Pharmacological Investigation of the Glucoside and Aglucone Isolated from *Caccinia glauca*

By H. R. K. ARORA† and R. B. ARORA‡

A glucoside and its aglucone isolated from *Caccinia glauca* were studied for their pharmacological activity. Both compounds exhibited a diuretic action which appeared to be due to an increase in glomerular filtration. The dose which induced diuresis did not have any other significant effects. Isolated tissue preparations were not affected except by very high concentrations. The compounds were effective by the oral as well as parenteral route and showed a large safety margin.

CACCINIA GLAUCA Savi., commonly known as gaozaban (1, 2), is frequently used in the indigenous system of medicine as a diuretic, in irritation of the bladder, strangury, and in several other conditions. Various uses for this plant have been described by ancient writers (3, 4).[1] Recently, a glucoside, caccinin, and its aglucone, caccinetin, which is the dimethylallyl ester of caffeic acid, have been isolated from it (5). In view of the reputation and extensive use of the drug by practitioners of the indigenous system of medicine, an investigation was undertaken to elucidate the pharmacological properties of the pure compounds.

METHODS AND RESULTS

Extraction and purification was done as reported earlier (5) and the chemical identity of the pure compounds was confirmed.[2] The glucoside was used as 1–5% solution in normal saline and the aglucone, because of its low solubility in water and saline, as a 1–2% solution in 0.5% sodium bicarbonate.

Cardiovascular Actions.—The effects of these compounds on intact circulation were studied on mongrel dogs of both sexes and male albino rats. The dogs weighed 5–8 Kg. and were anesthetized with an i.v. dose of pentobarbital sodium equivalent to 30 mg./Kg. of body weight. The rats weighed 175–250 Gm. and were anesthetized with an i.p. dose of 25% urethane in water equivalent to 0.6–0.8 ml./100 Gm. of body weight. Blood pressure was recorded from the carotoid artery on a smoked drum. Drugs were injected through a cannulated vein. Electrocardiogram, bipolar limb lead II, was recorded in all experiments.

A fall in blood pressure was recorded, both in rats and dogs, during administration of the glucoside and lasted for 1–3 minutes. Following this, the arterial pressure rose in rats but not in dogs (Fig. 1). The duration and extent of the rise depended upon the dose (Table I). An increase in heart rate was noted in rats but not in dogs. Electrocardiogram remained unchanged with doses up to 200 mg./Kg. of body weight on the glucoside and 50 mg./Kg. of body weight of the aglucone.

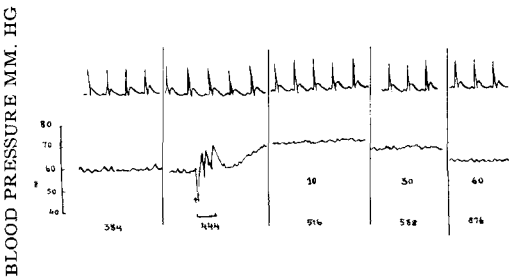

Fig. 1.—From above downwards the records are: electrocardiogram; blood pressure; time in minutes after drug administration; and heart rate per minute showing the effects of the glucoside in the rat. The drug was administered at the time indicated by arrow in a dose of 200 mg./Kg. of body weight intravenously.

The cardiac effects were studied on failing papillary muscle preparation of cat (6), isolated perfused rabbit heart (7), perfused frog ventricle (Straub-Fuehner preparation) (8), and heart-lung preparation of dog (9).

A positive inotropic action was noted on the cat papillary muscle preparation but high concentrations were required to elicit this effect (Table I). Isolated perfused rabbit heart was not significantly affected by doses up to 5 mg. of the glucoside or the aglucone added to the perfusing fluid. Similarly, perfused frog ventricle was unaffected by concentrations of 10^{-4} Gm./ml. of the glucoside or 10^{-3} Gm./ml. of the aglucone, although a concentration of 10^{-3} Gm./ml. of the glucoside reduced heart rate by 45 % ± 9.0 S.E. without producing any other effect.

