Study on the hypoglycemic effect of mango leaf extract grown in Son La

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Abstract

Mango leaves were extracted by Soxhlet with 60% ethanol, the obtained solution was distilled to remove the solvent under reduced pressure. The results showed that when using the maximum allowable dose, there were no dead mice. Experimenting on blood glucose-lowering effects on diabetic white mice obtained the results: with a dose of 500 mg/kg giving the best therapeutic effect, even slightly better than the positive control drug gliclazide, the blood glucose level decreased immediately after three days. After 21 days reduced to 52.5% and brought the blood glucose index to normal. This is a new direction in the use of drugs of plant origin in general and Mango leaves in particular.

Keywords: *Mango leaves, diabetes, in vivo.*

1. INTRODUCTION

Mango - *Mangifera indica* L. belongs to the family Anacardiaceae. The main ingredient in mango leaves is mangiferin. In Vietnam, mango is grown everywhere in the country with more than 10 species of mango and about 30 different names, distributed from North to South. Some provinces that grow a lot of mangoes include Tien Giang, Vinh Long, Dong Nai, … Son La is the province that grows the most mangoes in the North of Vietnam. According to folk experience, mango leaves are used for the treatment of a number of diseases such as diabetes, itchy skin, digestive disorders, and lower blood pressure, ...

Up to now, in the world, there have been studies on the effects of Mango leaves such as a study on the anti-diabetic effects of Mangifera indica stem bark and leaves on a diabetic mouse model with type 1 and type 2 by Bhowmik A et al. [3] or study on the effect of Mangifera indica on blood glucose and total lipid levels of normal disease rabbits and rabbits with alloxan-induced hyperglycemia by Wadood N, Abmad N, Wadood A [5]. In Vietnam, author Nguyen Thi Ai Lan et al [1] tested the hypoglycemic effect on diabetic rats by AM of young mango leaves collected in Tra Vinh.

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In this study, we extracted mango leaves grown in Son La with ethanol at different concentrations to find the optimal concentration for the extraction process through the obtained mangiferin content, then the extraction is tested for acute toxicity and hypoglycaemic effect in mice induced hyperglycemia by streptozotocin (STZ).

2. MATERIALS AND METHODS

2.1. Research Materials

- Mango leaves are collected in Son La from February to May. After removing old leaves, the leaves were washed, dried at 110°C for 10 min to kill yeast, then, they were dried at 60°C until the humidity was not excess 13% according to Appendix 9.6 of Vietnamese Pharmacopoeia V. The leaves were stored in a cool place.

- Swiss strain white mice (20 ± 2 g) provided by the Institute of Hygiene and Epidemiology were stabilized in the laboratory.

2.2. Method

2.2.1. Extraction process

Mango leaves were crushed and then extracted Soxhlet with ethanol with different concentrations. The obtained solution was distilled under reduced pressure. The residue was dissolved with distilled water to obtain the extract. The extract was taken to determine the chemical composition by characteristic reactions.

2.2.2. Oral toxicity test LD_{50}

Mice were given increasing amounts of extract to determine LD_{50}: the lethal dose of 50% mice.

White mice regardless of breed, healthy, weight 18 - 22 g, 5 - 6 weeks old were stably raised in the laboratory, divided into 5 groups, each group of 6 - 7 animals:

+ Control group: Drink distilled water.

+ DCEtOH 1 group: Mice drank ethanol extract with the highest possible dose up to 30 g/kg body weight.

+ DCEtOH 2 group: Mice drank ethanol extract at a dose of 20 g/kg body weight.

+ DCEtOH 3 group: Mice drank ethanol extract at a dose of 10 g/kg body weight

Before giving the mice the extract, the mice were starved for 16 h. The extract was mixed with distilled water so that the volume for mice to drink was 0.1 mL/10 g of body weight. Mice were raised normally and monitored for locomotion, eating, fur, and skin,... and determined the death rate in 72 hours after drinking.

2.2.3. Method to evaluate the hypoglycemic effect in mice

- Hyperglycemia by streptozotocin (STZ) in mice:

White mice regardless of breed, healthy, weight 18 - 22g, 5 - 6 weeks old were stably raised in the laboratory.

