

Hypoglycemic effect of *Curcuma comosa* Roxb. (Nanwin-ga) rhizomes on rabbits model

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This study was carried out to determine the phytochemical constituents, acute toxicity and hypoglycemic effect of crude powder and 80% ethanolic extract of dried rhizomes of *Curcuma comosa* Roxb. (Nanwin-ga). Adrenaline-induced hyperglycemic rabbits were used to study the hypoglycemic effect. Oral route of administration was used in this study. The phytochemical studies of the crude powder and 80% ethanolic extract of this plant showed that both contained alkaloids, flavonoids, glycosides, steroids, saponins, tannins and amino acid. In acute toxicity study in mice, it was observed that the crude powder of the rhizomes was not toxic up to the maximal feasible dose of 5g/kg. But, 80% ethanolic extract of the dried rhizomes showed mild acute toxic effect and median lethal dose (LD₅₀) was determined to be 5.2 g/kg and its confident limit was 4 g/kg - 6.76 g/kg. The results showed that the 80% ethanolic extract of the rhizomes at the dose level of 1.5 g/kg had significant hypoglycemic effect at 2 hr, 3hr and 4 hr (p<0.01- p<0.05) after oral administration when compared with those of the controls. But, the crude powder of the rhizomes at the dose level of 3 g/kg showed no significant hypoglycemic effect. It was observed that the hypoglycemic effect of 80% ethanolic extract was inferior to that of the standard drug glibenclamide. Therefore, it can be concluded that the 80% ethanolic extract of the dried rhizomes of *Curcuma comosa* Roxb. (Nanwin-ga) possessed significant hypoglycemic effect on adrenaline-induced hyperglycemic rabbits model and the effect was inferior to that of glibenclamide.

INTRODUCTION

Diabetes mellitus is a common clinical syndrome and complications of diabetes mostly effect on the eyes, the kidney, nervous system and vascular system [1]. Diabetes mellitus is worldwide in distribution and the estimated worldwide prevalence of diabetes in 1997 derived from WHO data was 124 million people and the number of people with diabetes is expected to increase to 221 millions in 2010 [2]. The present day drugs including insulin and oral hypoglycemic agents are used to control the blood sugar level in diabetes mellitus. But these drugs have considerable side effects and toxicities.

Even today a large number of herbal drugs are being used for the treatment of diabetes mellitus in different regions of the world. Mya Bwin and Sein Gwan [3] mentioned a large numbers of herbal drugs which are reputed for the treatment of diabetes mellitus.

Curcuma comosa Roxb. (Nanwin-ga) (Family-Zingiberaceae) grows widely in Myanmar and Thailand. In Myanmar traditional medicine, this plant is known to be useful in the treatment of diabetes mellitus, hypertension, fever, hyperlipidemia and stress [4]. It also contains in some Myanmar traditional medicine formulations for hypertension and diabetes

mellitus. But, there is no scientific report of hypoglycemic effect of *Curcuma comosa* Roxb. in experimental animal model and human in Myanmar. Therefore, this study was performed to investigate the hypoglycemic effect of the rhizomes of *Curcuma comosa* Roxb. on rabbits model.

MATERIALS AND METHODS

Type of study and study design

Type of the study was experimental animal study. Parallel study design for acute toxicity study in mice and cross over study design for hypoglycemic activity study in rabbit model were used. Site of the study was Pharmacology Research Division, Department of Medical Research (LM).

Ethanol extraction of the rhizomes of Curcuma comosa Roxb.

The rhizomes of *Curcuma comosa* Roxb. collected from Yangon were air dried in the shade and then powdered by electric grinder. One-hundred grams of the air-dried rhizomes powder were put into conical flask of 2 litres capacity and 80% ethanol was added up to 500 ml capacity. Then, the conical flask was put onto the water bath and heated at 70°C for 12 hours. After 12 hours of extraction, the resultant extract was cooled down to room temperature and the supernatant solution was decanted. The supernatant solution was filtered through the cheese cloth. The filtrate was placed in the petridish and evaporated to dryness on a boiling water bath [5].

Phytochemical studies and physicochemical tests

Phytochemical studies of both crude powder and 80% ethanolic extracts of the rhizomes of *Curcuma comosa* Roxb. were performed qualitatively for the presence of alkaloids, flavonoids, glycosides, steroids, saponins, tannins, resin, amino acid, phenol and cyanogenic glycoside using the method of Physicochemical standards of Unani formulations [6]. The physicochemical tests

of the crude powder of the rhizomes of this plant were done by the method of WHO, 1998 [7].

