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Safety Assessment of Whole and Hulled Djulis (*Chenopodium formosanum* Koidz)

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Chenopodium formosanum Koidz is a traditional crop of the aborigines in Taiwan, and is commonly called "Djulis". Dlulis is rich in nutrients and functional ingredients, and research has shown that it has various biological effects, such as decrease of cholesterol and enhancement of immunity. Because Djulis is not listed as a food crop in many countries including Taiwan, it is difficult to promote its cultivation and product development. Therefore, there is an urgent need to determine the safe intake level of Djulis by safety assessment. In this study, male and female rats were divided into 8 groups: blank (AIN-76A diet), control (high-gluten flour), low-, medium-, and high-dose whole Djulis (WD) or hulled Djulis (HD). To prepare the diets, WD or HD was pre-mixed with 2× water, heated, mixed with rat chow, then dried to form 7.5, 14.1, and 25.2% WD or HD diets. Results revealed that after feeding for 28 days, the behavior, organ/tissue weights, histological stain, and blood and urine biochemistry of rats fed WD or HD were all in the normal range and not statistically different from those of rats fed the blank or control diets. However, plasma glutamic pyruvic transaminase activities in both female and male rats of high-dose HD groups were significantly increased, as compared with those of rats fed the blank or control groups, and were higher than the normal range, suggesting that H-HD may potentially cause liver damage. Based on the medium dose (14.1%), a safe daily intake of HD for human adults is estimated to be 70.5 *g* (dry weight) while the safe daily intake for WD is at least beyond 126 *g* (estimated based on 25.2% WD or higher intake).

Key words: *Chenopodium formosanum* Koidz, Djulis, Safety assessment, Safe dose.

中文題目

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臺灣藜 (*Chenopodium formosanum* Koidz.) 為原住民傳統之糧食作物，又稱為"Djulis"，具有相當高的營養價值及機能成分以及某些生理功能，如：降膽固醇及提高免疫力等。臺灣藜並非我國農政單位表定之糧食作物，使政府在推動種植及產品開發上受到相當大的限制，因此急需進行安全性評估證實其食用安全劑量。本研究將雌、雄大鼠各分為對照組 (AIN-76A飼料)、控制組 (高筋麵粉)、帶殼與不帶殼臺灣藜低、中、高劑量共八組。將以2倍水加熱糊化之蒸熟帶殼 (WD) 或脫殼 (HD) 臺灣藜混入大鼠飼料 (AIN-76A) 加熱乾燥定型後，製備成含有7.5、14.1及25.2%之WD及HD飼料。結果顯示，大鼠連續28天食用WD、HD後，所有臺灣藜組別雌雄大鼠之動物行為、組織/器官重量、組織染色、血液及尿液生化值等，多在正常值範圍內，也與空白組或控制組無顯著差異 ($p > 0.05$)。但餵食高劑量HD組之公、母鼠之血清Glutamic pyruvic transaminase均顯著高於對照組與控制組 ($p < 0.05$)，且高於正常範圍，此種結果顯示餵食高劑量HD具有潛在的肝臟毒性。故本研究以中劑量 (14.1%) 之臺灣藜為攝食安全劑量，換算出成人每日食用臺灣藜之安全劑量範圍約為70.5 *g* (乾重)，而WD之每日攝食安全劑量至少可達約126 *g* (以25.2%之高劑量WD或更高攝取量計算)。

關鍵字：臺灣藜，安全性評估，安全劑量。

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1. Introduction

The goosefoot Family (Chenopodiaceae) consists of about 110 genera and 1500 species, and *Chenopodium* L., which contains about 120 species, is a representative and the most primitive genus of *Chenopodiaceae*[1]. A *Chenopodiaceae* species, *C. Quinoa*, is native and unique to Taiwan that is commonly known as red quinoa, or 'Djulis' in the indigenous language[2]. In 2008, Djulis was officially given the scientific name *Chenopdium formosanum* Koidz. Traditionally, Djulis has been used to produce a rice wine as an important cultural heritage of the Taiwanese indigenous people[2].

It has been shown that Quinoa has high quality composition of essential amino acids similar to that of casein, the protein of milk[3]. Djulis possesses such properties as high adaptability, easy planting, and versatility, and is often referred to as 'ruby cereals' that is worth exploitation because its whole plant is rich in diverse colors including red, orange, magenta, pink, and yellow, and thus is a good source of natural pigments and a good garden flower[4]. In addition, Djulis can be used as a traditional Chinese medicine for prevention or treatment of symptoms and diseases such as pain, diarrhea, hypertension, and stroke[5,6].

Internationally, some relatives of Djulis species (*Chenopodium* spp.), such as the *Andean* quinoa that originated from South America, have become important and nutritious food crops because of their high nutritional values. Quinoa has a biological value similar to that of milk and higher than that of fish, beef, soybean, and wheat[7-9]. Quinoa provides all of the essential amino acids; therefore, it does not need to be combined with other protein sources. Additionally, quinoa contains high amounts of iron, calcium, and zinc[10]. Indeed, Quinoa has been recommended as part of a gluten-free diet[11], and *Andean* quinoa has been listed as a vital crop in the CELSS (Controlled Ecological Life Support System) program by the US National Aeronautics and space Administration (NASA)[12].

Noteworthy, in vitro data have suggested that quinoa prolamins can stimulate innate and adaptive immune responses in celiac patients[11]. Furthermore, as Djulis is not a tradition crop and has not been listed as an official agricultural crop in some countries including Taiwan, it is difficult to vigorously promote the cultivation and uses of Djulis, although there are various Djulis products such as bread, biscuits, and cakes in the market currently. Therefore, there is an urgent need for safety assessment and for setting the safe intake levels of Djulis.

