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Toxicity Study of Antidiabetics Functional Drink of *Piper crocatum* and *Cinnamomum burmannii*

MEGA SAFITHRI[1]*, SEDARNAWATI YASNI[2], MARIA BINTANG[1], ANNA SETIADI RANTI[3]

[1]Departement of Biochemistry, Faculty of Mathematic and Natural Science, Bogor Agricultural University, Darmaga Campus, Bogor 16680, Indonesia
[2]Departement of Food Science and Technology, Faculty of Agricultural Tehcnology, Bogor Agricultural University, Darmaga Campus, Bogor 16680, Indonesia
[3]Departement of Natural Cosmetics Development, Faculty of Farmacy, Pancasila University, Srengseng Sawah, Jagakarsa, Jakarta 12640, Indonesia

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Piper crocatum* and *Cinnamomum burmannii* formulations is known to be a new diabetes functional drink. Thus, its toxicological profile needs to be studied. At present, the formulation was evaluated for the repeated dose toxicity study. The *Sprague dawley* albino rats were treated with *P. crocatum* and *C. burmannii* formulations (0, 630, 1260, and 1890 mg/kg) and administered orally for a period of 28 days in albino rats. The effects on body weight, food and water consumption, organ weight, hematology, clinical biochemistry as well as histology were studied. There were no significant differences in the body weight, organ weights and feeding habits between control and treated animals. Hematological analysis showed no marked differences in any of the parameters examined in either the control or treated groups. There were no significant changes that occurred in the blood chemistry analysis including glucose, cholesterol, triglycerides, creatinine, SGPT, and SGOT in experimental animals. Pathologically, neither gross abnormalities nor histopathological changes were observed. The formulation of *P. crocatum* and *C. burmannii* was found safe in repeated dose toxicity studies.

Key words: *Piper crocatum, Cinnamomum burmannii*, repeated dose toxicity

INTRODUCTION

Indonesia is ranked fourth for having the highest number of diabetics (5.6 million in the year 2001 and is predicted to increase to 8.2 million in the year 2020) in the world after India, China, and USA (Boyle *et al*. 2001). Diabetics can suffer from chronic complications of nephropathy (renal failure), neuropathy (nerve dysfunction) and retinopathy (retinal eye disorders), cardiovascular disorders, and can cause hypertension due to free radicals produced during states of hyperglycemia (Ceriello 2003). Antioxidants are important inhibitors of lipid peroxidation as a defence mechanism of living cells against oxidative damage (Mahdi *et al*. 2003; Ghosh *et al*. 2008). Plants are potential sources of natural antioxidants. Researches in traditional medicine for appropriate hypoglicemic agents have been focused on plants due to the fact that traditional medicines give better treatments than drugs (Grover *et al*. 2002; Gbolade 2008; Erejuwa *et al*. 2010).

Traditionally in diabetes treatment, people used decoctions of *Piper crocatum* (Piperaceae). According to previous study, ten days of treatment with various doses of decocted extract of *P. crocatum* prevented body weight reduction of up to 5-52% and reduced blood sugar level by 10-38%. The phytochemicals assay of *P. crocatum* showed that decoctions contained alkaloid, flavonoid and tanin (Safithri & Fahma 2008). They were known to be bioactive anti-diabetic and antioxidant compounds (Satyanarayana 2006; Battu *et al*. 2007; Tapas *et al*. 2008). *P. crocatum* extract, however, has bitter taste, so it is not preferred by consumers. To overcome that, it can be combined with *Cinnamomum burmannii*, in order to obtain a more acceptable flavor and at the same time preserve the functionality of the product.

Cinnamomum burmannii showed relatively high inhibitory activities against the five foodborne pathogenic. It could be seen from the diameter of the clear zone of each of the bacteria observerd. They were *B. cereus* (15.4 mm), *L. monocytogenes* (11.5 mm), *S. aureus* (15.7 mm), *E. coli* (8.7 mm), and *S. anatum* (12.1 mm). It had antioxidant capacity (107.7 mmol trolox/100 g dry weight) (Shan *et al*. 2007). Cinnamon is used for flavor and taste in food preparation, however cinnamon may also have additional roles in glucose metabolism and blood pressure regulation (Preuss *et al*. 2006). It was reported that 1, 3, and 6 grams of cinnamon per day had beneficial effects on blood glucose, cholesterol and triglycerides of people with type 2 diabetes (Khan *et al*. 2003). *Cinnamomum* has been granted generally recognized as safe (GRAS) status as a food additive by the U.S. food and drug administration (FDA), whereas there are many limitations regarding the safety and efficacy of these formulations. In this study,

*Corresponding author. Phone/Fax: +62-251-8423247, E-mail: mega_safithri@yahoo.com

repeated dose oral toxicity study of *P. crocatum* and *C. burmanniii* formulation, were investigated in experimental animals.