Acute toxicity studies of Curcuma comosa Roxb. rhizomes on albino mice

The acute toxicity test was done to determine the symptomatology consequent, degree of toxicity to administration of the drug and to find out the median lethal dose (LD₅₀) by the method of Litchfield & Wilcoxon, 1949 [8].

Fifty albino mice of both sexes, weighing 25-35 gm were used in this study. The mice were separated into 5 groups and each group contained 10 mice. Food was withheld for the period of 18 hours before the experiment but they were allowed free access to water. One group served as the control and only drug vehicle (i.e 10% tween 80) was administered orally. 80% ethanolic extract of *Curcuma comosa* Roxb. was dissolved in 10% tween 80 and the required doses were administered orally with intragastric needle to every mice. Each group of mice received each dose level of the 80% ethanolic extract (i.e 2g/kg, 4g/kg, 6g/kg and 8g/kg body weight). Then, the mice were observed for behavior, neurologic and autonomic signs 1 hourly up to 6 hours after administration of the extracts and daily up to two weeks. The crude powder of the dried rhizomes of *Curcuma comosa* Roxb. was suspended in 1% methyl cellulose and the same procedure described above was applied for acute toxicity study on albino mice at the dose levels of 1g/kg, 2g/kg, 4g/kg and 5g/kg body weight.

The effect of the crude powder of Curcuma comosa Roxb. rhizomes on blood glucose level in adrenaline-induced hyperglycemic rabbits

Six adult healthy rabbits (JW strain) of both sexes weighing 2.73±0.07 Kg were used in this study. The rabbits were kept without food for 18 hours with free access to water before the experiment.

In the control group, only drug vehicle (1% methyl cellulose 10ml/kg) was administered orally to each rabbit by using the intragastric tube connected to a plastic syringe containing the calculated volume of 1% methyl cellulose. Before the drug vehicle administration, 0.1ml of blood was collected from marginal ear vein as the baseline blood sample. Then, 1% methyl cellulose was administered orally and just after that, the rabbit was made hyperglycemic by injecting the adrenaline (0.2 mg/kg) (MPF) subcutaneously [9]. Similar sample of 0.1ml of blood was collected at 1 hr, 2 hr, 3 hr and 4 hr intervals after subcutaneous injection of adrenaline. Blood glucose levels were determined at 0 hr, 1 hr, 2 hr, 3 hr and 4 hr by using the glucometer (Elite, Bayer Company limited, USA). After drug free interval of one week, the same 6 rabbits were used again and the same procedures were performed as above for determination of the blood glucose levels of the rabbits after oral administration of the crude powder of *Curcuma comosa* Roxb. rhizomes (suspended in 1% methyl cellulose) at the dose of 3g/kg.

The effect of 80% ethanolic extract of Curcuma comosa Roxb. rhizomes on blood glucose level in adrenaline-induced hyperglycemic rabbits

Six adult healthy rabbits (JW strain) of both sexes weighing 2.16 ± 0.329 kg were used in this study. The rabbits were kept without food for 18 hours with free access to water before the experiment. In the control group, only drug vehicle (i.e 10% tween 80 10ml/kg) was administered orally to each rabbit by using the intragastric tube. The same procedures were performed as above for induction of hyperglycemia with adrenaline, collection of blood and determination of blood glucose levels. After drug free interval of one week, the same 6 rabbits were used again and the same procedure was repeated before the experiment. Then, the 80% ethanolic extract of this plant was dissolved in 10% tween 80 and the same procedures were performed as above for

determination of blood glucose levels of the rabbits after oral administration of the extract at the dose levels of 0.375g/kg and also for 0.75g/kg and 1.5g/kg.

The effect of standard drug glibenclamide on blood glucose level in adrenaline-induced hyperglycemic rabbits

After drug free interval of one week, the same six rabbits were used again and the same procedure was repeated. Then, the standard drug glibenclamide 4mg/kg (Hovid Company Ltd, Malaysia) dissolved in distilled water was administered to the rabbit orally. Then, the same procedure was performed as above for determination of blood glucose levels.

Data analysis

The results were shown in (mean \pm standard error). Student 't' tests were used for calculation of statistical significance between control and treated groups.

