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Safety assessment of a new developed fruit juice product - mixed fruit Juice in experimental rats

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Abstract

The interest in dietary antioxidants which are mainly found in fruits, has prompted research in the field of commercial high antioxidant juice for healthy purposes. Fruits also are rich with antioxidants that help in reducing of degenerative diseases such as cancer, arthritis, cardiovascular disease and inflammation. Based on the health claims from the natural antioxidants, a new healthy juice called Mixed Fruit Juice (MFJ) has been developed by using three combinations of local fruits (soursop, mango and kasturi lime). In order to promote the commercial use of this product, the safety evaluation is needed to be carried out. The 28-days repeated toxicity test has been conducted in female and male rats for pre-clinical safety assessment prior to human study. There was no mortality observed when varying doses of the MFJ (5, 10 and 20%) administered to all rats. Hematological analysis showed no significant differences in most parameters examined. There were no significant changes observed in the liver and kidney functions tests of all treated-rats as compared to control normal rats. Furthermore, lipid profiles and blood glucose level were also within the normal range as noted in control rats. The present data demonstrate that the supplementation of MFJ did not produce adverse effects on the body system of experimental rats. This is the first documented report on the safety assessment of MFJ in rats.

Introduction

Fruits contain high concentrations of bioactive compounds including antioxidants that may be beneficial to health. It is suggested that regular consumption of fruit juices rich in polyphenols can enhance the protective effects against numerous degenerative diseases. Fruit juice is a nutritious beverage that is also included as an option within the fresh fruit servings. One glass of fruit juice is important source of fluids and provides vitamin C, folate, potassium and antioxidants (Landon, 2007)

The interest in the role of dietary antioxidants in human health has prompted research in the field of food science. There are a number of commercial fruit juices that base on their marketing strategies on antioxidant potency. Based on this, MARDI as a research institute in the agricultural area, carry the responsibility to utilize local resources such as tropical fruits to develop a high antioxidant juice product for human benefits. As a result, a new functional juice has been developed namely `Mixed Fruit Juice` (MFJ), which was a combination of soursop (*Anona muricata* Linn), mango (*Mangifera indica* var Chokanan) and kasturi lime *(Citrus microcarpa)*. The selections of these fruits were based on the other previous project related to the screening of various local tropical fruits for high antioxidant sources.

This product is categorized as functional juice that may have beneficial effects to human. However, there is no toxicological study been carried out on this product to investigate any adverse effect on the certain blood biochemical parameters that commonly used as safety biological marker.

Toxicological study using normal healthy rats is part of the safety assessment to investigate any adverse effects of the product before it can be consumed by human. A sub-chronic toxicity study was conducted to investigate the effect of MFJ in the body's system. In addition, the study was performed to further support the safety of MFJ upon its potential use as a functional beverage.

Thus, the aim of the present study was to determine the effect of supplementation of MFJ on the body weight, organ relative weight and blood biochemistry profiles using experimental rats. This is the first time, the potential adverse effects, if any, of this product in rats following sub-chronic administration. The

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scientific information is very important to validate its safety for human consumption and as a part of marketing strategy of MFJ in the future market.

Materials and Methods

MFJ sample preparation

Mixed Fruit Juice (MFJ) product was obtained from Food Technology Research Centre (MARDI) in Serdang, Selangor. This product has been developed successfully with established processing parameters. The MFJ was prepared using three combinations of fruits (mango, soursop and kasturi lime). The puree (soursop and mango) and juice from kasturi lime were mixed and heated until 80˚C for 15 min. Honey and water were added at a specific ratio.

Experimental animals

Male and female Sprague-Dawley (SD) rats at 8 weeks of age (160-220 g) were used for the sub-chronic toxicology studies (28 days repeated dose). The rats were obtained from the Animal House, University National of Malaysia (UKM). The animals were acclimatized to laboratory conditions at Animal House, MARDI (Serdang) for 7 days prior to the experiments. The rats were housed individually in polycarbonate cages, with free access to normal pellet (48% carbohydrate, 23% crude protein, 3% crude fat, 8% crude ash, 5% crude fiber and 13% moisture, AIN93 diet) and distilled water ad libitum. The rats were maintained at 22 ± 2°C under light/dark cycle of 12 hrs and relative humidity 70 ± 10%. The food pellets for the experimental animals were purchased from Gold Coin Holdings Sdn. Bhd. (Malaysia). All procedures in this study were performed according to the Animal Ethics Committee, International Islamic University of Malaysia (IIUM) document: IIUM/310/G/13/4/4.

