	-	-	-
	Thymus		-	-	-	-

[a] C: control was distilled water, DW; TN-2: Tainung No. 2; 16-0-1 and 18-2-4: GM papaya fruits.
[b] Degree of lesion was graded from one to five depending on severity: minimal (< 1%); slight (1 - 25%); moderate (26 - 50%); moderate/ severe (51 - 75%); severe/high (76 - 100%). Incidence: affected rats/ total examined rats. -: no significant lesion.

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