PHARMACOLOGICAL STUDIES OF ZIZYPHUS SEEDS

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Abstract—Pharmacological properties of fractions obtained from Zizyphus seeds were estimated by a screening method consisting of 5 tests: 1) neuropharmacological observations in mice, 2) tests on the respiratory and cardiovascular systems in the rat, 3) tests on the isolated guinea pig ileum, 4) potentiation of hexobarbital anaesthesia, and 5) inhibition of writhing syndrome induced by acetic acid. Z. No. 2, Z. No. 3 and Z. No. 5 were confirmed to have sedative activity as a result of tests I and IV, and were estimated to have analgesic and anti-inflammatory properties from test V. Z. No. 5a was determined to have the strongest activities. The arterial blood pressure showed a prolonged elevation by fat and oil fractions, which was not eliminated by Phentolamine and hexamethonium. Water soluble fractions showed a transient hypotension, which was partially eliminated by atropine but not by diphenhydramine and propranolol. Increase of the contraction induced by nicotine could be seen in all fractions. Increase of the contraction induced by serotonin could be seen in fat and oil fractions. Water soluble fractions showed cholinergic activity. Papaverine-like activity was noted in Z. No. 3.

Zizyphus seeds (Sansonin) have been utilized for thousands of years in the Far East and India as sedatives and nervine tonics. Chemical research on Zizyphus seeds, leaves and bark have been reported by a number of investigators, especially by Indian and South American chemists (1-11). Shibata and his co-workers (12) have investigated the chemical structure of saponins and sapogenins of Zizyphus seeds.

In the pharmacological fields, only a few papers concerning the extracts Zizyphus seeds, leaves and bark have been reported. Taran (13) reported the anaesthetic effects of Zizyphus leaves on the taste buds. Barros et al. (14) reported that both aqueous and ethanolic extracts of stem-barks of Zizyphus joaferio Mart. (Rhamnaceae) have a stimulating effect on respiration and a depressor effect on blood pressure in cats, and a stimulating effect on toad rectus abdominis muscle and heart preparations. A spasmogenic activity on both isolated guinea-pig ileum and rabbit duodenum was also exhibited, as well as oxytocic activity on isolated rat uterus. Nakano (15) reported that betulic acid from Zizyphus seeds was useful as a hypnotic.

In our laboratory, systematic analysis of pharmacological properties of various chemical compounds have been studied (16). The present study is an attempt to estimate pharmacological properties of Zizyphus seeds (Zizyphus vulgaris Lam. var. spinosus Bunge), and to differentiate pharmacologically active substances. A number of relatively simple tests were employed in order to obtain a spectrum of the pharmacological activities of the fractions obtained from Zizyphus seeds extract.
METHODS AND MATERIALS

Pharmacological properties of the extracts of Zizyphus seeds were estimated by blind screening which consisted of three tests; 1) neuropharmacological observations in mice based on the work of Irwin (17), 2) tests on the respiratory and the cardiovascular systems in rats, and 3) tests on the isolated guinea-pig ileum. Effects on hexobarbital anesthesia and inhibition of writhing syndrome produced by 0.7% acetic acid were also examined. Details of these five tests were described by Takagi et al. (16).

Preparation of the extracts from Zizyphus seeds:

Extractions of Zizyphus seeds were prepared by Shibata et al. (12) as shown in Fig. 1. Twenty mg/kg of Z. No. 1, Z. No. 2, Z. No. 3, Z. No. 6 and betulic acid fractions were suspended in saline with the aid of tween-80. The other fractions were dissolved in physiological saline.

