



# *Tribulus Terrestris* Induces the Relaxation of Porcine Coronary Artery

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**Objectives:** Angina pectoris is usually due to spasm or obstruction of the coronary arteries that furnish blood to the heart muscle. Clinical symptoms include chest pain and discomfort, and the discomfort also can happen in patient's jaw, shoulders, arms, neck, or back. The medications in traditional Chinese medicine are a few for angina pectoris in clinical practice. Furthermore, the effect of *Tribulus terrestris* (*T. terrestris*) on coronary artery is unclear.

**Methods:** *T. terrestris* and its active ingredients including kaempferol, ruscogenin, protodioscin, protogracillin, and harmine were used in this study to examine their effect on porcine coronary artery.

**Results:** The results showed *T. terrestris* can induce relaxation of porcine coronary artery in a dose dependent manner after U46619-induced contraction.

**Conclusions:** The results provide a potential to develop *T. terrestris* for the treatment of angina pectoris in traditional Chinese medicine.

**Key words:** angina, *Tribulus terrestris*, coronary artery, relaxation

## Introduction

Angina pectoris is defined as chest pain, pressure, or discomfort. These symptoms are typically precipitated by strain and/or emotional stress, lasting for more than 30 to 60 seconds, and are palliated by rest and nitroglycerin.<sup>1</sup> Clinical symptoms include chest pain and discomfort, and the discomfort also can happen in the epigastric area, back, neck area, jaw, or shoulders. Major risk factors for angina include tobacco use, diabetes, high blood cholesterol or elevated triglyceride levels, high blood pressure, lack of exercise, and family

history of heart disease.<sup>2</sup> The common specific medications for angina pectoris include  $\beta$ -blockers, nitrates, calcium channel blockers, and the late sodium current blocker ranolazine.<sup>2</sup> Though some traditional Chinese herbal products are used in patients with angina pectoris, the effect of the use of traditional Chinese herbal products for angina is still not fully clarified.<sup>3</sup> Hence, it is very important to find more efficacious and safe herbal medication for the treatment of angina pectoris.

*Tribulus terrestris* (*T. terrestris*) is an annual herb of the Zygophyllaceae family and is commonly known as Gokshur, Gokharu, or puncturevine. This plant has been applied

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in traditional medicine to treat a variety of diseases for centuries. The main active plant components of this plant include flavonoids, alkaloids, saponins, lignin, amides, and glycoside. Different plant parts have different pharmacological activities. The extracts of the aerial parts and fruits have been applied for tonic, aphrodisiac, and diuretic effects. In addition, it also has an anti-inflammatory, antimicrobial and antioxidant effect. *T. terrestris* is most commonly applied for loss of libido and infertility. Furthermore, it has hepatoprotective, hypolipidemic, immune-modulating anthelmintic and anti-cancer activities.<sup>4-6</sup>

To the best of our knowledge, there has been no research on the effect of *T. terrestris* on coronary artery in pigs. The purpose of this study was to study the effect of *T. terrestris* on coronary artery in pigs. In addition, the study is expected to develop *T. terrestris* to treat angina pectoris in traditional Chinese medicine.

## Materials and Methods

### Materials

This study was exempted from the review of Institutional Animal Care and Use Committee of E-DA Hospital because we involved no live animals using in this study and the coronary artery we used were mainly obtained from local slaughterhouses. These specimens were obtained from pigs with a net weight of approximately 110 kg and these pigs were stunned and exsanguinated in a regulated slaughterhouse overseen by Council of Agriculture, Executive Yuan, R.O.C. (Taiwan). After taking the specimens, we stored them in ice-cold oxygenated Krebs-Henseleit solution and transferred them back to the laboratory as soon as possible. The delivery time was about 30 minutes. The Krebs-Henseleit buffer solution consists of the following: 1.2 mM  $\text{NaH}_2\text{PO}_4$ , 4.7 mM KCl, 118 mM NaCl, 25 mM  $\text{NaHCO}_3$ , 1.8 mM  $\text{CaCl}_2$ , and 14 mM glucose, and maintained at a constant pH of 7.4. Kaempferol,

ruscogenin, protodioscin, protogracillin, and harmine were purchased from ChemFaces Biochemical Co., Ltd. (Hubei, PRC).