Study design

The experiment was conducted according to the protocols describes by OECD Guideline 407 (OECD, 2008). The duration of the toxicological study (28 days) was reported to be enough for the safety study of food in human (Hor *et al.*, 2012). 48 SD male and female rats were randomly assigned into four groups each sexes; a control group and three treatment groups (low, medium and high dose of MFJ) with six rats for each group (female and male). The MFJ was dissolved in distilled water and administration orally on a daily basis for 28 consecutive days at single dose of 5%, 10% and 20% juice, respectively. The selection of this dosage was based on the previous toxicological studies on other fruit juices (Wang *et al*., 2002; Boateng *et al*., 2007; Hadijah *et al*., 2008). The control group received only distilled water for the comparison purposes (100 mL). All rats were supplied with the same amount of normal rat pellet (40 g per day) throughout the study.

The behavior of the rats was observed daily, and their weights were recorded once per week. At the end of the experiment (28 days), all rats were killed by using diethyl ether after an overnight fasting (13-15 hrs). 10 mL of fresh blood samples were drawn from the posterior vena cava into three types of blood tubes, i. plain (for clinical biochemistry analysis); ii. EDTA (whole blood was for haematological analysis); iii. Heparine (whole blood sample was for antioxidant enzyme analysis; plasma extract was for total antioxidant status). Serum or plasma samples were obtained after blood centrifugation at 3000 rpm for 10 min (4°C) using Blood Centrifuge (Mikro 22R, Hettigh, Germany). Serum and plasma were stored at -80°C for further analysis.

Vital organs weight and gross pathology

All surviving animals at the end of the study were subjected to complete necropsy. The weights of major organs such as the liver, kidney, spleen, lungs, heart and ovaries were recorded after the removal of peripheral fat tissue. All organs were examined macroscopically. The shapes, sizes and colors of these internal organs were visually observed for signs of gross lesions. Histopathology of vital organs (liver and kidney) was considered only in case of evidence of any gross pathological lesions. Absolute organ weights were measured and the relative organ weights were calculated as (organ/final body weight) X 100%.

Clinical biochemistry measurement

Serum samples were used to analyze clinical biochemistry parameters such as aspartate aminotransferase (AST), alanine aminotransferase (ALT), blood urea nitrogen (UREA), creatinine (CREA), total cholesterol (TCHO), LDL-Chol, HDL-CHOL, triglyceride (TG), total protein (TP), albumin (ALB) and total bilirubin (TBIL).

All parameters determined using Fully Automated Chemistry Analyzer (Vitalab, Selectra-E, Italy). Diagnostic kits used were from Randox (UK) supplied by All Eight Sdn. Bhd. (Malaysia).The reagent kits are as follows: AB 361 (albumin), AL 1200 (ALT), AS 1267 (AST), BR 411 (total bilirubin), CH 200 (cholesterol), CH 2652 (HDL-cholesterol), CR 510 (creatinine), TP 245 (total protein), TR 1697 (triglyceride), UR 220 (Urea) and UA 1613 (uric acid). Glucose value was obtained from rat's

blood tail sample and measured using Glucometer (Precision, Roche, UK).

Haematological parameters measurement

Haematological analysis parameters such as white blood cells count (WBC), red blood cells count (RBC), haemoglobin (HGB), haematocrete (HTC) and platelet (PLT) were determined using Haematology Anayzer (Medonic Vet, CA 503, UK). Diluent and lyser reagents were from Medonic that supplied by All Eight Sdn. Bhd. (Malaysia). Whole blood from EDTA-blood tube was used in this analysis and performed within 4 hrs after blood collection.

Statistical analysis

The significant differences between the control and treated groups were determined using ANOVA followed by Duncan New Multiple Range Test (DMRT) using SAS System ver. 9. All values are expressed as group mean ± standard error of mean (S.E.M.) with n = 6 rats. The minimal level of significance accepted was $p < 0.05$.

Results

Body weight and organs relative weight

There were no animal deaths in any of the groups (female and male rats) throughout the 28-days study. Physical and behavioral examinations did not reveal any treatment-related adverse effects after dosing. Normal body weight gains were observed in rats of all dose groups and control group as seen in Table 1. No significant different were observed between the control group and MFJ treatment groups for every sex.

