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Original Article

Functional Beverages from Blends of Ficus *Deltoidea* Leaves and Brown Rice Powders: Physico-Phytochemical Properties, Antioxidant Activities, Sensory Evaluation and Acute Toxicity Study

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ABSTRACT

Background: *Ficus deltoidea* (Ficus: Moraceae) has great potential as a functional food. Administration of *F. deltoidea* has been reported to reduce hyperglycemia, oxidative stress and increase insulin secretion in diabetic rats and humans. However, the poor bioavailability and intestinal absorption of *F. deltoidea* impede its therapeutic effectiveness at a lower dosage, thus integrating *F. deltoidea* into brown rice will provide additional advantages. This study aimed to examine the phyto-physicochemical profile, antioxidant properties, consumer acceptance, and safety of beverages formulated from fine powder mixtures of *F. deltoidea* leaves and brown rice. **Methods:** The new beverage formulations were prepared by mixing the fine powders of *F. deltoidea* leaves with brown rice at ratios of 1:6 and 1:13, respectively. Physicochemical, phytochemical, and antioxidant analyses were performed to characterize the prepared beverages. Consumer acceptance was assessed utilising a 9-point hedonic scale and an acute toxicity study was employed to determine the safety of *F. deltoidea*-added formulations. **Results:** *F. deltoidea* decreased the pH and increased the moisture content, ash, and viscosity of a brown rice beverage. The total phenolic, flavonoid, and tannin content as well as antioxidant activities increased significantly in both *F. deltoidea*-added formulations. The oral LD_{50} of the *F. deltoidea*-added formulation was higher than 2000 mg/kg body weight. **Conclusions:** These results suggest that adding *F. deltoidea* leaves to brown rice beverages is safe to consume and improves the phyto-physicochemical profile, antioxidant activities, and consumers' acceptance of the formulation.

Key words: Animal study, DPPH assay, FRAP assay, Functional beverages, 9-point hedonic scale.

INTRODUCTION

Nutrition is an important aspect of living a healthy lifestyle. According to World Health Organization, good nutrition has been linked to better health at all ages, a lower risk of disease, and a longer life. Concerns about health, particularly during the COVID-19 epidemic, have spurred interest in functional foods and beverages that promote good health.[1,2] Functional beverages contain phytochemicals derived from plants that function in the body to prevent the onset of certain non-communicable diseases.[3] These natural functional beverage products are in limelight because of their therapeutic potential due to an array of phytochemical compounds which protect against various diseases.[4] Functional beverages derived from plants are not only intended to satisfy hunger, and provide humans with necessary nutrients but also have a role in health promotion and disease prevention due to antioxidant compounds present in plants.[5]

Ficus deltoidea Jack is one of the most well-known natural plants locally known as mas cotek in Malaysia.[6] It is traditionally used by the Malay community for health maintaining purposes where different parts of the plant are used for the treatment of several conditions such as relief of headache (fruit part), toothache (fruit part), and sores and wound (roots and leaves).[7] The decoction of boiled leaves of *F. deltoidea* is traditionally used as an antidiabetic treatment and an after-birth tonic to contract the uterus and vaginal muscles, to treat disorders of the menstrual cycle, and also to treat leucorrhoea.[8] The anti-inflammatory,[9] antinociceptive,[10] antioxidant,[11,12] antimicrobial,[13] anti-proliferative,[11] anti-photoaging,[14] wound healing,[15] anti-cancer,[12,16] anti-diabetic,[17,18] and cytotoxicity effects[19] of *F. deltoidea* has also been extensively reported. It has been demonstrated that *F. deltoidea* leaves contain an array of phytochemical compounds such as flavonoids, tannins, phenols and terpenoids.[20,21] The use of this plant as alternative traditional medicine for non-communicable diseases is gaining attention with the sale of tea bags and capsules of *F. deltoidea* in the local market.[8,22] Nevertheless, it has been reported that some of the active compounds have poor bioavailability and intestinal absorption, thus a higher dosage of *F. deltoidea* is required to obtain the therapeutic effects.[23] Consuming more than one litre of *F. deltoidea* tea per day has been reported to cause hypermagnesemia and manganese toxicity.[24]

Brown rice is one of the whole-grain food in Asian countries. It can be used as a functional food or beverage to satisfy dietary and nutritional demands. It has been shown that brown rice can counteract

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oxidation and antioxidants, reduce inflammation, and lower blood lipids.[25] Brown rice contains high levels of fibre and bioactive phytochemicals including tocopherols, tocotrienols, oryzanols, dietary fibres, vitamins, and phenolic compounds, which are beneficial to humans health and well-being.[26] Intake of brown rice helps regulate bowel function, which may further enhance the absorption of nutrients. However, it has been reported that brown rice is less popular in daily life consumption since it takes longer to cook, has an unappealing color and has a rigid texture.[27] Based on these considerations, combining *F. deltoidea* with brown rice as a novel functional drink will offer more benefits. However, little is known regarding the synergistic and bioactivities effects. This prompted us to examine the physicochemical profile, phytochemical constituents, antioxidant activity, sensory evaluation and the acute toxicity testing of a newly developed functional beverage formulated from fine powder blends of *F. deltoidea* leaves and brown rice. A combination of chemical analysis, sensory testing, and toxicity studies helps to provide more insight into the benefits of a new beverage formulation.[28]

MATERIALS AND METHODS

Materials

Raw *F. deltoidea* (FD) leaves were procured from Moro Sri Utama Enterprise, which owned a plantation in Batu Pahat, Johor. Other materials required for the preparation and production of formulated beverage (FB) were purchased from the local market in Shah Alam, Selangor. Brown rice powder was supplied from Abrand Manufacturing Sdn. Bhd. DPPH, 2,4,6-tri-(2-pyridyl)-1,3,5-triazine, Folin–Ciocalteu's phenol reagent, potassium iodate, ascorbic acid, tannic acid, gallic acid and quercetin were sourced from Sigma-Aldrich (St Louis, MO, USA). Materials needed for the sensory evaluation test of FB were bought from the local market in Shah Alam, Selangor.

Preparation of formulated beverages

The dried leaves of *F. deltoidea* were ground into finer powdery particles using a grinder and were fractioned into fine and coarse powder fractions with a 40-micron sieve.[29] The FB with different FD content ranging from 2.5 g to 5.0 g was further prepared by manually mixing the ingredients stated in Table 1. The base formulation is referred to as 'control' (NBR) and had no FD powder. All the ingredients were weighed and added to a mixing bowl. The mixture was then homogenized using a laboratory homogenizer (IKA T25 digital Ultra-Turrax) and was stored in a sealed bag at 4°C until further analysis.

