Chemical and Pharmacological Studies on *Argemone mexicana*

By B. C. BOSE, R. VIJAYVARGIYA, A. Q. SAIFI, and S. K. SHARMA

Chemical and pharmacological studies of Argemone mexicana have been carried out. Chemical analysis revealed the presence of total alkaloids 0.125 per cent, consisting of protopine 0.084 per cent and berberine 0.041 per cent, tannin 1.10 per cent, resin 1.75 per cent, and a toxic principle in argemone oil. The main activity was related to the alkaloids, the protopine fraction stimulating heart, respiration, skeletal muscles, and blood pressure. The total alkaloidal fraction stimulated all the smooth muscles and antagonized the actions of acetylcholine, histamine, and 5-hydroxytryptamine. Its oxytocic action resembled that of pitocin. Atropine, adrenergic blocking agents, and antihistaminics did not modify the response on blood pressure. The carotid occlusion reflex was inhibited, but responses to acetylcholine and catecholamines remained unaffected. It antagonized barbiturate-induced depression and potentiated methamphetamine stimulation of spontaneous motility in mice. It produced a mild neuromuscular block of the diaphragm and showed antiacetylcholine action on the frog rectus.

ARGEMONE MEXICANA (*Papaveraceae*), an American plant naturalized in India, grows widely and is commonly known as a pivla dhatura or satyanashi. The yellow, milky juice has long been used in indigenous systems of medicine for dropsy, jaundice, skin diseases, indolent or syphilitic ulcers, eye conditions, respiratory disorders, and constipation. Schlotterbeck (1) isolated and identified two alkaloids, berberine and protopine, from the plant. Berberine was tried by the early physicians in malaria and leishmaniasis (2–4). The seeds of this plant yield a fixed oil, argemone oil (22 to 36%), which, when consumed in adulterated mustard oil for cooking, was found responsible for the epidemic dropsy in certain areas (2). Mukerji (5) isolated a toxic substance, with an empirical formula $C_{19}H_{15}NO_4$, responsible for this. Pharmacological actions of berberine, obtained from the berberis family, have been reported (6, 7). Since no investigations on the effects of total alkaloids, and especially the protopine fraction of this plant have so far been reported, the present study was undertaken.

EXPERIMENTAL

The air-dried powdered roots and stems of the plant were subjected to successive extractions with the following solvents in a soxhlet apparatus and the residues found to be: alcohol—10.5, chloroform—2.1, petroleum ether—2.7, benzene—0.9, ether—1.7, acetone—3.3, and ethylacetate—1.6%.

On incineration, the total, acid soluble, acid insoluble, and water soluble ashes were found to be 8.56, 85.3, 14.7, and 50.8%, respectively. Inorganic constituents detected were potassium, calcium, sodium, aluminum, sulfates, nitrates, and carbonates.

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The paper chromatographic studies of alcohol and chloroform extracts, using Whatman No. 1 filter paper and water-saturated *n*-butanol:glacial acetic acid (25:1) as solvent, gave two distinct spots with R_f values of 0.35 and 0.23 and golden yellow and bluish fluorescence, respectively. The chromatograms, when sprayed with modified Dragendorff's reagent, showed them to be due to alkaloids. These were found to agree with berberine and protopine, respectively.

The plant was also found to contain tannins (1.10%) and resin (1.75%) by potassium permanganate titration and water precipitation methods, respectively. All the extracts gave a positive Libermann-Burchard's test for sterol bodies which was later found to be due to the alkaloids themselves.

The alkaloids were quantitatively estimated by the method described by Alfredo Santos (8). Much variation was observed from sample to sample, but the average total alkaloidal content was 0.125%. Of these, protopine formed 0.084%. The presence of these alkaloids was confirmed by the melting points and maximum light absorption spectra.

The toxic principle from the seed was separated and identified from the oil according to the method described by Mukerji (5).

Most of the pharmacological experiments were conducted with a total alkaloidal solution with the hydrochloride salt. In a few cases, both berberine and protopine were used for comparative evaluation of differences in actions.

Ten dogs, (five anaesthetized with urethane, 1.8 Gm./Kg., and five with diallyl barbituric acid, 0.7 ml./Kg.), weighing between 6–13 Kg., were utilized in the present study for observations on the effects of alkaloids on blood pressure, respiration, auriculo-ventriculogram, and intestine by the usual techniques.

Blood Pressure.—The total alkaloidal fraction was studied in a dose range of 0.05 to 5.0 mg./Kg. Smaller doses either had no effect or occasionally produced a slight rise, but a dose above 0.1 mg./Kg. produced a definite fall in blood pressure (Fig. 1). Atropine, adrenergic blocking agents, or antihistaminics did not modify the response. The hypotensive effect was more marked in experimentally induced hypertension with norepinephrine.