2. Material and Methods

2.1. Materials

Djulis seeds were provided by Taitung District Agricultural Research and Extension Station, Council of Agriculture (Jinfeng Township, Taitung, Taiwan) in Feb. 2014.

2.2. Experimental animals and housing conditions

Male and female healthy Wistar rats (6-week-old, weighing 200-225 *g*) were obtained from the National Laboratory Animal Center (NLAC) of Taiwan. The mice were housed in plastic cages and allowed to adapt to the conditions of the animal house for 10 days before the experiments began. The animals were maintained on a 12 *h* dark/light cycle at about 22 ± 3℃ and allowed free access to standard laboratory diet (AIN-76A chow diet) and tap water during the experiments. All experimental procedures involving animals were conducted in accordance with National Institutes of Health (NIH) guidelines. This experiment

was approved by the Institutional Animal Care and Use Committee (IACUC, no. 033) of the Chung Chou University of Technology (Zhanghua, Taiwan).

2.3. Sample preparation and animal groups

2.3.1. Preparation of whole Djulis (WD) or hulled Djulis (HD) feed

The amount of Djulis used in the feed of rats was estimated from the calories contributed daily from rice, the main staple food, in Taiwanese population, i.e., 500 Kcal daily from two bowls of rice (a bowl of rice has 250 *kcal*). This amount of calories (500 Kcal) accounts for about 25% of total calories required daily (2,000 *kcal*). We design approximate 25% of WD and HD as a high dose, and continuous halved to 8.5 and 4.25 *g* to be a medium and low dose, respectively. The WD and HD diets were prepared first by mixing 4.25, 8.5, and 17 *g* WD or HD with two parts of water, followed by cooking in a rice cooker until the Djulis were completely gelatinized. After cooling, the cooked Djulis was mixed with 50 *g* AIN-76A commercial rat chow to form the low-, medium-, and high-Djulis diets. Then, the mixed diets were shaped into strips by hand and oven-dried at 50℃ for 3 *h*. The dry weights thus obtained were 56.4, 60.3 and 67.4 *g* for low-, medium-, and high-Djulis diets, respectively. Thus, the contents of WD and HD in the low-, medium-, and high-Djulis diets were 7.5% (4.25 *g*/56.4 *g* × 100%), 14.1% (8.5 *g*/60.3 *g* × 100%), and 25.2% (17 *g*/67.4 *g* × 100%), respectively. The blank-control rats were fed AIN-76A diet only, while the control rats were fed a diet containing 25.2% high-gluten flour containing about 13% protein (close to the protein content of Djulis, 15%), to replace Djulis by mixing one part of the flour with two parts of water in a rice cooker, followed by mixing with AIN-76A rat chow and drying, as described above. All diets containing Djulis were freshly made daily.

2.3.2. Animal grouping

Female or male rats were randomly divided into 8 groups (6 animals in each group): 1) blank control (rat chow AIN-76A; Blank); 2) control (25.2% high-gluten flour group; CON); 3) low-dose whole Djulis (7.5%, L-WD); 4) medium-dose whole Djulis (14.1%, M-WD); 5) high-dose whole Djulis (25.2%, H-WD); 6) low-dose hulled Djulis (7.5%, L-HD); 7) medium-dose hulled Djulis (14.1%, M-HD); and 8) high-dose hulled Djulis (25.2%, H-HD). The diets and water were provided *ad libitum*, and the intakes of diet and water were recorded daily for 28 days.

2.4. Clinical examinations

Clinical signs were observed and weights were recorded once daily in the study. The recorded items included three categories: cage side observation, neurological examination, and physical examination.

2.5. Hematological examinations

Various hematological parameters including red blood cells(RBC), white blood cells (WBC), platelet counts (PLT), hematocrits (Hct), erythrocyte mean corpuscular volumes (MCV), hemoglobin (HB), mean corpuscular hemoglobin (MCH), mean corpuscular hemoglobin concentration (MCHC), and platelet counts were determined using an automatic serum biochemical analyzer (Chiron Diagnostics Corporation, Oberlin, OH, USA).

2.6. Biochemical assays

2.6.1. Urine biochemical tests

Urine biochemical markers were examined using an automatic biochemical analyzer

(Chiron Diagnostics Corporation, Oberlin, OH, USA). Test items included: total urine (TU), blood urea nitrogen (BUN), uric acid (UA), creatinine (CRE), proportion of the value of specific gravity (SG), urine pH, urobilinogen (URO), color (COL), clarity (CLA), urine glucose (GLU), urinary protein (PRO), bilirubin (BIL), ketones (KET) and leukocytes (LEU).

2.6.2. Serum biochemical tests

Blood was collected in serum separator tubes and centrifuged at 775 × *g* for 15 *min* to obtain blood serum. Serum biochemical tests were conducted using an automatic biochemical analyzer (Chiron Diagnostics Corporation, Oberlin, OH, USA), and the test items included: alkaline phosphatase (ALP), blood urea nitrogen (BUN), creatinine (CRE), glucose (GLU), albumin (ALB), cholesterol (CHOL), triglyceride (TG), total protein (TP), glutamic-oxaloacetate transaminase (GOT),and glutamic-pyruvic transaminase (GPT).

2.7. Histopathological studies

Organ weights (liver, kidney, lung, heart, spleen, brain, thymus, testes, ovaries, and adrenal) were recorded when the animals were sacrificed at the end of experiment. For histopathological examinations, the tissues were fixed in 10% buffered formalin and dehydrated in graded series of alcohol, cleared in xylene and embedded in paraffin wax. Multiple sections from each block were prepared at 5 *μm* and stained with haematoxylin and eosin (H&E).

