

Acute and subchronic toxicity studies of an aqueous extract of *Morinda citrifolia* fruit in rats

[Kajian ketoksikan secara akut dan subkronik ekstrak berair buah mengkudu (*Morinda citrifolia*) terhadap tikus]

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Key words: *Morinda citrifolia*, acute toxicity, subchronic toxicity, kidney function test, liver function test, *Sprague-Dawley* rats

Abstrak

Eksperimen ini dijalankan untuk menilai ketoksikan ekstrak berair buah *Morinda citrifolia* atau mengkudu terhadap tikus *Sprague-Dawley* jantan melalui kajian akut (ujian DM₅₀) dan subkronik. Dalam ujian DM₅₀, tikus telah diberikan tiga dos ekstrak *M. citrifolia* (1.0, 2.0 dan 3.0 g/kg berat badan). Kesemua tikus tersebut diperhatikan tanda-tanda ketoksikan terutamanya kematian dalam masa 24 jam sehingga 14 hari. Manakala dalam kajian subkronik pula, kesan pemberian *M. citrifolia* (0.25, 0.50 dan 1.00 g/kg berat badan) terhadap tikus ditentukan selama 6 minggu dengan mengukur parameter biokimia darah seperti glukosa, kolesterol, trigliserida, protein jumlah, albumin, urea, kreatinin dan aktiviti enzim (aspartat transaminase, alanin transaminase, alkalin fosfatase, laktat dehidrogenase dan γ -glutamil transferase).

Semua tikus hidup sepanjang tempoh pemerhatian dalam ujian DM₅₀. Pemberian dos *M. citrifolia* secara berulang dalam kajian subkronik pula tidak memberikan kesan terhadap kebanyakan parameter biokimia darah pada tikus berbanding dengan tikus kawalan. Kesimpulannya, pemberian ekstrak *M. citrifolia* pada dos rendah dan tinggi tidak menunjukkan tanda-tanda ketoksikan terhadap tikus.

Abstract

This experiment was conducted to evaluate the toxicity of an aqueous extract *Morinda citrifolia* fruit on *Sprague-Dawley* male rats through acute (LD₅₀) and subchronic studies. In the LD₅₀ test, rats were given three dosages of *M. citrifolia* extracts (1.0, 2.0 and 3.0 g/kg of body weight). They were observed for any toxic signs, especially death for the first 24 hours and continued up to 14 days. In the subchronic study, effects of *M. citrifolia* extracts in rats (0.25, 0.50 and 1.0 g/kg of body weight) were determined for 6 weeks by measuring the blood biochemical parameters such as plasma glucose, cholesterol, triglyceride, total protein, albumin, urea, creatinine and enzymes (aspartate transaminase, alanine transaminase, alkaline phosphatase, lactate dehydrogenase and γ -glutamyl transferase).

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All rats survived throughout the observation study in LD₅₀ test. The administration of repeated dose of *M. citrifolia* extracts in subchronic study did not affect most of the blood biochemical parameters in rats as compared to the control rats. In conclusion, low and high doses of *M. citrifolia* extracts showed no toxicity signs in rats.

Introduction

There has been a wide increase in the consumption of herbs in many countries in the world. Bodekar (2000) reported that 40% of population in the USA and UK, 60% in Australia and 60–80% in most developing countries have used herbs as traditional medicine. Plant materials have been known for their medicinal properties since long ago. International organizations such as WHO also advocates the usefulness of traditional or complementary medicines (Ahmad Fadzil 2000). In Malaysia, one of the famous herbs is *Morinda citrifolia* Linn. (locally known as 'mengkudu') that has been traditionally used to treat various diseases. It is believed to reduce high blood pressure, alleviate pain, stimulate the immune system and increase body energy (Heinicke 1985; Muhammad and Mustafa 1994; Solomon 1998; Indu and Ng 2000). However, there are not many data available in the literature on its toxicity or side-effects that might ensure its safe use.