The sinus response due to carotid occlusion was inhibited by this fraction, but responses due to acetylcholine, epinephrine, or norepinephrine remained unaltered (Fig. 2). With smaller concentrations, a potentiation of acetylcholine-induced fall in blood pressure was observed.

Protopine fraction produced a rise in the blood pressure in smaller concentrations and a fall in doses greater than 1 mg./Kg. Berberine produced a fall in all the concentrations tried.

Heart.—The total alkaloidal fraction (1.0 mg./Kg.) inhibited both auricles and ventricles *in situ* in dogs. Rabbit heart, both *in situ* and isolated, was also depressed by the alkaloids in a concentration of 1×10^{-5} to 4×10^{-4}. Isolated amphibian heart was depressed even in a concentration of 1×10^{-6}. It also antagonized acetylcholine and epinephrine responses in a concentration of 1×10^{-5} to 2×10^{-4} (Fig. 3). Whereas the protopine fraction produced a slight stimulation even in a dose of 25 mcg./Kg., the berberine fraction was consistently found to be a myocardial depressant.

Respiration.—The total alkaloidal fraction, when given intravenously, stimulated respiration. It antagonized barbiturate-induced respiratory depression at higher concentrations (Fig. 4). The protopine fraction was very potent in this respect, whereas berberine produced an initial stimulation followed by depression. Both atropine and dihydroergotamine did not affect these actions.

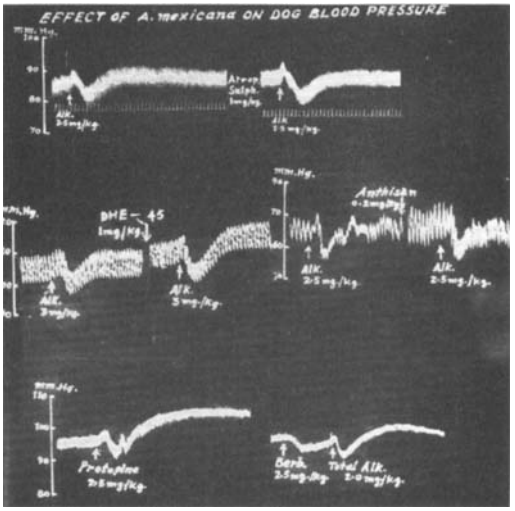

Fig. 1.—Effect of total alkaloid, protopine and berberine on blood pressure in the dog.

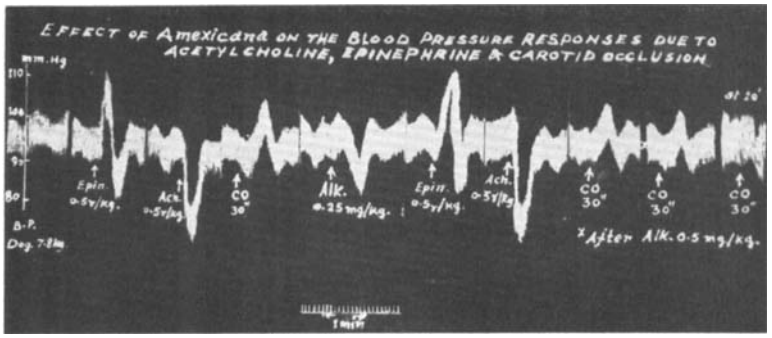

Fig. 2.—Effect of *A. mexicana* on the blood pressure responses to acetylcholine, epinephrine, and carotid occlusion responses.

Intestine.—In anesthetized dog and rabbit, both total and individual alkaloidal fractions produced an immediate stimulation of tone and peristaltic movements of the gut in a dose of 0.05 mg./Kg. Atropine inhibited the increase in tone but did not affect the augmentory action of the alkaloid on peristalsis.

Segments of ileum and uterus from freshly killed rats, guinea pigs, and rabbits, were mounted in an isolated organ bath and the effect of the alkaloids studied by the usual method. The intestine was stimulated in a concentration of 1×10^{-7} to 2×10^{-6}, with a concentration of 5×10^{-5}, though tone was increased; but there was a decrease in the amplitude of rhythmic contractions. In a concentration of 2×10^{-6} to 1×10^{-5} the total alkaloidal fraction administered half a minute before or simultaneously with spasmogens produced antiacetylcholine, antihistamine, and anti-5-hydroxytryptamine actions, even though it produced stimulation of its own. But when these spasmogens were allowed to remain in contact for some time and a persistent increased tone level obtained, the alkaloid produced an immediate relaxation. In atropinized tissue preparations the alkaloid still produced stimulation, though not markedly.