MATERIALS AND METHODS

Plant Material. *P. crocatum* and *C. burmannii* were collected from Indonesian Medicinal and Aromatic Crops Research Institute (IMACRI). The material was identified in the Herbarium Bogoriense Indonesian Institute of Sciences (LIPI) , Bogor.

Preparation of Functional Drink from *P. crocatum* and *C. burmannii.* Dried leaves of *P. crocatum* (10 g) were boiled in water (200 ml), and the process was terminated when the volume reached up to 100 ml. Dried bark of *C. burmannii* (20 g) were boiled in water (200 ml), and the process was terminated when the volume reached up to 100 ml. The decoction was filtered through a Whatman no. 4 filter paper and the resulting extract were used in the biological assays. *P. crocatum* extract was mixed with the extract of *C. burmannii* with a ratio of 1:0.6. Then, it was added the sweetener, stevia, as much as 0.67% (v/v).

Experimental Animals. *Sprague dawley* albino male and female rats (210-260 g) were obtained from the experimental animal facility of The National Agency of Drugs and Food Control (BPOM) Republic of Indonesia. Four weeks prior and during the experiment, rats were fed with a standard pellet diet (CP Rodent, Thailand; 18% protein, 3% fat, 13% water, 10% ashes, 9% fiber, 9000 IU/kg Vit A, 1800 IU/kg Vit D_3, 80 IU/kg Vit E, and 800 mg/kg Vit C). Randomly chosen rats acclimatized for 7 days in 24 $\pm$ 1 ^{o}C with 12- h light/dark cycle and 55-75% relative humidity [Organisation for Economic Co-operation and Development (OECD) Guideline for the Testing of Chemicals 1995].

Experimental Design. Fourty *Sprague dawley* rats (210-260 g) were randomly divided into 4 groups: the control group was given distilled water (5 males and 5 females), groups 2, 3, and 4 were given a drink of functional drink with each dose group given 0, 630, 1260, and 1890 mg/kg bw, respectively, for 28 days. The number of rats for each group was set at 5 males and 5 females. The maximum volume administered to each rat was not greater than 2 ml/100 g body weight. The animals had free access to food and water *ad libitum*.

All rats were observed daily for toxic manifestations and mortality. Body weight, food and water consumption for all the rats were measured on day -7, 0, 7, 14, 21, 28. Blood samples (2 males and 2 females from each group) were obtained from the tail vein in fasting rats for 18 hours on day 0 and 28. Necropsy and histopathological examination were done on day 28 (2 males and 2 females in each group). The experimental protocols were approved by the Institutional Animal Ethics Committee (Animal Care and Use Committee of PT. Bimana Indomedical R.02-11-IR).

Hematology. Hematological analysis was performed using an automatic hematological analyzer (Celltac α, Automated Hematology Analyzer MEK-6450, Nihon Kohden, Japan). Hemoglobin, hematocrit, total red blood corpuscles (RBC), total white blood corpuscles (WBC), platelets and red cell indices viz., packed cell volume (PCV), mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH) and mean corpuscular hemoglobin concentration (MCHC) of blood samples were recorded.

Clinical Biochemistry. Glucose, triglycerides, cholesterol, creatinine, serum glutamate pyruvate transaminase (SGPT), and serum glutamate oxaloacetate transaminase (SGOT), were analyzed, using an auto-analyzer (Clinical Chemistry Analyzer Selectra Yunior 69.154).

Pathological Examination Gross Necropsy. All animals in the study were subjected to a full, detailed gross necropsy which included careful examination of the external surface of the body, all orifices, and the cranial, thoracic, and abdominal cavities and their contents. The brain, heart, liver, kidney, spleen and pancreas of all animals were removed and their wet weights were taken immediately after dissection to avoid drying.

Histopathology. The livers and kidneys were fixed immediately in 10% formalin for routine histopathological examination. The tissues were embedded in paraffin, then sectioned and stained with haematoxylin and eosin, and were examined under a light microscope. Photomicrographs of the microscopical sections were taken with the help of Nikon Eclipse 80i. DS Fi1, Japan.

Statistical Analysis. The differences among experimental and control groups were determined using the MNITAB 14 statistical software for Windows. Comparisons among different groups were performed by analysis of variance using ANOVA test. Significant differences between control and experimental groups were assessed by Tukey *t*-test. All data are expressed as a mean $\pm$ standard error of mean (S.E.M.). *p*-values less than 0.05 were considered to be significant.

RESULTS

Body Weight, Food and Water Intake. In repeated dose toxicity study, administration of functional drink created from *P. crocatum* and *C. burmannii* tended to maintain the body weight of the rats that were tested (Table 1). The rats showed no significant change ($P < 0.05$) in body weights, both male and female, during the trial period. Similarly, no significant change ($P < 0.05$) in food and water intake (Table 2 & 3), both male and female, were observed between control and treatment groups during the trial period. However, male and female rats treated to 1890 mg/kg bw of functional drink for 28 days showed the largest degradation of body weight (2.2 and 3.9%).

