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Thirteen-week toxicity study of mengkudu juice (*Morinda citrifolia*): Effect on the blood analysis

[Kajian ketoksikan jus mengkudu (*Morinda citrifolia*) selama tiga belas minggu: Kesan terhadap analisis darah]

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Key words: *Morinda citrifolia*, liver function test, kidney function test

Abstract

The study was conducted to evaluate the subchronic toxicity of an aqueous extract of mengkudu (*Morinda citrifolia*) fruit on *Sprague-Dawley* male rats. The rats were given three dosages of mengkudu extracts (5, 10 and 20%) in drinking water. No significant changes in haematology values such as red blood cell (RBC), white blood cell (WBC), haematocrit and haemoglobin in all mengkudu-treated rats. However, the platelet count was increased significantly in high dose group of rats, indicative of liver injury. In kidney function test, rats treated with the highest dose of mengkudu juice showed significant increase (p <0.05) in the urea and creatinine values, suggestive of renal injury. Moreover, the ALT and GGT (both enzyme markers for liver injury) also increased significantly (p <0.05) in the highest dose of mengkudu juice. Thus, this study found that a dosage of 20% mengkudu juice (highest dose) was not safe for the rats as it indicated renal as well as liver injuries. However, lower dosages of the juice (5% and 10% respectively) showed no significant changes in any aspect of the study, hence were considered safe.

Introduction

There has been a wide increase in the consumption of herbs in many countries in the world. Plant materials have been known for their medicinal properties. However, anti-nutritional and toxic compounds are also present in many plants (Guil et al. 1997). Several substances in plants express cytotoxic and show correlation with the incidence of tumours (Ames 1983). In addition, a number of highly toxic compounds have been isolated from plants (Ur-Rahman and Zaman 1989). Therefore, understanding the health benefits and the potential toxicity of medicinal plants is very important. Although most of the herbs have been used for many generations and are considered safe, scientific toxicological trials are still necessary.

In Malaysia, one of the famous herbs is *Morinda citrifolia* (locally known as mengkudu) that has been traditionally used for lowering high blood pressure, alleviating pain, stimulating the immune system and increasing body energy (Muhammad and Mustafa 1994; Solomon 1998). However, there was not much data available on its toxicity or side-effects that could compromise the safe use of this herb. This experiment was conducted to evaluate the

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toxicity of mengkudu juice through long-term toxicity study for 13 weeks. Long-term toxicity study is very useful tool rather than short-term study to evaluate the sign of toxicity by measuring the physical changes and blood analysis. This is because some of the toxicants will not show the toxic effects in blood or target organ in short term period. Thus, the 13-week toxicity study is normally conducted to evaluate the toxicity effects in small animal such as rat (WHO 1993).

Scientific findings from small animal studies may be used as a guide in dictating the amount of safe dosage. This safety study also will increase the acceptability and commercial viability of local herbs, being widely used in alternative medicine both locally and internationally.

Materials and methods

Preparation of mengkudu juice

The fruits of mengkudu were collected randomly from MARDI Station Mu'adzam Shah, Pahang. Only the fully mature fruits (yellowish in colour) were used in this study. According to Rohani and Rosalizan (2005), the maturity index of *M. citrifolia* fruit is at the yellow-white stage which is 15–16 weeks after fruit set. The fresh fruits were blended with distilled water at a ratio of 1:1 (w/v) and kept at 4 ºC until used. The puree was then mixed with distilled water to prepare the three doses of extracts (5, 10 and 20%) which would be used for feeding trials on rats as suggested by Wang et al. (2002) in the previous study.

Experimental animals

A total of 28 male *Sprague-Daw*ley rats (age about 2 months) each weighing between 200 g and 250 g were used for the study. They were fed with a standard rat chow diet and water *ad libitum* (free access water for 24 h). All rats were acclimatized to the animal facility for a week before starting the experiment. The acclimatization period is very important to the rats for an adaptation in new environment or cages (WHO 1993).

The rats were divided into four groups (I, II, III and IV) which consisted of seven rats per group. According to the guidelines (WHO 1993), at least five rats per group for a certain sex (male or female) should be used for toxicity study to be statistically validated. In this study, Group I rats were given distilled water and served as control. Groups II–IV rats were given the mengkudu juice in their drinking water at doses of 5, 10 and 20%, respectively. All rats were given 100 ml of drinking water every day.

Analysis procedures

After 13 weeks of feeding trials, rats were made to fast overnight (at least for 15–16 hr.) and anesthetized using ethyl ether. About 2.0 ml of blood was collected from the posterior vena cava and transferred into blood tube. The blood was then centrifuged at 4 °C (3,000 rpm, 10 min) to get the serum fraction. The serum was used to determine the blood chemistry values such as urea, creatinine, alanine aminotransferase (ALT), alkaline phosphatase (ALP), gamma-glutamyl trasferase (GGT) and total protein. All blood parameters weren measured by using Blood Chemistry Analyzer (Selectra E, Vitalab – Sweden) in Food Technology Research Centre, MARDI..