In addition, Ur-Rahman and Zaman (1989) reported that a number of highly toxic compounds have been isolated from plants. The administration of high dose of onion and garlic extracts in rats has caused toxic effects on liver and lung (Alnaqeeb et al. 1996; Thomson et al. 1998). Although most of the herbs have been used for many generations and are considered safe, scientific toxicological trials are still necessary. This experiment was conducted to evaluate the toxicity of an aqueous extract of *M. citrifolia* through acute (LD₅₀ test) and subchronic studies on rats.

Materials and methods

Preparation of extract

The fruits of *M. citrifolia* were collected randomly from MARDI Station in Serdang,

Selangor. The fresh fruits were blended with distilled water at a ratio of 1:1 (w/v). The cloudy juice was centrifuged at 2000 rpm for 10 min to get a clear supernatant. The supernatant was then stored at 4 °C.

Experimental animals

A total of 48 male *Sprague-Dawley* rats each weighing between 200 g and 250 g were fed a standard rat chow diet and water *ad libitum*. All rats were acclimatized to the animal facility for a week before starting the experiment.

In the acute study, rats were divided into four groups of six rats per group. Group 1 was given distilled water by an oral feeding and served as control. Groups 2–4 were given an aqueous extract of *M. citrifolia* orally at doses of 1.0, 2.0 and 3.0 g/kg of body weight, respectively. Rats were then observed for toxic signs, especially death, for the first 24 hours until the 14th day.

Likewise, in the subchronic study, rats were also divided into four groups of six rats per group. Group 1 was served as control. Groups 2–4 were given an aqueous extract of *M. citrifolia* orally at doses of 0.25, 0.50 and 1.0 g/kg of body weight, respectively, for 6 weeks. Body weights of all rats were recorded at the initial and final stages of the experiment.

Analytical procedures

After 6 weeks of oral feedings, rats were fasted overnight and anesthetized using ethyl ether. Blood was collected from the posterior vena cava and transferred into tube containing anticoagulant solution, EDTA to get the plasma fraction. The plasma was used to determine glucose, cholesterol, triglyceride, urea and creatinine, while urea and creatinine were used for kidney function

test. Serum was obtained by collecting blood in non-EDTA tube. The serum was used for liver function test by measuring total protein, albumin and enzyme activities such as alanine transaminase (ALT), aspartate transaminase (AST), alkaline phosphatase (ALP), lactate dehydrogenase (LDH) and γ -glutamyl transferase (GGT). Plasma and serum samples were kept at -20°C . The reagents used to perform biochemical analyses were supplied by Roche Diagnostic. All analyses were done using Cobas Mira Blood Chemical Analyzer (Roche, UK) in Universiti Putra Malaysia.

Organ relative weight

After taking the blood samples, the liver, heart and kidney were quickly excised, rinsed in 0.9% cold saline to remove blood, blotted and immediately soaked in liquid nitrogen as suggested by Sachan and Yatim (1992). The organ relative weight (% body weight) was obtained by dividing the final weight of organ to final body weight.

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 \pm standard error of mean. The minimal level of significance accepted was $p < 0.05$.

Results and discussion

Acute study

Oral administration of *M. citrifolia* extract did not induce mortality up to the highest dose which was 3.0 g/kg body weight. All treated rats did not show any toxic signs (such as bleeding from nose, fur loss, frequent urination and diarrhoea) throughout the observation period. According to WHO (1992), if the administration of the highest dose of herbal extracts used in certain experiment does not show any toxicity effects, it is considered safe. Thus, the result may suggest the LD_{50} for *M. citrifolia* extract is more than 3.0 g/kg. Similar results

were also reported by Qureshi et al. (1992) in acute toxicity studies of *Alpinia galanga* and *Curcuma longa*. In addition, the root of *M. citrifolia* has been tested previously for LD_{50} and produced no toxicity effects (Younos et al. 1990).

Subchronic study

Effect of *M. citrifolia* extract on body and organ weights Body weight measurement is important as it determines the health status of animal groups (Heywood 1983). Supplementation of *M. citrifolia* extracts had significantly increased the final body weight of all rats ($342.58 \pm 8.10 - 363.40 \pm 14.25$) as compared to the initial (*Table 1*). All rats gained positive weight, indicating good health status. Organs' relative weights (liver, kidney and heart) were not affected by the administration of *M. citrifolia* extracts. They were not significantly different compared to the control ($p > 0.05$).