2.8. Statistical analysis

All statistical analyses were performed using SPSS for Windows, version 17 (SPSS, Inc., Chicago, IL, USA). Data are expressed as means ± SD and analyzed using one-way ANOVA followed by Duncan's multiple range tests. $p < 0.05$ is considered statistically significant.

3. Results

3.1. Behavioral observation and measurements of body weight and food and water intakes

The behavior of rats was observed at least twice a day, and we found no abnormality in behavior by cage side observations (cage activity, feces amount, feces color, feces consistency), neurological examinations (tail elevation, abnormal gait, ataxic gait, and head position), and physical examination (hair coat, mucus membrane/eye/skin color, body temperature, respiratory rate, respiratory character) during the 28-day feeding period (data not shown). In addition, we found no significant differences in the body weight, food intake, and water intake among all groups of either male or female rats after continuous feeding for 28 days (Table 1).

3.2. Clinical pathology tests

3.2.1. Urine biochemical tests

Table 2 shows the urine biochemical index of renal function in male and female rats fed WD or HD. Results revealed that the levels of TU, BUN, UA, and CRE in rats fed low-, medium-,or high- dose WD and HD diets were not significantly different from those in rats fed the blank or control diets ($p > 0.05$). This was true for both male and female rats.

Table 3 shows that SG, pH, and URO of male and female rats fed low-, medium-, and high-dose WD and HD diets for 28 days were not significantly different from those of rats fed the blank or control diet ($p > 0.05$). The urine of all groups of either male or female rats exhibited clear and yellowish color, without the presence of glucose, protein, bilirubin, ketone, or white blood cells (data not shown).

Table 1. Body weights and feed and water intakes in male and female rats fed low, medium, and high levels of whole Djulis or hulled Djulis diets for 28 days

Groups	Body Weight (*g*)		Dietary intake (*g*)		Water intake (*mL*)	
	male	female	male	female	male	female
Blank[1]	431.3 ± 27.6[3]	259.8 ± 16.6	27 ± 2	20 ± 3	56 ± 6	42 ± 14
Control	442.9 ± 15.1	245.8 ± 26.6	30 ± 2	20 ± 3	57 ± 5	29 ± 6
WD[2]						
L-dose	457.8 ± 20.7	254.5 ± 14.3	30 ± 3	20 ± 2	53 ± 10	37 ± 17
M-dose	423.6 ± 31.5	269.0 ± 10.9	30 ± 3	20 ± 2	54 ± 9	35 ± 10
H-dose	398.6 ± 35.6	234.8 ± 15.8	29 ± 4	19 ± 6	49 ± 8	36 ± 9
HD						
L-dose	440.9 ± 27.8	256.9 ± 15.7	29 ± 2	18 ± 3	62 ± 9	35 ± 6
M-dose	441.2 ± 19.7	260.1 ± 17.1	29 ± 2	19 ± 3	48 ± 9	31 ± 5
H-dose	414.8 ± 31.1	257.9 ± 18.6	28 ± 3	20 ± 3	49 ± 10	31 ± 10

1: Blank group was fed AIN-76A diet and control group fed AIN-76A diet + 25.2% high grade flour diet.
2: WD: whole Djulis; HD; hulled Djulis.
3: The statistical analysis exhibited no significant difference among groups.

Table 2. Urine and kidney biochemical markers in male and female rats fed low, medium, and high levels of whole Djulis or hulled Djulis diets for 28 days

Groups	TU (*mL/day*)		BUN (24 *h*) (*g/day*)		UA (24 *h*) (*mg/day*)		CRE (24 *h*) (*mg/day*)	
	male	female	male	female	male	female	male	female
Blank[1]	41 ± 17[a3]	51 ± 9[b]	0.26 ± 0.02[a]	0.23 ± 0.03[a]	3.7 ± 0.2[a]	1.6 ± 0.0[a]	10.2 ± 0.1[a]	7.3 ± 0.3[a]
Control	43 ± 1[a]	42 ± 3[ab]	0.38 ± 0.04[a]	0.31 ± 0.06[a]	3.9 ± 0.3[a]	1.9 ± 0.1[a]	10.9 ± 0.3[a]	6.7 ± 0.1[a]
WD[2]								
L-dose	34 ± 6[a]	46 ± 9[ab]	0.26 ± 0.05[a]	0.25 ± 0.01[a]	3.3 ± 0.3[a]	1.8 ± 0.1[a]	9.7 ± 0.7[a]	8.1 ± 0.9[a]
M-dose	44 ± 9[a]	43 ± 1[a]	0.28 ± 0.06[a]	0.27 ± 0.29[a]	3.0 ± 0.4[a]	1.6 ± 0.2[a]	10.0 ± 1.0[a]	7.1 ± 0.6[a]
H-dose	40 ± 6[a]	46 ± 18[b]	0.30 ± 0.08[a]	0.23 ± 0.05[a]	2.8 ± 0.3[a]	1.3 ± 0.0[a]	11.9 ± 0.2[a]	7.6 ± 0.4[a]
HD								
L-dose	45 ± 8[a]	45 ± 8[b]	0.30 ± 0.10[a]	0.26 ± 0.11[a]	3.6 ± 1.0[a]	1.4 ± 0.2[a]	11.2 ± 3.3[a]	6.1 ± 0.6[a]
M-dose	37 ± 10[a]	37 ± 10[b]	0.27 ± 0.28[a]	0.30 ± 0.25[a]	2.8 ± 0.5[a]	1.3 ± 0.2[a]	8.1 ± 1.8[a]	6.3 ± 0.7[a]
H-dose	36 ± 7[a]	36 ± 7[ab]	0.34 ± 0.03[a]	0.34 ± 0.03[a]	3.3 ± 0.1[a]	1.6 ± 0.1[a]	11.9 ± 0.7[a]	6.5 ± 0.6[a]

1: Blank group was fed AIN-76A diet and control group fed AIN-76A diet + 25.2% high grade flour diet.
2: WD: whole Djulis; HD; hulled Djulis.
3: Value (means ± SD, n = 6 for the test groups) in each column not sharing a superscript letter are significantly different ($p < 0.05$).

