Diuretic Activity of Roots of *Clitoria ternatea* L. in Dogs

Recently SINGH and GUPTA[1] reported on their chemical investigation of *Clitoria ternatea* Linn., a plant which belongs to the subfamily Papilionoideae, family Leguminoseae. This tropical plant is found in India, where the roots or root bark are used as cathartics and diuretics[2,3]; the root juice used in the treatment of chronic bronchitis[3] has also been reported to cause nausea and vomiting.

We recently tested the powdered form of the dried whole root and an ethanol extract thereof for diuretic activity in unanesthetized and anesthetized dogs. The pharmacological procedures employed have been described previously[4].

One group of 4 unanesthetized hydrated dogs received a single oral dose of powdered whole root, 1.25 g/kg; another group of 4 dogs received 0.31 g/kg. All 4 dogs at the higher dose level and one of the 4 dogs at the lower dose level vomited shortly after dosing. For the 3 remaining dogs, the 6 h urine volumes and the urinary Na and K concentrations were within the normal response range.

A 95% ethanol-soluble extract of the powdered whole root was prepared by Miss MILDRED MOORE of our Biological Chemistry Section. 500 g of root were extracted with 3 l of 95% ethanol by mechanical stirring for $^1/_2$ h at room temperature. The ethanol-soluble portion was filtered and the filtrate was evaporated to dryness by vacuum distillation at 60°C. The residual roots were then extracted 3 more times with a total of 9 l of 95% ethanol and the ethanol-soluble portions were dried similarly and pooled. The total of 30.7 g of extract was used in subsequent studies. For the oral tests in mice and dogs, 9.1 to 50 mg of extract was suspended in an aqueous solution of propylene glycol (2.5 to 10% v/v) and agar (0.25% w/v). For intravenous dosage in dogs, 20 or 30 mg of extract was dissolved per ml of propylene glycol.

Single oral doses of the ethanol extract were administered to albino mice; doses of 91, 182, and 364 mg/kg of extract failed to produce acute symptoms or mortality within a 5-day holding period. At least 5 mice were used at each dose level.

Six unanesthetized, hydrated dogs received single oral doses of 19 mg/kg of the extract; another 6 dogs received 76 mg/kg. 6 h urine volumes as well as urinary Na and K excretions were within the normal response range for these 12 dogs. No emesis occurred in any of these 12 dogs, but it did occur in one additional dog which received the higher dose level of extract.

A crossover comparison of the intravenously administered extract and its solvent, propylene glycol (0.2 ml/kg), was also conducted in 10 unanesthetized, hydrated dogs. 4 mg/kg of the extract produced 4 h excretions of Na and K that were six-fold and three-fold greater, respectively, than those produced by the solvent alone, but the urine volume was unaffected. In 4 more dogs, 6 mg/kg (i.v.) of the extract increased the urinary output of Na and K five-fold and three-fold respectively, without appreciably altering the urinary volume. Glycosuria and traces of albuminuria were detected in all urine samples collected for 24 h after each i.v. dose of the extract; urine glucose and albumin were determined semi-quantitatively (Clinistix and Albustix reagent strips, Ames Co., Elkhart, Indiana). The glycol solvent alone did not produce glycosuria or albuminuria.

In a pentobarbitalized dog, infused continuously with buffered 5% glucose, 6 mg/kg of the extract injected intravenously halved the glomerular filtration rate. The % of filtered Na excreted in the urine was increased from a pre-drug level of 1.3% to a post-drug level of 2.8%; the % of filtered K excreted in the urine was also increased from 13.2% (pre-drug) to 36.5% (post-drug). The % of filtered Cl that was excreted, however, fell from 2.1% (pre-drug) to 1.5% (post-drug). Presumably the major anion excreted was HCO_3, for the urinary pH increased sharply. Furthermore, glycosuria and appreciable hematuria were evident within 2 h after injection of the extract. Intravenous dosage of the same dog with equal volumes of the solvent propylene glycol, under similar conditions on a different day, caused no glycosuria, hematuria, or reduced glomerular filtration rate. The solvent increased the % of filtered Na excreted from 0.9% (pre-solvent) to 1.2% (post-solvent) and the % of filtered K excreted from 10.1% (pre-solvent) to 13.5% (post-solvent).

Four mg/kg, given i.v. to another pentobarbitalized dog, did not affect either the glomerular filtration rate or the urinary pH but did enhance slightly the excretion of Na, K, and Cl. Glycosuria was also observed after this dose. A kidney function test (PSP excretion) performed 24 h after dosing these two animals yielded no indication of renal dysfunction.

These results indicate that sub-emetic, single oral doses of either ground whole root of *Clitoria ternatea* or of a 95% ethanol extract thereof failed to produce any appreciable diuretic or natriuretic effects in unanesthetized dogs. Single intravenous doses of the extract in unanesthetized and anesthetized dogs, however, produced moderate increases in urinary excretion of Na and K, decreases in Cl excretion, and little or no change in urine volume. The natriuretic effects were accompanied by one or more of the following undesirable effects: glycosuria, albuminuria, hematuria, and decreased glomerular filtration rate. We conclude that any safe, oral, diuretic activity remains to be demonstrated for the roots of *Clitoria ternatea*[5].

Zusammenfassung. Zermahlene *Clitoria ternatea*-Wurzeln oder deren Extrakt in 95% Alkohol zeigten bei oraler Verabreichung in nicht-toxischer Dosis keine nennenswerte diuretische oder natriuretische Wirkung beim Hund. Intravenöse Gaben des Extraktes führten zu mässiger Steigerung der Ausscheidung von Natrium und Kalium im Harn, zeigten aber gleichzeitig auch Anzeichen von Nierenschädigung.

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[5] This investigation was carried out in collaboration with Sarabhai Chemicals Research Institute, Ahmedabad (India). The whole root used in this study was supplied by them and the identity of the root was verified by Dr. P. VARADARAJAN.