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The Effect of *Morinda citrifolia* on Isolated Rat Hearts

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Abstract: The effects of *Morinda citrifolia* (Rubiceae) extract on coronary perfusion pressure, contractility and heart rate of isolated rats hearts were studied by using the technique of Langendorff system. Sodium nitroprusside (SNP) was used as a positive control and standard while saline was used as negative control. The extract at doses of 1.0 and 10 mg, respectively showed a significant decrease in coronary perfusion pressure compared with saline. The extract dose of 10 mg showed a maximum decrease in coronary perfusion pressure, developed tension and heart rate. The next part of the study was to investigate the involvement of nitric oxide in the coronary vasodilatory effects of the extract. The inhibitor of nitric oxide synthesis, N^G-nitro-L-arginine methyl ester (L-NAME) 300 µM was perfused together with Krebs-Henseleit solution in the heart for test group, while the time-matched control group without L-NAME. L-NAME did not abolish the decrease of coronary perfusion pressure and developed tension by the second injection. So, nitric oxide is not involved in the mechanism of coronary vasodilation. In conclusion, the *M. citrifolia* extract decreased coronary perfusion pressure and developed tension.

Key words: *Morinda citrifolia*, effect, isolated heart, developed tension, L-NAME, SNP

Introduction

Morinda citrifolia is a wild plant grouped under the family of Rubiceae. In Malaysia it is known as mengkudu and being widely used in traditional medicine to ease pain, improve blood circulation and to treat diabetes (Rukayah, 1999). *M. citrifolia* is also popular among the people in South Pacific Islands, China and Caribbean Islands. It is also important as a diet in health care among the aborigines in Australia and Burma (Solomon, 1999).

Although it is believed to be a potential therapeutic source, only a few studies have been reported to confirm some of its beneficial effects in Malaysia. The study by Ridzwan *et al.* (2000) revealed that its fruit juice has an antinociceptive effect on mice. Besides, these reports others belief, which need to be proved scientifically, include the potential of this plant in treating hypertension. Thus in this study we tried to observe its effects on cardiovascular system, especially on the heart. The main objective was to determine the effect of its fruit extract on an isolated heart rat based on changes in coronary perfusion pressure, contractility and heart rate without the influence of baroreceptor reflex and autonomic nervous system. If the extract had some effects on these three parameters, further objective was to determine whether this change did involve the stimulation of nitric oxide production or vice versa.

Materials and Methods

***Morinda citrifolia* fruits:** *Morinda citrifolia* or 'mengkudu' fruits were obtained locally from the wild in Pahang, a state on the East Coast of the Peninsular Malaysia.

Animals: Male Sprague-Dawley rats with the average of 275 g body weight were obtained from Animal Unit, Medical Research Institute, Kuala Lumpur.

Methods

Preparation of *Morinda citrifolia* extract: The *M. citrifolia* fruits (150 g) were cleaned, pitted, cut into small pieces later homogenized with 200 ml distilled water and then filtered. The filtrate (50 ml) was froze overnight prior to freeze-dry for 24 h to turn into a powder form. Its weight was determined before being stored at 4° C kept for further use.

Preparation of modified Krebs-Henseleit solution: A modified Krebs-Henseleit solution was prepared with chemical composition (mM); NaCl (118), C₆H₁₂O₆ (11.6), NaHCO₃ (25.0), KCl (4.7), KH₂PO₄ (4.7), KH₂PO₄ (1.2), MgSO₄·7H₂O (1.2) and CaCl₂·6H₂O (1.23). These substances were dissolved in distilled water to obtain either 2 or 5 L solution. Calcium chloride, was added to prevent the solution from cloudiness.

Preparation of sodium nitroprusside: The stock of 10 mM sodium nitroprusside was prepared by diluting 0.9% w/v sodium chloride to the required concentration.

Preparation of dose extract of *Morinda citrifolia*: The extract powder of *M. citrifolia* (0.3 g) was diluted with 3 ml distilled water as a stock extract solution of 0.1 g/ml. This stock extract solution was further diluted to the doses of 10, 1, 0.1 and 0.001 mg/ml.