3.2.2. Hematological tests

As shown in Table 4, no significant differences were found in RBC, WBC, HB, HCT, MCV, PLT, MCH, and MCHC of female rats fed low-, medium-,or high-dose WD and HD, as compared with those of female rats fed the blank or control diets ($p > 0.05$). Similarly, no significant differences were found in RBC, WBC, HB, HCT, MCV, PLT, MCH, and MCHC of male rats fed low-, medium-,or high-dose WD and HD, as compared with those of male rats fed the blank or control diets ($p > 0.05$, Table 5).

3.2.3. Biochemical assays

In both male and female rats fed WD and HD for 28 days, the levels of BUN (13-22 *mg/dL*), CRE (0.4-0.7 *mg/dL*), ALB (4.3-5.0 *g/dL*) were all within the normal ranges of the rats, and there were no significant

Table 3. Urine specific gravity (SG), pH, and urobilinogen (URO) in male and female rats fed low, medium, and high levels of whole Djulis or hulled Djulis diets for 28 days

Groups	SG		pH		URO	
normal range	1.005-1.030		5.0-8.0		1 *mg/dL*	
	male	Female	male	Female	male	Female
Blank[1]	1.02 ± 0.01[3]	1.00 ± 0.00	7.0 ± 0.7	6.8 ± 0.4	1 ± 0	1 ± 0
Control	1.02 ± 0.01	1.00 ± 0.00	6.5 ± 0.5	6.3 ± 0.4	1 ± 0	1 ± 0
WD[2]						
L-dose	1.02 ± 0.01	1.00 ± 0.00	6.8 ± 0.3	6.3 ± 0.4	1 ± 0	1 ± 0
M-dose	1.02 ± 0.00	1.02 ± 0.01	6.7 ± 0.3	6.7 ± 0.3	1 ± 0	1 ± 0
H-dose	1.02 ± 0.01	1.02 ± 0.01	7.0 ± 0.5	6.5 ± 0.0	1 ± 0	1 ± 0
HD						
L-dose	1.02 ± 0.00	1.02 ± 0.01	6.8 ± 0.6	6.8 ± 0.8	1 ± 0	1 ± 0
M-dose	1.02 ± 0.01	1.02 ± 0.00	6.5 ± 0.0	6.2 ± 0.3	1 ± 0	1 ± 0
H-dose	1.03 ± 0.01	1.02 ± 0.01	6.8 ± 0.3	6.0 ± 0.0	1 ± 0	1 ± 0

1: Blank group was fed AIN-76A diet and control group fed AIN-76A diet + 25.2% high grade flour diet.
2: WD: whole Djulis; HD; hulled Djulis.
3: The statistical analysis exhibited no significant difference among groups.

Table 4. Blood composition in male rats fed low, medium, and high levels of whole Djulis or hulled Djulis diets for 28 days

Groups	RBC	WBC	HB	HCT	MCV	PLT	MCH	MCHC
	$10^6/uL$	$10^3/uL$	*g/dL*	*%*	fL[4]	$10^3/uL$	pg[5]	*g/dL*
Blank[1]	8.3 ± 0.6[3]	10.2 ± 1.0	15.4 ± 1.1	45.8 ± 2.8	55.2 ± 1.3	758 ± 58	18.6 ± 0.5	33.6 ± 0.6
Control	8.2 ± 0.2	12.9 ± 1.8	15.3 ± 0.7	45.9 ± 2.0	55.8 ± 1.1	810 ± 190	18.6 ± 0.4	33.4 ± 0.4
WD[2]								
L-dose	7.9 ± 0.2	12.6 ± 1.9	14.9 ± 0.5	44.5 ± 1.1	56.4 ± 1.7	800 ± 170	18.9 ± 0.8	33.4 ± 0.4
M-dose	8.3 ± 0.4	12.4 ± 2.2	15.1 ± 0.8	45.0 ± 2.4	54.2 ± 2.6	801 ± 160	18.1 ± 0.7	33.5 ± 0.4
H-dose	8.2 ± 0.2	12.5 ± 1.6	14.6 ± 0.4	44.3 ± 1.3	54.3 ± 1.7	886 ± 134	17.9 ± 0.5	33.0 ± 0.4
HD								
L-dose	8.5 ± 0.4	11.5 ± 2.5	15.4 ± 0.6	45.8 ± 1.1	53.9 ± 2.6	760 ± 110	18.1 ± 1.0	33.5 ± 0.7
M-dose	8.3 ± 0.4	12.4 ± 1.7	15.1 ± 0.3	45.4 ± 0.7	54.9 ± 2.1	857 ± 117	18.3 ± 0.5	33.3 ± 0.3
H-dose	8.2 ± 0.3	13.8 ± 2.6	15.3 ± 0.6	46.1 ± 1.8	56.1 ± 1.4	934 ± 135	18.6 ± 0.3	33.2 ± 0.4

1: Blank group was fed AIN-76A diet and control group fed AIN-76A diet +25.2% high grade flour diet.
2: WD: whole Djulis; HD; hulled Djulis.
3: The statistical analysis exhibited no significant difference among groups.
4: $fL = 10^{-15}\ L$.
5: $pg = 10^{-12}\ g$.

differences among all groups of rats (Table 6). All values in all male and female rats were also within the normal rang (62-230 *U/L*)[13]. However, the ALP values of male and female rats fed WD and HD were increased, although only the ALP value of female rats fed H-WD group was significantly different from that of the blank and the control rats. No significant differences were found for CHOL, TG, TP, and GOT levels in male and female rats fed WD and HD diets, as compared with those of the blank and the control rats (Table 7). However, the GPT levels of the HD group in both male and female rats (70 and 72 *U/L*, respectively) exceeded the standard value (18-45 *U/L*) and were significantly higher than those of the blank and control rats ($p < 0.05$).

