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Comparison of the sedative and hypnotic effects of flavonoids, saponins, and polysaccharides extracted from Semen *Ziziphus jujube*

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Semen *Ziziphus jujube* (SZJ), the seeds of *Ziziphus jujuba* Mill. var. *spinosa*, is a kind of traditional Chinese medicine used for its action on insomnia. In order to analyze the effective component, we investigated and compared the sedative and hypnotic effects of three kinds of compounds, flavonoids, saponins, and polysaccharides. Flavonoids, saponins, and polysaccharides were extracted from SZJ and orally administered to mice separately at $17\,g\,kg^{-1}$ per day for certain days before animal tests. Spontaneous motility and coordinated movement tests were used to observe the effects of the three kinds of compounds on the mouse behavior, and sodium barbital-induced sleeping time of mouse were tested to analyze the effects of the three kinds of compounds on the sleep of mouse. Results show that flavonoids and saponins caused a significant reduction of walking time and coordinated movement ability of mouse, significantly prolonged its sleeping time at $40\,mg\,kg^{-1}$, ip, subthreshold dose and increased the sleeping number of animals at $50\,mg\,kg^{-1}$, ip, superthreshold dose induced by coeliac injection of sodium barbital. Polysaccharides did not show any significance in all animal tests. Comparative analysis showed that saponins had a more effective sedative and hypnotic function than that of flavonoids, polysaccharides did not show a sedative and hypnotic effect.

Keywords: Flavonoids; Saponins; Polysaccharides; Semen *Ziziphus jujube*; Sedative and hypnotic effects

1. Introduction

Ziziphus jujuba Mill. var. *spinosa* (Bunge) Hu ex H. F. Chou is a wood plant belonging to the family Rhamnaceae [1,2]. The seed of *Ziziphus jujube* (SZJ), Suanzaoren in Chinese, is one of the commonly used Chinese medicines. In traditional Chinese medicine, SZJ has been used for its action on insomnia and anxiety as a sedative and hypnotic medicine [3]. Its action of mechanism was as a result of decreasing the monoaminergic system activity [4]. Semen *Z. jujube* was first recorded in the medicine

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monograph Shen Nong's Herbal known as Classic on the Herbal (Ben Cao Jing) or The Herbal (Ben Cao), the earliest book on medica in China, which appeared more than 2500 years ago. In this book SZJ was ranked as high grade, and in Chinese medicinal formulae SJZ is often used as principal drug. Besides the sedative and hypnotic effect, SZJ has been tested to possess hypotensive, antihypoxia, antihyperlipidemia, hypothermic, and anxiolytic effects [5].

Insomnia is a common subjective complaint of inadequate sleep that affects 15–40% of the general population. Fewer than 15% of patients with insomnia receive treatment. There is compelling evidence that insomnia is under-recognized, underdiagnosed, and undertreated; a longitudinal study found that practitioners in general practice settings were unaware that patients had difficulty sleeping in more than half the reported cases of insomnia, the overall prevalence of insomnia increases with age [6]. There are many deficiencies in the therapeutic effects of established medicinal drugs [7] and the incidence and nature of side-effects with them, the latter generally being of a more benign nature with herbal products [8]. The treatment of insomnia provides a prime example of this divide between natural and unnatural (by definition) remedies. The latter provide a whole range of benzodiazepine and other hypnotics of varying durations of action, to suit all varieties of sleep disturbance, from brief delay of onset to repeated night-long wakings, but at the cost of potential dependence for many. It is hoped that herbal remedies can fill this gap [9].

Unlike synthetic chemical drugs that contain single entities of known composition and specific therapeutic actions, herbal drugs are derived from plant extracts of mixed composition. Although the constituents of the latter have usually been identified, it is often not known which are the active compounds and which are simply adjuvants. Therefore, dosage directions are based on amounts of the whole extract [9]. In recent years, more and more studies have been conducted on the components and their pharmacological analysis of plant medicines [10–12]. An extensive literature exists on the chemical components and pharmacological properties of components have been described [13–18]. In the pharmacological studies of SZJ, the flavonoids, saponins, oil, and unsaturated fatty acid, were used to investigate the sedative and hypnotic effects [19–22]. In this study, we extracted the flavonoids, saponins, and polysaccharides from SZJ to compare their sedative and hypnotic bioactivities by the use of animal testing. Among these components, the first two are thought to have sedative and hypnotic effects, and the polysaccharides have never been studied before.