Modified Langendorff isolated perfused heart Preparation: Male Sprague-Dawley rat was anaesthetized with sodium pentobarbitone (60 mg/ml i.p.), then killed by cervical dislocation. The heart was immediately isolated by cutting the aorta and placed in the petri dish, which contained cold Krebs-Henseleit solution. The heart was then fixed with the cannula by typing the aorta to it. This allowed the flows of the ready Krebs-Henseleit solution at the rate of 10 ml/minute, at 37° C. Then the Krebs-Henseleit solution was gassed with a mixture of 95% of oxygen and 5% of carbon dioxide until it reached a pH of 7.4.

The cannula was connected to a pressure transducer that measured the coronary perfusion pressure. The hook was placed at the apex of the heart, which was also connected to the tension isometric transducer. This isometric transducer measured the contractility and the heart rate by a fixed pulley at the rest tension of 2 g. Then the drainage needle was placed in the left ventricle. The signals from both transducers were recorded using a Grass Model 7D Polygraph machine. The heart was stabilized for approximately 20 min before the experiment started. The dose of sodium nitroprusside, the extract and the distilled water were injected via the rubber tube to the nearest cannula.

Determination of sodium nitroprusside dose response: The stabilized isolated heart was injected with the doses of sodium nitroprusside that were increased in its concentration (µM); 0.0001, 0.001, 0.01, 0.1 and 1.0. Observation was made on its effects on the coronary perfusion pressure, contractility (developed tension) and heart rate. The response versus dose curves were plotted and the doses of sodium nitroprusside that gave the maximum effect were determined as the positive control. This experiment was repeated for another 4-isolated rat hearts (n= 4).

Determination of maximum effective dose range of *Morinda citrifolia* extract: The isolated heart was injected with the

respective doses (mg/ml) of *M. citrifolia* extract; 0.001, 0.01, 0.1, 1 and 10 for approximately 5 to 10 min for each injection. The distilled water and sodium nitroprusside, which acts as a negative control and a positive control respectively, were injected to the heart. The three experimental parameters; coronary perfusion pressure, contractility (developed tension) and heart rate were observed. This experiment was repeated on another six isolated hearts. The dose-response curves for each parameter were plotted and the extracted dose that gave a maximum effect was determined.

Determination of nitric oxide involvement in vasodilatation mechanism of *Morinda citrifolia* extract

Time-matched control group (-L-NAME): After the heart was stabilized for approximately 20 min, it was injected with bolus dose 10 mg *M. citrifolia* extract (first dose). The effect of the extract was allowed to disappear for approximately 15 min and the next first dose of 1 µM sodium nitroprusside acted as a positive control and a comparison standard dose were given. Its effect was let to disappear (10 minutes). Then the heart was perfused with modified Krebs-Henseleit solution without L-NAME for approximately 30 min. The second dose of injection (10 mg of *M. citrifolia* extract and 1 µM of sodium nitroprusside) was injected to the heart. The coronary perfusion pressure, the developed tension for contractility and the heart rate were observed for each and every time the injection were given to another five isolated rat hearts (n= 5).

Test group (+ L-NAME): The same protocol as the time-matched group was repeated for the test group. For this group, the heart was perfused with modified Krebs-Henseleit solution, which contain 300 mM L-NAME for approximately 30 min after the first dose of sodium nitroprusside was injected (n= 5). If no vasodilatation effect was observed at the second extract dose, it can be concluded that nitric oxide was not involved in this vasodilatation mechanism.

Data analysis: The effect of sodium nitroprusside, *Morinda citrifolia* extract and distilled water on the experiment parameters (coronary perfusion pressure, developed tension and heart rate) were measured accordingly. The statistical analysis that were used for this data analysis were paired and non-paired student-t test. Paired student-t test was used to evaluate the extract effect, the negative control effect and the positive control effect for the same isolated heart group. While the non-paired student-t test was used to evaluate the extract effect, the negative control effect and the positive control effect for different isolated heart groups. The probability value, P< 0.01 (**), and P< 0.05 (*) were shown if there was significant difference between the extract and the control effect.

Results

The effect of sodium nitroprusside on isolated rat heart

Decreased coronary perfusion pressure: The coronary perfusion pressure decreased when the dose of sodium nitroprusside increased. The maximum coronary perfusion pressure decreased to approximately 35± 7 mmHg from the basal coronary perfusion pressure which was 120± 14.47 mmHg at the dose of 1 µM (Table 1).