The heart-lung preparation of dog was unaffected by concentrations of 1.5×10^{-4} Gm./ml. and 6×10^{-4} Gm./ml. of the glucoside. Caffeine, as caffeine citrate, used for comparison, slightly increased venous pressure and decreased competence of the heart when given in a concentration of 1.5×10^{-4} Gm./ml. In a concentration of 6×10^{-4} Gm./ml. it caused an acute fall in blood pressure and a marked rise in venous pressure.

Effect on blood vessels was studied by perfusing rabbit ear vessels (7) with Ringer-Locke solution. Doses of 5 mg. and above of the glucoside or the aglucone reduced the flow through ear vessels by 16 – 20% or more. Lower doses were ineffective.

Received September 29, 1961, from the Department of Pharmacology, Maulana Azad Medical College, New Delhi, India.

Accepted for publication March 6, 1962.

† Present address: Department of Pharmacology, Maulana Azad Medical College, New Delhi, India.

‡ Present address: All India Institute of Medical Sciences, New Delhi, India.

[1] Original references in Arabic were obtained through the kind courtesy of Hakim Abdul Wahid Saheb of Hamdard Dawakhana, New Delhi, India.

[2] Dr. T. R. Seshadri, Professor of Chemistry, Delhi University, kindly confirmed the chemical identity.

Urinary Actions.—Diuretic activity was studied on conscious and anesthetized rats (10, 11) and anesthetized dogs (12).

In studies on conscious rats, the animals were used in groups of three. All compounds were administered orally. Hourly urine output for the first 5 hours, and the total urine output for the next 19 hours (hereafter referred to as the 24-hr. urine) were noted. The urine outputs of groups receiving test compounds were compared with groups receiving urea or saline only. Chloride and uric acid contents of the total urine excreted during the first 5 hours and the subsequent 19 hours were separately estimated (13, 14).

The glucoside and the aglucone significantly increased the total urine outputs at 5 hours ($0.05 > P > 0.02$) as well as at 24 hours ($0.01 > P > 0.001$) when compared with groups receiving saline only (Table II). The 5- and 24-hr. urine outputs of groups receiving urea were not significantly greater than those receiving the test compounds ($0.5 > P > 0.4$). Chloride excretion was not altered but uric acid excretion was apparently increased. The compounds, however, gave false positive test for uric acid by the method employed.

In anesthetized rats the compounds were injected intravenously and the urine collected every 5 minutes through a polythene catheter placed in the bladder. In this preparation also, urine output was increased by the glucoside as well as the aglucone to the extent of 104% ± 23.8 S.E. and 62% ± 12.8 S.E., respectively. Similarly urine output increased by 85% ± 12.4 S.E. when a dose of 100 mg./Kg. of body weight of the glucoside was injected intravenously in dogs. Chloride excretion in dogs was not affected but endogenous creatinine excretion showed an increase of 92% ± 11.9 S.E. (Table II). Phenol red excretion in dogs was not altered by the glucoside.

Other pharmacological properties.—Intraperitoneal administration of doses up to 400 mg./Kg. of body weight of the glucoside in albino rats failed to

TABLE I.—EFFECT OF GLUCOSIDE AND AGLUCONE ON BLOOD PRESSURE AND HEART RATE OF INTACT ANIMALS AND CAT PAPILLARY MUSCLE[a]

Intact Animals

Animal	No. of Experiments	Drug	Dose, mg./Kg. Body Wt.	Increase, %: Blood Pressure ± S.E.	Increase, %: Heart Rate ± S.E.
Rat	10	Glucoside	50	11.0 ± 2.1	6.0 ± 1.3
Rat	10	Glucoside	100	20.0 ± 2.7	14.0 ± 1.7
Rat	10	Glucoside	200	42.0 ± 2.6	39.0 ± 2.0
Rat	10	Aglucone	100	10.0 ± 1.7	8.0 ± 1.0
Dog	6	Glucoside	100	No rise, only initial fall	8.0 ± 1.9
Dog	6	Aglucone	50	No rise, only initial fall	6.0 ± 2.1