2. Materials and methods

2.1. *Plant material*

Ziziphus jujuba was produced and SZJ medicine material was made in Province Shanxi of China. The processing drugs of SZJ were made in Chinese traditional medicine plant of Guangzhou, Guangzhou City, China. SZJ samples for the present research were purchased from a drug store in Wushan, Guangzhou. The quality of the samples was measured up to Pharmacopeia of P. R. China. The license number of the drug store is Y200500100.

2.2. *Animals*

All experiments were performed using mice (20 ± 2 g) of 7–9 weeks of age which were obtained form the Experimental Animal Centre of the Guangdong Province. They were housed in groups of four or five animals in standard cages containing a supply of food pellets and water.

2.3. *Extraction of chemical constituents*

For the purpose that the three kinds of constituents (flavonoids, saponins, and polysaccharides) are extracted from the same batch of raw material and the extracts meet the requirements of the following animal test; no toxical reagents or those possibly influenced on hypnotic activity are introduced to the extraction process and no residual toxicity in products. The following extraction programs were designed:

For Saponins, SZJ samples were shattered and screened with 40 mesh. After being evaporated at 60°C, it was Soxhlet extracted in 10 times of petroleum benzin for 4 h at 60°C, and the extraction was repeated three times. After drying, levigation and screen with 40 mesh, the residues were inverse flow extracted in 25 times of 70% ethanol for 3 h at 85°C, then were filtrated and the residue (residue I) was conserved for the following use. The alcohol solution was evaporated to dryness with reduced pressure at 60°C, and then dissolved with water. After filtration and discarding of extraneous component, the alcohol solution was extracted by adding water-saturated *n*-butanol (1 : 1 v/v), the *n*-butanol phase was then treated by 1 M KOH, alkaline-water phase (water phase II) was conserved for the following use. The *n*-butanol phase was evaporated to dryness under pressure, and the raw saponin was obtained. Three times of recrystallization was conducted to get the saponin sample.

For flavonoid, the previous water phase II was neutralized by 1 M HCl, and then extracted by acetic ester (1 : 1 v/v). The acetic ester phase was evaporated to dryness with reduced pressure and the raw flavonoid was obtained. Three times of recrystallization was conducted to get the flavonoid sample.

For polysaccharide, the residue I was added to water (1 : 30 v/v) and decocted in water bath at 65°C for 0.5 h, the solution after sucking filtration was precipitated by adding 95% ethanol (1 : 2 v/v), and it was placed in 4°C to stay overnight. After that, it was centrifuged and flocculent precipitate was evaporated to dryness at room temperature, and the raw saponin was obtained. Three times of recrystallization was conducted to obtain the polysaccharide sample.

2.4. *Mouse behavior studies*

2.4.1. Subjects. Mice were housed in groups of 10 in their home cages, where ambient temperature was 20–25°C, and a 12 h light/dark cycle was in effect. Mice were allowed food and water *ad libitum*. All studies were conducted during the light cycle.

2.4.2. Drug administration. Total of $10\,\text{mg}\,\text{mL}^{-1}$ physic liquors of flavonoids, saponins, and polysaccharides extracted from SZJ were made respectively.

All compounds were given by oral gavage as suspensions. The physic liquors of flavonoids, saponins, and polysaccharides were given at doses 0.15 mL 10 g^{-1} per day, equivalent to 17 g kg^{-1} per day of crude drug. Control group was treated with a 0.9% NaCl solution in the same conditions, continuous administration once a day for 3 days. Mice were kept on fasting 24 h before the last administration.

2.4.3. Spontaneous motility. The sedative properties of flavonoids, saponins, and polysaccharides were compared using spontaneous activity as an index of sedation. Five minutes after dosing, mice were placed into cages that were similar to their home cages. After being kept in this room for 5 min, a 5 min test was given, during this time two parameters (ambulation time and times of open and close arms) were recorded.