Hematology and Clinical Biochemistry. The effect of the repeated dose of oral administration of functional drink from *P. crocatum* and *C. burmannii* on hematological parameters (Table 4) showed no significant changes ($P < 0.05$) in red blood cells (RBC), white blood cells (WBC), haemoglobin, hematocrit, platelet, mean corpuscular hemoglobin (MCH), mean corpuscular haemoglobin concentration (MCHC), mean corpuscular volume (MCV),

Table 1. The effect of 28 days treatment with functional drink form *P. crocatum* and *C. burmannii* on male and female rats body weight

Day	Avarage body weight (g)							
	Control		Functional drink from *P. crocatum* and *C. burmannii*					
	0 mg/kg bw		630 mg/kg bw		1260 mg/kg bw		1890 mg/kg bw	
	Male	Female	Male	Female	Male	Female	Male	Female
-7	233.4 ± 11.2a	235.8 ± 16.7a	225.6 ± 5.9a	228.4 ± 7.5a	232.4 ± 5.9a	233.2 ± 13.9a	237.2 ± 15.3a	234.0 ± 9.5a
0	221.1 ± 12.1a	235.9 ± 14.8a	213.8 ± 7.8a	220.8 ± 11.1a	219.7 ± 9.2a	234.2 ± 7.4a	228.1 ± 14.9a	230.9 ± 13.6a
7	219.7 ± 11.5a	229.4 ± 20.4a	205.4 ± 8.0a	227.8 ± 6.0a	214.0 ± 10.1a	228.0 ± 6.4a	220.8 ± 14.9a	226.0 ± 17.7a
14	216.9 ± 11.8a	230.3 ± 22.0a	203.7 ± 8.5a	226.3 ± 6.8a	214.9 ± 11.9a	226.9 ± 7.6a	217.1 ± 16.5a	222.5 ± 9.9a
21	215.1 ± 12.1a	228.6 ± 22.5a	206.8 ± 9.0a	225.8 ± 5.8a	216.4 ± 13.2a	226.4 ± 7.2a	215.1 ± 17.5a	220.4 ± 10.3a
28	220.7 ± 14.5a	230.7 ± 24.7a	212.1 ± 9.1a	227.9 ± 6.8a	222.3 ± 10.1a	229.1 ± 10.2a	223.0 ± 19.6a	221.9 ± 10.2a

The same letter(s) indicated not significant difference on P < 0.05 in the same colom.

Table 2. The effect of 28 days treatment with functional drink form *P. crocatum* and *C. burmannii* on male and female rats food consumption

Day	Avarage food consumption (g)							
	Control		Functional drink from *P. crocatum* and *C. burmannii*					
	0 mg/kg bw		630 mg/kg bw		1260 mg/kg bw		1890 mg/kg bw	
	Male	Female	Male	Female	Male	Female	Male	Female
-7	11.7 ± 2.2a	11.8 ± 5.6a	11.3 ± 1.2a	11.4 ± 1.5a	11.6 ± 1.2a	11.7 ± 2.8a	11.9 ± 3.1a	11.7 ± 1.9a
0	11.1 ± 2.4a	11.8 ± 2.9a	10.7 ± 1.6a	11.1 ± 2.2a	11.1 ± 1.8a	11.7 ± 1.5a	11.4 ± 2.9a	11.5 ± 2.7a
7	11.1 ± 2.3a	11.5 ± 4.1a	10.3 ± 1.6a	11.4 ± 1.2a	10.7 ± 2.1a	11.4 ± 1.3a	11.1 ± 3.1a	11.3 ± 3.5a
14	10.8 ± 2.4a	11.5 ± 4.4a	10.2 ± 1.7a	11.3 ± 1.4a	10.8 ± 2.4a	11.3 ± 1.5a	10.9 ± 3.3a	11.1 ± 1.9a
21	10.9 ± 2.4a	11.4 ± 4.5a	10.3 ± 1.8a	11.3 ± 1.2a	10.8 ± 2.6a	11.3 ± 1.4a	10.8 ± 3.5a	11.1 ± 2.1a
28	11.1 ± 2.9a	11,5 ± 4.9a	10.6 ± 1.8a	11.4 ± 1.4a	11.2 ± 2.1a	11.5 ± 2.1a	11.2 ± 3.9a	11.1 ± 2.2a

The same letter(s) indicated not significant difference on P < 0.05 in the same colom.