Physicochemical properties

The pH was measured by using the pH meter (Mettler Toledo, Germany).[30] Solubility and swelling power were analyzed by mixing an amount of 0.35 g of the samples with 12.5 mL of distilled water before being heated in a water bath of 60°C for 30 min with constant agitation. After centrifugation at 3500 x gm for 20 min (Model 5420, Kubota, Japan), the supernatant was decanted in a pre-weighed evaporating dish and dried at 100°C for 20 min. The difference in weight of the evaporating dish was used to calculate the solubility. Swelling power was calculated by weighing the residue after centrifugation and divided by the original weight of the samples.[31] Water and oil absorption capacities (WAC and OAC) were calculated using a method proposed by.[32] Two g of samples were mixed with 20 mL distilled water for WAC and refined soybean oil for OAC. The mixture was allowed to stand at ambient temperature (32°C) for 30 min followed by centrifuging for 30 min at 3000 rpm or 2000 x g Model 5420, Kubota, Japan). WAC and OAC were expressed as percent water or oil bound per gram of sample such as follow:

$$Water\ or\ oil\ absorption\ capacity = \frac{Weight\ of\ water\ or\ oil\ absorbed}{Weight\ of\ sample}$$

Moisture content was estimated by the oven drying method. Ash content was quantified using the method AOAC (2003). The viscosity of NBR, FB1 and FB2 was measured by mixing 35 g of each sample with 250 mL of hot water (80-90°C). The solutions were stirred thoroughly to avoid any lump formation. Viscosity was measured by inserting the solution into the Brookfield DV-I viscometer (U.S.A) with spindle No.1 at 100 rpm. The viscosity reading in centipoises (cP) after 30 seconds of rotation was recorded (Fmna and Zailan, 2019). The color of samples was measured by chromameter CR400 (Konica Minolta, Japan). L*, a* and b* parameters indicate lightness (100 = white; 0 = black), greenness (+ red; − green) and yellowness, (+ yellow; − blue), respectively. The white calibration plate CMA101 was used for calibration. Measurement was performed in triplicate.[33]

Table 1: Composition of FB formulas.

Ingredients	Different Formulas of FB		
	NBR	FB1	FB2
FD (g)	0.0	2.5	5.0
NBR (g)	29.6	27.1	24.6
Inulin (g)	5.1	5.1	5.1
Sodium (mg)	154.8	154.8	154.8
Magnesium (mg)	18.0	18.0	18.0
Calcium (mg)	113.4	113.4	113.4

Qualitative phytochemical screening

Phytochemical screening of samples (NBR, FB1 and FB2) was performed according to the standard method described by[34] to ascertain the presence of phenolics, flavonoids, tannins, cardiac glycosides, alkaloids, terpenoids and steroids.

Quantitative analysis of phenolics, flavonoids and tannins contents

A chemical test was carried out on the beverages using a standard procedure to identify the constituents as total phenolics, total flavonoids and total tannins. The total phenolic contents of NBR, FB1 and FB2 were determined using Folin-Ciocalteau reagent by a method of[35] with some modification. A 30 µL aliquot of the infusion samples was mixed with 150 µL of the Folin-Ciocalteau reagent in a 96-well microtiter plate. After 3 min at room temperature, 50 µL of saturated sodium carbonate was added. The mixture was incubated in a water bath at 37°C for 30 min. The absorbance was measured triplicate at 750 nm using a UV-Vis spectrophotometric microplate reader. Gallic acid was used as the reference standard and the results were expressed as mg of gallic acid equivalents (0.0005 – 0.5 mg/mL).

A method proposed by[36] was used as a guideline with some modifications to determine total flavonoid contents using aluminium chloride ($AlCl_3$) complex-forming assay. Each infusion sample (30 µL), distilled water (125 µL) and 5% sodium nitrite (7.5 µL) were added to a 96-well microtiter plate. After 6 min, 15 µL of 10% $AlCl_3$ solution was added and the mixture was allowed to stand for 5 min. Sodium hydroxide (1M 50 µL) and distilled water (22.5 µL) were added to bring the total volume to 250 µL. Absorbance was read at 510 nm (Biochrom Libra S22, Santa Barbara, CA, USA). Quercetin was used as standard. Results were expressed in quercetin equivalents per gram of dry beverage (QE/g), through a calibration curve of quercetin (0.005 – 5.0 mg/mL).

The total tannins contents were determined using the potassium iodate (KIO_3) test adopted from.[37] Tannic acid was prepared in concentrations of 0, 0.005, 0.05, 0.5 and 5 mg/mL in distilled water as standard solutions for calibration. Five mL of 2.5% KIO_3 solution, preheated for seven min at 30°C were mixed with one mL of tenfold diluted extracts. The mixture was placed in a thermostatic bath at 30°C for 2 min and the absorbance was measured at 550 nm (Biochrom Libra S22, Santa

Barbara, CA, USA). The total tannins contents were expressed as mg tannic acid equivalent (TAE) per g dry weight. All samples were analyzed in triplicate.

Antioxidant activities

The antioxidant capacity of NBR, FB1 and FB2 was quantified using ferric reducing antioxidant power assay.[36] FRAP reagent was freshly prepared using 300 mM sodium acetate buffer pH 3.6, 20 mM ferric chloride hexahydrate and 10 mM 2,4,6-Tris (2-pyridyl) s-triazine (TPTZ), Fluka, Ireland; in 40mM HCl, Fisher-Scientific, Ireland, in the ratio of 10:1:1, v/v/v) and were incubated in a water bath at 37°C for 5 min. In a 96-well microtiter plate, 100 µL of FRAP reagent were added to 50 µL of infusion samples. The mixture was incubated for 10 min at 25°C followed by a reading of the absorbance at 593nm. Gallic acid was used as standard. Results were expressed in Gallic acid equivalent per gram of dry sample (GA/g), extrapolated from a calibration curve of Gallic acid (0.0005-0.5 mg/mL).