Statistical analysis

The significant differences between the control and extract-treated groups were determined using ANOVA followed by Duncan New Multiple Range Test (DMRT). All values are expressed as group mean ± standard error of mean (SEM). The minimal level of significance accepted was $p < 0.05$.

Results and discussion

Effect of mengkudu juice on haematology values

Red blood cell (RBC), white blood cell (WBC), haematocrit (HCT), platelet (PLT) and haemoglobin (HGB) are normally used for the screening of haematotoxicity (Goldstein 1988). This screening is

important to determine any adverse effects caused by the administration of drugs or herbal preparations.

In human, haematology analysis is normally most recommended in the Pathology Service Unit to determine any disease or toxic effect (Halimah 2001). The RBC is tested for any disorders that are grouped either into anaemias (severe reductions in circulating red cells) or polycythemias (abnormal increases in circulating red cells). The WBC is tested for the leukocytosis (an increased number of cells) or leucopenia which is a decreased number of cells (Fishbach 1980). In this study, the results showed that there were no signs of anaemias, polycythemias, leukocytosis as well as leucopoenia occurred in the treated rats as compared to control rats. There was no significant different in the RBC and WBC values in all treated and control rats (*Table 1*).

The measurement of haematocrit and haemoglobin were also important because both parameters are related to the concentration of RBC. Haematocrit is a measure of the relative volume of plasma, the RBC mass and the concentration of RBC (as a percentage of the total volume of blood). The decreased values will indicate anaemia or leukaemia. Meanwhile, haemoglobin is an iron-containing protein that is found in RBC whose primary function is to transport oxygen from the lung to the body. The decreased values are normally found in anaemia or severe haemorrhage or bleeding (Vaughn 1999). Results showed that there were no significant changes in the haematocrit and haemoglobin values in treated rats as compared to control rats (*Table 1*).

Platelet (or thrombocyte) is the smallest element in the blood. Normally, platelet activity is necessary for blood clotting, thus this measurement is helpful in evaluating bleeding disorders. A deficiency of platelet (or thrombocytopenia) leads to prolonged bleeding time or impaired clot retention. While the increased value of platelet (or thrombocythemia) is frequently associated with the liver disease such as cirrhosis. In this study, the platelet count was increased significantly ($p < 0.05$) in the high-dose treated rats as compared to control rats (*Table 1*), suggesting that liver injury had occurred.

Effect of mengkudu juice on liver function test

Serum enzymes such as alanine aminotransferase (ALT), alkaline phosphatase (ALP) and gamma-glutamyl transferase (GGT) concentrations are commonly used to evaluate the status of liver function (Lamela et al. 1986; Frank 1996). Liver is the central organ in the detoxification of compounds (Heywood 1983). Organ or tissue damage causes the release of increased amounts of many enzymes into the blood stream (Marshall 2000). The leakage of large quantities of enzymes into blood stream is associated with

Table 1. Effect of mengkudu juice on haematology values

Treatments	Red blood cell (106/mm3)	Haematocrit (%)	Platelet (103/mm3)	White blood cell (103/mm3)	Haemoglobin (g/dl)
Control (distilled water)	9.31 ± 0.21	47.12 ± 0.95	705.92 ± 54.54	8.80 ± 1.65	16.23 ± 0.32
Low dose (5% juice)	9.27 ± 0.27	48.08 ± 1.48	678.00 ± 135.94	7.88 ± 0.33	16.74 ± 0.41
Medium dose (10% juice)	8.85 ± 0.10	45.23 ± 0.80	745.83 ± 57.94	9.24 ± 0.59	15.72 ± 0.23
High dose	9.23 ± 0.40	47.78 ± 2.0	851.75 ± 201.02*	10.78 ± 2.0	16.35 ± 0.64

*p <0.05, as compared to the control rats

massive centrilobular necrosis, ballooning degeneration and cellular infiltration of the liver (Wang et al. 2004).

Total protein value is also very important. A healthy liver is crucial for protein metabolism since liver disease is frequently associated with alterations in protein metabolism (Marshall 2000). Total protein will be decreased by inadequate synthesis due to liver disease (Datta et al. 1999).

As shown in *Table 2,* rats treated with the highest dose of mengkudu juice showed significant increase (p <0.05) in the ALT as well as GGT concentrations. The increased value of these enzymes in the serum normally will reflect active liver damage (Irfan and Namik 2002). However, no significant changes observed in the ALP values as compared to control rats. Total protein had decreased significantly (p <0.05) as compared to control rats, suggesting liver injury.