Decreased in developed tension: The decreased percentage of the developed tension started to increase when the dose of sodium nitroprusside increased until it reached the maximum decrease of 34.38± 5.96 % from the basal developed tension 7.0± 1.84 g at the dose of 1 µM (Table 1).

Decreased heart rate: The decrease of the heart increased with the increase in sodium nitroprusside dose. The heart rate reached the maximum decreased of 13.75± 1.08 beats/minute from the basal heart rate 212.50± 11.39 beats/min at the dose of 1 µM (Table 1).

The effect of *Morinda citrifolia* extract on the isolated rat heart

Changes in coronary perfusion pressure: The coronary perfusion pressure increased from the basal (122.5± 10.4 mmHg) for approximately 24.2± 11.6 mmHg at the extract dose of 0.001 mg, 18.3± 10.0 mmHg at the dose of 0.01 mg and 15.0± 10.1 mmHg at 0.1 mg (Table 2). The coronary perfusion pressure decreased from the basal to -15.0± 7.7 mmHg at the dose of 1 mg. The maximum decrease was achieved at the value of -37.9± 13.4 mmHg at the dose of 10 mg. The decrease in coronary perfusion pressure at the dose of 1 mg showed a significant different at P< 0.05. On the other hand, at the dose of 10 mg, the result showed a significant difference at the level P< 0.01. The above values were obtained when the samples were compared with the distilled water as a negative control (Table 2).

Decrease in developed tension: At the dose of 0.001 mg, the developed tension dropped suddenly to 26± 9% from the basal developed tension which was 6.88± 0.48 g. The decrease was steadily increased when the extract dose was increased from 0.01 up to 10 mg. The maximum decrease in the developed tension was at 32.3± 5 % at the extract dose of 10 mg. This showed a significant difference when compared with distilled water at the level of P< 0.01 (Table 2).

Decrease in the heart rate: The heart rate was decreased to approximately 10 to 22 beats/minute from basal heart rate of 239.2± 13.4 beats/minute with the increment of the extract dose. The maximum decrease in the heart rate was achieved at the dose of 10 mg with the value of -21.9± 6.7 beats/minute. However, this decreasing tendency did not show any significant difference when compared with saline (Table 2).

The results revealed that *M. citrifolia* extract did show a clear vasodilation by the maximum decrease of coronary perfusion pressure at -37.9± 13.34 mmHg at the dose of 10 mg. Hence, the study of mechanism reaction was done in order to determine whether the nitric oxide was responsible in stimulating this effect or not.

The study of the mechanism reaction

The involvement of nitric oxide in vasodilatation mechanism by *Morinda citrifolia* extract: The *Morinda citrifolia* extract showed a decrease in the coronary perfusion pressure at the value of

Table 1: Summary of the effect of sodium nitroprusside on coronary perfusion pressure (CPF), developed tension (contractility-DT) and heart rate (HR) of isolated rat hearts

Parameters	Normal	Dose of sodium nitroprusside (µM)				
		0.0001	0.001	0.01	0.1	1.0
CPF (mmHg)	12.00± 14.47	23.75± 2.07	20.00± 4.33	26.25± 4.80	33.88± 2.02	35.13± 7.31
DT (g)	7.00± 1.84	10.08± 3.09	14.93± 4.74	20.33± 4.33	27.60± 7.91	34.38± 5.96
HR beats/min	212.50± 11.39	5.75± 1.29	7.50± 1.25	0	12.63± 2.13	13.75± 1.08

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Table 2: Summary of the effect of *Morinda citrifolia* extract on coronary perfusion pressure (CPF), developed tension (contractility-DT) and heart rate (HR) of isolated rat hearts

Parameters	Dose of <i>M. citrifolia</i> (μ M)							
	Control	0.001	0.01	0.1	1.0	10	Saline	SNP
CPF (mmHg)	122.5 \pm 10.4	242.0 \pm 11.6	18.3 \pm 10.0	15.0 \pm 10.1	-15.0 \pm 7.7	-37.9 \pm 13.4	0.8 \pm 7.2	37.9 \pm 5.2
DT (g)	6.9 \pm 0.5	-25.6 \pm 9.1	-7.7 \pm 3.3	-6.5 \pm 3.3	-7.6 \pm 1.7	-32.3 \pm 5.0	-4.6 \pm 1.9	-1.9 \pm 5.2
HR beats/min	239.2 \pm 13.4	-9.6 \pm 3.0	-10.4 \pm 2.3	-9.6 \pm 2.4	-13.8 \pm 3.7	-21.9 \pm 6.7	-8.5 \pm 3.5	-13.0 \pm 2.5