Mice were injected peritoneally with STZ (mixed in citrate buffer pH = 4.5) at a dose of 100 mg/kg, after 24 hours of injection at a dose of 50 mg/kg. After 72 hours, measure blood glucose, and select mice with blood glucose ≥ 10 mmol/L to include in the study.

- *Methods to evaluate the blood glucose lowering effect of the samples:*

Rats were divided into groups (n = 6 - 7) and given the following test samples or distilled water as follows:

+ Control group: normal mice, drink distilled water.

+ Diabetic control group: Mice caused hyperglycemia, drink distilled water.

+ Reference group: Mice caused hyperglycemia, drink gliclazide (60 mg/kg body weight/time/day).

+ DCEtOH- dose 1 group: Mice caused hyperglycemia, drank extraction of 250 mg/kg body weight.

+ DCEtOH- dose 2 group: Mice caused hyperglycemia, drank extraction of 500 mg/kg body weight.

+ DCEtOH-dose 3 group: Mice caused hyperglycemia, drank extraction of 1,000 mg/kg body weight.

The extract was mixed with distilled water so that the volume for rats to drink was 0.1 mL/10 g of body weight. Rats were given extracts daily at a certain time. Then, mice were taken blood and measure glucose after 3, 7, 14, 21 days. The mice were starved for 12 h before sampling. Blood was taken from the tail vein of the mice to determine glucose by a rapid test kit.

3. RESULTS AND DISCUSSIONS

3.1. Extraction and determination of mangiferin

3.1.1. Determination of mangiferin in mango leaf extract by thin layer chromatography

- *Test solution (T)*: extraction solution 5 g/100 mL.
- *Standard solution (C)*: mangiferin 25 mg/100 mL.
- *Chromatographic condition:* silicagel GF 254 thin layer chromatography, solvent system: EtOAc - HCOOH - H_2O [10 : 1,5 : 1], reagent NH_4OH.

On the chromatogram of the mango leaf extract, 8 spots were found, of which 1 had R_f and the color was similar to that of standard mangiferin. It is shown that the extract contains mangiferin.

Figure 1. *The chromatogram of mango extract*

3.1.2. Determination of mangiferin in mango leaf extract by HPLC

- *Chromatographic condition:* BDS Hypersil C18 (250 × 4,6mm; 5µm) column, mobile phase: ACN - CH_3COOH 3% (14 : 86), detector UV 257 nm.

Mango leaves were extracted with ethanol at 3 concentrations of 90, 60, and 40%. The content of mangiferin obtained was 4.66, 6.31, and 5.90%, respectively. Thus, when using 60% ethanol solvent for extraction, the obtained mangiferin content was the highest. Therefore, 60% ethanol solvent was used for the extraction in this study.

3.2. Result of acute toxicity test (LD_{50}) by oral route

Mice were given oral doses of 10, 20, 30 g/kg body weight, respectively; no mice died. Thus, with the maximum allowable dose of 30 g/kg body weight, no mice died. Therefore, we have not determined the LD_{50} of oral mango leaf extract. This result showed that the extract of mango leaves administered orally was not toxic to mice.

3.3. Results of testing the effects of mango leaf extract on diabetic mice

3.3.1. Blood glucose levels during the days of taking extraction

Results of the experiment of 2.2.3 are presented in Table 1 to Table 6.

Physiological control group: normal mice, drink distilled water (Table 1).

Table 1. *Blood glucose level of Physiological standard group*

Physiological control group	***Glucose (mmol/L)***			
	Day 3	***Day 7***	***Day 14***	***Day 21***
1	6.2	6.1	6.2	6.3
2	5.9	5.8	5.8	5.9
3	5.7	5.5	5.6	5.7
4	6.6	6.3	6.4	5.7
5	6.0	5.7	6.0	6.1
6	6.4	6.4	6.3	6.4
7	6.2	6.2	6.4	6.4
Average ± SD	6.1 ± 0.3	6.0 ± 0.3	6.1 ± 0.3	6.1 ± 0.3

The results showed that the blood glucose level was relatively stable, the level of change was not significant.

Diabetic control group: Mice caused hyperglycemia, drink distilled water (Table 2).