Table 3. The effect of 28 days treatment with functional drink from *P. crocatum* and *C. burmannii* on male and female rats water consumption

Day	Avarage water consumption (ml)							
	Control		Functional drink from *P. crocatum* and *C. burmannii*					
	0 mg/kg bw		630 mg/kg bw		1260 mg/kg bw		1890 mg/kg bw	
	Male	Female	Male	Female	Male	Female	Male	Female
-7	23.3 ± 1.1a	23.6 ± 1.7a	22.6 ± 0.6a	22.8 ± 0.8a	23.3 ± 5.9a	23.3 ± 1.4a	23.8 ± 1.5a	23.4 ± 1.1a
0	22.1 ± 1.2a	23.6 ± 1.5a	21.4 ± 0.8a	22.1 ± 1.1a	22.1 ± 0.9a	23.4 ± 0.7a	22.8 ± 1.5a	23.1 ± 1.4a
7	22.1 ± 1.1a	22.9 ± 2.1a	20.6 ± 0.8a	22.8 ± 0.6a	21.4 ± 1.1a	22.8 ± 0.6a	22.1 ± 1.5a	22.6 ± 1.8a
14	21.7 ± 1.2a	23.1 ± 2.2a	20.4 ± 0.9a	22.6 ± 0.7a	21.5 ± 1.2a	22.7 ± 0.7a	21.7 ± 1.7a	22.3 ± 1.1a
21	21.5 ± 1.2a	22.9 ± 2.3a	20.7 ± 0.9a	22.6 ± 0.6a	21.7 ± 1.3a	22.6 ± 0.7a	21.5 ± 1.8a	22.1 ± 1.1a
28	22.1 ± 1.5a	23.1 ± 2.5a	21.2 ± 0.9a	22.8 ± 0.7a	22.2 ± 1.1a	22.9 ± 1.1a	22.3 ± 2.1a	22.2 ± 1.1a

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Table 4. The effect of 28 days treatment with functional drink from *P. crocatum* and *C. burmannii* on male and female rats hematological parameters

Hematological 1 parameters	Day	Control		Functional drink from *P. crocatum* and *C. burmannii*					
		0 mg/kg bw		630 mg/kg bw		1260 mg/kg bw		1890 mg/kg bw	
		Male	Female	Male	Female	Male	Female	Male	Female
RBC (10^6 µl)	0	8.7 ± 1.0a	8.1 ± 0.2a	9.5 ± 0.4a	7.8 ± 0.2a	9.2 ± 1.3a	8.0 ± 0.4a	10.1 ± 0.6a	8.5 ± 0.2a
	28	7.1 ± 0.8a	7.5 ± 0.2a	7.2 ± 0.9a	6.1 ± 0.6a	7.9 ± 0.1a	7.3 ± 0.3a	8.2 ± 0.4a	7.2 ± 0.2a
WBC (10^3 µl)	0	10.1 ± 2.0a	17.7 ± 1.1a	10.9 ± 0.4a	10.8 ± 4.0a	11.1 ± 2.1a	13.2 ± 0.3a	8.9 ± 1.6a	10.1 ± 2.1a
	28	7.4 ± 5.3a	12.0 ± 2.6a	7.3 ± 0.5a	6.9 ± 2.4a	10.2 ± 0.5a	10.6 ± 3.5a	7.7 ± 0.8a	11.8 ± 5.5a
Haemoglobin (g/dl)	0	18.4 ± 1.1a	16.4 ± 0.7a	18.6 ± 1.2a	16.3 ± 1.0a	17.6 ± 2.8a	16.5 ± 0.8a	19.2 ± 0.6a	17.7 ± 0.5a
	28	14.3 ± 1.6a	15.3 ± 0.3a	13.5 ± 1.0a	13.3 ± 1.1a	15.0 ± 0.4a	15.4 ± 0.8a	15.1 ± 0.1a	15.1 ± 0.1a
Hematocrit (%)	0	47.6 ± 4.5a	45.8 ± 3.0a	50.1 ± 1.1a	44.0 ± 3.3a	48.5 ± 7.1a	48.9 ± 3.0a	52.0 ± 1.0a	48.0 ± 0.2a
	28	38.7 ± 5.4a	41.1 ± 1.1a	36.9 ± 3.7a	35.8 ± 3.7a	40.9 ± 0.8a	41.6 ± 2.2a	42.4 ± 0.1a	40.7 ± 0.3a
Platelet (10^3 µl)	0	787.5 ± 21.9a	857.5 ± 47.4a	801.5 ± 64.4a	925.5 ± 14.9a	676.5 ± 43.1a	1039.0 ± 159.8a	696.0 ± 14.1a	843.0 ± 17.0a
	28	911.5 ± 403.8a	816.5 ± 94.0a	648.5 ± 71.4a	952.5 ± 248.2a	894.0 ± 328.1a	878.0 ± 39.6a	819.5 ± 248.2a	912.0 ± 297.0a
MCH (ρg)	0	21.3 ± 1.3a	20.2 ± 0.4a	19.6 ± 2.1a	20.9 ± 0.7a	19.2±0.2a	20.7 ± 0.2a	19.4 ± 2.1a	20.8 ± 0.2a
	28	20.2 ± 0.1a	20.3 ± 0.8a	18.8 ± 1.0a	21.8 ± 0.3a	19.0±0.5a	21.3 ± 0.3a	18.3 ± 0.8a	21.0 ± 0.9a
MCHC (g/dl)	0	38.7 ± 1.3a	35.9 ± 0.8a	37.1 ± 1.7a	37.0 ± 0.4a	36.2±0.3a	35.1 ± 0.6a	37.0 ± 1.8a	36.8 ± 0.9a
	28	36.9 ± 1.0a	37.2 ± 0.3a	36.6 ± 1.0a	37.2 ± 0.8a	36.6±0.1a	37.1 ± 0.1a	35.4 ± 0.1a	37.1 ± 0.2a
MCV (fL)	0	55.1 ± 1.2a	56.2 ± 2.1a	52.9 ± 3.3a	56.6 ± 2.4a	52.9±0.2a	58.9 ± 0.4a	51.4 ± 1.9a	56.4 ± 0.8a
	28	54.6 ± 1.2a	54.5 ± 2.7a	51.4 ± 1.3a	58.4 ± 0.1a	51.8±1.2a	57.4 ± 0.9a	51.7 ± 2.3a	56.6 ± 2.3a
MPV (fL)	0	3.8 ± 0.1a	4.3 ± 0.1a	3.8 ± 0.3a	4.6 ± 0.1a	3.7±0.1a	4.4 ± 0.4a	3.9 ± 0.2a	4.0 ± 0.6a
	28	4.9 ± 1.9a	4.2 ± 0.8a	4.2 ± 1.1a	4.8 ± 1.4a	3.8±0.5a	4.2 ± 0.7a	4.0 ± 0.6a	4.2 ± 0.4a
PDW (%)	0	16.2 ± 0.7a	17.1 ± 0.8a	16.4 ± 0.3a	15.9 ± 0.2a	16.6±0.1a	16.5 ± 0.6a	16.4 ± 0.1a	16.2 ± 0.4a
	28	16.2 ± 1.3a	16.1 ± 0.7a	15.9 ± 0.1a	16.7 ± 0.9a	15.9±0.1a	15.6 ± 0.7a	16.0 ± 0.8a	15.0 ± 0.1a
RDW (%)	0	13.5 ± 0.2a	12.9 ± 0.1a	13.1 ± 2.0a	12.8 ± 0.6a	13.2±0.2a	13.4 ± 0.1a	12.3 ± 0.4a	14,1 ± 1.5a
	28	12.4 ± 1.1a	12.6 ± 0.3a	14.5 ± 0.1a	13.7 ± 0.4a	13.4±1.3a	13.0 ± 0.6a	13.8 ± 0.1a	12.8 ± 0.6a