The data for organ relative weights in female and male rats is presented in Table 2. Results revealed no significant differences noted in treatment groups as compared to control group in female and male rats. Moreover, the daily treatment with MFJ at all doses for 28 days did not induce observable toxicopathologic lesions in organs of all treated rats.

Serum clinical biochemistry parameters

The clinical biochemistry parameters for liver and kidney function tests are presented in Table 3. Treatment with MFJ showed no significant alterations between treated rats and control rats for both sexes in both parameters.

Meanwhile, data for general biochemistry parameters (glucose and lipid profile) is presented in Table 4. There were no significant differences in glucose and lipid profile between the control group and MFJ-treated groups for both sexes. No significant differences observed in all biochemical parameters measured on rats.

Haematological parameters

Important haematology parameters of rats (WBC, RBC, HGB, HCT, PLT) is shown in Table 5 (male and female rats). No significant differences were noticed between the MFJ-treated rats and control rats for both sexes.

Discussions

The present study demonstrated for the first time, the safety profile of MFJ in sub-chronic toxicity study in rats. It should be noted that the primary objective of repeat-dose toxicity study in animals is to identify the organs and (or) systems that are the targets of the product's toxicity and the threshold dosages of producing toxic effects (Yin Fan *et al*., 2010).

Evaluation of oral toxicity via a repeated dose 28-day experiment has been advocated as a fundamental requirement for assessing safety and has been applied in many safety assessment studies (Rosidah *et al*., 2009; Mohamed *et al*., 2011; Hor *et al*., 2012). For instance, famous fruit juice namely MonaVie Active® (containing 19 fruits and berries) had been evaluated for its safety or identify any concerns before releasing to the public market (Schauss *et al*., 2010). Pomegranate juice that has received much attention related to its antioxidant compound, punicalagins, was also been assessed for potential adverse effects using rats (Patel *et al*., 2008).

With regards to the safety evaluation of MFJ, no deaths and no treatment-related signs were observed in rats of all groups (male and female). All rats at each dosage group continued to gain weight (positive pattern) throughout the 28-days study of both sexes of rats (Table 1). The absolute organ weights in all treated groups of rats (female and male) did not differ significantly from those of the control rats as shown in Table 2.

In general, the repeated exposure to potentially toxic substances will lead to a slight reduction in the body weight gain and internal organ weights (Teo *et al*., 2002). Organ weight measurement was also important to access general toxicity because any change in organs weight was related to toxic effects (Hadijah *et al*., 2003). In this study, results showed no changes detected in the internal organ of rats in any of the treatment groups. Furthermore, macroscopic examinations at necropsy had revealed no changes attributable to the administration of MFJ. This result suggested no grossly toxic effect from the

Table 1. Effect of MFJ on weekly body weight changes in female and male *Sprague-Dawle* rats

Week	Control Rats (Distilled Water)	Low Dose (10% MFJ)	Medium Dose (15% MFJ)	High Dose (20% MFJ)
Female Rats				
1	247.79 ± 23.26	253.59 ± 24.58	245.26 ± 17.56	247.86 ± 16.49
2	250.76 ± 21.74	252.84 ± 25.57	248.69 ± 16.01	255.35 ± 17.58
3	263.70 ± 25.18	263.70 ± 26.89	259.09 ± 20.31	269.88 ± 18.73
4	255.24 ± 25.95	256.15 ± 23.31	252.23 ± 17.97	260.72 ± 18.90
Male Rats				
1	293.41 ± 21.05	298.88 ± 26.09	290.98 ± 20.14	288.72 ± 22.77
2	329.34 ± 25.43	340.17 ± 27.29	323.08 ± 23.77	327.77 ± 25.81
3	335.70 ± 35.85	349.38 ± 25.25	328.14 ± 29.26	331.73 ± 23.57
4	365.95 ± 35.36	365.49 ± 19.67	349.81 ± 30.34	359.22 ± 25.38

Data based on the mean concentration ± Standard Error of Mean (S.E.M).
n=6 in control and MFJ-treated rat.
Not significantly different at $p > 0.05$ in treated rats as compared to the control rats.