### Preparation of *T. terrestris*

Concentrated preparations of traditional Chinese medicine *T. terrestris* was purchased from Kaiser pharmaceutical CO., Ltd. (Tainan, Taiwan). 30 mg powder of concentrated preparations of traditional Chinese medicine *T. terrestris* was dissolved in 1 mL 20% ethanol for this study.

### Measurement of *T. terrestris*-induced relaxation of porcine coronary artery after U46619-induced contraction

Anatomical structures of coronary artery between humans and pigs are similar. Therefore, pigs are often used to study the disorders or diseases related to human coronary artery.<sup>7</sup> First, the epicardium of the obtained specimen was cut off to expose the coronary artery, and then the coronary artery was extracted, and then surgical scissors was used to cut the coronary artery to segmental arterial tubule of about 1 cm long and 0.3 cm wide. Furthermore, these segmental arterial tubules were immersed in a 7 mL organ bath containing 5 mL Krebs-Henseleit buffer solution. The temperature of Krebs-Henseleit buffer solution in organ bath is maintained at 37°C with continuous injection of 95%  $\text{O}_2$  and 5%  $\text{CO}_2$ . Additionally, the segmental arterial tubules were fixed to the two the specific brackets, and one end of the bracket is tied to another bracket of the organ bath with silk surgical wire, and the other end is tied to an isometric transducer (FORT10g; Grass Technologies, RI, USA). The isometric transducer is connected to the amplifier (Gould Instrument Systems, OH, USA), and then signal is recorded by a computer recording system (BIOPAC Systems, CA, USA).<sup>8</sup> In this study, the vascular tension was adjusted to 1.0 g. After 30 minutes of equilibration, 60 mM KCl-contained Krebs-Henseleit solution

was added into the organ bath to cause contraction of arterial tubules, then 60 mM KCl-contained Krebs-Henseleit solution was wash off with Krebs-Henseleit buffer solution 3 times, and after a 30-minute equilibrium period again, 1  $\mu$ M U46619 was added to the organ bath to cause contraction of arterial tubules. 3 g/L, 9 g/L or 15 g/L of solution of concentrated preparations of *T. terrestris* to examine the effect of *T. terrestris* on coronary artery relaxation, respectively.

### Measurement of *T. terrestris* ingredients-induced relaxation of porcine coronary artery after U46619-induced contraction

The active ingredients of *T. terrestris* including kaempferol, ruscogenin, protodioscin, protogracillin, and harmine were applied in this study. The procedure is mentioned in last paragraph. After 30 minutes of equilibration, 60 mM KCl-contained Krebs-Henseleit solution was added into the organ bath to cause contraction of arterial tubules, then 60 mM KCl-contained Krebs-Henseleit solution was wash off with Krebs-Henseleit buffer solution 3 times, and after a 30-minute equilibrium period again, 1  $\mu$ M U46619 was added to the organ bath to cause contraction of arterial tubules. Afterward, 10  $\mu$ M, 30  $\mu$ M and 100  $\mu$ M of active ingredients of *T. terrestris* was added every 5 minutes respectively in organ bath after maximal contraction of coronary arteries induced by 1  $\mu$ M U46619 to examine the effect of active ingredients of *T. terrestris* on coronary artery.