Table 5. Blood composition in female rats fed low, medium, and high levels of whole Djulis or hulled Djulis diets for 28 days

Groups	RBC	WBC	HB	HCT	MCV	PLT	MCH	MCHC
	$10^6/uL$	$10^3/uL$	*g/dL*	*%*	*fL*[4]	$10^3/uL$	*pg*[5]	*g/dL*
Blank[1]	8.5 ± 0.4[3]	12.9 ± 2.4	15.6 ± 0.6	47.5 ± 2.0	56.0 ± 2.5	1,012 ± 132	18.4 ± 0.8	32.8 ± 0.8
Control	8.5 ± 0.3	13.6 ± 1.4	15.4 ± 0.6	46.4 ± 1.3	54.8 ± 1.9	851 ± 120	18.2 ± 0.6	33.2 ± 0.6
WD[2]								
L-dose	8.4 ± 0.4	12.8 ± 2.0	15.7 ± 0.7	47.3 ± 2.4	56.1 ± 1.5	840 ± 70	18.6 ± 0.4	33.2 ± 0.7
M-dose	8.4 ± 0.5	12.0 ± 2.2	15.5 ± 0.8	47.4 ± 2.5	56.5 ± 3.0	813 ± 70	18.5 ± 0.8	32.7 ± 0.5
H-dose	8.6 ± 0.5	12.6 ± 2.8	15.8 ± 0.8	48.5 ± 2.1	56.8 ± 3.1	919 ± 75	18.5 ± 1.1	32.6 ± 0.4
HD								
L-dose	8.7 ± 0.3	12.5 ± 1.9	15.9 ± 0.7	48.3 ± 1.9	55.8 ± 1.2	872 ± 159	18.4 ± 0.4	32.9 ± 0.3
M-dose	8.6 ± 0.3	12.6 ± 1.5	15.9 ± 0.4	48.3 ± 1.5	56.0 ± 2.4	875 ± 118	18.4 ± 0.6	32.9 ± 0.4
H-dose	8.2 ± 0.3	13.8 ± 2.6	15.3 ± 0.6	46.1 ± 1.8	56.1 ± 1.4[a]	934 ± 135	18.6 ± 0.3	33.2 ± 0.4

1: Blank group was fed AIN-76A diet and control group fed AIN-76A diet +25.2% high grade flour diet.
2: WD: whole Djulis; HD; hulled Djulis.
3: The statistical analysis exhibited no significant difference among groups.
4: $fL = 10^{-15}\ L$.
5: $pg = 10^{-12}\ g$.

Table 6. Blood biochemistry in male and female rats fed low, medium, and high levels of whole Djulis or hulled Djulis diets for 28 days

Groups (normal range)	ALP *U/L* (62-230)		BUN *mg/dL* (7-20)		CRE *mg/dL* (0.2-0.5)		GLU *mg/dL* (70-208)		ALB *g/dL* (3.4-4.8)	
	M	F	M	F	M	F	M	F	M	F
Blank[1]	116 ± 38[ab]	59 ± 5[a3]	17 ± 3[a]	13 ± 1[a]	0.4 ± 0.1[a]	0.6 ± 0.1[a]	549 ± 61[ab]	426 ± 93[a]	4.6 ± 0.1[a]	4.6 ± 0.1[ab]
Control	98 ± 36[a]	54 ± 9[a]	15 ± 2[a]	13 ± 2[a]	0.4 ± 0.1[a]	0.6 ± 0.1[a]	423 ± 69[a]	474 ± 72[a]	4.4 ± 0.1[a]	4.7 ± 0.3[ab]
WD[2]										
L-dose	129 ± 19[ab]	79 ± 12[ab]	19 ± 2[a]	16 ± 1[ab]	0.4 ± 0.1[a]	0.7 ± 0.1[a]	433 ± 59[a]	501 ± 73[a]	4.4 ± 0.2[a]	5.0 ± 0.2[b]
M-dose	134 ± 18[ab]	84 ± 14[ab]	18 ± 1[a]	16 ± 3[ab]	0.4 ± 0.1[a]	0.7 ± 0.1[a]	440 ± 52[a]	531 ± 46[a]	4.5 ± 0.2[a]	4.5 ± 0.2[a]
H-dose	173 ± 9[b]	94 ± 18[b]	19 ± 7[a]	17 ± 3[ab]	0.5 ± 0.1[a]	0.7 ± 0.1[a]	439 ± 136[a]	539 ± 75[a]	4.3 ± 0.2[a]	4.5 ± 0.2[ab]
HD										
L-dose	158 ± 38[ab]	100 ± 15[b]	22 ± 3[a]	20 ± 5[ab]	0.4 ± 0.1[a]	0.7 ± 0.2[a]	570 ± 79[b]	521 ± 136[a]	4.6 ± 0.3[a]	4.7 ± 0.1[ab]
M-dose	153 ± 29[ab]	107 ± 21[b]	20 ± 4[a]	19 ± 5[ab]	0.4 ± 0.1[a]	0.7 ± 0.1[a]	458 ± 81[a]	510 ± 42[a]	4.4 ± 0.1[a]	4.7 ± 0.2[ab]
H-dose	135 ± 23[ab]	101 ± 13[b]	20 ± 1[a]	17 ± 2[ab]	0.5 ± 0.1[a]	0.72 ± 0.0[a]	480 ± 78[a]	512 ± 58[a]	4.5 ± 0.2[a]	4.7 ± 0.1[ab]