RESULTS

1) Neuropharmacological observations in mice

From the number of survivals in each group, approx. i.p. LD₅₀'s of the extracts from Zizyphus seeds in mice were estimated to be more than 10 g/kg (Z. No. 1 and 4), between 5 and 10 g/kg (Z. No. 2), approx. 5 g/kg (Z. No. 5b), and between 2 and 5 g/kg (Z. No. 3, 5a, 6 and betulic acid fraction). The lethal doses produced the following common symptoms for 2 hr after administration: decrease of alertness and grooming, a slight decrease
of the following; touch response (Z. No. 2, 3, 4, 5a and betulic acid fraction), spontaneous movement, grip tone, body tone, body temp. and respiratory rate, and a slight increase in passivity, piloerection (all fractions except Z. No. 5a), ptosis (Z. No. 2, 3, 4 and 5a). Abnormal gait and writhing syndrome were observed approx. 30 min after administration. A slightly extended posture with abdomen touching floor was also observed. Disappearance of pinna reflex (Z. No. 3 and 5b), incapability of touching a wire with hind paws (traction test: Z. No. 2, 3, 5a and 5b) were observed during a 2 hr period. These changes in behavior continued for several hr and the mice died 12 to 48 hr after treatment.

In doses of less than LD₅₀, Z. No. 1 (0.5–10 g/kg), Z. No. 3 and Z. No. 6 (0.1–2 g/kg), Z. No. 4 (0.1–10 g/kg), Z. No. 5a and betulic acid fraction (0.2–2 g/kg), and Z. No. 5b (0.5–2 g/kg) produced almost the same effects as those caused by an approx. lethal doses. These effects appeared to be dose-dependent. Diarrhea was observed after the administration of Z. No. 1. Decreased urination were slightly observed with Z. No. 1, betulic acid fraction and Z. No. 5b, whereas increased urination with Z. No. 2. Z. No. 5b decreased fecal excretion for 2 hr. The peak effects were seen within 1 hr after administration of Z. No. 4, Z. No. 5b, Z. No. 6 and betulic acid fraction and the mice treated with them returned to normal states a few hr later. The peak effects by Z. No. 1, Z. No. 2,
Z. No. 3 and Z. No. 5a lasted from 30 to 180 min after administration to the mice showing renewed vitality 24 hr later. Table 1 shows the pattern of changes in general behavior induced by Z. No. 5a.

2) Tests on the respiratory and the cardiovascular systems in the rat

As the solution of some fractions was prepared with an addition of a drop of tween-80 to the physiological saline, the effects of tween-80 on blood pressure, heart rate and respiration in the rat were studied. The arterial blood pressure of the rat was elevated slightly and transiently with i.v. injections of higher doses of tween-80 (more than 20 mg/kg). Effects of acetylcholine chloride (ACh), epinephrine chloride (Epi), histamine dihydrochloride (His), vagal stimulation and carotid occlusion on cardiovascular responses due to tween-80 were tested, however, it did not alter the characteristic blood pressure responses to the five different stimuli.

Water insoluble fraction: The arterial blood pressure of the rat showed a prolonged elevation with the doses of more than 5 mg/kg of Z. No. 2 (Fig. 2), and more than 10 mg/kg of Z. No. 1 and Z. No. 6. In 5 rats given 10 mg/kg, the mean arterial blood pressure caused by Z. No. 1, Z. No. 2 and Z. No. 6 increased by 32, 15 and 9%, respectively, to that of normal. A slight decrease in heart rate was observed and an excitatory effect on respiration was produced in association with the hypertensive phase of blood pressure with Z. No. 1 and Z. No. 2, whereas Z. No. 6 had no effects on heart rate and respiration. Intravenous injection of phentolamine mesylate (Phent) or hexamethonium bromide (C6) did not eliminate the hypertensive response due to Z. No. 1, Z. No. 2 and Z. No. 6, but decreased the excitatory effect on respiration. Z. No. 3 and betulic acid fraction had no effects on blood pressure, heart rate and respiration with 10, 20 and 50 mg/kg.