RESULT

Extraction of the rhizomes of Curcuma comosa Roxb.

The 80% ethanolic extraction of dried rhizomes powder of *Curcuma comosa* Roxb. yielded 11.9 g ethanolic extract per 100g of dried rhizomes powder (i.e 11.9 %).

Phytochemical studies and physicochemical tests

The results are shown in Table 1.

Table 1. Phytochemical studies of the crude powder and 80% ethanolic extract of the rhizome of *Curcuma comosa* Roxb.

Pythochemical costituents	Crude powder	80% ethanolic extract
Alkaloids	(+)	(+)
Flavonoids	(+)	(+)
Glycosides	(+)	(+)
Steroids	(+)	(+)
Saponin	(+)	(+)
Tannin	(+)	(+)
Amino acid	(+)	(+)
Phenol	(-)	(-)
Resin	(-)	(-)
Cyanogenic glycoside	(-)	(-)

+ present - absent

Physicochemical tests of the crude powder of *Curcuma comosa* Roxb. rhizomes showed moisture content (11.8%), swelling index (0.6 cm), foaming index (<100%), watery extract (18.7%), ethanolic extract (7.3%), chloroform extract (8.8%) and petroleum ether extract (2.4%).

Acute toxicity study of the rhizomes of Curcuma comosa Roxb.

In the present study, it was found that the crude powder of the rhizomes of this plant showed no toxic effect and lethality up to the maximal feasible dose of 5g/kg body weight. In the acute toxicity study of 80% ethanolic extract of this plant's rhizomes, it was observed that the mice died within 7 days after single dose oral administration of the extract at the dose level of 4g/kg, 6g/kg and 8g/kg. It was observed that there were decrease in motor activity, sedation, reduction of screen grip, ataxia, piloerection and finally, they died at these dose levels. On the autopsy examination, the internal organs such as brain, heart, lungs, liver, stomach, intestine, kidneys, spleen and pancreas were found to be grossly normal. In this study, LD₅₀ of 80% ethanolic extract of *Curcuma comosa* Roxb. rhizomes was found to be 5.2 g/kg and its confidence limit was 4-6.76 g/kg.

The effect of the crude powder of Curcuma comosa Roxb. rhizomes on blood glucose level in adrenaline-induced hyperglycemic rabbits

In the control group, the significant increases in blood glucose levels were observed at 1hr, 2hr, 3hr and 4hr after subcutaneous injection of adrenaline tartrate (P <0.001) (Fig.1).

The mean blood glucose levels of the rabbits treated with the crude powder of *Curcuma comosa* Roxb. rhizomes 3g/kg at 0hr, 1hr, 2hr, 3hr and 4hr after subcutaneous injection of adrenaline tartrate were 78.5 ± 4.53mg/dl, 252.25 ± 24.13mg/dl, 321.67 ± 12.52mg/dl, 386.33 ± 16.39mg/dl and 366.5 ± 21.38mg/dl respectively. It was observed that there was no significant decrease in

blood glucose levels when compared with those of the controls. The results are shown in Fig. 1.

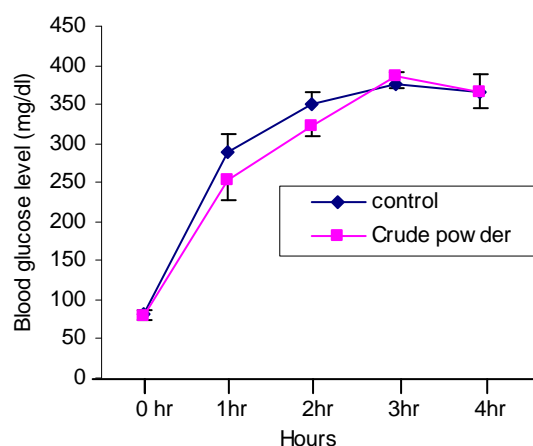


Fig. 1. Hypoglycemic effect of the crude powder of *Curcuma comosa* Roxb. rhizomes on adrenaline-induced hyperglycemic rabbits (n=6). The results in mean ± SE.

The effect of 80% ethanolic extract of Curcuma comosa Roxb. rhizomes on blood glucose level in adrenaline-induced hyperglycemic rabbits

In the control group, the significant increases in blood glucose levels were observed at 1hr, 2hr, 3hr, and 4hr after subcutaneous injection of adrenaline tartrate (P<0.001) (Fig. 2).