Cat Papillary Muscle

No. of Experiments	Drug	Bath Concentration, Gm./ml.	% Amplitude of Contraction after Drug, ± S.E.
6	Glucoside	10^{-3}	100 ± 11.75
6	Glucoside	2×10^{-4}	64 ± 10.0
6	Glucoside	10^{-4}	44 ± 7.5
6	Aglucone	10^{-3}	88 ± 9.0
6	Aglucone	10^{-4}	46 ± 6.8
6	Caffeine (as citrate)	10^{-3}	98 ± 10.8
6	Caffeine (as citrate)	10^{-4}	58 ± 8.0

[a] Failed to approximately 50% of control amplitude with pentobarbital sodium.
[b] Control amplitude of contraction, prior to inducing failure, was taken as 100 %.
[c] Concentrations are for caffeine base.

TABLE II.—EFFECT OF GLUCOSIDE AND AGLUCONE ON URINE OUTPUT OF RATS AND URINE OUTPUT AND ENDOGENOUS CREATININE EXCRETION OF DOGS

Effect on Rats

Serial No.	Animals, No. Used	Drug	Dose, mg./Kg. Body Wt.	5-hr. Total Urine Output ml./Kg. Body Wt., ± S.E.	24-hr. Total Urine Output, ml./Kg. Body Wt., ± S.E.
1	18	Control (saline only)	...	8.8 ± 1.68	15.5 ± 2.12
2	18	Urea	750	17.0 ± 1.93	22.5 ± 2.54
3	24	Glucoside	50	13.0 ± 1.56	23.0 ± 1.86
4	24	Aglucone	50	12.2 ± 1.70	21.0 ± 2.18

Effect on Dogs

Serial No.	Animals, No. Used	Drug	Dose, mg./Kg. Body Wt.	% Increase in Urine Output after Drug, ± S.E.	% Increase in Endogenous Creatinine Excretion after Drug, ± S.E.
1	6	Glucoside	100	85 ± 12.4	92 ± 11.9

elicit any gross sedation or excitation. Hexobarbital sleeping time in mice (15) was not altered by doses up to 200 mg./Kg. of body weight of the glucoside or 50 mg./Kg. of body weight of the aglucone given intraperitoneally.

No anticonvulsant activity against maximal electroshock and maximal metrazol seizures (16) could be elicited in albino rats with i.p. doses up to 400 mg./Kg. body weight of the glucoside or 50 mg./Kg. body weight of the aglucone. Also, no activity could be detected with these doses against pain induced by application of radiant heat to the rat's tail (17).

A concentration of 10^{-4} Gm./ml. of the glucoside or its aglucone did not have any effect on the movements of isolated rabbit intestine or rat uterus (7). Acetylcholine induced contractions of the isolated rabbit intestine, rat uterus, and dog tracheal muscle preparation (18), were also not influenced by this concentration of the two compounds. A concentration of 5% of the glucoside or 0.5% of the aglucone did not exhibit any mydriatic, miotic, or local anesthetic effect on the rabbit eye. Ciliary movements of the frog esophagus (7) and acetylcholine induced contractions of frog rectus abdominis muscle preparation (7) were also not affected by a concentration of 10^{-3} Gm./ml. of the glucoside or its aglucone This concentration failed to show any antibacterial activity against *Staph. aureus*, *E. coli*, *S. typhi*, and *P. vulgaris* by the agar plate method.

Intravenous LD_{50} of the glucoside in mice (19) was found to be 576 mg./Kg. of body weight. Because of its low solubility, only the oral toxicity of the aglucone, as a 10% suspension in 4% acacia, was studied in rats. No mortality could be recorded with doses upto 2 Gm./Kg. of body weight. Chronic administration of 200 mg./Kg. of body weight of the glucoside and 100 mg./Kg. body weight of the aglucone given subcutaneously to mice failed to alter body weight or induce any histological changes in the liver, kidney, heart, spleen, or brain.