DPPH radical-scavenging method was used to analyse the antioxidant capacity of NBR, FB1 and FB2.[38] A 50 µL aliquot sample and 200 µL of 0.5 mM methanolic DPPH were mixed in a 96-well microtiter plate. The mixture was thoroughly mixed and kept in the dark for 1 hour. The absorbance was measured at 517 nm using microplate reader spectrophotometers. Samples were measured in triplicate. The percentage of DPPH scavenging activity was calculated as below:

$$Percentage\ inhibition\ of\ DPPH\ (\%) = \frac{Abs\ control - Abs\ sample}{Abs\ control}\ x\ 100$$

Sensory evaluation test

An acceptance test was conducted on the sensory evaluation of formulated beverages in terms of appearance, colour, aroma, taste, aftertaste and overall liking. Fifty untrained panelists were invited to participate in this evaluation. All of them are undergraduate and postgraduate students from Universiti Teknologi Mara (UiTM) with ages ranging from 18 to 40. The evaluation was conducted in the Sensory Laboratory at the Faculty of Applied Sciences in UiTM under ambient temperature and fluorescent light. Tissue, plain water and spit cup were given to all panelists on a tray. The panelists were briefed on the definition of descriptive terms before sensory evaluation sessions. Three samples were given to panelists which consists of 1) control (NBR), 2) 2.5 g of *F. deltoidea* in NBR and 3) 5.0 g of *F. deltoidea* in NBR. The samples were given a random numerical code to avoid bias. Samples arrangements between panelists were also arranged differently to avoid positional or order bias. Each of the samples was served to them in paper cups with three-digit random numbers labelled to them. Panelists were required to rinse their mouths after each sample evaluation before going for the next sample. Panelists then had to answer a sensory evaluation form that had a 9-point hedonic scale for each sample for the attribute mentioned. The evaluation was based on their degree of like (scale 1-9) where 1 = dislike extremely and 9 = like extremely. Samples with mean scores of more than 5.00 for overall acceptability were considered acceptable.[39]

Acute toxicity testing

The acute toxicity study was conducted in accordance with Organization for Economic Co-operation and Development (OECD) Guideline 423 (OECD, 2001). The welfare and handling of the experimental animals were according to the OECD Guidelines for the Testing of Chemicals 420 (2001) and were approved by Animal Research and Ethics Committee, Universiti Teknologi Mara (UiTM CARE) with an approval number: UiTM CARE: 325/2020. All experiments were performed on female Sprague Dawley rats aged six weeks (mean body weight, 250 ± 5g) that were purchased from Chenur Supplier Sdn Bhd., Serdang, Selangor. A total of 12 female rats was divided into two groups with n=6 animals per group (were housed n=3 per cage according to OECD guidelines). The group was labelled as CT (control group) and FB2 (treatment group). FB2 group was selected based on the result of physicochemical, phytochemical, antioxidant activities and sensory evaluation tests among the NBR, FB1, and FB2. Animals in the CT group received saline as the treatment vehicle (1 ml/ 100 g bwt), while animals in the FB2 group received the functional beverages formulated with *F. deltoidea* leaves. The treatment was administered using oral gavage at a single dose of 2000 mg/kg bwt with a volume of 1 ml/ 100 g bwt. The further limit test was conducted at the same dosage when there is no animal died.

Animals were fasted from food for 12 hours prior to dosing. Animals were observed periodically during the first 24 hours after administration of the beverages and assessment was continued for 14 days. The toxic symptoms such as skin and fur changes as well as mortality were recorded. Throughout the experimental period, the physical parameters that indicate the toxicity effects of formulated beverages such as mortality, skin, tremors, convulsion, pupils, salivation, lacrimation, food intake and water intake were observed and recorded daily. Changes in body weight were measured weekly.

At the end of the acute study, animals were fasted overnight and anesthetized by intraperitoneal injection of ketamine and xylazine (were provided by Fakulti Perubatan Veterinar Universiti Putra Malaysia) at a dose of 0.01 ml/gram body weight. The blood samples (5-10 ml) were collected by cardiac puncture into EDTA-containing tubes for haematological analysis. The liver and kidney were excised and weighed. These organs were preserved in a fixation medium of 10% buffered formalin for histopathological study. The relative organ weight was calculated based on the following formula:

$$Relative\ organ\ weight\ (g) = \frac{Absolute\ organ\ weight\ (g)}{Weight\ of\ rats\ on\ sacrifice\ day\ (g)} \times 100$$

Statistical analysis

All data were analysed with the statistical package for the social sciences (SPSS) 21.0 software. An analysis of variance (ANOVA) was used to analyze data from the physicochemical properties, quantitative analysis of phenolics, flavonoids, tannins, antioxidant activities, sensory test and acute toxicity study. Differences between groups were performed on all variables using one-way ANOVA. Duncan's multiple comparison test was employed to elucidate significant means. Results were presented as the mean ± SEM. All analysis was performed at 95% confidence level.

RESULTS

Physicochemical properties

Table 2 summarizes the physicochemical properties of NBR, FB1, and FB2. It is noticeable that the FB2 had the lowest pH and L* values but recorded the highest value of moisture, ash, and viscosity. Meanwhile, FB1 only showed significant changes in pH, moisture, viscosity, L* and B* values as compared to NBR.

Qualitative phytochemical screening

Table 3 shows the result of qualitative phytochemical screening of NBR, FB1 and FB2. Phenolics, flavonoids, tannins, saponins, cardiac glycosides, alkaloids, terpenoids and steroids were strongly present in the FB2 but moderately and slightly present in the FB1 and NBR, respectively. However, steroids were only present in the FB1 and FB2.

Quantitative analysis of phenolics, flavonoids and tannins contents

The quantitative phytochemical analysis of NBR, FB1 and FB2 are shown in Figure 1. The total phenolics, flavonoids and tannins contents were recorded highest in the FB2 (TPC: 11.36 ± 0.12[a]; TFC: 27.40 ±

Table 2: Physicochemical properties of NBR, FB1 and FB2.

Parameters	NBR	FB1	FB2
pH	6.83 ± 0.04^{c}	6.50 ± 0.02^{b}	6.34 ± 0.00^{a}
Swelling Power (g)	2.52 ± 0.59	3.08 ± 0.09	3.33 ± 0.08
WAC (%)	182.33 ± 3.37	168.27 ± 12.45	170.43 ± 6.58
OAC (%)	146.80 ± 3.00	144.00 ± 9.41	144.70 ± 8.11
Solubility (%)	9.21 ± 0.76	10.22 ± 0.44	11.03 ± 0.16
Moisture (%)	6.92 ± 0.04^{a}	7.09 ± 0.07^{b}	7.27 ± 0.01^{c}
Ash (%)	2.43 ± 0.08^{a}	2.75 ± 0.09^{a}	3.51 ± 0.14^{b}
Viscosity (cP)	27.07 ± 0.15^{a}	40.93 ± 0.15^{b}	42.77 ± 0.09^{c}
Lightness (L*)	66.24 ± 0.38^{c}	60.19 ± 0.03^{b}	53.41 ± 0.26^{a}
Greenness (a*)	-1.96 ± 0.04^{a}	-1.88 ± 0.04^{a}	-1.09 ± 0.14^{b}
Yellowness (b*)	0.65 ± 0.32^{a}	14.72 ± 0.20^{b}	16.73 ± 0.10^{c}

Values are mean ±SEM for triplicate reading of samples. Values with different superscripts in a row differed significantly at $p < 0.05$. *Key: WAC = Water absorption capacity; OAC = Oil absorption capacity.

Table 3: Phytochemical screening of NBR, FB1 and FB2.