Table 2. Effect of MFJ on organs relative weight in female and male *Sprague-Dawley* rats

Week	Control Rats (Distilled Water)	Low Dose (10% MFJ)	Medium Dose (15% MFJ)	High Dose (20% MFJ)
Female Rats				
% liver	3.05 ± 0.24	3.19 ± 0.42	2.91 ± 0.20	3.02 ± 0.30
% kidney	0.65 ± 0.07	0.58 ± 0.12	0.60 ± 0.04	0.66 ± 0.07
% spleen	0.20 ± 0.04	0.19 ± 0.02	0.20 ± 0.04	0.21 ± 0.04
% heart	0.27 ± 0.07	0.30 ± 0.03	0.29 ± 0.02	0.29 ± 0.02
% lung	0.58 ± 0.04	0.55 ± 0.09	0.58 ± 0.05	0.52 ± 0.06
% ovary	0.27 ± 0.04	0.25 ± 0.04	0.23 ± 0.05	0.28 ± 0.09
Male Rats				
% liver	2.69 ± 0.31	2.52 ± 0.29	2.58 ± 0.23	2.67 ± 0.22
% kidney	0.55 ± 0.05	0.57 ± 0.04	0.49 ± 0.18	0.56 ± 0.04
% spleen	0.17 ± 0.04	0.18 ± 0.04	0.15 ± 0.02	0.17 ± 0.03
% heart	0.28 ± 0.03	0.28 ± 0.02	0.30 ± 0.03	0.29 ± 0.02
% lung	0.49 ± 0.11	0.52 ± 0.11	0.47 ± 0.09	0.46 ± 0.06
% testis	0.50 ± 0.14	0.57 ± 0.19	0.63 ± 0.12	0.54 ± 0.18

Data based on the mean concentration ± Standard Error of Mean (S.E.M).
n=6 in control and MFJ-treated rat.
Not significantly different at $p > 0.05$ in treated rats as compared to the control rats.

supplementation of MFJ in all rats.

With regards to the liver function parameters, the increase levels of liver enzymes such as AST, ALT and ALP in the blood are associated with damage of hepatic cells (Burger *et al*., 2005). For instance, the ALT and AST are specific enzyme markers of necrotic injury and cholestasis (Mohamed *et al*., 2011). Hepatotoxic substances cause damage to the liver cell membrane and these enzymes are leaked out into serum and shows increased activities that can be measured (Kumar *et al*., 2004). The increased value of these liver enzymes in the serum normally will reflect active liver damage (Irfan and Namik, 2002; Wang *et al*., 2004). The results in this study showed that there were no increased activities of liver enzymes (AST and ALT) in both sexes of rats after MFJ administration, indicating that the product is non-hepatotoxic to all rats (Table 3).

Besides liver enzymes, total protein, albumin and total bilirubin are among parameters used to evaluate the liver function status. A small elevation in plasma bilirubin is an important indicator of liver damage in laboratory animals or could be a sign of biliary duct obstruction (Rasekh *et al*., 2008). If the liver becomes irritated, the total bilirubin may rise in plasma (Mohamed *et al*., 2011). Moreover, determination of plasma protein like albumin can act as a criterion for assessing synthetic capacity of the liver since nearly all of them are synthesized in hepatocytes. Therefore, a decrease in plasma proteins will reflect chronic liver damage. In this study, data showed that there were no significant differences noted in total protein, albumin as well as bilirubin in all treated rats (female and male) as shown in Table 3. This indicates that these parameters were not affected by the MFJ supplementation.

Table 3. Effect of MFJ on liver and kidney function parameters in female and male *Sprague-Dawley* rats