2.4.4. Coordinated movement. Five minutes after the last dosing, mice were tested for their ability of active avoidance retention. A slippery glass plate (60 cm × 50 cm) was inclined on an angle 42.5°, and a mouse was placed on the upper end and the time it took to glide from upper end to lower end was recorded. If the drugs had sedative effect, the coordinated ability of mouse would be decreased, and the gliding time would be shortened.

2.5. *Mouse sleeping studies*

2.5.1. Suprathreshold and subthreshold dosage of pentobarbital sodium. A total of 50 mice were subdivided randomly into five groups ($n = 10$ animal/group). Pentobarbital sodium dissolved in physiological saline was administered by intraperitoneal injection at a dose of 35, 40, 45, and 50 mg kg^{-1} for each treated group, the control group was given physiological saline with same volume. The mice who slept within 60 min were numbered. Results showed that there were 0, 1, 0, 8, and 9 mice, respectively, who fell asleep in control group and each treated group, respectively. So the threshold of pentobarbital sodium is 45 mg kg^{-1}. The suprathreshold and subthreshold sleeping dosage of pentobarbital sodium were determined as 50 and 40 mg kg^{-1}, respectively.

2.5.2. Suprathreshold barbiturate induced sleeping time. After 30 min the four groups of animals were, respectively, given physic liquors of flavonoids, saponins, and polysaccharides at doses 0.15 mL 10 g^{-1} per day and physiological saline; each group was administered by intraperitoneal injection of pentobarbital sodium at dose of 50 mg kg^{-1}. The sleep duration was measured based on the loss and regaining of the righting reflex.

2.5.3. Subthreshold barbiturate induced sleeping time. Methods were kept the same as earlier, other than subthreshold sleeping dosage of pentobarbital sodium 40 mg kg^{-1} for intraperitoneal injection. The number of mice which fell asleep were observed and recorded.

2.6. *Statistical analysis*

The results were expressed as $\bar{x} \pm S$ [23]. Each group treated was compared with the corresponding controls by means of Student's t-test, and differences were regarded as significant when $p < 0.05$.

3. Results

3.1. *Spontaneous motility*

A total of 37 mice were subdivided into four groups (10, 8, 9, 10 animals for each group, respectively). The data for the individual animals are presented in table 1, and figure 1 shows the means. Based on the results, it can be seen that the mean motilities of animals treated by saponins and flavonoids were clearly lower than that of control group. The mean motility of animals treated by polysaccharide showed a little reduction of the motility compared with the control. The highest percentage (76%) for the reduction of motility was achieved by the group treated by saponins compared to the control values.

Table 1. Walking time and arm elevating time of animals within 5 min after 3-day of oral gavage.

	Walking time (s)				Arm elevating time			
Animals number	Control group	Polysaccharide group	Saponin group	Flavonoid group	Control group	Polysaccharide group	Saponin group	Flavonoid group
1	85.61	65.12	24.32	23.03	28	27	28	12
2	91.50	51.48	17.23	32.24	11	30	7	28
3	93.78	54.86	17.27	16.73	14	33	29	17
4	46.09	46.37	18.39	31.92	8	18	30	13
5	59.00	49.26	17.79	32.79	22	36	15	16
6	113.12	85.45	20.07	35.59	19	13	15	11
7	88.17	47.00	14.88	23.27	6	15	4	24
8	51.83	70.97	12.47	13.79	12	20	7	7
9	62.31		18.19	28.09	10		12	15
10	63.36			32.79	30			16

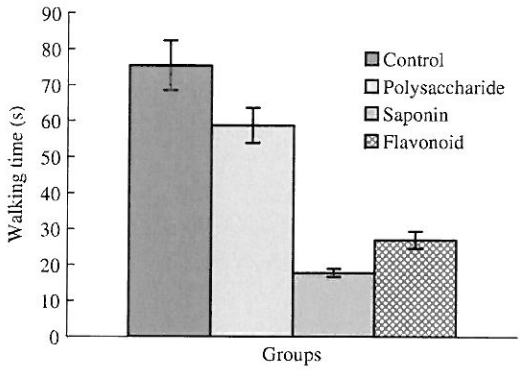

Figure 1. Effects of flavonoids, saponins, and polysaccharides on spontaneous activity.