1: Blank group was fed AIN-76A diet and control group fed AIN-76A diet + 25.2% high grade flour diet.
2: WD: whole Djulis; HD; hulled Djulis.
3: Value (means ± SD, n = 6 for the test groups) in each column not sharing a superscript letter are significantly different ($p < 0.05$).

3.4. Organ weights and histopathology records

3.4.1. Organ weights

The organ weights (brain, heart, thymus, liver, lung, spleen, kidney, testicles and adrenal gland) in either male or female rats were not significantly affected by feeding low-, medium-, or high-dose WD or HD for 28 days (Tables 8 and 9).

3.4.2. Histopathological findings

To the naked eye, all organs including adrenal gland, brain, heart, thymus, liver, lung, spleen, and kidneys, and

Table 7. Liver markers in male and female rats fed low, medium, and high levels of whole Djulis or hulled Djulis diets for 28 days

Groups (normal range)	CHOL *mg/dL* (37-85)		TG *mg/dL* (20-114)		TP *g/dL* (5.2-7.1)		GOT *U/L* (74-143)		GPT *U/L* (18-45)	
	M	F	M	F	M	F	M	F	M	F
Blank[1]	97 ± 17[a3]	79 ± 14[a]	170 ± 69[a]	103 ± 8[ab]	7.3 ± 0.2[ab]	6.9 ± 0.2[a]	69 ± 10[a]	71 ± 5[a]	33 ± 9[a]	35 ± 6[a]
Control	98 ± 9[a]	84 ± 8[a]	158 ± 56[a]	101 ± 46[ab]	6.9 ± 0.2[ab]	7.2 ± 0.4[a]	71 ± 11[a]	86 ± 16[a]	35 ± 10[a]	39 ± 9[a]
WD[2]										
L-dose	108 ± 22[a]	92 ± 12[a]	150 ± 43[a]	206 ± 70[b]	7.0 ± 0.3[ab]	7.5 ± 0.4[a]	83 ± 10[ab]	74 ± 7[a]	41 ± 8[a]	40 ± 9[a]
M-dose	95 ± 12[a]	94 ± 23[a]	183 ± 20[a]	129 ± 16[ab]	7.1 ± 0.2[ab]	6.9 ± 0.3[a]	66 ± 1[a]	83 ± 9[a]	37 ± 7[a]	47 ± 11[a]
H-dose	98 ± 13[a]	89 ± 6[a]	159 ± 43[a]	75 ± 21[a]	6.7 ± 0.3[a]	6.9 ± 0.3[a]	79 ± 11[ab]	72 ± 5[a]	56 ± 12[ab]	48 ± 7[a]
HD										
L-dose	116 ± 22[a]	105 ± 14[a]	265 ± 98[a]	207 ± 55[b]	7.4 ± 0.5[b]	7.3 ± 0.2[a]	74 ± 12[a]	84 ± 12[a]	52 ± 6[ab]	48 ± 8[a]
M-dose	106 ± 8[a]	101 ± 28[a]	217 ± 69[a]	170 ± 59[ab]	7.0 ± 0.2[ab]	7.1 ± 0.3[a]	88 ± 13[a]	88 ± 13[a]	54 ± 15[ab]	47 ± 7[a]
H-dose	95 ± 18[a]	93 ± 12[a]	134 ± 28[a]	169 ± 45[ab]	7.0 ± 0.3[ab]	7.0 ± 0.2[a]	91 ± 15[ab]	92 ± 17[a]	70 ± 11[b]	72 ± 8[b]

1: Blank group was fed AIN-76A diet and control group fed AIN-76A diet + 25.2% high grade flour diet.
2: WD: whole Djulis; HD; hulled Djulis.
3: Value (means ± SD, n = 6 for the test groups) in each column not sharing a superscript letter are significantly different ($p < 0.05$).

Table 8. Organ weights in male rats fed low, medium, and high levels of whole Djulis or hulled Djulis diets for 28 days

Groups	Weight (*g*)								
	Liver	Kidney	Lung	Heart	Spleen	Brain	Thymus	Testis	Adrenal
Blank[1]	16.5 ± 3.9[3]	3.5 ± 0.5	1.7 ± 0.1	1.4 ± 0.1	0.9 ± 0.1	1.9 ± 0.2	0.7 ± 0.1	3.4 ± 0.2	0.12 ± 0.02
Control	16.2 ± 1.5	3.6 ± 0.2	1.9 ± 0.1	1.4 ± 0.1	1.0 ± 0.1	2.0 ± 0.1	0.7 ± 0.1	3.6 ± 0.2	0.09 ± 0.01
WD[2]									
L-dose	16.9 ± 1.9	3.6 ± 0.4	1.9 ± 0.3	1.5 ± 0.1	1.0 ± 0.1	2.0 ± 0.1	0.8 ± 0.1	3.6 ± 0.4	0.14 ± 0.02
M-dose	15.7 ± 2.0	3.6 ± 0.2	1.7 ± 0.2	1.3 ± 0.1	1.1 ± 0.1	2.1 ± 0.1	0.9 ± 0.1	3.7 ± 0.2	0.13 ± 0.03
H-dose	13.5 ± 1.5	3.2 ± 0.3	1.7 ± 0.4	1.3 ± 0.1	0.9 ± 0.1	2.0 ± 0.1	0.8 ± 0.1	3.5 ± 0.4	0.09 ± 0.02
HD									
L-dose	18.9 ± 1.7	3.3 ± 0.3	1.7 ± 0.1	1.3 ± 0.2	1.1 ± 0.2	1.9 ± 0.1	0.9 ± 0.2	3.5 ± 0.2	0.13 ± 0.04
M-dose	17.6 ± 1.9	3.4 ± 0.1	1.9 ± 0.2	1.4 ± 0.1	0.9 ± 0.1	1.9 ± 0.1	1.0 ± 0.2	3.7 ± 0.3	0.12 ± 0.03
H-dose	16.1 ± 2.3	3.2 ± 0.2	2.0 ± 0.1	1.3 ± 0.1	1.0 ± 0.2	1.9 ± 0.1	1.0 ± 0.3	3.5 ± 0.4	0.13 ± 0.03