The same letter(s) indicated not significant difference on P < 0.05 in the same colom.

mean platelet volume (MPV), platelet distribution wide (PDW) and red distribution wide (RDW) in male and female rats for the treatment group, when compared to the control group. However, female rats' blood WBC, platelet, MCH, MCV, MPV levels tend to be higher than male rats'. Otherwise, male rats' blood RBC, haemoglobin, hematocrit, MCHC, PDW, RDW levels tend to be higher than female rats'.

The effect of the repeated dose of oral administration of functional drink from *P. crocatum* and *C. burmannii* on biochemical parameters (Table 5) showed no significant differences observed in any of the biochemical parameters (glucose, triglycerides, cholesterol, creatinine, SGPT, and SGOT) examined in either the control or treated group for the male and female rats. However, female rats' blood glucose, cholesterol, and SGPT level tend to be higher than those of male rats. Otherwise, male rats' blood triglycerides, creatinine, and SGOT level tend to be higher than those of female rats.

Pathological Examination. There were no significant differences between the control and treated groups in the organ weights of male and female rats (Table 6). However, liver, brain, lung, pancreas, spleen, adrenal, and thymus organ weight of female rats tend to be higher than those of male rats. Otherwise, heart, kidney, bladder, and salivary gland organ weight of male rats tend to be higher than those of female rats.

Table 5. The effect of 28 days treatment with functional drink from *P. crocatum* and *C. burmannii* on male and female rats biochemical parameters