Statistical analysis shows that the spontaneous motility values of the groups treated with saponins and flavonoids were significantly different from those of the control group ($p<0.01$), suggesting sedative effects of these compounds, whilst those treated with polysaccharide did not show a significant difference ($p>0.05$).

The walking time and the arm elevating times of mice were recorded simultaneously (table 1). The means are showed in figure 2. Differing from walking time test, the arm elevating times of the animals treated with the saponins, flavonoids, and polysaccharide did not show any differences from those of the control group (table 2).

3.2. *Coordinated movement*

After spontaneous motility tests, same administrations were continued for 3 days and an active avoidance retention test, slipping time on the glass plate was conducted (table 3). Results clearly show that the slipping times of groups control and treated with polysaccharides were longer than those of groups treated with saponins and flavonoids (figure 3). The percentages for the increasing time induced by saponins and flavonoids were 65 and 64%, respectively. The coordinated movement abilities of the groups treated with saponins or flavonoids were significantly different from those of the control group, and treatment with the polysaccharide did not show any differences from those of the control group (table 2).

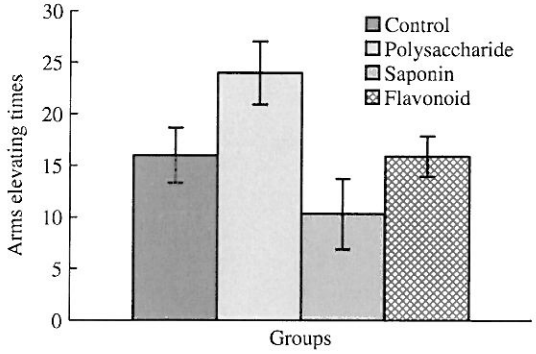

Figure 2. Effects of flavonoids, saponins, and polysaccharides on arm elevating times.

Table 2. Statistics analysis of spontaneous activity and coordinated movement ($\bar{x} \pm S$).

Groups	Number of animal	Walking time and arm elevating times after 3-day oral gavage: Walking time (second)	Walking time and arm elevating times after 3-day oral gavage: Arm elevating times	Sliping time on the glass plate after 6-day oral gavage (second)
Control	10	75.48 ± 21.83	16.00 ± 8.37	2.30 ± 1.53
Polysaccharide	8	58.81 ± 13.90, $p>0.05$	24.00 ± 8.68, $p>0.05$	2.33 ± 2.70, $p>0.05$
Saponin	9	17.85 ± 3.29, $p<0.01$	15.22 ± 9.00, $p>0.05$	0.81 ± 0.09, $p<0.01$
Flavonoid	10	27.02 ± 7.48, $p<0.01$	15.90 ± 6.10, $p>0.05$	0.837 ± 0.98, $p<0.05$

3.3. *Suprathreshold barbiturate sleeping time*

A total of 36 mice were subdivided into four groups (9, 8, 9, 10 animals for each group, respectively). The test was conducted 30 min after the last administration. Data were obtained in seconds and submitted to statistical analysis but, in order to simplify the visualization, the results were transformed into minutes in figure 4.

Table 3. Slipping time on the glass plate.

Animals number	Control group	Polysaccharide group	Saponin group	Flavonoid group
1	3.70	0.92	0.75	1.01
2	0.96	8.61	0.83	0.83
3	1.10	1.56	0.65	0.72
4	6.03	2.34	0.81	0.81
5	1.87	3.05	0.87	0.92
6	1.53	1.17	0.91	0.71
7	2.62	0.53	0.74	0.85
8	1.22	0.46	0.94	0.83
9	20.9		0.79	1.33
10	2.21			0.81

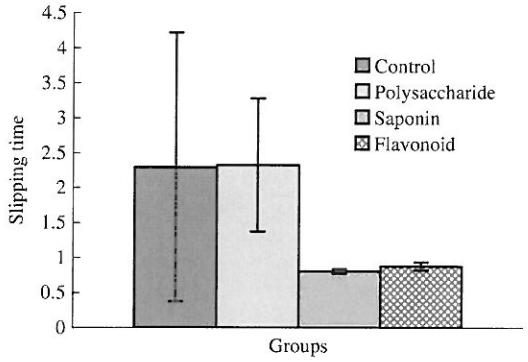

Figure 3. Effects of flavonoids, saponins, and polysaccharides on coordinated movement.