Table 2 . *Blood glucose level of diabetic control group*

Diabetic control group	***Glucose (mmol/L)***			
	Day 3	***Day 7***	***Day 14***	***Day 21***
1	26.2	33	33	33
2	28.7	33	33	33
3	29.4	33	33	33
4	28.7	33	33	33

5	29.7	33	33	33
6	24.8	33	33	33
Average ± SD	27.9 ± 2.0	33	33	33

The results showed that the blood glucose level after 3 days increased very high, from the 7th day, they were all higher than the defined threshold of the measurement device.

Reference group: Mice caused hyperglycemia, given gliclazide (dose 60 mg/kg body weight/time/day) (Table 3).

Table 3. *Blood glucose level of reference group*

Reference group	***Glucose (mmol/L)***			
	Day 3	***Day 7***	***Day 14***	***Day 21***
1	9.3	12.7	8.1	6.7
2	10.4	5.6	7.1	8.6
3	15.2	15.2	11.7	9.8
4	9.4	12.1	6.7	7.3
5	10.2	13.7	8.0	6.3
6	10.2	13.1	8.2	5.6
Average ± SD	10.8 ± 2.2	12.1 ± 3.3	8.3 ± 1.8	7.4 ± 1.5

The results showed that the blood glucose level increased up to day 7, then decreased on day 21.

DCEtOH- dose 1 group: Mice caused hyperglycemia, drank of 250 mg/kg (Table 4).

Table 4. *Blood glucose level of DCEtOH - dose 1 group*

DCEtOH - Dose 1	***Glucose (mmol/L)***			
Group	***day 3***	***day 7***	***day 14***	***day 21***
1	11.1	11.1	6.3	6.9
2	10.7	7.2	5.3	6.8
3	11.1	8.0	4.7	6.7
4	13.1	16.1	5.1	7.6
5	15.1	8.2	5.8	6.8
6	9.8	7.6	5.2	6.2
Average ± SD	11.8 ± 2.0	9.7 ± 3.4	5.4 ± 0.6	6.8 ± 0.5

The results showed that the blood glucose level decreased continuously from day 3 to day 14, but by day 21 the blood glucose level increased again.

DCEtOH- dose 2 group: Mice caused hyperglycemia, drank of 500 mg/kg (Table 5).

Table 5. *Blood glucose level of DCEtOH - dose 2 group*

DCEtOH - Dose 2 group	***Glucose (mmol/L)***			
	Day 3	***Day 7***	***Day 14***	***Day 21***
1	22.0	15.6	10.1	11.1
2	11.0	7.6	5.1	5.4
3	10.3	7.0	5.8	6.2
4	9.7	7.2	5.4	5.4
5	12.4	4.4	6.6	6.1
6	9.7	7.6	6.1	5.5
Average ± SD	12.5 ± 4.8	8.2 ± 3.8	6.5 ± 1.8	6.6 ± 2.2

The results showed that blood glucose decreased continuously from day 3 to day 14, to day 21 blood sugar increased but not significantly.

DCEtOH- dose 3 group: Mice caused hyperglycemia, drank of 1,000 mg/kg (Table 6).

Table 6. *Blood glucose level of DCEtOH - dose 3 group*

DCEtOH - dose 3 group	***Glucose (mmol/L)***			
	Day 3	***Day 7***	***Day 14***	***Day 21***
1	10.6	10.4	7.3	6.7
2	10.2	9.4	6.6	5.7
3	26.3	26.0	17.6	13.9
4	11.2	9.4	7.6	5.9
5	31.7	31.6	12.5	19.3
6	10.0	8.3	8.0	8.2
Average ± SD	16.7 ± 9.7	15.1 ± 10.9	9.9 ± 4.3	7.8 ± 3.1

The results showed that the first week of treatment with a high concentration of 1,000 mg/kg reduced blood glucose insignificantly when from day 3 to day 7 it only decreased by 9.04%. Until day 14, blood glucose decreased better (41.72%) and by day 21, blood glucose level was only 7.8 mmol/L, corresponding to a reduction rate of 53.3%.

Overall, the results show that the mango leaf extract has the ability to lower blood sugar in the experimental diabetes model by STZ. The blood glucose lowering effect of gliclazide (Diamicron®, Servier) was quite rapid, but the most effective in the model was the extract from mango leaves at the concentration of 500 mg/kg.

