DOI: 10.13656/j.cnki.gxkx.1999.03.019
广西科学 Guangxi Sciences 1999, 6 (3): 216~218

Pharmacological Action of Total Flavone of *Ampelopsis grossedentata* from Guangxi
广西产藤茶总黄酮的药理研究

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Abstract For studying the pharmacological action of total flavone of *Ampelopsis grossedentata* from Guangxi (GXTF), experimental observation on its antiplatelet aggregation, antithrombus, lipid-lowering and antioxidation was performed using animals experiment. The results showed that GXTF could signicantly inhibite the platelet aggregation in vitro and thrombus formation of rats in vivo, reduce the levels of serum lipid and blood glucose in hyperlipidemia model mice. In addition it also increased the superoxide dismutase (SOD) activity and decreased the malondialdehyde (MDA) content in serum and liver of aging model mice induced by D-galactose. All these suggests that GXTF has effects of anti-platelet aggregation, anti-thrombus formation, lipid-lowering and antioxidation.

Key words *Ampelopsis grossedentata*, anti-platelet aggregation, anti-thrombus formation, lipid-lowering, antioxidation, animal experiment

摘要　采用动物实验,探讨广西产藤茶总黄酮(GXTF)对抗血小板聚集,抗血栓,降血脂和抗氧化作用。结果表明,GXTF能明显抑制大鼠体外血小板聚集和体内血栓形成;降低高血脂症模型小鼠血脂和血糖水平;提高D-半乳糖所致小鼠衰老模型血清和肝脏中超氧化物歧化酶(SOD)活性,减少丙二醛(MDA)含量。由此表明GXTF具有抗血小板聚集和血栓形成,降血脂和抗氧化作用。

关键词　藤茶　抗血小板聚集　抗血栓形成　降血脂　抗氧化　动物实验
中图法分类号　R 961; R-332

The Teng Cha (*Ampelopsis grossedentata*) from Guangxi (GXTC), grows in Shangsi and Jinxiu counties of Guangxi. The tender stem and leaves of Teng Cha have been made into a healthy tea for treating common cold and pyretic fever, pain-swelling of pharynx and larynx as well as jaundice hepatitis[1], with a long history of several hundred years among Yao nationality people of Guangxi. It has been reported that decoction of Teng Cha exhibited the effects of diaphoretic and antipyretic, immunopotentiation[2]. Its water extract has the action of analgesia and anti-inflammatory[3]. Qin et al reported that a flavonoid (Ampelopsin) was extracted from GXTC, which has the actions of resolving sputum and reliving cough as well as protecting liver[4,5]. Total flavone of GXTC (GXTF), a potent component, has been extracted by Yuan Axing et al, but its pharmacological action has not been reported. According to the previous studies, flavonoid exhibits wide pharmacological activity on physiological systems of cardiovascular, respiratory and digest etc[6]. The aim of this study is to explore the drug for the prevention and cure of atherosclerosis, and the experimental observation on GXFT's anti-platelet aggregation, antithrombus, lipid-lowering, and antioxidation was performed.

1 Materials and Methods

1.1 Drugs and Reagents

GXTF, a kind of yellow powder crystals, was provided by the phytochemical department of Guangxi Institute of Traditional Medical and Pharmaceutical Sciences. The suspension was prepared by grinding GXTF together with a few drops of Tween-80 (Polysorbate 80) when used. Danshen injection (DSI) was from Ya'an Pharmaceutical Factory of Sichuan, Fufang danshen pian (FDSP) from Huacheng Pharmaceutical Factory of Guangzhou, vitamin E capsule from Guilin Pharmaceutical Factory of Guangxi, clofibrate from Yanzhou Pharmaceutical Factory of Jiangsu, adenosine diphosphate (ADP) from Shanghai Institute of Biochemistry of Chinese Academy of Sciences, both the kits of serum total cholesterol (TC), triglyceride (TG), high density lipoprotein cholesterol (HDL-C) and glucose from Dongou Biol Engin Co. Both the kits of superoxide dismutase (SOD) and malondialdehyde (MDA) were the product of Jiancheng Institute of Biol. Engin. of Nanjing.

1998-11-26收稿。

1.2 Animals

Both SD strain rat (180 g~ 220 g) and NIH strain mice (18 g~ 22 g), including both sexes, were from the animal department of Guangxi Institute of Traditional Medical and Pharmaceutical Sciences. All animals were sex-separately housed in the experiment room under air condition at 22± 2℃, and ate and drank by themselves during the experiment.