Parameters	NBR	FB1	FB2
Phenolics	+	++	+++
Flavonoids	+	++	+++
Tannins	+	++	+++
Saponins	+	++	+++
Cardiac glycosides	+	++	+++
Alkaloids	+	++	+++
Terpenoids	+	++	+++
Steroids	-	++	+++

Key: - (Absent); + (Present, low); ++ (Present, mild), +++ (Present, strong)

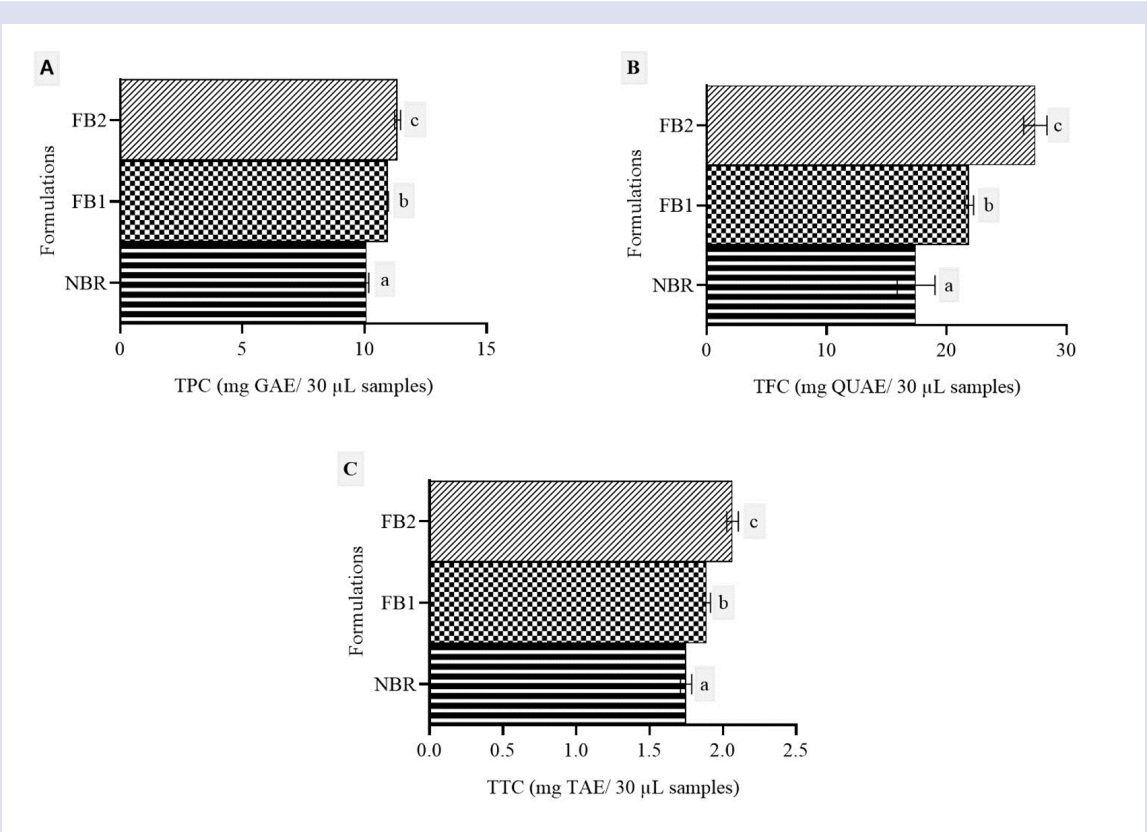

Figure 1: Quantitative analysis of phenolics, flavonoids and tannins contents of NBR, FB1 and FB2. (A) Total phenolic content of the samples. (B) Total flavonoid content of the samples. (C) Total tannins content of the samples. Data are presented as mean ± SEM. Values with different superscripts are significantly different at $p < 0.05$.

0.97[b]; TTC: 2.07 ± 0.04[c]), moderate in the FB1 (TPC: 10.95 ± 0.03[a]; TFC: 21.88 ± 0.38[b]; TTC: 1.89 ± 0.03[c]), and lowest in the NBR (TPC: 10.10 ± 0.08[a]; TFC: 17.46 ± 1.58[b]; TTC: 1.75 ± 0.04[c]).

Antioxidant activities

Antioxidant activities of NBR, FB1 and FB2 are shown in Figure 2. Results show that the FB2 (6.26 ± 0.04[a]) had the highest antioxidant activities as compared to the FB1 (6.24 ± 0.01[a]) and NBR (7.17 ± 0.03[b]). The highest value of IC_{50} was recorded in NBR (5.81 ± 0.04[d]).

Sensory evaluation test

The sensory characteristics of the functional beverage prepared from brown rice and *F. deltoidea* powder are shown in Figure 3. The analysis indicates that there was a statistical difference in taste and aftertaste between the control and formulated beverages. All the samples were considered acceptable due to the mean scores of more than 5.00 for overall liking parameters.

Acute toxicity testing

Administration of FB2 at a single dose of 2000 mg/kg bwt showed no adverse effects on the tested rats. Neither sign of toxicity nor mortality of rats were recorded during the 14 days of the experimental period. Morphological features such as skins, pupils, fur and nose appeared to be normal as shown in Table 4. No salivations, tremors, convulsion and lacrimation were observed. All the rats were in healthy condition until the end of the experimental period.

Food intake and water intake

The effects of FB2 administration on food and water intake were shown in Figures 4A and B respectively. The intake of FB2 beverages causes a significant reduction in food consumption in the treated group as compared to the control group. Meanwhile, there is no significant difference between the control and treated groups in the amount of water intake.

Changes in body weight

Figure 4C shows the effects of FB2 intake on body weight. Administration of FB2 beverage at 2000 mg/kg bwt causes no significant differences in the body weight.

Relative organ weight

The effects of FB2 administration on the relative organ weight were shown in Figure 4D. It was noted that there were no statistical

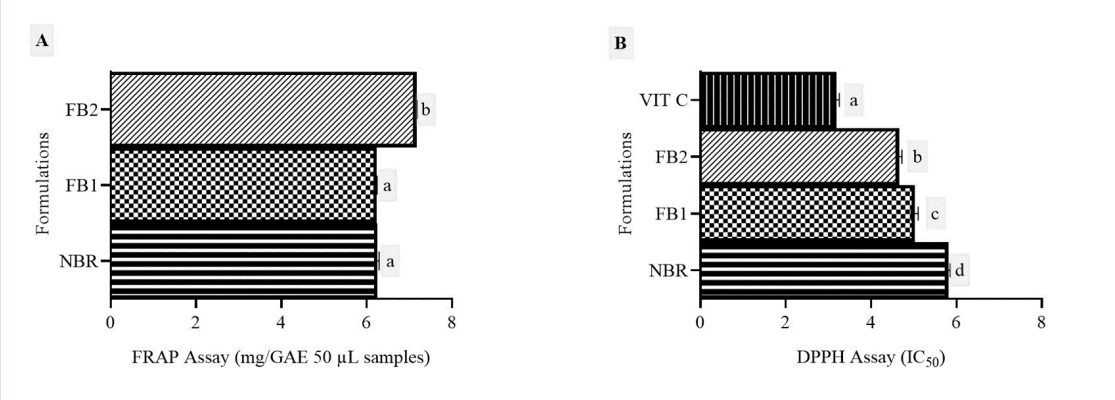

Figure 2: Antioxidant activities of NBR, FB1 and FB2. (A) FRAP assay of the samples. (B) DPPH assay of the samples. Data are presented as mean ± SEM. Values with different superscripts are significantly different at $p < 0.05$.