Water soluble fractions: The blood pressure showed a significant but transient fall by injection of more than 1 mg/kg of Z. No. 5a and Z. No. 5b, and 10 mg/kg of Z. No. 4. In 5 rats given 10 mg/kg, the arterial blood pressure decreased by 15, 32 and 31% of normal with Z. No. 4, Z. No. 5a and Z. No. 5b. A slight decrease of heart rate could be detected. Z. No. 4 had no effect on respiration. An excitatory effect on respiration in association with hypotensive phase by Z. No. 5a as well as an inhibitory effect, in association with

![Fig. 2. Effect of Z. No. 2 on the respiratory and the cardiovascular systems in rats.](image-url)
hypotensive phase by Z. No. 5b were detected. With previous administration of atropine sulfate (Atr), hypotensive responses and respiratory excitation of Z. No. 5a decreased significantly, but diphenhydramine hydrochloride (Diph) and propranolol hydrochloride (Prop) did not alter hypotensive responses caused by the extracts. Effects of ACh, Epi, His, vagal stimulation and carotid occlusion on cardiovascular response to all fractions were tested. The fractions did not alter the five different characteristic blood pressure responses. Tachyphylaxis could not be detected upon repetitive injection of all fractions.

3) Tests on the guinea pig isolated ileum

Effects of tween-80 on the ileum were first observed. Tween-80 produced no contraction. In a lower concentration ($10^{-6}$ g/ml) it induced a slight potentiation of contractions caused by ACh and His, whereas it slightly inhibited the contractions in a higher concentration ($10^{-4}$ g/ml). It also antagonized the contractions induced by nicotine bitartrate (Nic) and serotonin (5-HT) in a concentration of $10^{-4}$ g/ml.

**Water insoluble fractions:** They did not produce contraction of the ileum. Z. No. 6 had no effect on ACh contraction, but Z. No. 1, Z. No. 2, betulic acid fraction and Z. No. 3 inhibited the contraction in a higher concentration ($10^{-4}$ g/ml) by 10, 10, 21 and 17% respectively. Z. No. 2 and Z. No. 6 had no effect on His contraction, but Z. No. 1, betulic acid fraction and Z. No. 3 inhibited the contraction in a concentration of $10^{-4}$ g/ml by 25, 15 and 25%, respectively. In higher concentrations ($10^{-3}$-$10^{-4}$ g/ml), Z. No. 6, Z. No. 2 and betulic acid fraction significantly potentiated Nic contraction, for example, by 41, 17 and 30% in a concentration of $10^{-4}$ g/ml, respectively. Z. No. 3 also induced significant potentiation in concentration of more than $10^{-4}$ g/ml by more than 40%. Z. No. 1 induced slight potentiation of Nic contraction in a higher concentration ($10^{-4}$ g/ml). Z. No. 2 and betulic acid fraction ($10^{-6}$-$10^{-4}$ g/ml) and Z. No. 6 ($10^{-6}$-$10^{-4}$ g/ml) potentiated 5-HT contraction by 18, 20 and 25%, respectively. Z. No. 1 induced slight potentiation of 5-HT contraction in higher concentrations, and Z. No. 3 had no effect on the contraction.

**Water soluble fractions:** Three soluble fractions caused contractions of the ileum in higher concentrations ($10^{-3}$-$3 \times 10^{-4}$ g/ml). Contractions, produced at $3 \times 10^{-4}$ g/ml, were completely inhibited by $10^{-8}$ g/ml of Atr, but not by $10^{-8}$ g/ml of Diph. Z. No. 4 and Z. No. 5a ($10^{-9}$-$10^{-4}$ g/ml), and Z. No. 5b ($10^{-4}$ g/ml) induced a slight potentiation of ACh contraction. A significant potentiation of His contraction in more than $10^{-4}$ g/ml of Z. No. 4 and a slight potentiation of it with Z. No. 5a and Z. No. 5b, were induced. Z. No. 4 induced a potentiation of Nic contraction in $10^{-2}$-$10^{-4}$ g/ml, and Z. No. 5a and Z. No. 5b potentiated it in a lower concentration ($10^{-8}$ g/ml) by 32 and 24% respectively. They had no effect on 5-HT contraction.