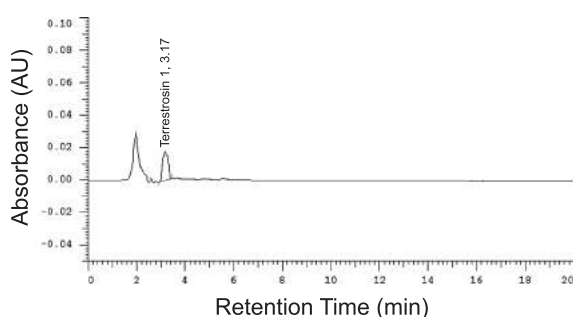


Fig. 1 HPLC of *Tribulus terrestris*. The retention time of terrestrosin 1 is 3.17 minutes.

### Data analysis

The data are expressed as the means  $\pm$  SEM. Statistical analysis of the results was performed by using Student's t-test or one-way ANOVA with post-hoc Tukey test. In all cases, differences were considered significant at  $p < 0.05$ . All analyses were performed using the SPSS statistical software version 24 (IBM Corp., NY, USA).

## Results

### HPLC analysis of *T. terrestris*

Figure 1 showed HPLC of concentrated preparations of *T. terrestris* that was provided by Kaiser pharmaceutical CO., LTD. Terrestrosin 1 was used as a standard compound and the retention time of terrestrosin 1 is 3.17 minutes.

### Measurement of *T. terrestris*-induced relaxation of porcine coronary artery after U46619-induced contraction

Figure 2A shows typical tracings of 9 g/L *T. terrestris*-induced relaxation of coronary arterial tubules. Relaxed responses were induced in coronary arterial tubules by the application of differently increasing concentrations of *T. terrestris*. As shown in Figure 2B, 3, 9 and 15 g/L of *T. terrestris* caused significant relaxation responses of  $21.64 \pm 1.80\%$ ,  $57.84 \pm 3.01\%$  and  $92.97 \pm 3.08\%$  in responses to 1  $\mu$ M U46619-induced contraction, in coronary arterial tubules, respectively ( $n = 10, 7, 4$  respectively). There was a significant difference between 3 and 9, 3 and 15, and 9 and 15 g/L of *T. terrestris*-induced relaxation in coronary arterial tubules (both  $p < 0.05$ ).

### Measurement of *T. terrestris* ingredients-induced relaxation of porcine coronary artery after U46619-induced contraction

As shown in Table 1, cumulative concentrations of 10  $\mu$ M, 40  $\mu$ M and 140  $\mu$ M of active ingredients of *T. terrestris* including kaempferol, ruscogenin, protodioscin, protogracillin, and

harmine caused significant relaxation responses after responding to 1  $\mu$ M U46619-induced contraction, in coronary arterial tubules, respectively. However, there was no significant difference between cumulative concentrations of 10  $\mu$ M, 40  $\mu$ M and 140  $\mu$ M of active ingredients of *T. terrestris*-induced relaxation and corresponding solvents in coronary arterial tubules (all  $p > 0.05$ ,  $n = 3$  in each group except  $n = 2$  in ruscogenin group and  $n = 5$  in corresponding vehicle of ruscogenin group).

## Discussion

In this study, *T. terrestris* caused the relaxation of porcine coronary artery after U46619-induced contraction in a dose-response manner. In addition, the onset of *T. terrestris*-induced relaxation of porcine coronary artery is immediate and the rapid effect of relaxation induced by *T. terrestris* plays a very important and helpful role for patients with acute coronary syndrome. However, the active ingredients of *T. terrestris* in this study cannot induce the relaxation of porcine coronary artery after U46619-induced contraction.