Biochemical parameters	Day	Control		Functional drink from *P. crocatum* and *C. burmannii*					
		0 mg/kg bw		630 mg/kg bw		1260 mg/kg bw		1890 mg/kg bw	
		Male	Female	Male	Female	Male	Female	Male	Female
Glucose (mg/dl)	0	60.5 ± 4.9a	74.5 ± 0.7a	57.0 ± 4.2a	84.0 ± 26.9a	72.5 ± 14.9a	74.0 ± 21.2a	70.0 ± 12.7a	79.0 ± 12.7a
	28	61.5 ± 10.6a	68.0 ± 22.6a	63.0 ± 4.2a	81.5 ± 19.1a	55.5 ± 3.5a	64.0 ± 19.8a	58.5 ± 10.6a	80.0 ± 2.8a
Triglycerides (mg/dl)	0	61.5 ± 0.7a	26.5 ± 0.7a	40.5 ± 9.9a	36.5 ± 9.2a	41.0 ± 1.4a	29.5 ± 4.9a	47.5 ± 2.1a	29.0 ± 1.8a
	28	44.0 ± 5.7a	29.0 ± 4.2a	30.5 ± 0.7a	32.5 ± 1.4a	40.0 ± 5.7a	30.0 ± 5.7a	39.5 ± 3.5a	33.0 ± 1.4a
Cholesterol (mg/dl)	0	70.0 ± 8.5a	87.0 ± 9.9a	66.0 ± 12.7a	84.5 ± 6.4a	78.5 ± 4.9a	96.5 ± 6.4a	70.5 ± 0.7a	91.5 ± 2.1a
	28	87.0 ± 8.5a	66.0 ± 18.4a	77.0 ± 4.3a	85.0 ± 8.5a	87.5 ± 0.7a	103.5 ± 2.1a	84.0 ± 1.4a	84.5 ± 7.8a
Creatinine (mg/dl)	0	0.66 ± 0.24a	0.58 ± 0.19a	0.51 ± 0.35a	0.82 ± 0.01a	0.51 ± 0.30a	0.61 ± 0.31a	0.41 ± 0.17a	0.63 ± 0.21a
	28	0.60 ± 0.14a	0.51 ± 0.06a	0.59 ± 0.17a	0.47 ± 0.06a	0.47 ± 0.09a	0.50 ± 0.01a	0.41 ± 0.17a	0.50 ± 0.03a
SGPT (U/L)	0	55.0 ± 5.7a	65.5 ± 4.9a	51.0 ± 5.7a	49.5 ± 0.7a	48.5 ± 7.8a	55.5 ± 16.3a	49.0 ± 8.5a	52.5 ± 13.4a
	28	34.5 ± 3.5a	54.5 ± 0.7a	40.0 ± 4.2a	58.5 ± 3.5a	45.5 ± 2.1a	52.5 ± 3.6a	44.5 ± 7.8a	56.0 ± 18.4a
SGOT (U/L)	0	60.0 ± 7.1a	55.0 ± 5.7a	55.0 ± 1.4a	42.5 ± 3.5a	64.0 ± 8.5a	52.0 ± 2.8a	54.0 ± 4.2a	49.0 ± 5.7a
	28	50.0 ± 11.3a	46.5 ± 0.7a	43.5 ± 0.7a	54.5 ± 0.7a	48.0 ± 5.7a	50.5 ± 4.9a	51.0 ± 57a	45.0 ± 4.2a

The same letter(s) indicated not significant difference on $P < 0.05$ in the same colom.

Table 6. The effect of 28 days treatment with functional drink from *P. crocatum* and *C. burmannii* on male and female rats organ weight