Other Smooth Muscles.—*In situ* experiments with rabbit uterii and isolated rat and guinea pig uterii showed that the alkaloid possessed a significant oxytocic activity in a concentration exceeding 5×10^{-7}; concentration of 5×10^{-6} produced, qualitatively, an effect similar to that of pitocin, 0.05 units/ml. (Fig. 5).

With frog blood vessel perfusion experiments, it was observed that the alkaloid produced vasodilatation in a concentration of 1×10^{-7} to 6×10^{-5}.

The effect on bronchioles was studied by both tracheal chain and lung perfusion experiments by the techniques detailed earlier (9). It was observed that though the total alkaloidal fraction produced a bronchoconstriction it exhibited antiacetylcholine and antihistaminic actions in a concentration of 5×10^{-6} to 3×10^{-4}.

Skeletal Muscle.—The protopine fraction, in a dose of 2.5 mg./Kg. or above, produced generalized muscle twitchings, more predominant in the neck and limb muscles, lasting for 2–5 minutes in dogs. This response usually corresponded with the respiratory stimulation. In a concentration of 2×10^{-5} to 2×10^{-4}, the total alkaloidal fraction showed an antiacetylcholine activity on frog rectus abdominis muscle studied in a leech muscle apparatus. A concentration of 2×10^{-4} evoked muscle twitchings. With experiments on a rat phrenic nerve diaphragm preparation by the method of

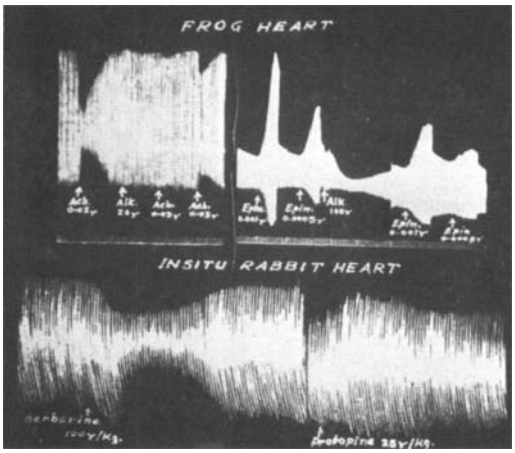

Fig. 3.—Effect of *A. mexicana* on frog and rabbit hearts.

Bulbring (10) it was observed that total alkaloidal fraction, in a concentration of 2×10^{-4}, produced a mild neuromuscular blocking effect which was partially antagonized by prostigmine. Concentrations of 2×10^{-5} or above produced muscle twitchings.

Effect of the total alkaloidal fraction on spontaneous motility was studied in mice (in groups of two each) with a simple instrument. It antagonized barbiturate-induced narcosis and potentiated the methamphetamine-induced hypermotility. The total alkaloidal fraction had no effect on rabbit cornea, conjunctival reflexes, or at the site of local injection in rats. But crude chloroform and petroleum ether extracts, suspended in normal saline, produced edema and necrosis at the site of injection in 80% of the cases within 2–3 days.

A few experiments conducted with the toxic substance isolated from argemone oil, showed hypotension, myocardial, respiratory, and intestinal inhibition. There was always a local action at the site of parenteral administration in rats.

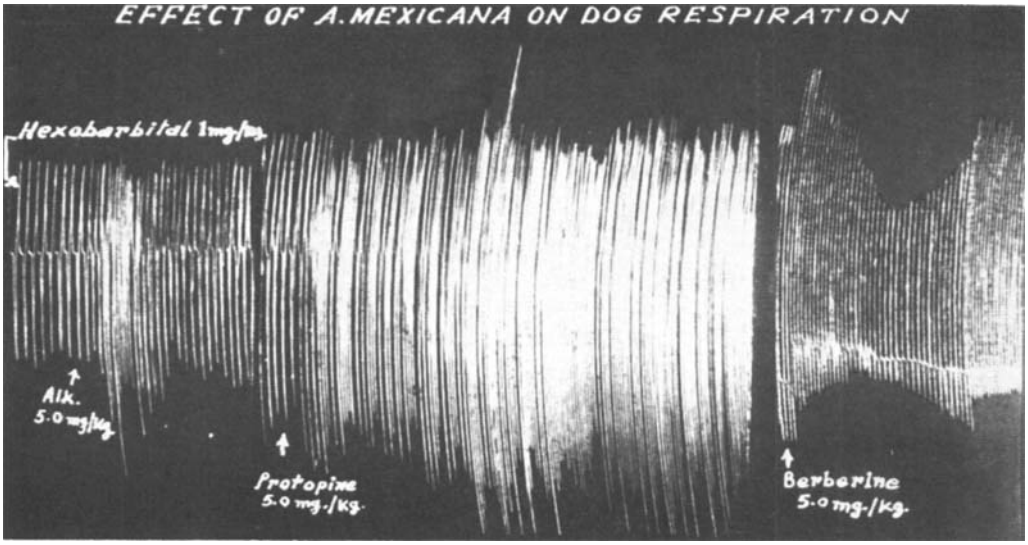

Fig. 4.—Effect of protopine and berberine on hexobarbital-depressed respiration of dog.