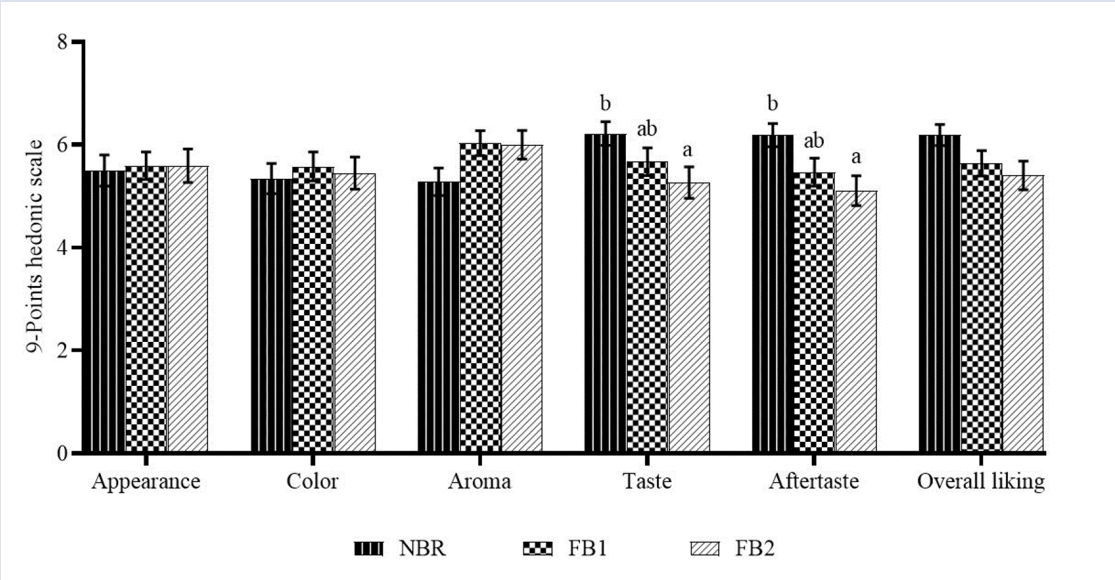

Figure 3: Sensory evaluation test of NBR, FB1 and FB2**.** Data are presented as mean ± SEM. Values with different superscripts are significantly different at $p < 0.05$.

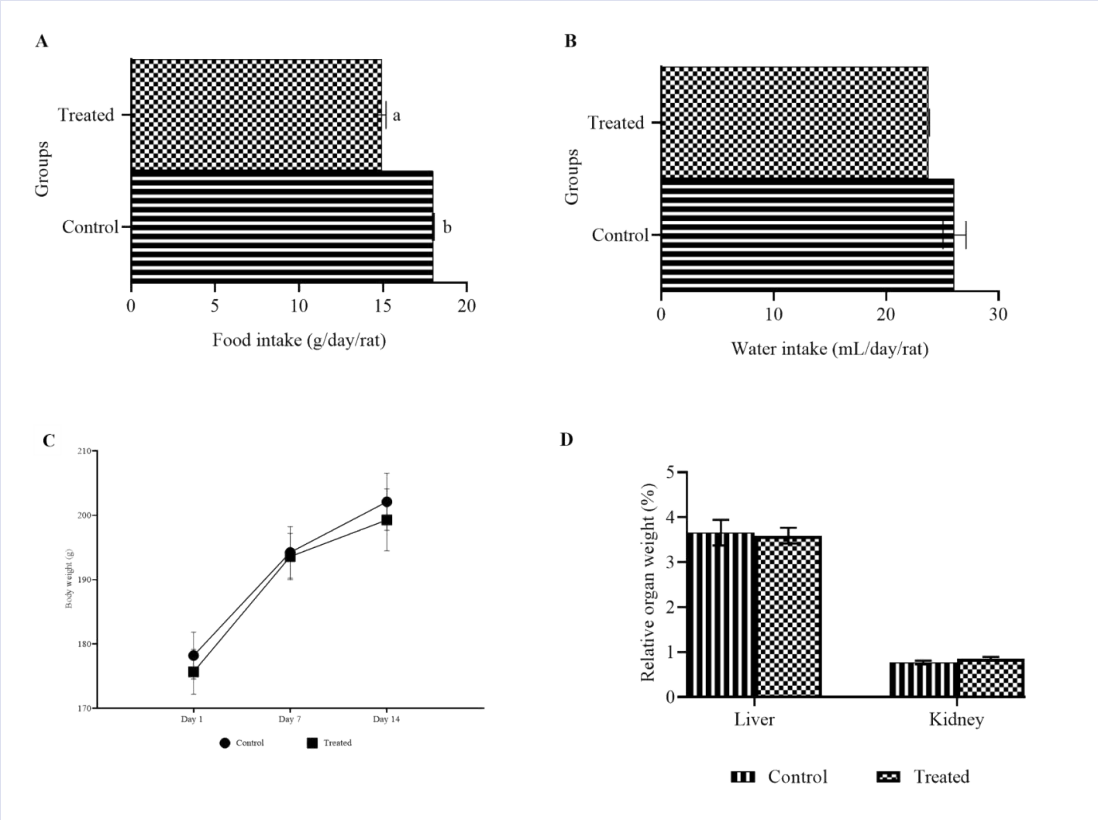

Figure 4: Effects of FB2 administration. A) Food intake B) Water intake. C) Body weight changes D) Relative organ weight. Data are presented as mean ± SEM. Values with different superscripts are significantly different at $p < 0.05$.