3.3.2. Blood sugar before and after 21 days of testing

Before treatment, the groups of diabetic mice had no statistically significant difference in blood glucose values. After 21 days of treatment, diabetic mice treated with mango leaf extract had blood glucose levels reduced to normal. At a dose of 250 mg/kg, blood glucose decreased by 48.5%. At a dose of 500 mg/kg, there is a potential for lowering blood glucose by 52.5%. At the highest concentration of 1,000 mg/kg, blood glucose concentration decreased by 45.8%. For diabetic mice treated with Gliciazide, after 21 days, the reduction was equivalent to 32.1%. The results was shown in Table 7.

Table 7. *Changes in blood glucose of mice before and after diabetes treatment*

Group	***n***	***Glucose (mmol/L) (percent hypoglycemia)***				
		Before treatment	***After treatment with mango extraction***			
			3 days	***7 days***	***14 days***	***21 days***
Control	7	6.0 ± 0.5	6.1 ± 0.3 (- 0.2%)	6.0 ± 0.3 (0%)	6.1 ± 0.3 (-0.2%)	6.1 ± 0.3 (-0.2%)
Diabetes + distilled water	6	10.7 ± 2.5	27.9 ± 2.0 (- 260.7%)	33	33	33
Diabetes + Gliclazide	6	10.9 ± 1.8	10.8 ± 2.2 (1.0%)	12.1 ± 3.3 (-11.0%)	8.3 ± 1.8 (23.8%)	7.4 ± 1.5 (32.1%)
Diabetes + extraction 250 mg/kg	6	13.2 ± 2.0	11.8 ± 2.0 (10.6%)	9.7 ± 3.4 (26.5%)	5.4 ± 0.6 (59.1%)	6.8 ± 0.5 (48.5%)
Diabetes + extraction 500 mg/kg	6	13.9 ± 4.8	12.5 ± 4.8 (10.1%)	8.2 ± 3.8 (41.0%)	6.5 ± 1.8 (53.2%)	6.6 ± 2.2 (52.5%)
Diabetes + extraction 1,000 mg/kg	6	14.4 ± 6.3	16.7 ± 9.7 (- 16.0%)	15.1 ± 10.9 (- 0.5%)	9.9 ± 4.3 (31.2%)	7.8 ± 3.1 (45.8%)

4. CONCLUSION

This study conducted extracting from mango leaves grown in Son La. We did not determine the LD_{50} of mango leaf extract in ethanol at 60°C, when using the maximum allowable dose, there was no death of mice. Successfully implemented a diabetic mice model by injecting STZ peritoneally with two doses, 100 mg/kg on day 1 and 50 mg/kg on day 2. Results of testing mango leaf extract on STZ model showed that 500 mg/kg dose gave the best therapeutic effect, even slightly better than gliciazide treatment, blood glucose level decreased immediately after 03 days of use and after 21 days reduced to 52.5% and brought the blood glucose level to normal.

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Nghiên cứu tác dụng hạ đường huyết của dịch chiết lá xoài trồng tại Sơn La

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Tóm tắt

Lá xoài được chiết Soxhlet bằng ethanol 60%, dịch thu được cất loại dung môi dưới áp suất giảm thu được cắn. Cắn được hòa tan bằng nước cất thu được dịch chiết rồi tiến hành cho các lô chuột uống dịch chiết. Kết quả cho thấy khi dùng liều tối đa cho phép vẫn không xuất hiện chuột bị chết. Tiến hành thử tác dụng hạ glucose huyết trên chuột nhắt trắng đái tháo đường (ĐTĐ) thu được kết quả: với liều 500 mg/kg cho hiệu quả điều trị tốt nhất, thậm chí còn nhỉnh hơn so với thuốc chứng dương gliciazid, mức đường huyết giảm ngay sau 03 ngày sử dụng và sau 21 ngày giảm đến 52,5% và đưa chỉ số glucose huyết về mức bình thường. Đây là một hướng mới trong việc sử dụng thuốc có nguồn gốc thực vật nói chung và lá xoài nói riêng.

Từ khóa: *Lá xoài, đái tháo đường, in vivo.*