4) Potentiation of hexobarbital anaesthesia

Z. No. 5a in doses of 0.2, 0.5 and 1 g/kg, Z. No. 3 in doses of 1 and 2 g/kg, and Z. No. 1, Z. No. 2 and Z. No. 5b in a dose of 2 g/kg, produced a significant prolongation of sleeping time by hexobarbital administered 30 min after injection of the fractions. Z. No. 4 and betulic acid fraction had no effect on sleeping time by hexobarbital (Fig. 3).
FIG. 3. Effects of Zizyphus seeds on hexobarbital anaesthesia in mice. Height of a column represents mean ± S.E. of 6 mice. 
*** : significantly different from control (P<0.01), **: (P<0.02) and *: (P<0.05)

FIG. 4. Effects of Zizyphus seeds on writhing induced by 0.7% acetic acid. Height of a column represents mean ± S.E. of 6 mice.
** : significantly different from control (P<0.01) and *: (P<0.05)
5) Writhing syndrome induced by 0.7% acetic acid

Z. No. 5a in doses of more than 0.1 g/kg, Z. No. 5b in doses of more than 0.2 g/kg, Z. No. 3 in a dose of 0.5 g/kg and Z. No. 2 in doses of 1 and 2 g/kg had a significant inhibiting effect on writhing induced by acetic acid, but Z. No. 1 and betulic acid had no inhibitory effect. Z. No. 4 increased the number of mice writhings significantly (Fig. 4).

DISCUSSION

Pharmacological properties of various fractions of Zizyphus seed extracts were estimated from the results of 5 tests. Data on neuropharmacological observation of these fractions in mice were compared with a large mass of data of known drugs, but drugs showing similar neuropharmacological spectra could not be found. Interpretation of possible pharmacological properties of extracts was then attempted.

CNS-depressant activity could be estimated in all fractions. Slightly decreased alertness, grooming and grip tone, reduced spontaneous movement, and increased passivity were observed. In addition, traction test (18) (Z. No. 2, 3, 5a and 5b), dose ratio of corneal to pinna reflex (19) (Z. No. 3), dose ratio of reduction in spontaneous movement to decrease in alertness (Z. No. 3), lowering of body temp. (Z. No. 5a) and ptosis (Z. No. 2, 3 and 5a) strongly indicate tranquillizing activity. Myorelaxant and antipyretic actions were also weak. Increase in urination (Z. No. 1 and 2) or decreases in urination and fecal excretion (Z. No. 5b and betulic acid fraction) could be seen for 2 hr after administration.

Zizyphus seeds were demonstrated to have two reverse effects on the arterial blood pressure of the rat, that is, prolonged hypertensive responses caused by Z. No. 1, Z. No. 2 and Z. No. 6, and transient hypotensive responses by Z. No. 4, Z. No. 5a and Z. No. 5b. The previous administration of Phent and C6 did not eliminate these hypertensive responses. Atr significantly decreased the hypotensive responses to Z. No. 4, Z. No. 5a and Z. No. 5b, and respiratory excitation with Z. No. 5a, but Diph and Prop did not eliminate these hypotensive responses. Z. No. 3 and betulic acid fraction had no effects on blood pressure, heart rate or respiration. From these results, Z. No. 1, Z. No. 2 and Z. No. 6 may act as a direct cardiovascular stimulant, and may have vasoconstrictive and/or MAO-inhibiting activities (18). Z. No. 4, Z. No. 5a and Z. No. 5b appear to have muscarinic, cardiovascular depressant and/or vasodilative activities.

Water insoluble fractions did not induce the contraction of the ileum, but water soluble fractions showed contractions in concentrations more than $10^{-5}$ g/ml. As the contractions induced by a concentration of $3 \times 10^{-4}$ g/ml, were antagonized by Atr but not by Diph, they appear to have cholinergic activity. Potentiation of Nic contraction of guinea pig ileums could be seen in all fractions. Z. No. 1, Z. No. 2, Z. No. 6 and betulic acid fraction increased 5-HT contraction. Betulic acid fraction showed an inhibition of His contraction and Z. No. 1 inhibited both ACh and His contractions. As Z. No. 3 antagonized ACh and His contractions in larger concentrations, it may have papaverine-like activity. Z. No. 4, Z. No. 5a and Z. No. 5b potentiated ACh and His contractions but had no effect on 5-HT. From the results of test III, it was confirmed that all fractions
may have Nic potentiating activity, water soluble fractions cholinergic and His potentiating activities, and water insoluble fractions papaverine-like and 5-HT potentiating activities.