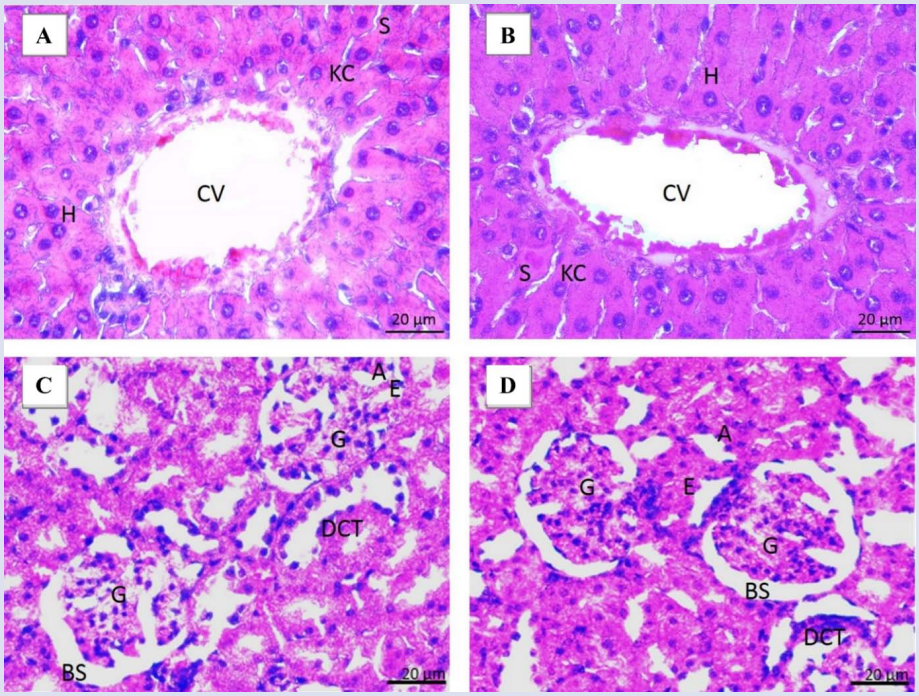

Figure 5: Light photomicrograph of liver and kidney sections from control and treated groups. Tissues were collected, processed and stained with hematoxylin-eosin. (A) Control group showed normal architecture of the liver. Hepatocytes: H, central vein: CV, sinusoids: S and Kupffer Cell: KC are in normal morphology. (B) Treated group showed normal architecture of the liver. Hepatocytes: H, central vein: CV, sinusoids: S and Kupffer Cell: KC are in normal morphology. (C) Control group showed a normal appearance of the kidney. Glomerulus: G, Bowman space: BS, distal convoluted tubule: DCT, afferent: A and efferent: E tubule are in normal morphology. (D) Treated group showing normal appearance of the kidney. Glomerulus: G, Bowman space: BS, distal convoluted tubule: DCT, afferent: A and efferent: E tubule are in normal morphology. Images are representative of six animals per experimental group (magnification 40X).

Table 4: Morphological features for control and treated groups.

Observation	Control group				Treated group			
	30 m	4 h	24 h	14 d	30 m	4 h	24 h	14 d
Skins	N	N	N	N	N	N	N	N
Pupils	N	N	N	N	N	N	N	N
Fur	N	N	N	N	N	N	N	N
Nose	N	N	N	N	N	N	N	N
Salivations	N	N	N	N	N	N	N	N
Tremors	N	N	N	N	N	N	N	N
Convulsion	N	N	N	N	N	N	N	N
Lacrimation	N	N	N	N	N	N	N	N
Mortality	N	N	N	N	N	N	N	N

Key: m = minute, h = hour, d = day, N = normal.

Table 5: Effects of FB2 intake on haematological parameters.

Parameters	Control group	Treated group
Platelet (s)	154.17 ± 31.13	120.83 ± 227.21
Haematocrit (%)	46.96 ± 1.46	43.27 ± 1.84
Haemoglobin (%)	232.65 ± 0.00	236.23 ± 3.58
Neutrophil (%)	31.67 ± 7.78	44.00 ± 1.71
Eosinophil (%)	4.33 ± 0.71	4.33 ± 0.56
Basophil (%)	1.50 ± 0.43	1.17 ± 0.31
Lymphocyte (%)	53.83 ± 8.69	42.50 ± 1.54
Monocyte (%)	8.67 ± 0.49	7.83 ± 0.95

Values are mean ±SEM for triplicate reading of samples.

differences for both liver and kidney relative weight between the control and treated groups.

Haematological analysis

The assessment of the haematological parameters of the experimental animals revealed no significant differences between the control and treated groups. Table 5 shows the effects of FB2 intake on haematological parameters.

Histological analysis

Histological evaluations of the liver and kidney sections are shown in Figure 5. The results of the treated group demonstrated no abnormalities were detected in the pathological examinations of the tissues as compared to the control group. Liver sections of animals treated with FB2 beverages (Figure 5B) showed normal architecture with a normal appearance of the central vein: CV, sinusoids: S, Kupffer cell: KC and hepatocytes: H similar to the control group (Figure 5A). The cross-section of kidneys from both the control and treated groups demonstrated the appearance of the glomerulus: G, Bowman space: BS, distal convoluted tubule: DCT, afferent: A and efferent: E tubule structures were normal (Figure 5C and 5D).

DISCUSSIONS

The physicochemical analyses of the samples demonstrated that the incorporation of *F. deltoidea* leaves into brown rice resulted in a significant decrease in the pH value and an increase in the moisture and ash (Table 2). Similar findings have been reported in several herbal tea beverages commonly found in markets such as Lipton ice lemon tea, traditional blackcurrant tea, and raspberry mix (strawberry and loganberry).[40] Beverages with a pH below 7.0 have been reported can help the digestion of food and ensure the proper absorption of mineral elements in an acidic environment in the stomach.[41,42] This finding suggests that a higher amount of *F. deltoidea* added to brown rice would improve intestinal absorption. The study also revealed that the moisture content of FB2 beverage was significantly increased with higher proportions of *F. deltoidea* leaves as compared to NBR and FB1. This data was in accordance with the previous study by[43] on the instant Sorrel (Zobo) drinks and[44] on the instant mango drink powder. It has been demonstrated that the moisture content below 14% help in preventing bacterial, fungal, and yeast growth.[45] Minimisation of moisture level inhibits the growth of bacteria and ensures the longer preservation of food, which is an ideal solution when appropriate storage is not available.[46] It is interesting to suggest that the addition of *F. deltoidea* leaves into beverage make it suitable for the prevention of microbial growth and chemical changes for safe storage. A significant increase in ash content in FB2 was also noted by the addition of *F. deltoidea* into brown rice. This result was in agreement with the previous report on the composite biscuit made of brown rice flour and wheat flour.[47] It has been reported that ash content has a correlation with the presence of minerals and could reflect the nutritional value of the product.[48] It is conceivable that the addition of *F. deltoidea* leaves into brown rice could improve the amount of high dietary fiber and mineral content in the FB2 as compared to NBR and FB1. The high nutritional content of the beverage would be of nutritional value in most developed nations, where many residents are unable to afford well-nutritional foods due to high prices.[42]

Viscosity determines the acceptability of beverages and is regarded as the "mouth feeling" parameter.[39,49] The data in the present study show that the viscosity of formulated beverages increased significantly with the higher addition of *F. deltoidea* leaves into brown rice. Similar findings have been obtained in milk barberry drinks with higher incorporation of the pectin concentration.[50] It has been reported in the earlier studies that the viscosity of beverages can decline impact on hunger.[51] The greater viscosity enabled the consumers better bolus control and time to prepare for the onset of the pharyngeal swallow to engage the airway protective system, thereby reducing the risk of aspiration.[52] Indeed, these findings are in good accordance with the ability of incorporation of *F. deltoidea* leaves into brown rice to increase the perceived satiety and reduced the effect of starvations.