Week	Control Rats (Distilled Water)	Low Dose (10% MFJ)	Medium Dose (15% MFJ)	High Dose (20% MFJ)
Female Rats				
(Liver Function Test)				
ALT (U/L)	43.86 ± 5.68	43.30 ± 8.77	46.00 ± 6.98	36.36 ± 10.59
AST(U/L)	157.00 ± 43.00	151.00 ± 33.00	139.00 ± 28.00	109.00 ± 14.00
TOTAL PROTEIN (g/L)	80.13 ± 4.96	78.31 ± 2.22	79.52 ± 3.26	78.00 ± 3.89
ALBUMIN (g/L)	44.20 ± 2.34	43.81 ± 1.12	42.01 ± 1.28	43.53 ± 2.53
TOTAL BILIRUBIN (µmol/L)	3.37 ± 1.12	3.32 ± 0.21	3.71 ± 0.74	3.20 ± 1.19
(Kidney Function Test)				
CREATININE (µmol/L)	74.9 ± 1.65	78.07 ± 5.54	76.98 ± 5.25	70.35 ± 5.47
UREA (mmol/L)	6.82 ± 0.86	6.82 ± 0.97	6.79 ± 0.88	6.02 ± 0.67
URIC ACID (mmol/L)	0.22 ± 0.07	0.25 ± 0.14	0.24 ± 0.05	0.27 ± 0.14
Male Rats				
(Liver Function Test)	71.71 ± 3.20	69.00 ± 6.98	65.67 ± 5.20	65.29 ± 12.11
ALT (U/L)	129.00 ± 58.00	149.00 ± 23.00	154.00 ± 32.00	146.00 ± 34.00
AST(U/L)	82.80 ± 4.66	78.59 ± 2.18	80.49 ± 3.22	80.69 ± 2.95
TOTAL PROTEIN (g/L) ALBUMIN (g/L)	42.06 ± 3.62	39.24 ± 1.81	40.13 ± 3.04	40.94 ± 1.09
TOTAL BILIRUBIN (µmol/L)	3.22 ± 1.36	2.03 ± 1.3	2.79 ± 0.58	3.24 ± 0.29
(Kidney Function Test)				
CREATININE (µmol/L)	67.26 ± 4.01	65.61 ± 5.00	63.97 ± 4.67	66.55 ± 4.65
UREA (mmol/L)	5.08 ± 1.05	5.40 ± 0.42	5.65 ± 0.44	5.34 ± 0.75
URIC ACID (mmol/L)	0.17 ± 0.07	0.16 ± 0.07	0.16 ± 0.04	0.17 ± 0.04

Data based on the mean concentration ± Standard Error of Mean (S.E.M).
n=6 in control and MFJ-treated rat.
Not significantly different at $p > 0.05$ in treated rats as compared to the control rats.

Table 4. Effect of MFJ on lipid profiles and glucose parameters in female and male *Sprague-Dawley* rats

Week	Control Rats (Distilled Water)	Low Dose (10% MFJ)	Medium Dose (15% MFJ)	High Dose (20% MFJ)
Female Rats				
CHOLESTROL(mmol/L)	2.10 ± 0.28	1.81 ± 0.34	2.04 ± 0.29	2.20 ± 0.37
TRIGLYCERIDE(mmol/L)	0.66 ± 0.16	0.60 ± 0.25	0.55 ± 0.16	0.62 ± 0.13
HDL(mmol/L)	0.79 ± 0.12	0.65 ± 0.08	0.77 ± 0.08	0.79 ± 0.09
LDL(mmol/L)	0.30 ± 0.13	0.26 ± 0.06	0.33 ± 0.14	0.36 ± 0.13
GLUCOSE (mmol/L)	4.84 ± 0.46	4.97 ±0.75	4.67 ± 0.45	4.83 ± 0.56
Male Rats				
CHOLESTROL(mmol/L)	1.69 ± 0.14	1.84 ± 0.26	1.65 ± 0.20	1.81 ± 0.22
TRIGLYCERIDE(mmol/L)	0.68 ± 0.16	0.62 ± 0.19	0.63 ± 0.21	0.58 ± 0.18
HDL(mmol/L)	0.47 ± 0.05	0.56 ± 0.09	0.49 ± 0.08	0.55 ± 0.07
LDL(mmol/L)	0.91 ± 0.13	1.00 ± 0.15	0.87 ± 0.20	1.00 ± 0.21
GLUCOSE (mmol/L)	4.50 ± 0.72	3.98 ± 0.33	4.20 ± 0.77	4.50 ± 0.82

Data based on the mean concentration ± Standard Error of Mean (S.E.M).
n=6 in control and MFJ-treated rat.
Not significantly different at $p > 0.05$ in treated rats as compared to the control rat.

Kidney was the second organ most frequently affected by any plant-based compounds (Hadijah *et al.*, 2003). Kidney functions were evaluated by means of urea, uric acid and creatinine in rat's serum (Hor *et al*., 2012). It is well known that almost all drugs, chemicals and xenobiotics are eliminated through renal excretion; hence it is necessary to estimate the effects of MFJ on kidney functions (Biswas *et al*., 2010). Increase blood creatinine and urea is a good indicator of the negative impact in kidney functions (Hassan *et al*., 2007). Furthermore, elevated serum urea is as a result of toxic effects on the renal tubule and renal parenchyma (Evan, 2009). In the present study, the data showed that there were no significant differences in the urea, creatinine and uric acid levels of the rats (female and male) supplied with MFJ as compared to the rats in control group (Table 3). The results suggested that the MFJ did not cause nephtrotoxicity effects to all rats of both sexes.