Table 3: The effect of *Morinda citrifolia* (MC) extract and sodium nitroprusside upon changes in coronary perfusion pressure of isolated rat hearts Time-matched control group (-L-NAME) versus test group (+ L-NAME). BCPP (basal coronary perfusion pressure), number of isolated hearts used per group are indicated in ()

Groups	BCPP1		Extract MC (10 mg)		BCPP2		SNP (10-6 M)	
	mmHg		Dose 1	Dose 2	mmHg		Dose 1	Dose 2
Control (4)	126 \pm 21.3	151 \pm 17.7	-22.0 \pm 2.0	-7.0 \pm 11.9	134 \pm 19.4	157 \pm 16.6	-44.0 \pm 6.2	-38.0 \pm 9.4
Test (5)	126 \pm 17.7	165 \pm 17.8	-27.0 \pm 8.0	-13.5 \pm 9.7	132 \pm 19.7	168 \pm 18.6	-45.3 \pm 9.0	-41.5 \pm 6.0

Table 4: Changing in coronary perfusion pressure of isolated rat hearts for time matched control group (L- NAME) and test group (+ L-NAME). BCPP (Basal coronary perfusion pressure). Number of isolated rat hearts used are indicated in ()

Groups	BCPP (mmHg)	Changes BCPP (mmHg)
Control (5)	125 \pm 20.7	26.0 \pm 8.7
Test (5)	142 \pm 25.5	17.5 \pm 11.7

-22 \pm 2.0 mmHg at the first dose (basal coronary perfusion pressure = 126 \pm 21.3 mmHg). As for the second dose, the coronary perfusion pressure was -7 \pm 11.9 mmHg and the basal coronary perfusion pressure was 151 \pm 18 mmHg. No significant difference between the effect of first dose and second dose was observed at the level P= 0.45 (Table 3).

At the first dose of sodium nitroprusside, it decreased the coronary perfusion pressure to -44 \pm 6.2 mmHg from its basal 134 \pm 9.4 mmHg while the decrease of -38 \pm 9.4 mmHg for the second dose (basal perfusion pressure = 157 \pm 16.6 mmHg). No significant difference was observed between the effect of first dose of SNP with the second dose at the level P= 0.68.

Test group(+L-NAME): The *M. Citrifolia* extract showed a decrease in the coronary perfusion pressure of 27 \pm 8 mmHg (basal perfusion pressure = 126 \pm 17.7 mmHg) for the first dose and -13.5 \pm 9.7 mmHg (basal perfusion pressure = 165 \pm 17.8 mmHg) for the second dose. No significant difference between the effect of the first dose with the second dose was obtained at the level p= 0.26 (Table 3). The presence of L-NAME caused the increment of the coronary perfusion pressure to 17.5 \pm 11.7 g from the basal perfusion pressure which was 142 \pm 25.5 mmHg. No significant difference was observed between the increments of coronary perfusion pressure with the presence of L-NAME compared with the time-matched control group (Table 4).

The mean comparison of coronary perfusion pressure for the first dose between time-matched control group with the test group revealed that there was non significant difference at the value of P= 0.56. The same result was obtained for the effect of extract on the coronary perfusion pressure for second dose compared with the time-matched control group and the test group (P= 0.97).

The SNP showed a decreasing tendency in the coronary perfusion pressure to 45 \pm 9 mmHg for the first dose (basal perfusion pressure = 132 \pm 20 mmHg) and the decreased of 42 \pm 6 mmHg for the second dose from its basal perfusion pressure 168 \pm 19 mmHg. There was non significant difference between the first and second dose of SNP at P= 0.66 (Table 3).

The mean comparison of the coronary of the coronary perfusion pressure between the first dose of the time-matched control group with the first dose of the test group revealed that there was no significant difference at the value of P= 0.91. As for the second dose, non significant difference was observed at P= 0.76.

