

## PRIMARY PHARMALOGICAL STUDY ON ETHYL ACETATE EXTRACT OF ROSA ROXBURGHII

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Rosa Roxburghii is an unique wild fruit growing in Guizhou, Sichuan and Yun'nan Provinces. It is growing at sides of mountains, at sides of road, near trees or in bushes. Its fruits are sweet and sour in taste with special scent. Compendium of Materia Medica says that its fruits can alleviate mental depression and remove stagnated food. Luo Qian Yi in Gui Yang Agricultural Institute had made a lot of research from the 1940's on threpsological, biochemical and biological aspects of Rosa Roxburghii. Their research approved that there is a high content of Vitamin C. But, there is not any detailed report on materials of its phyto-chemical and pharmalological aspects. In order to make good use of the wild plant and present scientific basis for developing new products, we made preliminary research both toxicologically and pharmacologically on ethyl acetate extracted from Rosa Roxburghii. The results are as follows:

### EXPERIMENTAL MATERIALS

**Medicine:** The ethyl acetate extract from Rosa Roxburghii, Yellow – colored powder, solvable in water. The juice of rosa roxburghii produced in Guangxi Province is extracted by use of ethyl acetate and dried, for later use, by the phytochemical group of the Chinese Medical Herbs Research Office of Gui Lin Medical School. Every milligram of the powder is equivalent to 0.315 milliliters of the original juice. The powder and sterilized water are mixed into test solutions of different concentration for use in the experiment.

**Animals:** Healthy mice, 17~23g each; large mice, 70~125g each, of both sexes, presented by the animal office of Gui Lin Medical School.

### Method and Results.

#### I. Toxicological Test

##### a. Acute Toxicity

**Method:** Choose 50 small mice, male and female, weighing 17-23 grams. They are equally classified at random into five groups with 10 mice in each according to weight. The intraperitoneal injection of different doses of ethyl acetate extract of Rosa roxburghii is taken once. Observe three – day toxic reaction and the death toll. Then find out the acute half lethal dose (LD50).

**Result:** The symptoms of acute intoxication of ethyl acetate extract are decreasing activity, quick breathing, akinesia, coma, tic, etc., Then breathing ceases, heart beating ceases and the mice decease at last. The dose of ethyl acetate injected into mice for LD50 is  $273.0 \pm 31.7$ mg/kg (confidence limit: 95%).

##### b. Subacute Toxicity

**Method:** Choose 30 large mice weighing 70-125 grams, male and female. They are randomly divided into three groups. The intraperitoneal injection dose of the ethyl acetate of Rosa Roxburghii is 40mg/kg and 20mg/kg respectively for the first and second groups. The third is a control group injected with physiological saline. Inject once a day for 30 days. Weigh the mice in each group once and check the hemogram once at every six-day interval before injection and during the experimental process. The next day after the injection, take blood sample and check their hemogram, liver function (SGPT) and kidney function (NPN). Have a histopathological checkout on their main organs (heart, liver, spleen, lung and kidney) after they are killed.

**Result:** In the course of medicinal application, the animals in every group have normal activities, appetite and weight growth. In comparison to the control group, the animals in experimental groups have no clear difference in blood platelet and erythrocyte count, SGPT and NPN besides a little higher growth of leucocyte.

From histopathological observation, there is not any difference in the form of their heart, liver, spleen and kidney besides mild interstitial pneumonia in the lung of the animals in each experimental group.

Since the lungs of all animals both in the control group and in the experimental groups are influenced, and the interstitial pneumonia of the mice is caused mostly by viral injection, it is clear that the pneumonia is not caused by this medicine used.

**II. Effect on Tolerance of Acute Anoxia of Mice**

- a. **Test on Tolerance of Anoxia of Mice in Constant Pressure Method:** Choose 30 small mice weighing 18-23 grams. Divide them into three groups with 10 in each group. Give the first group peritoneal injection of 40mg/kg of ethyl acetate extract of *Rosa Roxburghii*. The second group, ip of 20mg/kg of propanolol. The third group, ip of physiological saline as a control group. Forty minutes later, put the small mice of all groups into 250ml wide-mouth bottles with 15g soda lime in them, two in each bottle. Then tighten the lid, and record the survival time of the mice.

**Result:**

See Table 1

TABLE 1					
Group	Mice Number	Dose (ip)	Survival Time (Minutes) X ± sd % longer		P-Value
First	10	40mg/kg	70.7 ± 17.4	56.1	<0.01
Second	10	20/mg/kg	64.6 ± 17.1	42.6	<0.05
Third	10	20ml/kg	45.3 ± 13.5	-	-

It is clear from the above diagram that the ethyl acetate extract and propanolol both can help the mice survive longer for anoxia in constant pressure.

**b. Test of Mice's Tolerance on anoxia in Decompression**

**Method:** Choose 30 small mice weighing 17-22 grams. Divide them at random into three groups with 10 in each. Forty minutes later, put two mice into each 250ml wide-mouth bottle. Test their tolerance on anoxia in a tight-sealed decompression device. Record their survival time.