To the best of our knowledge, there is no study about *T. terrestris* on the treat-

ment of angina pectoris in traditional Chinese medicine. In traditional Chinese medicine for angina pectoris, Danshen (*Salvia miltiorrhiza*) preparations, puerarin, *Crataegus hawthorn*, *Crataegus oxyacantha*, *Panax notoginseng*, *Terminalia arjuna*, and Xuefu Zhuyu Tang were reported to applied in patients with angina pectoris.<sup>9-13</sup> A meta-analysis study shows *Panax notoginseng* can reduce cardiovascular events, relieve symptoms of angina, and improve electrocardiogram finding and the frequency of angina.<sup>14</sup> Puerarin is used in combination with conventional Western medicine, compared with conventional Western medicine alone, and the effect of combine therapy to treat unstable angina pectoris is better.<sup>15</sup> Although a large number of clinical trials have shown that certain Danshen products in China can effectively and safely treat cardiovascular diseases, the quality of most products is insufficient.<sup>16</sup> In this study, the results might provide potential effect of *T. terrestris* for the treatment of angina pectoris by promoting relaxation of coronary artery rapidly.

There are few studies about the effect of *T. terrestris* on smooth muscle. In vascular smooth muscle cells, total saponin extracted from *T. terrestris* protects against atheroscle-

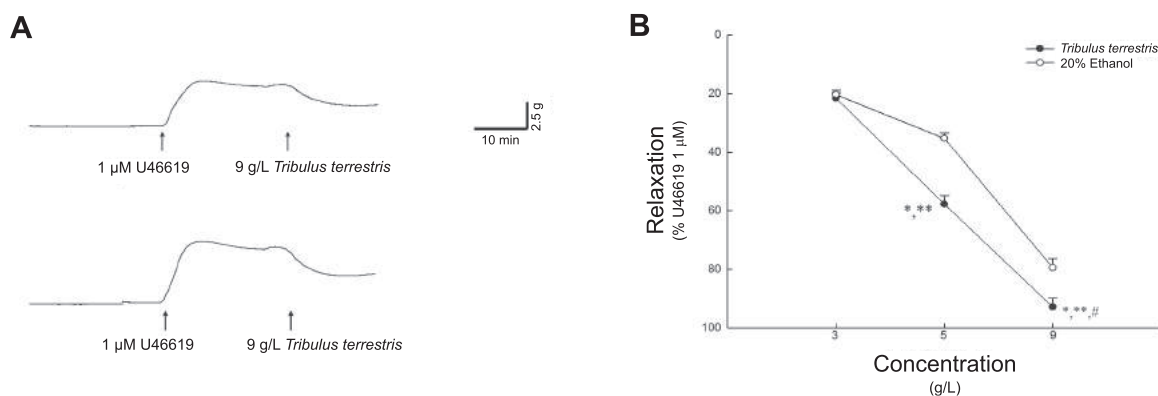


Fig. 2 The *Tribulus terrestris* (*T. terrestris*) or ethanol (vehicle)-induced relaxation of porcine coronary artery. (A) Typical tracings of 9 g/L *T. terrestris*-induced relaxation of coronary arterial tubules. (B) *T. terrestris* caused significant relaxation in the coronary artery in a dose-dependent manner. These values are expressed as the percentage of contraction induced by 1  $\mu$ M U46619. These results come from at least four experiments. The vertical bars represent  $\pm$  standard error of the mean (SEM). The \* indicates a significant difference compared with the same volume and concentration of corresponding ethanol (vehicle) ( $p < 0.05$ ). The \*\*, \* indicates a significant difference compared with 3 g/L *T. terrestris* that induced relaxation ( $p < 0.05$ ). The  $\pi$  indicates a significant difference compared with 9 g/L *T. terrestris* that induced relaxation ( $p < 0.05$ ).



rosis by significantly suppressing the increase in cells proliferation.<sup>17</sup> In addition, *T. terrestris* improved the sexual function of male rats by activating the Nrf2/HO-1 pathway and simultaneously inhibiting NF- $\kappa$ B levels.<sup>18</sup> Furthermore, *T. terrestris* can cause lower blood pressure by decreasing serum and different tissues including aorta, heart, kidney and lung in 2K1C hypertensive rats.<sup>19</sup> *T. terrestris* L. fruit methanol extract including ferulic acid, phloridzin and diosgenin improved ischemic insult in H9c2 cells by maintaining mitochondrial function via its antioxidant potential.<sup>20</sup> Therefore, *T. terrestris* might provide a protective effect against angina pectoris by antioxidant effect and relaxing vessels.