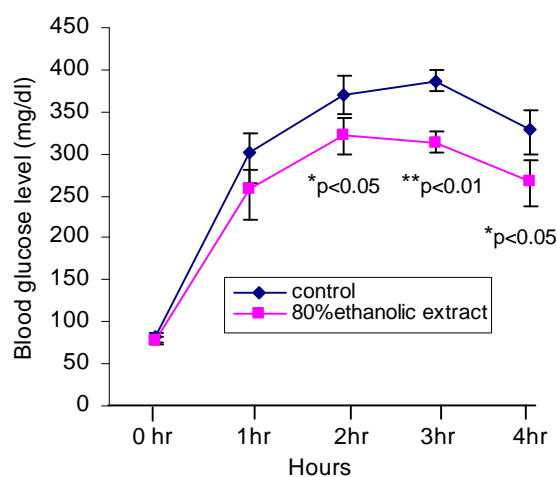


Fig 2. Hypoglycemic effect of 80% ethanolic extract of *Curcuma comosa* Roxb. rhizomes (1.5g/kg) on adrenaline-induced hyperglycemic rabbits (n=6). The results in mean ± SE. *p< 0.05, **p<0.01, ***p<0.001

The mean blood glucose levels of the rabbits treated with 80% ethanolic extract of *Curcuma comosa* Roxb. rhizomes (0.375g/kg) at 0 hr, 1 hr, 2 hr, 3 hr and 4 hr after subcutaneous injection of adrenaline tartrate were 77 ± 3.54 mg/dl, 279.83 ± 34.61 mg/dl, 334.83 ± 37.38 mg/dl, 327.33 ± 40.64 mg/dl and 310.83 ± 54.91 mg/dl, respectively. It was found that there was no significant decrease in blood glucose levels when compared with those of the controls. The mean blood glucose levels of the rabbits treated with 80% ethanolic extract of the rhizomes (0.75mg/kg) at 0 hr, 1 hr, 2 hr, 3 hr and 4 hr after subcutaneous injection of adrenaline tartrate were 71.5 ± 6.07 mg/dl, 263.33 ± 35.29 mg/dl, 343.17 ± 25.62 mg/dl, 333.67 ± 25.24 mg/dl and 306.83 ± 40.78 mg/dl, respectively. It was found that there was no significant decrease in blood glucose levels when compared with those of the controls.

The mean blood glucose levels of the rabbits treated with 80% ethanolic extract of *Curcuma comosa* Roxb. rhizomes (1.5g/kg) at 0hr, 1hr, 2hr, 3hr and 4hr after subcutaneous injection of adrenaline tartrate were 77.33 ± 5.33 mg/dl, 258 ± 35.96 mg/dl, 321.17 ± 26.65 mg/dl, 313.83 ± 12.01 mg/dl and 268.33 ± 36.54 mg/dl, respectively. Significant decreases in blood glucose levels were found at 2hr ($P < 0.05$), 3hr ($P < 0.01$) and 4hr ($P < 0.05$) when compared with those of the controls (Fig. 2).

The effect of standard drug (glibenclamide) on blood glucose level in adrenaline-induced hyperglycemic rabbits

The mean blood glucose levels of the rabbits treated with standard drug, glibenclamide (4mg/kg) at 0hr, 1hr, 2hr, 3hr and 4 hr after subcutaneous injection of adrenaline tartrate were 75 ± 2.46 mg/dl, 245.5 ± 39.73 mg/dl, 271.33 ± 19.35 mg/dl, 241.5 ± 26.74 mg/dl and 216 ± 33.76 mg/dl, respectively. Significant decreases in blood glucose levels were found at 1 hr ($P < 0.05$), 2 hr ($P < 0.001$), 3 hr ($P < 0.01$) and 4 hr ($P < 0.05$) when compared with those of the controls. The mean percent reductions of

blood glucose levels with glibenclamide were 25.53 ± 9.93 %, 32.14 ± 3.69 %, 44.61 ± 9.35 % and 44.29 ± 12.45 % for 1 hr, 2 hr, 3 hr and 4 hr, respectively. The mean percent reductions of blood glucose levels with ethanolic extract of *Curcuma comosa* Roxb. rhizomes were 15.12 ± 4.74 %, 22.05 ± 3.39 %, and 25.32 ± 9.97 % at 2 hr, 3 hr and 4 hr, respectively. The results are shown in Fig. 3.