Discussion

The direct effect of *M. citrifolia* extract on the isolated rat heart was studied by using Modified Langendorff isolated perfused heart preparation. The advantage of this preparation is than, any effect caused by the extract on the heart could be seen clearly without the influence of compensatory processes such as baroreceptor reflex and stimulation of autonomic nerves via *in vivo*.

In this study sodium nitroprusside (SNP) was used as a positive control. This compound is a vasodilator that acts directly to decrease the blood pressure by dilation of smooth muscle of artery and vein and by reducing the vascular systemic resistance (Benowitz, 1998), thus decreases the coronary perfusion pressure. SNP is metabolized by the smooth muscle cells to its active metabolite, nitric oxide reported to be involved in vasorelaxation (Katzung & Chatterjee, 1998). The dose-response curves of SNP showed that the maximum decrease in the coronary perfusion pressure was -15 \pm 7.7 mmHg at the dose level of 1 μ M. Thus, this dose was chosen as a positive control and standard comparison in order to determine the effect of *M. citrifolia* extract. In this study, *M. citrifolia* extract (dose 1 or 10 mg) was shown to decrease significantly the coronary perfusion pressure but slightly less than produced by SNP. Since the vasodilation effect of SNP is related to nitric oxide, further experiment was carried out to determine whether similar mechanism involved using *Morinda* extract. The effect of this vasorelaxation due to nitric oxide can be inhibited by various analogues of L-arginine substrate such as *n*-monomethyl L-arginine (L-NMMA) and Nitro-L-arginine methyl ester (L-NAME) (Rees *et al.*, 1989; Moore *et al.*, 1990). L-NAME was used in this experiment in order to determine whether the effect of vasorelaxation of smooth muscle at coronary artery vessel was due to the production of nitric oxide as consequences of the stimulation of *M. citrifolia* extract on that smooth muscle injection was given. Andriambelosen *et al.* (1997) reported that polyphenol compound with a specific structure in red wine stimulate the vasorelaxation of rat aorta with the increasing nitric oxide endothelial synthesis. This reaction could be inhibited by L-NAME. Therefore, the study towards the vasorelaxation mechanism of *M. citrifolia* extract was based on the same concepts and the results of the study revealed that L-NAME was not responsible in inhibiting the vasodilatation effect that have been produced by this product. This was due to the decreasing effect of coronary perfusion pressure which still occurred after the isolated rat heart was perfused with Krebs-Henseleit solution which contain nitric oxide inhibitor that is L-NAME. Hence, this result suggested that the effect of coronary artery vasodilatation, which was produced by this extract, did not involve the production of nitric oxide. The extract although decreased the heart rate, the effect, however, was non significant (P> 0.05) when compared

with SNP (-3.8 ± 3.7 beats/min). SNP gave its chronotropic effect through its active metabolites, nitric oxide but this was the case with *M. citrifolia* extract as shown by the inhibitor L-NAME.

In this study SNP, dose 10 μ M was also shown to decrease to the maximum developed tension. This decrease could be related to the inhibition of calcium influx. Calcium is required for muscle contraction by the reaction of the nitric oxide towards the activation of PKG on cardiac muscles.

The extract showed the negative inotropic effect with decrease in developed tension percentage from the lower dose (0.001 mg) to the higher one (10 mg), 26 ± 9 and $32 \pm 5\%$, respectively. This higher dose was almost similar to the maximum effect of sodium nitroprusside which was $34 \pm 6\%$. However, since there is no report about the effect of *M. citrifolia* extract on the art contractility, the results obtained

cannot be compared. The *M. citrifolia* extract could decrease the coronary perfusion pressure as well as the developed tension. The maximum effective dose of *M. citrifolia* extract was at the dose of 10 mg, which was obtained from dose-response curve. This dose was used to study the mechanism of vasodilatation effect and contractility. However, the mechanism did not involve nitric oxide production. Further study therefore is suggested to determine the components or phytochemicals in *M. citrifolia* fruits by identifying compounds or active components which produced effect towards the coronary perfusion pressure and the contractility of isolated heart of rat. Therefore, other studies are needed to determine the mechanism of vasodilatation reaction and the decrease in developed tension induced from this extract by a suitable inhibitor or antagonist such as determinant of prostaglandin involvement.

Furthermore, the study of other parts of this plant might also produce the same effect on the isolated heart of the rat.

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