The color of formulated beverages is significantly affected by the amount of *F. deltoidea* added to brown rice. As shown in Table 2, the higher

ratio of *F. deltoidea* leaves to brown rice had significantly increased the darkness, greenness and yellowness of the beverages. This observation is in line with a previous study reported by[53] on the color of *Cosmus caudatus* herbal tea prepared from young leaves. The relationship between color and antioxidant properties has been reported in an earlier study.[54] The color of the sample is influenced by the antioxidant activities of the sample which the higher the antioxidant activity, the darker the color of the sample.[55-57] have demonstrated that the darker samples were found to have higher antioxidant activity due to the presence of the higher phenolics, flavonoids and carotenoids content in the sample. The darkness of the color somehow seems to be correlated to an elevation of the concentration of the phytochemical compounds. Therefore, it is reasonable to suggest that the incorporation of *F. deltoidea* leaves into brown rice could affect its color and improve the antioxidant capacity.

The qualitative and quantitative phytochemical screening was conducted to identify and quantify the bioactive compounds found in beverage-plant based.[58,59] The addition of *F. deltoidea* leaves into brown rice improved the presence of phenolics, flavonoids, tannins, saponins, cardiac glycosides, alkaloids, and terpenoids as compared to NBR. This finding was consistent with data reported by that detected the presence of tannins, phlobatannins, saponins, flavonoids, steroids, terpenoids, cardiac glycosides, alkaloids, anthraquinones and polyphenols in leaves and callus of *F. deltoidea* Jack var. *kunstleri*. It was also noted that the addition of *F. deltoidea* leaves contributed to the presence of steroids in the FB1 and FB2. This observation was similar to the study demonstrated by[60] that showed the presence of steroids in milk and dairy food product. Previous work had demonstrated that the presence of steroids may exert physiological and pharmacological activities by producing an inhibitory effect on inflammation disorder.[61,62] The consumption of plant steroids has been shown can decrease bile acid secretion which may lead to a decreased risk for colon cancer development.[63] However, long-term treatment of steroids has been reported to produce adverse effects such as immunosuppression, hypertension, osteoporosis, and metabolic disturbances including the increased susceptibility to infections and adrenal insufficiency.[64,65] Such harmful side effects limited the use of steroids in food and beverage for daily use which contradicts our findings. Indeed, steroids are frequently the most effective therapy available, and their use is only constrained by systemic side effects.[65,66] Further toxicity tests of the new functional beverage formulated from fine powder mixtures of *F. deltoidea* leaves and brown rice is, therefore, necessary to overcome these undesirable consequences.

The total phenolics, flavonoids and tannins content of the commercialized beverage has been increased upon the addition of *F. deltoidea* leaves into FB1 and FB2 as compared to NBR (Figure 1). These data were consistent with the previous study that reported that some phytochemicals like phenolic compounds and flavonoids are higher in *F. deltoidea* compared to *Eurycoma longifolia* and *Labisia pumila*, and lower compared to *Azadirachta indica, Centella asiatica* and *Hibiscus rosa-sinensis*.[67] Higher contents of these phytochemicals may be associated with their antioxidant activity which could play an important role in the prevention and treatment of chronic diseases.[68] There is growing evidence that consuming a variety of phytochemical components found in foods may reduce the risk of certain health problems.[69] Phenolics, flavonoids and tannins are highly effective as free radical scavengers and antioxidants that were used to prevent the formation of free radicals which can cause various chronic diseases.[70,71] Therefore, it is reasonable to propose that the incorporation of a higher amount of *F. deltoidea* into brown rice beverages could improve the amount of phytochemical components and possess stronger antioxidant activity.

As shown in Figure 2, the addition of *F. deltoidea* leaves into brown rice significantly increased the antioxidant activities of FB1 and FB2 as compared to NBR. This result corresponded to the findings of[72] on the seven varieties of *F. deltoidea*. Some researchers reported that high antioxidant activity is associated with high phenolic content and high flavonoid contents that seem to have an important role in stabilizing lipid oxidation.[73,74] The presence of coumaric acid and vitexin in the extracts of *F. deltoidea* may also contribute to the antioxidative action of the plant, suggesting that the phenolic and flavonoid compounds present in the extracts could be responsible for its beneficial effects.[19] This data may suggest that the antioxidant effects of FB2 could be attributed to the presence of high phenolic and flavonoid contents due to the higher ratio of *F. deltoidea* added. In the DPPH assay, it was found that the scavenging activity of the formulations was directly proportional to the ratio of *F. deltoidea* added and phytochemical content. The lower the IC_{50} value of an antioxidant the higher would be its free radical scavenging power. It has been reported that antioxidant substances scavenge free radicals such as peroxide, hydroperoxide, and lipid peroxyl, thus suppressing the oxidative pathways that lead to degenerative disorders.[75] This finding was parallel with a previous study that reported a good percentage of antioxidant activity of DPPH was found in the methanolic extract of *Ficus deltoidea*, var. *trengganuensis, Ficus deltoidea* var. *angustifolia,* and *Ficus deltoidea* var. *deltoidea*.[9] A previous study also revealed that the cultivated and wild leaves extract of *F. deltoidea* exhibit higher DPPH scavenging activity than the stem extract.[76] It is noteworthy to suggest that there was a positive correlation between the strong antioxidant and scavenging activity of FB2 with the higher phytochemical content.

The analysis of the acceptance of the beverage demonstrated that the mean scores of taste and aftertaste of formulated beverages were significantly different as compared to NBR (Figure 3). The mean scores for taste and aftertaste were highest in NBR, moderate and lowest in FB1 and FB2, respectively. These data are in agreement with recent work by[77] that has shown the addition of a higher concentration of cardamon rhizome spices into drinks reduce the preference of panellist on taste and aftertaste. The lower significant value of the taste and aftertaste in FB2 as compared to NBR and FB1 could be due to the addition of *F. deltoidea* leaves into the beverage. This condition might be the consequence of an unfamiliar sensation perceived by the panellist when tasting the beverage added *F. deltoidea* leaves. It has been reported that functional beverages enriched with medicinal plants and herbs are usually prepared using raw materials with high polyphenolic compounds.[78] A high amount of polyphenolic components in food are perceived as being bitter, tart, or astringent.[79,80] Even so, taste and aftertaste are not used as criteria for functional food products since products claiming to be healthy beverages are normally tasteless and perhaps a little bitter.[77] Therefore, the selection of FB2 for further study on toxicological profiles might be considered as there is no significant difference in appearance, color, aroma and overall testing parameters. Incorporation of *F. deltoidea* not only improves the acceptance of the FB2 but also its physicochemical, phytochemical and nutritional value. The consistency of higher antioxidant activity shown in each antioxidant assay may also support the selection of FB2 for toxicity study.