DISCUSSION

The most prominent property of the compounds isolated from *Caccinia glauca* is their diuretic activity. This property is exhibited after oral as well as parenteral administration to conscious and anesthetized rats and anesthetized dogs. Activity is manifested in doses which do not have other pharmacological actions. Thus diuretic activity could be elicited with a dose of 50 mg./Kg. of body weight of the compounds while the same dose given intravenously failed to influence blood pressure, heart rate or electrocardiogram. Only very high doses had any significant cardiovascular activity in the intact preparations. Isolated tissues also were affected by very high concentrations only. Since phenol red and chloride excretions were not affected while endogenous creatinine excretion in dogs was increased in proportion to the increase in urine output, it appears that these compounds act by increasing glomerular filtration. This very much resembles the action of xanthines, which have their main value in cases resistant to mercurial diuretics due to a low glomerular filtration (20) and are the only agents available at present which effectively increase glomerular filtration. The xanthines, however, possess undesirable side effects, especially in large doses, on the cardiovascular, gastrointestinal and central nervous systems. On the contrary, the isolated glucoside and the aglucone, even in large doses, do not exhibit any such undesirable actions as shown by this study. They may, therefore, be considered to have some promise for further studies.

REFERENCES

(1) Watts, G., "A Dictionary of Economic Products of India," Vol. V, Govt. of India publication, Calcutta, 1891, p. 487.
(2) Chopra, R. N., "Indigenous Drugs of India," 1st ed., Art Press, Calcutta, 1933.
(3) Dioscorides Pedanios, cited by Ibn-al-Baitar in "Jamuel Mufredate Adviya," Matbe Amra, Egypt, (1197–1248 A.D.).
(4) Avicenna, cited by Md. Ali Tehrani in "Kanune Sheikh," Persia, 1295 Hijri.
(5) Banerjee, N., and Seshadri, T. R., *J. Sci. Ind. Res. (India)*, **18B**, 493(1959).
(6) Bennet, D. R., Anderson, K. S., Andersen, M. V., Jr., Robertson, D. N., and Chenoweth, M. B., *J. Pharmacol. Exptl. Therap.*, **122**, 489(1958).
(7) Burn, J. H., "Practical Pharmacology," 1st ed., Blackwell, London, 1952, pp. 1, 7, 25, 62, and 65.
(8) Sollmann, T., "A Laboratory Guide in Pharmacology," 1st ed., Saunders, Philadelphia, Pa., 1917, pp. 35 and 191.
(9) Krayer, O., and Mendez, B., *J. Pharmacol. Exptl. Therap.*, **74**, 350(1942).
(10) Little, J. M., "Methods in Medical Research," Vol. V, Year Book Publishers, Chicago, Ill., 1952, p. 206.
(11) Ames, R. G. and VanDyke, H. B., *Endocrinology*, **50**, 350(1952).
(12) Bhide, N. K., Mehta, D. J., and Lewis, R. A., *Indian J. Med. Sci.*, **12**, 141(1958).
(13) Hutchison, R., and Hunter, D., "Clinical Methods," 12th ed., Cassell, London, 1952, p. 229.
(14) Hepler, O. E., "Manual of Clinical Laboratory Methods," 4th ed., Thomas, Springfield, 1958, pp. 283 and 287.
(15) Winter, C. A., *J. Pharmacol. Exptl. Therap.*, **94**, 7 (1948).
(16) Goodman, L. S., Grewal, M. S., Brown, W. C., and Swinyard, E. A., *J. Pharmacol. Exptl. Therap.*, **108**, 168 (1953).
(17) Gujral, M. L., and Khanna, B. K., *J. Sci. Ind. Res. (India)*, **16C**, 11(1957).
(18) Das, P. K., and Arora, R. B., *J. Pharmacol. Exptl. Therap.*, **121**, 149(1957).
(19) Burn, J. H., "Biological Standardization," 1st ed. Oxford, London, 1937, p. 37.
(20) Fishberg, A. M., *Postgrad. Med.*, **23**, 396(1958).