As for the general biochemistry parameters (lipid profile and glucose), the supplementation of MFJ also did not give any significant differences when compared to control rats, indicating that the lipid and glucose metabolism were not affected (Table 4). The glucose concentration is in concern when relates to the fruit juice consumption. This is due to the previous reports indicated the high intake of fruit beverages with the increase risk of diabetes (Schulze

Table 5. Effect of MFJ on haematology parameters in female and male *Sprague-Dawley* rats

Week	Control Rats (Distilled Water)	Low Dose (10% MFJ)	Medium Dose (15% MFJ)	High Dose (20% MFJ)
Female Rats				
RBC(10^{12}/L)	8.91 ± 0.49	9.27 ± 0.61	9.08 ± 0.43	9.15 ± 0.52
PLT(10^9/L)	1098.43 ± 103.66	981.5 ± 183.33	1233.36 ± 89.14	1145.14 ± 78.74
WBC(10^9/L)	4.793 ± 1.48	5.108 ± 1.42	4.864 ± 0.55	5.486 ± 1.53
HCT(%)	48.08 ± 2.43	49.33 ± 2.78	47.29 ± 2.06	47.49 ± 3.03
HGB(g/L)	171.57 ± 8.08	175.83 ± 10.89	171.43 ± 7.54	173.50 ± 10.69
Male Rats				
RBC(10^{12}/L)	9.19 ± 0.55	9.39 ± 0.59	9.94 ± 1.12	9.93 ± 0.41
PLT(10^9/L)	1095.29 ± 84.27	1017.71 ± 291.78	1032.50 ± 263.1	1167.93 ± 194.56
WBC(10^9/L)	5.24 ± 1.37	6.48 ± 1.38	5.84 ± 1.589	6.44 ± 2.19
HCT(%)	47.63 ± 3.48	48.29 ± 3.76	50.58 ± 6.02	49.71 ± 1.93
HGB(g/L)	165.64 ± 10.66	169.07 ± 12.46	177.86 ± 18.98	178.00 ± 5.97

Data based on the mean concentration ± Standard Error of Mean (S.E.M).
n=6 in control and MFJ-treated rat.
Not significantly different at $p > 0.05$ in treated rats as compared to the control rats.

et al., 2004; Palmer *et al*., 2008). However, study by Eshak *et al*. (2013) suggested that the 100% fruit and vegetable juices were not associated with the increased risk of type 2 diabetes in Japanese women. The naturally occurring sugars in the 100% fruit / vegetable juices may have different metabolic effects than those of high fructose corn syrup added to non-100% juice or fruit beverages. On the other hand, fruit juices have a low glycemic index (GI) which is help people to manage diabetes (Landon, 2007). In addition, a greater quantity of fruits and vegetables intake was associated with 21% lower hazard of type 2 diabetes in human intervention study conducted by Cooper *et al*. (2012).

Meanwhile, an analysis of whole blood parameters is relevant to risk evaluation as the changes in the haematological system have a higher predictive value for human toxicity (Olson *et al.*, 2000). In human, haematology analysis is normally most recommended in the Pathology Service Unit to determine any diseases or toxic effects (Halimah, 2001). In this study, the haematological profiles (RBC, WBC, platelet counts, hemoglobin and hematocrit) of treated rats showed no significant differences with rats in control group as presented in Table 5. The results showed no deleterious effects on blood counts and haemoglobin content, thereby suggesting that MFJ had no toxic effect on haematopoiesis or leukopoiesis in rats. Haematopoises is the formation of blood cellular components. Leukopoiesis is a form of haematopoiesis in which white blood cells are formed in bone marrow located in bones in adults and hematopoietic organs in the fetus (Evan, 2009).

Conclusions

In this present study, the 28-days sub-chronic toxicological test showed no systemic toxicity attributable to MFJ administration in all rats. This study did not result in mortality and was not associated with adverse effects on the general condition, body weight, relative organ weight, or haematological and biochemical parameters, nor did it show abnormalities in macroscopic findings. Thus, daily oral administration of MFJ for 28-days did not cause any adverse effects in female and male Sprague-Dawley rats. Therefore, this oral toxicity study provides considerable evidence of the safety of MFJ as the results relates to continuous daily intake.

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