Organ	Average organ weight (g)							
	Control		Functional drink from *P. crocatum* and *C. burmannii*					
	0 mg/kg bw		630 mg/kg bw		1260 mg/kg bw		1890 mg/kg bw	
	Male	Female	Male	Female	Male	Female	Male	Female
Liver	7.23 ± 1.15a	7.51 ± 0.25a	6.21 ± 0.18a	6.89 ± 0.81a	7.57 ± 1.30a	7.44 ± 0.91a	6.91 ± 0.59a	7.24 ± 0.67a
Heart	0.85 ± 0.21a	0.83 ± 0.04a	0.96 ± 0.2a	0.77 ± 0.01a	0.81 ± 0.06a	1.12 ± 0.24a	0.88 ± 0.05a	0.76 ± 0.04a
Left kidney	0.84 ± 0.04a	0.81 ± 0.01a	0.79 ± 0.05a	0.79 ± 0.09a	0.80 ± 0.10a	0.73 ± 0.05a	0.76 ± 0.06a	0.76 ± 0.01a
Right kidney	0.86 ± 0.00a	0.80 ± 0.02a	0.84 ± 0.04a	0.82 ± 0.07a	0.88 ± 0.11a	0.72 ± 0.09a	0.78 ± 0.05a	0.78 ± 0.10a
Brain	1.90 ± 0.03a	1.98 ± 0.05a	1.91 ± 0.04a	1.92 ± 0.05a	1.93 ± 0.25a	2.00 ± 0.06a	1.97 ± 0.00a	1.85 ± 0.00a
Left lung	0.55 ± 0.25a	0.79 ± 0.48a	0.75 ± 0.50a	0.63 ± 0.26a	0.55 ± 0.19a	0.78 ± 0.15a	0.95 ± 0.34a	0.71 ± 0.12a
Right lung	0.97 ± 0.19a	1.53 ± 0.76a	1.38 ± 0.57a	1.29 ± 0.28a	1.11 ± 0.20a	1.39 ± 0.02a	1.64 ± 0.51a	1.25 ± 0.10a
Pancreas	0.99 ± 0.07a	1.01 ± 0.02a	0.87 ± 0.01a	0.97 ± 0.11a	1.07 ± 0.08a	1.12 ± 0.04a	0.85 ± 0.28a	0.92 ± 0.01a
Spleen	0.45 ± 0.01a	0.51 ± 0.05a	0.45 ± 0.05a	0.44 ± 0.06a	0.44 ± 0.08a	0.52 ± 0.09a	0.42 ± 0.00a	0.49 ± 0.03a
Left adrenal	0.04 ± 0.01a	0.05 ± 0.02a	0.05 ± 0.00a	0.07 ± 0.01a	0.04 ± 0.00a	0.08 ± 0.04a	0.04 ± 0.00a	0.07 ± 0.00a
Right adrenal	0.04 ± 0.00a	0.05 ± 0.02a	0.03 ± 0.00a	0.06 ± 0.00a	0.04 ± 0.02a	0.05 ± 0.00a	0.04 ± 0.00a	0.06 ± 0.01a
Thymus	0.20 ± 0.08a	0.30 ± 0.03a	0.20 ± 0.01a	0.30 ± 0.05a	0.30 ± 0.05a	0.31 ± 0.07a	0.28 ± 0.09a	0.29 ± 0.06a
Left Thyroid gland	0.01 ± 0.00a	0.01 ± 0.00a	0.01 ± 0.00a	0.01 ± 0.00a	0.01 ± 0.00a	0.01 ± 0.00a	0.01 ± 0.00a	0.01 ± 0.00a
Right Thyroid gland	0.01 ± 0.00a	0.01 ± 0.00a	0.01 ± 0.00a	0.01 ± 0.00a	0.01 ± 0.00a	0.01 ± 0.00a	0.01 ± 0.00a	0.01 ± 0.00a
Bladder	0.24 ± 0.08a	0.14 ± 0.06a	0.36 ± 0.09a	0.08 ± 0.01a	0.33 ± 0.24a	0.23 ± 0.11a	0.18 ± 0.09a	0.28 ± 0.21a
Left salivary gland	0.33 ± 0.08a	0.30 ± 0.06a	0.29 ± 0.02a	0.33 ± 0.04a	0.28 ± 0.05a	0.32 ± 0.04a	0.28 ± 0.01a	0.25 ± 0.03a
Right salivary gland	0.42 ± 0.06a	0.34 ± 0.01a	0.35 ± 0.08a	0.31 ± 0.03a	0.26 ± 0.01a	0.33 ± 0.02a	0.26 ± 0.05a	0.30 ± 0.03a
Pituitary gland	0.01 ± 0.00a	0.01 ± 0.00a	0.01 ± 0.00a	0.01 ± 0.00a	0.01 ± 0.00a	0.01 ± 0.00a	0.01 ± 0.00a	0.01 ± 0.00a
Prostate gland	0.35 ± 0.04a	-	0.42 ± 0.01a	-	0.19 ± 0.05a	-	0.21 ± 0.06a	-
Left testes	1.67 ± 0.08a	-	1.53 ± 0.05a	-	1.11 ± 0.34a	-	1.16 ± 0.27a	-
Right testes	1.64 ± 0.22a	-	1.48 ± 0.08a	-	1.08 ± 0.28a	-	1.14 ± 0.19a	-
Seminal vesicles	11.1 ± 2.9a	-	10.6 ± 1.8a	-	11.2 ± 2.1a	-	11.2 ± 3.9a	-
Uterus	-	0.88 ± 0.02a	-	0.91 ± 0.18a	-	0.45 ± 0.13a	-	0.59 ± 0.21a
Left ovary	-	0.12 ± 0.01a	-	0.12 ± 0.02a	-	0.08 ± 0.00a	-	0.10 ± 0.04a
Right ovary	-	0.16 ± 0.01a	-	0.11 ± 0.01a	-	0.10 ± 0.01a	-	0.08 ± 0.02a

The same letter(s) indicated not significant difference on $P < 0.05$ in the same raw.

No alterations were detected in pathological examinations of the tissues during the microscopic examination of the internal organs. The findings were generally consistent with the expected pattern for *Sprague dawley* rats of this particular age. Histopathological observations further support the safety of functional drink from *P. crocatum* and *C. burmannii* (Figure 1 & 2).

DISCUSSION

Several biological activities of *P. crocatum* leaves extract have been reported to contain anti-hyperglycemic (Safithri *et al*. 2008), antioxidant (Alfarabi *et al*. 2010), inhibitor α-glucosidase (Alfarabi 2010), anticancer (Wicaksono *et al*. 2009) substances, while *C. burmannii* bark extract have been reported to contain anti-microbial and antioxidant substance (Shan *et al*. 2007). So, this showed that functional drink play an important role in the management of diabetics. Nevertheless, there were no scientific reports that highlited the safety of the functional drink from *P. crocatum* and *C. burmanii* in relation to long term administration, specifically in terms of a 28 days repeated dose toxicity study.