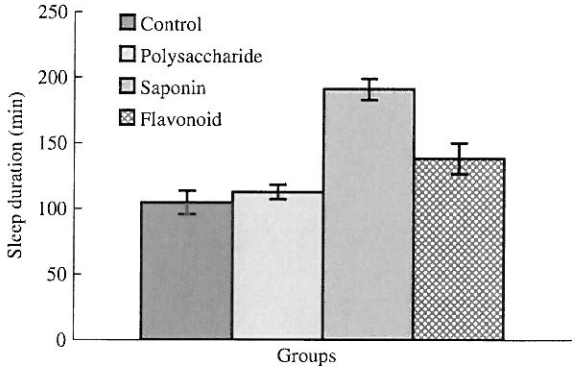

Figure 4. Effects of flavonoids, saponins, and polysaccharides on sleeping time induced by superthreshold sodium pentobarbital.

Table 4. Sleeping time (min) of superthreshold dose (50 mg kg^{-1}) of sodium barbital induced.

Animals number	Control group	Polysaccharide group	Saponin group	Flavonoid group
1	89	103	177	154
2	85	111	192	123
3	82	106	189	97
4	117	127	203	150
5	72	107	220	142
6	93	101	232	134
7	129	143	154	90
8	125	132	178	210
9	150		174	104
10				178

Table 5. Statistics analysis of suprathreshold ($\bar{x} \pm S$) and subthreshold barbiturate induced sleeping.

		Suprathreshold barbiturate (50 mg kg^{-1}) induced sleeping after 9-day oral gavage	Subthreshold barbiturate (40 mg kg^{-1}) induced sleeping after 12-day oral gavage (second)
Groups	Number of animals	Duration time of sleep	Number of sleep
Control	9	104.67 ± 26.37	2
Polysaccharide	8	112.65 ± 15.61, $p > 0.05$	3, $p > 0.05$
Saponin	9	191.00 ± 24.49, $p < 0.01$	8, $p < 0.01$
Flavonoid	10	138.20 ± 39.18, $p < 0.05$	7, $p < 0.05$

In table 4, the individual data for all animals are presented, and figure 4 shows the means. It can be concluded from the results that the mean sleep duration of the control group was 104.67 min, while the sleep duration was prolonged by 84 and 32% in the group which were administered with saponins or flavonoids, respectively (figure 4). Statistics analysis shows that groups treated with saponins or flavonoids produced a significant increase in hypnotic effect induced by pentobarbital. The result obtained with polysaccharides had no statistical significance ($p > 0.05$).

3.4. *Subthreshold barbiturate sleeping time*

Sodium barbiturate is catabolized by enzyme in liver. Any medicaments that have inhibitory function on the enzyme can prolong the sleeping time of mouse as well. The enhanced sleeping time may be due to interference with barbiturate enzymatic metabolism [24]. In order to exclude the situation, a subthreshold sodium barbiturate test is necessary.

After suprathreshold barbiturate (50 mg kg^{-1})-induced sleeping tests, amimals of the groups were continuously treated with the same doses of saponins, flavonoids, or polysaccharides for 12 days. Thirty minutes after the last administration subthreshold sodium barbiturate (40 mg kg^{-1}) was injected. Fifteen minutes after the injection of sodium barbiturate, the loss and regaining of the righting reflex for 1 min or more was determined as positive in the animals. The number of sleeping animals are recorded in table 5.