1.3 Instruments

Platelet aggregometer (model BS 631) was made by Beijing Biochemical Instrument Factory. Grating spectrophotometer (mode 722) by Shanghai Analytical Instrument Factory.

1.4 Platelet Aggregative Test[7]

Fasting rats were used. Blood samples were collected from abdominal aorta under anesthesia with sodium pentobarbital (30 mg/kg), anticoagulating with 3.8% sodium citrate. Platelet-rich plasma (PRP) and platelet-poor plasma (PPP) were prepared by routine method. The range of light through rate was adjusted by PPP to 100%, then by PRP to 0% on platelet aggregometer. Each test tube was accurately added with 0.3 mL of PRP, then the control tube was added with 0.05 mL of 0.2 mol PBS, and the drug tube was added with 0.05 mL of GXTF or DSI of different concentrations, respectively. After pre-incubation of 3 min at 37℃, both control and drug tubes were added with 0.02 mL of 2 μmol ADP (platelet aggregative-induced agent), and then traced platelet aggregative curve and millimetre number of curve lowering were determined. Inhibited percentage of aggregation was counted by comparing drug group with control group.

1.5 Examination of Thrombus Formation in Rat Body

Thirty-two rats were divided into 4 groups and were orally administered once a day for 14 days by the drug and dose in Table 2. The rats were anesthetized with sodium pentobarbital (30 mg/kg) one hour after last administration and platelet thrombus model was formed by method of neck total artery-extra-neck vena blood stream bypass[8]. Blood stream was opened 5 min after completion of surgical operation and suspended after 15 min. Silk thread was taken from blood stream pipe immediately, blotted on a filter, and then weighed as soon as possible. The wet weight of thrombus was obtained by substrating silk thread weight from total weight.

1.6 Hyperlipoidemia Model Mice

The hyperlipoidemia model method was described by Shan AL et al[9]. Hyperlipoidemia emulsion was prepared from composition of following components: 10% cholesterol, 2% sodium deoxi-cholic acid, 1% methylthiouracil, 20% lard, and then 20 mL each of Tween-80, propylene glycol were added to aid dissolution in emulsion, and distilled water was added to 100 mL in volume and poured the emulsion into bottle until use.

1.7 Aging Model Mice Induced by D-galactose

Fifty mice were divided into 5 groups and were orally given once a day for 45 days with the drug and dose shown in Table 4. Except the control group, All other groups were subcutaneously injected with D-galactose (40 mg/kg) to cause the aging model of mice one hour after oral given[10,11]. After last administration of 12 hours, blood samples were collected from retro-ocular venous plexus of mice, and 0.2 g liver were took out from dissected mice, and homogenized in 9 volume of ice-cold saline using a glass homogenizer to obtain the 10% liver homogenate. The SOD activity (xanthine oxidase method) and MDA content (thiobarbituric acid colorimetric method) in serum and liver homogenate were determined using the kits of clinical examining.

2 Results

2.1 Effect of GXTF on Platelet Aggregation of Rat In Vitro

The results were shown in Table 1. Comparing with control group, GXTF at 1.47 mg/mL and 2.94 mg/mL could significantly inhibited platelet aggregation induced by ADP to 50.2% and 77.9% respectively. GXTF (2.94 mg/mL) was strong than DSI (5 mg/mL).

Table 1 Effect of GXTF on platelet aggregation in rat (n= 8)

Group	Concentration (mg/mL)	Platelet aggregation curve $\bar{x}\pm s$ (mm)	Inhibition (%)
Control		93.0± 14.8	
DSI	5.00	45.5± 12.8*	51.1
GXTF	1.47	46.3± 13.2*	50.2
GXTF	2.94	20.5± 11.7*	77.9

Compared with control group* $P < 0.001$.

2.2 Effect of GXTF on Thrombus Formation in Rat

The inhibited rate of thrombus formation in drug groups was shown in Table 2. GXTF of 0.5 g/kg and 1.0 g/kg could inhibited thrombus formation, and the inhibition of GXTF at 1.0 g/kg was similar to FDSP.