Organ weight measurement is also important to assess general toxicity because any change in organ weight is a sensitive indicator of toxicity (Frank 1996). Liver is the target organ because most toxicants enter the body via the gastrointestinal tract, and after absorption the toxicants are carried by the hepatic portal vein to the liver. In theory, organ weight will be affected by the suppression of body weight as described by Heywood (1983). In this study, the administration of *M. citrifolia* extract did not give any significant changes in the organs' relative weights of treated rats compared to the control.

Effect of *M. citrifolia* extract on plasma glucose and lipids concentrations

Changes in the concentrations of glucose and lipids (cholesterol and triglyceride) in the plasma and liver are summarized in *Table 2*. The concentrations of plasma glucose and triglyceride were not affected by the administration of *M. citrifolia* extracts in rats. On the other hand, the medium dose of extract (0.50 g/kg) significantly reduced

Table 1. Effects of *Morinda citrifolia* extracts on body and percentage of organ weights in rats (n = 6)

	Control	Low dose (0.25 g/kg)	Medium dose (0.50 g/kg)	High dose (1.0 g/kg)
Body weight				
Initial weight (g)	261.45 ± 14.37a	282.33 ± 6.68a	276.45 ± 7.62a	271.03 ± 11.80a
Final weight (g)	360.93 ± 9.01b	352.70 ± 10.73b	342.58 ± 8.10b	363.40 ± 14.25b
Organ weight				
Liver				
Weight (g)	7.93 ± 0.28	8.08 ± 0.26	7.48 ± 0.16	7.89 ± 0.29
% Body weight	2.20 ± 0.08 ns	2.29 ± 0.06	2.19 ± 0.07	2.18 ± 0.05
Kidney				
Weight (g)	2.10 ± 0.06	2.15 ± 0.10	2.11 ± 0.05	2.19 ± 0.06
% Body weight	0.58 ± 0.01 ns	0.61 ± 0.02	0.62 ± 0.02	0.61 ± 0.02
Heart				
Weight (g)	0.99 ± 0.04	0.96 ± 0.04	0.92 ± 0.01	0.94 ± 0.05
% Body weight	0.28 ± 0.01 ns	0.27 ± 0.01	0.27 ± 0.01	0.26 ± 0.01

Mean values with different letters in the same column are significantly different ($p < 0.05$)

ns = not significant

Table 2. Effects of *Morinda citrifolia* extracts on plasma glucose, cholesterol and triglyceride in rats

	Glucose (mmol/L)	Cholesterol (mmol/L)	Triglyceride (mmol/L)
Control	6.91 ± 0.28 ns	2.72 ± 0.22a	0.56 ± 0.06 ns
Low dose (0.25 g/kg)	6.62 ± 0.84	2.18 ± 0.09ab	0.67 ± 0.08
Medium dose (0.50 g/kg)	6.07 ± 0.25	2.01 ± 0.10b	0.68 ± 0.04
High dose (1.00 g/kg)	6.31 ± 0.36	2.16 ± 0.30ab	0.59 ± 0.04

Mean values with different letters in the same column are significantly different ($p < 0.05$)

ns = not significant

($p < 0.05$) the cholesterol level (2.01 ± 0.10) as compared to the normal control rats (2.72 ± 0.22). However, the higher dose of extract did not change the cholesterol concentration significantly as the activity appeared not to be dose-dependent. These results suggested the potential of *M. citrifolia* extract in lowering plasma cholesterol concentration. Goh et al. (1995) and Solomon (1998) also have claimed that *M. citrifolia* can reduce cholesterol concentration.

Effect of *M. citrifolia* extract on kidney function test Kidney is the second organ most frequently affected by any compound (Marshall 2000). Therefore, renal functions can be assessed by measuring the concentration of creatinine and urea in plasma (Moshi et al. 2001). Previous report

showed that some of herbal preparations used in long period are associated with kidney injuries (Kadiri et al. 1999).

There were no significant changes in urea concentrations in all groups of rats as shown in *Table 3*. This indicates that *M. citrifolia* did not affect the normal concentrations of urea and creatinine. Plasma urea and creatinine concentrations are often used as an index of renal glomerular function and will be increased in renal injuries (Marshall 2000). Urea is synthesized in the liver, primarily as by-product of the deamination of amino acids. Creatinine is a by-product from muscle as any changes in muscle mass will affect its concentration in blood (Vaughn 1999).