In the repeated dose toxicity study done, the functional drink was given orally at doses up to 1890 mg/kg in rats. The changes in body weight were used as an indicator of adverse effects of drug and chemicals (Mounnissamy *et al*. 2010). the present results suggest that at the dose levels administered, the functional drink created from *P. crocatum* and *C. burmanii* is non-toxic in rats. In addition, determination of food consumption is important in the study of safety of a product with therapeutic purpose, as proper intake of nutrients are essential to the physiological status of the animals and to the accomplishment of the proper response to the drug tested instead of a false response due to improper nutritional conditions (Sateesh & Veeranjaneyulu 2009). Another plant, *Caesalpinia bonducella* (L) Fleming, reported to posses antidiabetic substances, showed that no significant variation in the body weights between the control and the treated group was observed after 28 days of treatment (Pillaia & Suresh 2011).

Analysis of blood parameters is relevant to risk evaluation as the changes in hematological system have a higher predictive value for human toxicity, when data are translated from animal studies. The results indicate that functional drink from *P. crocatum* and *C. burmanii* was not toxic to the circulating blood cells, nor interfered with their production. Hematopoiesis and leucopoiesis were also not affected even though the haematopoietic system is one of the most sensitive targets for toxic compounds and an important index of physiological and pathological status in man and animals (Arawwawala *et al*. 2011). Therefore, it is possible to assume that the functional drink from *P. crocatum* and *C. burmanii* is not hematotoxic.

Glucose, triglycerides, cholesterol, SGOT and SGPT plasma level remained normal after administration of functional drink of *P. crocatum* and *C. burmanii* for 28 days. This result represents a normal liver function with no impairment (Sateesh & Veeranjaneyulu 2009). Renal functions markers like urea and creatinine plasma levels remained normal after administration of functional drink of *P. crocatum* and *C. burmanii* at all selected dose levels. Thus it can be stated that functional drink of *P. crocatum* and *C. burmanii* does not show any renal toxicity (Tembhurne & Sakarkar 2010). However, subacute toxicity of *Alstonia congensis* Engler (Apocynaceae) bark and *Xylopia aethiopica* (Dunal) A. Rich (Annonaceae) fruits mixtures used in the treatment of diabetes revealed a tendency to cause kidney problems on a long-term use (Ogbannia 2008).

In conclusion, at the oral doses tested, the functional drink of *P. crocatum* and *C. burmannii* was well tolerated and neither produced overt signs of clinical toxicity nor any signs of hepato-, nephro or haematotoxicity. Thus, functional drink of *P. crocatum* and *C. burmannii* was found to be non-toxic when oral repeated dose toxicities were performed. Overall, this study provides valuable data on toxicity profile of functional drink of *P. crocatum* and

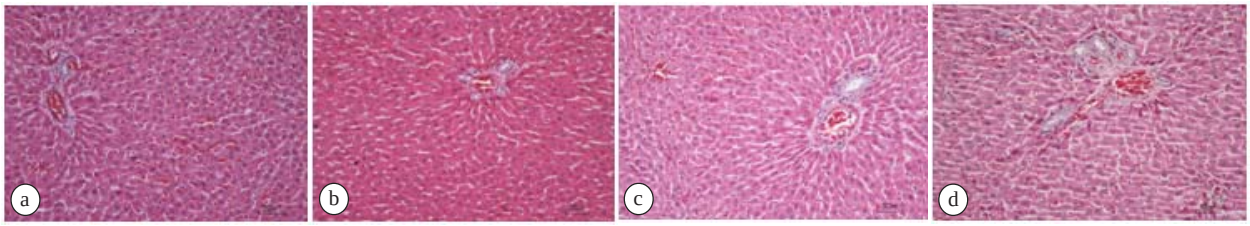

Figure 1. Histopathological analysis of liver organ treated with functional drink from *P. crocatum* and *C. burmanii*: a. 0 mg/kg bw, b. 630 mg/kg bw, c. 1260 mg/kg bw, d. 1890 mg/kg bw.

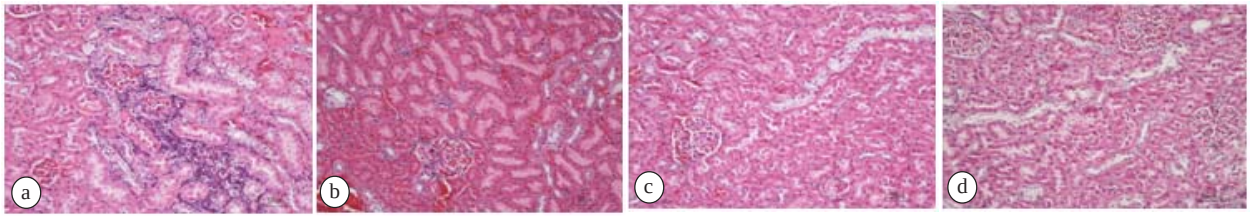

Figure 2. Histopathological analysis of kidney organ treated with functional drink from *P. crocatum* and *C. burmanii*: a. 0 mg/kg bw, b. 630 mg/kg bw, c. 1260 mg/kg bw, d. 1890 mg/kg bw.

C. burmanii that should be useful for the planning of future preclinical and clinical studies of the formulation.

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