**Result:**

See Table 2

TABLE 2						
Group	Animal	Medicine (IP)	Dose (IP)	Survival Time X ± SD min) % Longer		P Value
1 <sup>ST</sup>	10	Ethyl Acetate	40mg/kg	8.9 ± 6.1	132.2	<0.05
2 <sup>nd</sup>	10	Propanolol	20mg/kg	7.90 ± 3.7	107.9	<0.05
3 <sup>rd</sup>	10	Saline	20ml/kg	3.8 ± 3.5	-	-

We know from the diagram that ethyl acetate and propanolol can both prolong the survival time of mice on the condition of anoxia in decompression.

**c. Swimming Test on Rosa Roxburghii's effect on Mice's Tolerance on Fatigue**

**Method:** Choose 25 mice weighing 18-20 grams. Divide them randomly into two groups, one experimental group with IP 50mg/kg of ethyl acetate and the other control group with IP of the same amount of physiological saline. Forty minutes later, put them into a plastic jar in which the water is 40cm deep and the water temperature is 15° Centigrade, at the room temperature. Count their swimming time until they fall to the bottom and die.

**Result:**

See Table 3

<b>TABLE 3</b> Rosa's Effect on Mice's Swimming Time				
Group	Mice	Dose (ip)	Swimming time	P Value
Control Group	12	20ml/kg	5.2 ± 1.4	-
Experimental Group	13	40mg/kg	7.1 ± 2.7	<0.05

The result indicates that ethyl acetate can expand their swimming time.

**d. Effect on the Growth of Infant Mice**

**Method:** Choose 20 small mice weighing about 10 grams. Divide them at random into two groups. Inject the experimental group with 40mg/kg of ethyl acetate and the control group with the same amount of physiological saline, once every day for four weeks. Weigh them once every week. Observe their growth.

**Result:**

See Table 4.

<b>Table 4</b>							
Group	Mice	Dose (ip)	Weight (g)				
			Prior to Test	First Week	Second Week	Third Week	Fourth Week
Control Group	10	20ml/kgqdx28	11.0± 3.1	14.6± 2.6	15.9± 2.6	19.8± 5.5	16.3± 7.2
Test Group	10	40mg/kgqdx28	10.1± 0.8	14.8± 0.8	17.2± 2.7	22.4± 4.2	17.9± 5.5

From the results we know that the effect of promoting the growth of infant mice is not certain.

**e. Test on the effect on the Immune Organs of Little Mice**

**Method:** Choose 20 little mice weighing 13-17grams. Divide them into two groups. Inject the test group with Ip 40mg/kg of ethyl acetate and the control group with ip the same amount of physiological saline once every day for five days. On the sixth day kill all the mice. Take out their thymus glands and spleens.

Dry the surface of these organs with filter paper. Weigh the organs instantly. Count the weight of the thymus glands and spleens every 10grams of body weight.

**Result:**

See Table 5

TABLE 5						
Group Type	Mice	Dose (ip)	Thymus Gland (mg/10g)		Spleen (mg/10g)	
			X ± SD	P Value	X ± SD	P Value
Control	10	20ml/kg	23.7± 7.3	-	71.3± 50.6	0
Test	10	40mg/kg	28.4± 18.3	70.5	67.3± 38.9	>0.05

The results show that injection of 40mg/kg of ethyl acetate for five days continuously has no obvious effect on the weight of the immune organs of the mice

**CONCLUSION**

Rosa roxburghii is very rich in resource and high in nutrition. There is much room for development and application. Our experimental results show that the ethyl acetate extract of this wild fruit if injected to mice, can obviously prolong the survival time of mice in constant pressure and in decompression, as well as the swimming time in water. It is evident that the extract plays a role in tolerance on anoxia and fatigue. But this is only the first step of our research, and the method is a kind of nonspecific whole body anoxia. Among the factors effecting the mice’s tolerance on anoxia, some central depressants decrease the mouse activity and thus prolong its survival time; besides, its heart and brain tissues are specially sensitive to anoxia. But we have not found in this medicine any central inhibitory effect. So it is still unknown whether the mechanism of ethyl acetate which prolongs the survival time of mice in anoxia is concerned to lowering the heart’s oxygen consumption or increasing the coronary blood flow and so improving ability of the cardiac muscle to tolerate anoxia, but it needs further testification.

It is believed that one of the signs of decrepitude of the organic body is the progressive decrease of adaptive capacity to the changing environment. Since this medicine is effective to some extent in tolerance of anoxia and fatigue, the value of Rosa Roxburghii and its extract in preventing senile decay is worth a further study.

Besides, the experiments show that ethyl acetate has no obvious effect on mice’s growth and the weight of their thymus glands and spleens, but we have not enough supporting evidence to deny that the herb may have nonspecific immunity strengthening effect. In our acute and subacute toxicity tests, the rosa roxburghii extract has no obvious toxicity. This means that the syrup made by diluting the rosa roxburghii is not toxic but safe for drinking.