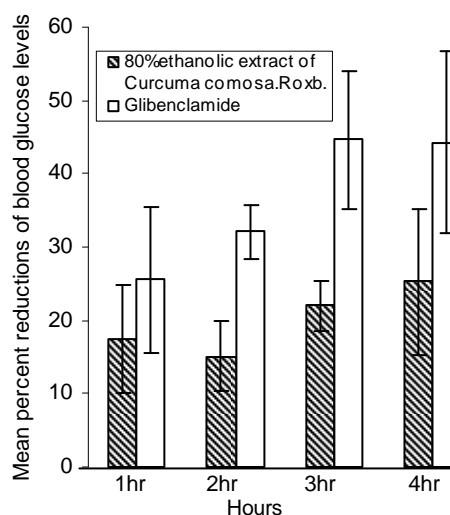


Fig 3. Mean percent reductions of blood glucose levels with 80% ethanolic extract of *Curcuma comosa* Roxb. rhizomes (1.5g/kg) and glibenclamide (4mg/kg) in adrenaline-induced hyperglycemic rabbits

DISCUSSION

In the phytochemical studies of the crude powder and 80% ethanolic extract of the rhizomes of this plant, both did not contain toxic plant compound like cyanogenic glycoside. In the acute toxicity studies of the crude powder and 80% ethanolic extract of the rhizomes of *Curcuma comosa* Roxb. oral route of administration was used because this route of administration is the intended route for use in man. In this study, it was found that the crude powder of the rhizomes of the plant showed no toxic effect and lethality up to the maximal feasible dose of 5g/kg. So, the LD₅₀ of the crude powder was more than 5g/kg.

In the acute toxicity study of 80% ethanolic extract of rhizomes of the plant, it was

found that median lethal dose (LD₅₀) was 5.2 g/kg and its confidence limit was 4 g/kg -6.76 g/kg. So, 80% ethanolic extract of the dried rhizomes of *Curcuma comosa* Roxb. showed mild acute toxic effect on mice. In this study, adrenaline-induced hyperglycemic rabbit model was used as the transient hyperglycemic model because adrenaline can produce hyperglycemia by three mechanisms. They are by inhibiting the release of insulin from pancreas, by reducing the peripheral utilization of glucose and by causing glycogenolysis in liver [10]. This adrenaline-induced hyperglycemic rabbits model is also the commonly used model.

In the study of hypoglycemic effect of the crude powder, at the maximal feasible dose of 3g/kg, it was found that there was no significant hypoglycemic effect. So, the higher dose of the crude powder was needed to show the significant hypoglycemic effect. In this study, it was observed that 80% ethanolic extract of *Curcuma comosa* Roxb. rhizomes (1.5g/kg) showed the significant hypoglycemic effect at 2 hr (P<0.05), 3 hr (P<0.01) and 4 hr (P<0.05) after oral administration of the extract when compared with those of the controls. Mean percent reduction of blood glucose level with 80% ethanolic extract ranged from 15.12% to 25.32%. But there were no significant hypoglycemic effects at the lower dose levels of 0.375 g/kg and 0.75 g/kg of the extracts. In the present study, the standard drug, glibenclamide 4mg/kg showed significant hypoglycemic effect from 1hr up to 4hr (P<0.05-P<0.001) after oral administration of the drug. Mean percent reduction of blood glucose level with glibenclamide ranged from 25.53% to 44.61%. In the comparison between hypoglycemic effect of 80 % ethanolic extract of *Curcuma comosa* Roxb. rhizomes with that of standard drug, glibenclamide, it was found that the effect of 80% ethanolic extract was less than (ie. 56.85%) that of glibenclamide (P<0.05). Oliver Bever & Zahnd [11] reported that the chemical

constitutions of the hypoglycemic principles from the plants are flavonoids, tannin, alkaloids, steroids and glycosides. Therefore, in this study, hypoglycemic action of 80% ethanolic extract of the rhizomes of *Curcuma comosa* Roxb. may be due to one or more compounds (i.e alkaloids, flavonoids, glycosides, tannin, steroids) obtained in this extract. It can be concluded that 80% ethanolic extract of rhizomes of *Curcuma comosa* Roxb. could produce significant hypoglycemic effect on adrenaline-induced hyperglycemic rabbit model and the effect was inferior to that of glibenclamide.

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