1: Blank group was fed AIN-76A diet and control group fed AIN-76A diet + 25.2% high grade flour diet.
2: WD: whole Djulis; HD: hulled Djulis.
3: The statistical analysis exhibited no significant difference among groups.

testicles (or ovarian) exhibited no enlargement or other abnormalities. In addition, histopathological examinations revealed no obvious pathological changes in the adrenal, brain, and testis/ovary in all groups of rats fed WD, HD, blank, or control diet (Table 10), although a slight bile duct hyperplasia was found in the liver of 1/6 of male rats fed the H-WD diet, and a slight inflammation was found in the kidney of 1/6 of female rats fed the same diet. However, pathological changes, such as local mononuclear inflammatory cell infiltration, were found in heart, liver, and kidneys of 1/6 to 2/6 of male rats in the blank and control groups. As each group of rats had some occasional and minor lesions, no specific changes can be attributed to the effect of the test substances.

Table 9. Organ weights in female rats fed low, medium, and high levels of whole Djulis or hulled Djulis diets for 28 days

Groups	Weight (g)								
	Liver	Kidney	Lung	Heart	Spleen	Brain	Thymus	Testis	Adrenal
Blank[1]	8.7 ± 1.1[3]	2.2 ± 0.3	1.3 ± 0.4	0.9 ± 0.1	0.7 ± 0.1	1.8 ± 0.1	0.5 ± 0.1	0.18 ± 0.03	0.11 ± 0.02
Control	8.0 ± 1.0	1.9 ± 0.3	1.2 ± 0.1	0.8 ± 0.1	0.6 ± 0.1	1.7 ± 0.2	0.5 ± 0.1	0.18 ± 0.03	0.10 ± 0.01
WD[2]									
L-dose	9.3 ± 1.5	2.2 ± 0.2	1.2 ± 0.1	0.9 ± 0.1	0.7 ± 0.0	1.8 ± 0.1	0.5 ± 0.1	0.16 ± 0.03	0.11 ± 0.02
M-dose	9.1 ± 0.5	2.3 ± 0.1	1.3 ± 0.2	0.8 ± 0.1	0.7 ± 0.1	1.8 ± 0.1	0.6 ± 0.1	0.19 ± 0.04	0.10 ± 0.03
H-dose	7.9 ± 0.7	2.1 ± 0.2	1.3 ± 0.2	0.8 ± 0.1	0.6 ± 0.1	1.8 ± 0.1	0.5 ± 0.1	0.18 ± 0.03	0.09 ± 0.02
HD									
L-dose	9.3 ± 1.3	2.0 ± 0.2	1.2 ± 0.1	0.9 ± 0.1	0.6 ± 0.1	1.7 ± 0.1	0.5 ± 0.2	0.17 ± 0.04	0.11 ± 0.02
M-dose	9.3 ± 0.8	2.0 ± 0.1	1.3 ± 0.2	0.8 ± 0.0	0.6 ± 0.1	1.8 ± 0.01	0.5 ± 0.2	0.20 ± 0.10	0.10 ± 0.03
H-dose	8.8 ± 1.0	1.9 ± 0.2	1.3 ± 0.2	0.9 ± 0.1	0.6 ± 0.1	1.9 ± 0.1	0.5 ± 0.1	0.16 ± 0.03	0.09 ± 0.02

1: Blank group was fed AIN-76A diet and control group fed AIN-76A diet + 25.2% high grade flour diet.
2: WD: whole Djulis; HD: hulled Djulis.
3: The statistical analysis exhibited no significant difference among groups.

Table 10. Histopathological lesions in male and female rats fed low, medium, and high levels of whole Djulis or hulled Djulis diets for 28 days

Organ	Histopathological lesions	Lesion rate							
		Male				Female			
		B[1]	C	H-WD[2]	H-HD	B	C	H-WD	H-HD
Adrenal	-	-	-	-	-	-	-	-	-
Brain	-	-	-	-	-	-	-	-	-
Heart	Focal and slight infiltration in mononuclear cell	-	2/6	-	-	-	-	-	-
Kidney	Focal or slight mineralization, tubule nephrosis, interstitial fibrosis, and tubular crystal diffuse	-	-	-	-	-	-	1/6	-
Liver	Focal or slight necrosis, infiltration, fatty and bile duct proliferation	-	-	1/6	-	-	-	-	-
Lung	Moderate collapse and diffuse	1/6	1/6	-	-	-	-	-	-
Testis/ovary	-	-	-	-	-	-	-	-	-

1: Blank group was fed AIN-76A diet and control group fed AIN-76A diet + 25.2% high grade flour diet.
2: H-WD: high-dose whole Djulis; H-HD: high-dose hulled Djulis.