The percentage of animals falling asleep in the groups is shown in figure 5. Compared to the control group, the number of sleeping animals in the groups treated with

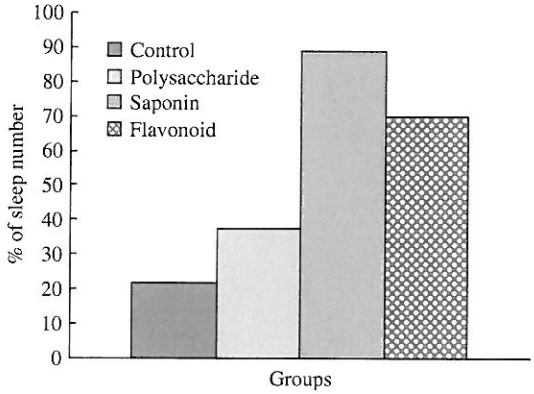

Figure 5. Percentage of sleeping animals in each group treated with flavonoids, saponins, or polysaccharides on the subthreshold sodium pentobarbital.

saponins or flavonoids increased significantly ($p<0.01$, $p<0.05$, respectively), whilst the group treated with polysaccharides did not show a significant increase in the number of sleeping animals.

4. Discussion

Semen *Z. jujube* has long been used as a prescription in traditional Chinese medicine for its anxiolytic effect. Its action of mechanism is related to the decrease in the monoaminergic activities in the brain [25]. Taking orally the aqueous extracts from SZJ is the traditional administration method and its ethanolic extract exhibits anxiolytic effect at lower dose and sedative effect at higher dose [25].

Using single type of compound, such as flavonoids, saponins, oils, or alkaloids extracted from SZJ, some sedative and hyponic studies have been reported by different authors, but the active effects of these compounds cannot be compared from their results. No research has been conducted to explore which compound of SZJ plays a major role for its action on insomnia. Siekwoo [26] thought that the spinosin are possibly one of the effective sedative components. Some authors indicated that flavonoids of SZJ also are the effective sedative and hyponic component [27], but oils did not have the fuction [19]. Zhao *et al.* [21] studied the effects of oils of SZJ on autonomic action and hypnosis induced by pentobarbital sodium and suggested that oils could be a potential active component in sedation and hypnosis. However, Wu *et al.* [20] found that Jujuboside A exerts no inhibiting effect, but a synergism with phenylalanine on central nervous system function. They hence thought that Jujuboside A is possibly not an effective sedative component. Guo *et al.* [22,28] reported respectively in 1996 and 1998 that total flavonoids or total saponins of SZJ both have the hypnotic activity to mouse and rat. There must be further studies to clarify the major sedative components of SZJ [25].

A major aim of the present study was to make a side-by-side comparison between the sedative and hyponic effects of saponins, flavonoids, and polysaccharides extracted from SZJ, and this has yielded some interesting results. Spontaneous motility and

coordinated movement tests showed that compared to control group, polysaccharide group did not show any differences. Flavonoid group had no significant difference ($p>0.05$) in arm elevating times, but had significant difference in coordinated movement ($p<0.05$) and extremely significant difference ($p<0.01$) in walking time, with those of control group. The mice in the Saponin group also had no significant difference in arm elevating times ($p>0.05$), but had extremely significant difference in coordinated movement walking time ($p<0.01$) compared with those of the control group. Sodium pentobarbital-induced sleeping time tests showed that polysaccharide group had no differences in suprathreshold barbiturate-induced sleeping time and subthreshold barbiturate sleeping animal number ($p>0.05$). Compared to control group, flavonoid group showed a significant difference in suprathreshold barbiturate-induced sleeping time ($p<0.05$), and the number of sleeping animals increased 48% ($p<0.05$). Saponin group had extremely significant difference in suprathreshold barbiturate-induced sleeping time ($p<0.01$), and the number of sleeping animals increased 67% ($p<0.01$) compared with those of the control group.

In conclusion, when assessed over all of the assays, saponins of SZJ emerge as having the most effective sedative and hyponic functions. Flavonoids also have the functions but their effects are not as great as saponins. Polysaccharides of SZJ have no real sedative or hyponic functions. The present research shows that the saponins extracted from SZJ have high sedative and hyponic effects, but as mentioned earlier, some of the saponins, such as Jujuboside A may not have the sedative and hyponic effects. The major active components of saponins and its precise anxiolytic mechanisms need to be further identified in the future.

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