Assessment and evaluation of the toxicological profile are required in screening the new beverage product development. There were no adverse toxicity signs and mortality recorded on the tested rats up to 14 days observation after administration of the FB2 at a single dose of 2000 mg/kg bwt. This study indicates that FB2 does not cause acute toxicity effects at the dose tested and with the LD_{50} value greater than 2000mg/kg bwt. This data was aligned with a study conducted by[81] reporting that there is no overt symptoms of acute toxicity or death were observed in mice and rats upon treatment of methanol extract of *F. deltoidea* up to the dose of 6400 mg/kg bwt. According to[82] substances with LD_{50} values higher than 5000 mg/kg bwt by the oral route are regarded as being safe or practically nontoxic. Hence, the absence of lethality and sign of

toxicity in rats suggest that the FB2 beverage is well tolerated and safe to be used when administered orally. Our acute toxicity results also confirm earlier reports showing that consumption of a plant sterol is safe to use over a long period.[83]

The food consumption of FB2-treated rats decreased significantly as compared to the control group. It was observed that the animals treated with FB2 consumed less food than the control group. However, there were no differences between treated and control groups for water consumption. A similar finding has been reported by[84] on the food intake of rats treated with 4000 mg/kg bwt of Red Hawn Kefir Powder. Reduce food intake in animals after FB2 administration could be attributed to the presence of brown rice in the beverage formula. Brown rice is one of the whole-grain foods that contain carbohydrates with a low glycaemic index which helps to reduce the blood glucose level.[85,86] Foods with a low glycaemic index are regarded to have a higher satiating capacity.[87] Whole-grain foods contain dietary fibre that is capable to influence glucose metabolism, gastrointestinal transit, and gastrointestinal hormone secretions, all of which can influence appetite by preventing hunger and stimulating lower food intake.[88,89] It has been revealed in a study that higher consumption of whole-grain food is associated with a lower risk of weight gain and incident overweight or obesity.[90] Therefore, it is relevant to suggest that FB2 is not only safe to consume but can reduce appetite and food intake which can lower the risk of obesity.

Animals in both treated and control groups presented a progressive increase in body weight throughout the study period (Figure 4C). There is no statistical difference between control and treated groups in the body weight changes. The results also showed that the relative organ weight was not significantly different between the control and treated groups. The present finding is supported by previous research that stated that the relative organ weight of mice treated with 2000 mg/kg bwt ethanolic extract of *F. deltoidea* leaves did not show a significant difference.[91] The haematological parameters (platelet, haematocrit, haemoglobin, neutrophil, eosinophil, basophil, lymphocyte and monocyte) showed that administration of FB2 beverage did not induce toxicity, as we did not observe any significant difference in this blood sample between control and treated groups. Analysis of blood parameters is relevant to risk assessment as the changes in the haematological system have a higher predictive value for human toxicity when data are transposed from animal studies.[92] It is therefore plausible to suggest that the FB2 beverage is not haematotoxic. The histopathological examination confirmed there were no changes seen at a single dose of 2000 mg/kg bwt in liver and kidney morphology harvested from both control and treated groups. In general, any injury to the parenchymal liver or kidney cells causes a change in the blood parameters.[93] Normal architecture seen on the liver and kidney tissues of treated rats suggests that there are no apparent adverse effects or morphological abnormalities caused by the oral administration of a single dose of 2000 mg/kg bwt of FB2 beverage.

CONCLUSIONS

The present study showed that the new functional beverage formulated from fine powder mixtures of *F. deltoidea* leaves and brown rice contains high amounts of phytochemical constituents and antioxidant activities. Consumers accepted the new beverage formulation with an overall acceptability rating of more than 5.0. The study also demonstrated that the newly developed beverage is safe to be used up to the tested concentration limit of 2000 mg/kg bwt.

ETHICAL ISSUES

All experimental procedures were approved by the ethics committee of the Animal Research and Ethics Committee, Universiti Teknologi Mara (UiTM CARE) with an approval number: UiTM CARE: 325/2020.

CONFLICTS OF INTEREST

All authors state there are no conflicts of interest.

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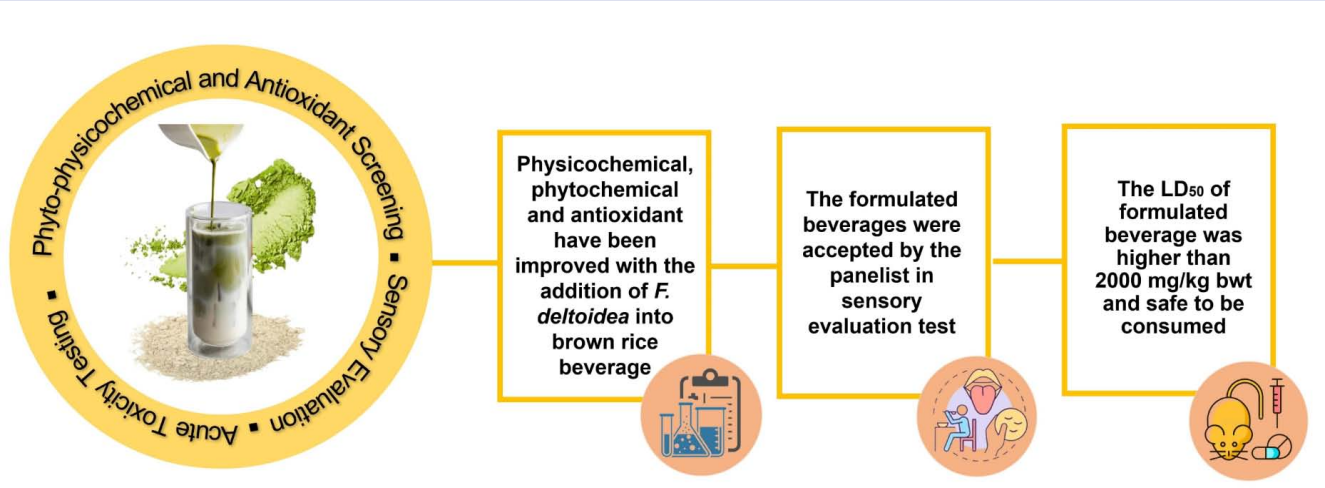
GRAPHICAL ABSTRACT



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