4. Discussion

Djulis is a traditional crop of Taiwanese aborigines. At present, the use of different processing methods (cooking, microwave, baking, frying and extrusion leavening) has developed different types of Djulis products in Taiwan, such as Taiwan Djulis oatmeal crackers, Djulis-microwave rice, Djulis-steamed rice, and Djulis-fried potato balls. In addition, a GABA-Djulis drink tea bag is available in market that claims to have reducing power or free-radical scavenging ability[14]. Furthermore, hulled Djulis has been used to make cooked starch or flour, bread, pasta, muffins, biscuits, pastry, and even fermented wine. However, Djulis has not been listed as a staple food crop by the Taiwanese agricultural authorities, although consumers often cook whole Djulis (un-hulled) for direct consumption. Therefore,

there is an urgent need to determine the safe consumption level of Djulis in order to promote its cultivation and product development as well as for the wellbeing of Taiwanese people.

In this study, both male and female rats were fed low-, medium-, and high-dose WD or HD diets for 28 days. The results revealed no significant changes in the behavior, body and organ weights, urinary biochemical parameters, and blood biochemistry values. In addition, no specific histopathologic lesions were found in either male or female rats fed WD or HD diets. These data indicate that no overt toxicity occurred in rats fed WD or HD diets for 28 days. However, the level of ALP and GPT in serum increased significantly in the male and female rats fed a high-dose HD diet for 28 days, although all ALP values were still within the normal range.

Both of the levels of GLU and CRE of the female rats in the blank and the control group were higher than the standard value, but there was no significant difference between HD, WD-treated female rats and the control group and the blank group ($p > 0.05$). Although ALP levels of the L-, M-, H-WD-treated female rats were higher than that of the control group; however, their values were within the standard range in rats (62-230 *U/L*). ALP serum biochemical marker is used to help detect liver disease or bone disorders. There was no obvious liver lesion but 1/6 renal lesion in histopathological analysis of H-WD female rats. This is not associated with a higher ALP, because the same ALP levels increased but no renal disease in the H-HD female rats. It is hypothesized that H-WD group of a female rat kidney disease should be congenital abnormality rather than feeding caused by WD.

Serum GPT, a more liver-specific indicator than GOT[15], in both male and female rats fed the high-dose HD diet (70 for male, 72 *U/L* for female) exceeded the standard value (18-45 *U/L*) and was significantly higher than that in rats fed the blank or control diet. These data suggested that feeding the high-dose HD diet may cause some liver damage in rats. In contrast, feeding high-dose WD diet to either male or female rats did not significantly increase serum GPT level. Whole grains are good sources of dietary fiber; and they contain minerals, vitamins, essential fatty acids, and phytochemicals such as polyphenols (phenolic acids, flavonoids and resorcinols)[16]. Most polyphenols occur in the outer layers of the grain, and they are largely lost during refining. Arranz *et al.*[17] found that extractable polyphenols in wheat bran are six times higher than in wheat flour.

It has been suggested that the shell of whole Djulis may have some protective effects against liver damage, possibly due to the presence of trace elements (minerals) and phytochemicals such as resveratrol, anthocyanins, -amino butyric acid (GABA) and betanin[18] in Djulis shell. Recent studies demonstrate that resveratrol has many therapeutic effects on liver disorders. For instance, resveratrol significantly increases survival after liver transplantation, decreases fat deposition, necrosis, and apoptosis induced by ischemia in Wistar rats[19]. Anthocyanin protects against acetaminophen-induced hepatotoxicity by blocking CYP2E1-mediated APAP bioactivation, up-regulating hepatic GSH levels, and acting as a free radical scavenger[20]. Similarly, GABA protects against cytotoxicity of ethanol in isolated rat hepatocytes by maintaining intracellular polyamine levels[21]. Importantly, Ali *et al.*[22] have shown that germinated and fermented mung bean exert better effects on liver injury than normal mung bean, indicating that the increase in amino acids, GABA, phenolic content, and other bioactives during germination and fermentation processes contributes to the hepatoprotective effects of mung bean

to ameliorate liver injury. These results suggest that resveratrol, anthocyanins, and GABA may, at least partly, be responsible for the protective effect of quinoa against chronic liver inflammation in rats.

5. Conclusions

This 28-day feeding study revealed that the HD and WD diet were relatively safe for consumption in rats. However, feeding the high-dose HD diet (25.2% dry weight), but not the high-dose WD diet, resulted in significantly increased serum GPT in both male and female rats. The results indicate that the consumption of whole Djulis is safe, whereas the daily consumption of hulled Djulis should be less than 25% in the diet to avoid liver damage. Using the medium dose of hulled Djulis (14.1%) and the average daily food intake (~500 *g* dry weight) in planned balanced diets for Taiwanese people[23]; we estimated that the safe daily consumption level of hulled Djulis for humans is 14.1% × 500 *g* = 70.5 *g*. This estimated daily intake for Djulis (70.5 *g*) may seem somewhat conservative but is on the safe side. In contrast, since the WD diet did not produce any noticeable side effects in rats, a safe daily intake for WD is estimated to be no less than 126 g (25.2% WD × 500 *g* = 126 *g*). This safety dose for human is higher than that suggested by Zevallos *et al.*[24] that a daily intake of 50 *g* quinoa for 6 weeks can be safely tolerated by celiac patients. Thus, Djulis has the potential to serve as a staple food and, particularly, a gluten-free diet for celiac patients.

Declaration of Conflicting Interests

The author(s) declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

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