Table 3. Effects of *Morinda citrifolia* extracts on plasma creatinine and urea concentrations in rats

	Creatinine (mg/dl)	Urea (mmol/L)
Control	0.54 ± 0.04 ns	9.18 ± 0.41 ns
Low dose (0.25 g/kg)	0.53 ± 0.04	8.62 ± 0.23
Medium dose (0.50 g/kg)	0.51 ± 0.02	9.03 ± 0.70
High dose (1.00 g/kg)	0.50 ± 0.03	8.67 ± 0.52

ns = not significant

Table 4. Effects of *Morinda citrifolia* extracts on total protein, albumin and serum enzymes activities in rats

	Control	Low dose (0.25 g/kg)	Medium dose (0.50 g/kg)	High dose (1.0 g/kg)
Total Protein (g/L)	68.01 ± 1.28 ns	64.00 ± 5.95	63.09 ± 3.12	62.31 ± 2.70
Albumin (g/L)	40.60 ± 3.76 ns	36.40 ± 1.84	36.76 ± 1.41	36.63 ± 1.96
AST (U/L)	192.56 ± 27.13 ns	180.03 ± 16.51	181.37 ± 19.44	179.48 ± 17.95
ALT (U/L)	89.17 ± 11.81 ns	80.63 ± 7.50	81.75 ± 7.89	77.72 ± 8.31
ALP (U/L)	121.63 ± 11.34 ns	107.60 ± 9.98	105.21 ± 8.61	106.35 ± 16.52
LDH (U/L)	295.40 ± 103.19 ns	328.00 ± 46.09	334.80 ± 61.77	177.80 ± 37.77
GGT (U/L)	2.58 ± 0.65 ns	2.49 ± 0.60	2.41 ± 1.04	2.42 ± 0.54

ns = not significant

AST = Aspartate transaminase; LDH = Lactate dehydrogenase

ALT = Alanine transaminase; GGT = γ -Glutamyl transferase

ALP = Alkaline phosphatase

Effect of *M. citrifolia* extract on liver

function test The activities of serum enzymes (AST, ALT, ALP, LDH and GGT), total protein and albumin concentrations are summarized in *Table 4*. These parameters are commonly used to evaluate the status of liver function (Lamela et al. 1986). Liver function test is crucial because liver is the central organ in the detoxification of compounds (Heywood 1983). There are a number of circumstances that the measurement of enzyme activities in body fluids such as blood, may be of diagnostic values.

In general, enzymes provide an excellent markers of tissue damage. Organ or tissue damage causes the release of increased amounts of many enzymes into the blood stream (Marshall 2000). In this study, no significant changes ($p > 0.05$) in the activities of the measured enzymes were observed in all groups of rats. Vaughn (1999) reported that the activities of most

enzymes normally detectable in blood remains fairly constant in healthy and normal person.

The results of total protein and albumin concentrations were also not affected by the administration of *M. citrifolia* extracts (*Table 4*). This shows that the synthesis of protein in the rat's liver is not influenced by the administration of *M. citrifolia* extracts. Similar results were also obtained in the toxicity studies of *Centella asiatica* (Lucia et al. 1997). A healthy liver is so crucial for protein metabolism since liver disease is frequently associated with alterations in proteins and disturbances of protein metabolism (Marshall 2000). Total protein and albumin concentrations will be decreased by inadequate synthesis due to liver disease (Datta et al. 1999).

Conclusion

An aqueous extract of *M. citrifolia* showed no toxicity in acute toxicological test. The

LD₅₀ for *M. citrifolia* extract was stated as >3.0 g/kg of body weight. The administration of repeated dose of *M. citrifolia* extracts ranging from 0.25–1.0 g/kg of body weight for 6 weeks also did not produce any abnormalities in blood biochemical parameters. Thus, it is concluded that *M. citrifolia* extract is considered safe in this study. Another toxicological study involves feeding trials on rats for 90 days duration will be conducted in future.

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