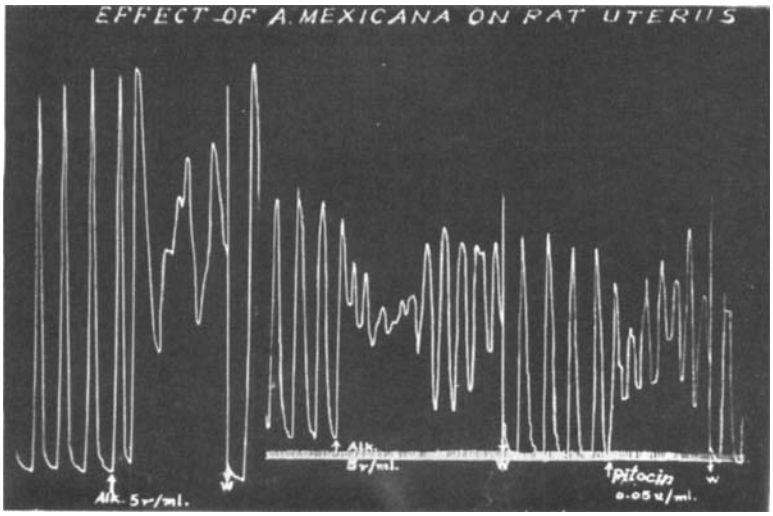

Fig. 5.—Effect of total alkaloid on isolated rat uterus.

Our findings with berberine on the gastrointestinal, cardiovascular, and respiratory systems are in agreement with those of other workers (6).

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Behavior of Erythrocytes in Various Solvent Systems I

Water-Glycerin and Water-Propylene Glycol

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The hemolytic behavior of rabbit and human erythrocytes in water–glycerin and water–propylene glycol solutions was investigated. Complete hemolysis of erythrocytes took place in all propylene glycol solutions and most glycerin solutions. Aqueous solutions containing 50, 60, and 70 per cent glycerin prevented complete hemolysis of rabbit erythrocytes but not human erythrocytes. The addition of sodium chloride to various glycerin solutions prevented hemolysis. The addition of sodium chloride to propylene glycol solutions prevented hemolysis of rabbit erythrocytes in 5–30 per cent solutions and of human erythrocytes in 5–40 per cent solutions. When possible, the data were used to calculate van't Hoff *i* values for sodium chloride in various water-glycerin and water–propylene glycol solutions. Unusual behavior was displayed by erythrocytes in 40–50 per cent propylene glycol solutions. The addition of sodium chloride to solutions containing 50 per cent or more of propylene glycol did not prevent complete laking of red blood cells.

SINCE THE DEVELOPMENT of the hemolytic method by Husa and co-workers (1, 2), many investigations have been carried out (3–12) to study the behavior of erythrocytes to various compounds. In the aforementioned investigations, water was used as the solvent for all of the substances studied. Water, however, is not the only solvent used for intravenous preparations. To prepare a safe, stable, and efficacious injection, it is sometimes necessary to employ a mixed solvent system consisting of water and a nonaqueous co-solvent. Two nonaqueous solvents that are used in the formulation of parenteral preparations are glycerin and propylene glycol.

Husa and Adams (1) showed that glycerin and propylene glycol did not prevent hemolysis at concentrations which were calculated to be isotonic according to physicochemical data. They also reported that 0.3 to 0.5% sodium chloride would prevent hemolysis when added to hypo-osmotic concentrations of the polyhydric alcohols in water. Hammarlund and Pedersen-Bjergaard (13) demonstrated that complete hemolysis of blood takes place in iso-osmotic concentrations of glycerin and propylene glycol.

Hemolytic studies with glycerin and propylene glycol solutions were carried out by Zanowiak and Husa (8). They found that complete hemolysis took place in each 10% polyhydric alcohol–water solution, even though this concentration was well above the iso-osmotic concentration of each substance. They also reported that the addition of 0.2% sodium chloride did not prevent hemolysis of blood in the 10% glycerin or propylene glycol solutions. The presence of 0.6% sodium chloride, however, in these 10% solutions did prevent hemolysis. The purpose of this investigation was to conduct experiments to study further the behavior of red blood cells in aqueous glycerin and propylene glycol solutions. The hemolytic method was employed, and the experiments were designed so that standard hemolysis curves of human and rabbit blood obtained from experiments using sodium chloride–water solutions could be compared to hemolysis curves obtained from experiments using sodium chloride–water–polyhydric alcohol solutions. From these

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