*T. terrestris* contains flavonoids, alkaloids, saponins, lignin, amides, and glycoside. Among them, saponins come from a variety of plants and they have a positive effect on the function of the heart, or they are helpful for the treatment of related diseases. It can also inhibit the formation of lipid peroxides in the myocardium or liver, affect the function of the enzymes involved in that, reduce blood coagu-

lation, cholesterol and sugar levels, and stimulate the immune system.<sup>21</sup> In this study, we choose kaempferol, ruscogenin, protodioscin, protogracillin, and harmine. Kaempferol is a natural flavonoid, ruscogenin is a saponin, protodioscin is a steroidal saponin, protogracillin is a steroid saponin, and harmine is a harmala alkaloid originally extracted from *T. terrestris* compound. In this study, kaempferol, ruscogenin, protodioscin, protogracillin, and harmine cannot cause significant relaxation of coronary artery compared with corresponding solvents for dissolving these compounds. Wider selection of active ingredients of *T. terrestris* might be tested to determine which compound is responsible to this relaxant effect.

The mechanism of relaxation of vessel is related to potassium channel, nitric oxide, cyclic adenosine monophosphate (cAMP), cyclic guanosine monophosphate (cGMP), protein kinase A (PKA), protein kinase G (PKG), Ca<sup>2+</sup>-ATPase pump and L-type Ca<sup>2+</sup> channel.<sup>22</sup> Therefore, further research to determine the mechanism of relaxation of coronary artery induced by *T. terrestris* will be necessary

Table 1. The active ingredients of *Tribulus terrestris*-induced relaxation of porcine coronary artery compared with corresponding vehicle ( $n \geq 2$ ).

	Ingredient or vehicle	Cumulative concentrations of ingredients			<i>p</i> value
		10 $\mu$ M	40 $\mu$ M	140 $\mu$ M	
Relaxation of coronary artery induced by ingredients or corresponding vehicles (% U46619 1 $\mu$ M)	Kaempferol	16.5 $\pm$ 2.2%	55.2 $\pm$ 2.0%		both > 0.05
	The same volume of vehicle of kaempferol	16.1 $\pm$ 0.1%	68.5 $\pm$ 3.9%		
	Ruscogenin	17.3 $\pm$ 3.4%	56.3 $\pm$ 3.5%		both > 0.05
	The same volume of vehicle of ruscogenin	20 $\pm$ 3.1%	85.4 $\pm$ 6.0%		
	Protodioscin	31.3 $\pm$ 7.4%	74.6 $\pm$ 3.6%		both > 0.05
	The same volume of vehicle of protodioscin	16.1 $\pm$ 0.1%	68.5 $\pm$ 3.9%		
	Protogracillin	6.02 $\pm$ 1.1%	26.2 $\pm$ 2.2%		both > 0.05
	The same volume of vehicle of protogracillin	7.7 $\pm$ 0.2%	29.2 $\pm$ 5.4%		
	Harmine	22.6 $\pm$ 1.8%	67.5 $\pm$ 1.2%	78.4 $\pm$ 3.4%	all > 0.05
	The same volume of vehicle of harmine	16.1 $\pm$ 0.1%	68.5 $\pm$ 3.9%	82.3 $\pm$ 7.0%	

to ensure the effectiveness, safety, and better understanding of this action.

## Conclusions

In this study, concentrated preparations of *T. terrestris* can induce the immediate relaxation of porcine coronary artery in dose-dependent manner. However, the main compounds of *T. terrestris* in this study cannot cause relaxation of porcine coronary artery compared with corresponding solvent. Hence, the results suggest *T. terrestris* may provide a potential therapy for angina pectoris in traditional Chinese medicine.

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## Conflicts of Interest

The authors declare no conflict of interest or competing financial interests.

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