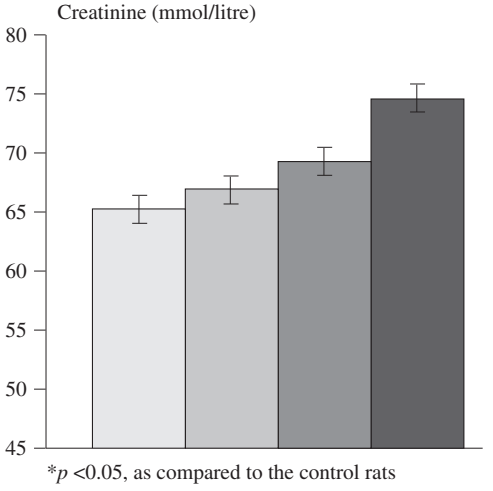
Effect of mengkudu extracts on kidney function test

Kidney is the second organ most frequently affected by any compound. Serum urea and creatinine concentrations are often used as an index of renal glomerular function and will be increased in renal injuries (Moshi et al. 2001). Previous report showed that some of herbal preparations used in long period were associated with kidney injuries (Kadiri et al. 1999).

Results showed that rats treated with highest dose of mengkudu juice showed significant increase in the urea and creatinine values (*Figure 1*), indicating kidney injury might had occurred in rats.

Conclusion

The administration of repeated different doses of mengkudu juices ranging from 5–20% for subchronic toxicity study (13 weeks) did not produce any abnormalities in the red blood cell (RBC), white blood cell (WBC), haematocrit (HCT) and haemoglobin (HGB). However, the platelet count was increased significantly, showing that the liver could be injured. In addition, the liver function tests indicating both ALT and GGT (markers for liver injury) had increased significantly in the highest dose of mengkudu juice. The administration of mengkudu juice (20%) also might be associated with the kidney injuries due to the increment of the urea and creatinine values in kidney function test. Thus, this study found that a dosage of 20% mengkudu juice (highest dose) was not safe in rats. However, lower dosages of the juice (5%



*p <0.05, as compared to the control rats

Figure 1. Effect of mengkudu juice on the urea and creatinine concentrations in rats

Table 2. Effect of mengkudu juice on liver function test

Blood parameters	Control – Distilled water	Low dose (5% juice)	Medium dose (10% juice)	High dose (20% juice)
TProtein (g/litre)	65.74 ± 3.88	68.59 ± 2.69	66.22 ± 4.26	62.32 ± 1.56*
Alanine aminotransferase (U/l)	84.60 ± 45.44	118.00 ± 15.38	119.75 ± 4.11	138.00 ±15.6*
Alkaline phosphate (U/l)	24.00 ± 48.51	33.85 ± 46.96	33.40 ± 53.46	24.71 ± 46.1
Gamma-glutamyl transferase (U/l)	6.50 ± 1.87	8.86 ± 1.86	10.40 ± 0.55	12.57 ± 8.92*

*p <0.05, as compared to the control rats

and 10% respectively) showed no significant changes in any aspect of the study, hence were considered safe in rats.

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Abstrak

Kajian ini dijalankan untuk menilai kesan ketoksikan subkronik jus buah mengkudu (*Morinda citrifolia*) terhadap tikus jantan *Sprague-Dawley*. Tikus telah diberi tiga dos jus mengkudu (5, 10 dan 20%) melalui air minuman. Tiada perubahan yang signifikan dalam nilai hematologi seperti sel darah merah (RBC), sel darah putih (WBC), hematokrit dan hemoglobin pada semua tikus yang dikaji. Walau bagaimanapun, kiraan platelet telah meningkat dengan signifikan dalam tikus yang diberi mengkudu dos tinggi, menandakan berlaku kecederaan hati. Dalam ujian fungsi ginjal, tikus yang diberi dos jus mengkudu paling tinggi telah menunjukkan peningkatan aras urea dan kreatinin yang signifikan (p <0.05) menandakan berlakunya kecederaan ginjal. Tambahan pula, enzim ALT dan GGT (kedua-duanya petunjuk kepada kecederaan hati) telah meningkat dengan signifikan (p <0.05) dalam tikus yang diberi jus mengkudu pada dos tinggi. Oleh itu, kajian mendapati pemberian jus mengkudu 20% (dos tinggi) tidak selamat bagi tikus kerana berlakunya kecederaan pada ginjal dan hati. Walau bagaimanapun, dos yang lebih rendah (5% dan 10%) tidak menunjukkan perubahan dalam semua parameter yang dikaji dan dianggap selamat.

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