2.3 Effect of GXTF on the Levels of Lipid and Blood Glucose in Serum of Hyperlipoidemia Mice

The results were shown in Table 3. Comparing with model group, GXTF at 0.5 g/kg and 1.0 g/kg could significantly lower the levels of TC, TG and BG in hyperlipoidemia model mice, and increase somewhat of HDL-C level.

Table 2 Effect of GXTF on thrombus formation in rat (n= 8)

Group	Doses (g/kg p.o)	Wet weight of thrombus $\bar{x}\pm s$ (mg)	Inhibition (%)
Control		29.1± 4.5	
FDSP	0.66	17.8± 5.5*	38.8
GXTF	0.50	25.4± 6.9	12.7
GXTF	1.00	18.4± 4.6*	36.8

Compared with control group* $P < 0.01$.

Table 3 Effect of GXTF on the levels of lipid and glucose in serum of hyperlipoidemia mice (n= 10, $\bar{x}\pm s$)

Group	Doses (g/kg p.o)	TC (mmol/L)	TG (mmol/L)	HDL-C (mmol/L)	BG (mmol/L)
Control		3.44± 0.94	1.37± 0.42	1.74± 0.38	3.95± 1.36
Model		10.87± 5.86###	2.16± 0.48###	1.29± 0.51	3.61± 1.29
Chlorfenisate	0.05	5.52± 1.52**	1.68± 0.12**	1.67± 0.94	2.24± 0.67**
GXTF	0.50	8.06± 4.20	2.00± 0.20	1.56± 0.33	1.83± 0.91**
GXTF	1.00	5.75± 2.18**	1.72± 0.10	1.64± 0.44	1.49± 0.93***

Compared with control group### $P < 0.001$; Compared with model group* $P < 0.05$,** $P < 0.01$,*** $P < 0.001$.

Table 4 Effect of GXTF on SOD activity and MDA content in serum and liver of aging mice induced by D-galactose (n= 10, $\bar{x}\pm s$)

Group	Doses (g/kg)	SOD		MDA	
		Serum (nu/mL)	Liver ($\times 10^3$ nu/g)	Serum (nmol/mL)	Liver (nmol/g)
Control		185.07± 10.41	2.11± 0.06	6.51± 1.09	293.41± 61.23
Model		183.32± 8.26	1.92± 0.20	9.66± 1.24###	322.58± 63.42##
Vitamin E	0.05	201.12± 9.85**	2.69± 0.08	7.86± 0.74**	192.50± 20.57***
GXTF	0.5	194.6± 7.05**	2.06± 0.10	8.15± 0.96	240.39± 35.90**
GXTF	1.0	203.3± 7.24**	2.73± 0.10	7.48± 0.86**	211.20± 38.14***

Compared with control group# $P < 0.05$,## $P < 0.01$,### $P < 0.001$; Compared with model group* $P < 0.05$,** $P < 0.01$,*** $P < 0.001$.

2.4 Effect of GXTF on SOD Activity and MDA Content in Serum and Liver of Aging Mice Induced by D-galactose

The results were shown Table 4. Comparing with model group, GXTF of 0.5 g/kg and 1.0 g/kg could significantly increase SOD activity and decrease MDA content in serum and liver of aging mice induced by D-galactose. They were similar to vitamin E (as positive drug) in action.

3 Discussion

Atherosclerosis (AS) is a common and frequent occurring diseases. The flavonoids in plants have been used as drugs for preventing and curing AS, such as ginkgentin, quercetin, silymarin[6].

In this study, we employed several animal models to study the pharmacological action of GXTF on AS. The result showed that GXTF could produce a significant inhibition of platelet aggregation, and showed a effectiveness-dose relation. The wet weight of thrombus in rat decreasing show GXTF has anti-thrombus property. GXTF could also lower the levels of TC, TG and BG in the serum of hyperlipoidemia mice. In addition, increasing SOD activity and decreasing MDA content in serum and liver of aging mice induced by D-galactose exhibits GXTF to have anti-oxidation. From these findings, it can be considered that antithrombus action of GXTF is related to inhibition of platelet aggregation. The lipid lowering might be result of the inhibition of absorption or accelerating excretion of cholesterol and lipid in hyperlipoidemia emulsion. The anti-oxidation of GXTF might be of the increasing of SOD activity in body. The results above-mentioned only is our initial research on GXTF, the pharmacological action in other aspects still needs to be further researched.

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(责任编辑: 蒋汉明)