As Z. No. 1, Z. No. 2, Z. No. 3, Z. No. 5a and Z. No. 5b induced a prolongation of sleeping time, they may have sedative and/or tranquillizing activities. Z. No. 2, Z. No. 3, Z. No. 5a and Z. No. 5b have an inhibitory effect on writhing, therefore they may have analgesic and anti-inflammatory activities based on the results of test I.

Z. No. 6 consisted of a mixture of fat and oil, which is thought to be divided into Z. No. 1, Z. No. 2 and betulic acid fraction. Betulic acid fraction includes betulic acid as a main component with traces of betulin and β-sitosterol. Hypnotic activity of betulic acid fraction with the test I and IV could not be confirmed in spite of the fact that Nakano (15) reported it in 1967. Z. No. 3, consisting of mainly saponins and phenolic-glycosides, was estimated to have the strongest tranquillizing activity among all fractions in test I.

Table 2. A summary of effects of Zizyphus seeds reported in this paper.

| Tests               | Z. No. 1 | Z. No. 2 | Z. No. 3 | Z. No. 5a | Z. No. 5b |
|---------------------|----------|----------|----------|-----------|-----------|
| Sleeplessness       |          |          |          |           |           |
| Sensitivity         |          |          |          |           |           |
| Motor coordination  |          |          |          |           |           |
| Touch response      |          |          |          |           |           |
| Body posture        |          |          |          |           |           |
| Grid tone           |          |          |          |           |           |
| Glucose reflex      |          |          |          |           |           |
| Respiration         |          |          |          |           |           |
| Blood pressure      |          |          |          |           |           |
| Heart rate          |          |          |          |           |           |
| Respiration         |          |          |          |           |           |
| Antagonism with 10^-9 M | |          |          |           |           |
| ACN                 |          |          |          |           |           |
| HCl                 |          |          |          |           |           |
| L-HCl               |          |          |          |           |           |
| Potentiation of sleeping time | ** | | | | ** |
| Antagonism to writhing | ** | | | | ** |

Test I: Symbols indicate the change in behavior produced in the following dose ranges. 1/10-1/5 of LD50, 1/5-1/2 of LD50, and approx. LD50.

Test II: Symbols indicate the significant difference from control (P<0.05) in the following dose ranges. 1/10-1/5 of LD50, 1/5-1/2 of LD50, and approx. LD50.

Test III: Symbols indicate the significant difference from control (P<0.05) in the following dose ranges. 10^-6 g/ml, 10^-5 g/ml, and 10^-4 g/ml of Zizyphus seed extracts increased (decreased) against contraction.

Test IV and V: Symbols indicate the significant difference from control (P<0.05) in the following dose ranges. 1/10-1/5 of LD50, 1/5-1/2 of LD50, and approx. LD50.
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Z. No. 4 consisted mainly of sugars and amino acids. Pharmacological properties of Z. No. 5a extracted with cold water were compared with those of Z. No. 5b extracted with hot water. Although approx. similar, cholinergic, sedative and analgesic activities of Z. No. 5a were somewhat stronger than those of Z. No. 5b. Z. No. 5a was estimated to have the strongest sedative and analgesic activities among all fractions. Results are summarized in Table 2. Cholinergic and hypotensive substances were present in the water soluble fraction, and hypertensive substances were present in fat and oil fractions, although not present in the betulic acid fraction. 5-HT potentiating and papaverine-like substances were also contained in the insoluble fraction, especially in Z. No. 3. Sedative and/or tranquillizing activity was seen in three fractions, and Nic potentiating activity was noted in all fractions. It is thought that at least three different substances have sedative and/or tranquillizing activity in the Zizyphus seed extracts (fat and oil fraction, Z. No. 3 and Z. No. 5), or one substance which was distributed into those fractions. The same distribution was noted for Nic potentiating activity.

In Chinese medicine, it is said that Sansonin has nervine tonic, sedative, tranquillizing, hypnotic and stomachic activities, and is used to treat insomnia or sleeping sickness. Though hypnotic activity of Zizyphus seeds could not be demonstrated, tranquillizing and/or sedative activity was confirmed in the three fractions.

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