

Weiner, M., Exp. Med. Surg., 1959, v17, 219.

3. Tukey, J. W., Biometrics, 1949, v5, 99.

4. Technical Bull. TB 6052 A, Beckman/Spinco

Division, Palo Alto, Calif.

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Effect of Asiaticoside on Wound Healing in the Rat. (32070)

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The mechanisms of wound healing and the agents which promote wound healing have received considerable attention in recent years (1-3). Prudden and co-workers(4,5) reported that powders prepared by acid-pepsin digestion of bovine tracheal cartilage effectively increase the tensile strength of wounds in experimental animals and humans and promote healing of chronic wounds in humans. Another healing agent reported as effective(6,7) is Asiaticoside,* a triterpene glycoside obtained from *Centella asiatica*.

The work reported here was performed with Asiaticoside. All results were obtained by measuring the tensile strength (tension for disruption) of healing linear wounds in rat skin.

Methods. The method of Fenton and West (8) with some modification, was used in the experiments. Male rats (Charles River C-D 1 strain) were selected that ranged in weight from 222 to 264 g; however, the range on any one day of testing was usually narrower.

Under ether anesthesia, the back of each rat was close-clipped and a midline incision 4 cm long was made through the whole skin thickness with a sharp scalpel. Each wound was closed with 3 equally spaced interrupted sutures of 4-0 silk. On the sixth day post wounding, each rat was sacrificed with sodium pentobarbital and a strip of skin 20×60 mm, with the wound in the middle, was excised and placed on a horizontal board. Skin on one side of the wound was firmly fastened with pins to the board. On the other side, a

hook was passed through the skin at a point opposite the center of the wound. A string tied to this hook was passed horizontally over a pulley and thence downward to a suspending platform. The wound sutures were then removed, a plastic bottle placed on the platform, and mercury run into the bottle at a constant rate until the wound disrupted. The mercury was run from a 125 ml leveling bulb by way of a tube fitted with a one way stop-cock and a 19 gauge hypodermic needle. The head was 25 cm and rate of flow was 370 g/minute. The total weight of mercury, container and platform in g, divided by length of wound in cm, gave a tensile strength value of g/cm wound.

The wounds were medicated with one local application (5-10 mg) of Asiaticoside, or a dilution of the glycoside, before being sutured. Dilutions were made by adjusting the undiluted glycoside with the 2% powder.

Three groups of 10 rats each were used at one time. One group with no medication served as control and each of the other 2 groups was treated with 2 different concentrations of the medicament.

The sixth day was selected for tensile strength testing because preliminary experiments had shown this to be a good time to detect either decreased or increased strength resulting from drug treatment.

On occasion a wound was observed to be infected; such wounds were discarded. No cultures or histologic sections were made.

Results. Table I shows the results obtained. The undiluted Asiaticoside gave statistically significant increases of tensile strength in 7 of 8 test groups. When the glycoside was diluted to 50%, 3 of 4 tests were positive, the increases being statistically significant. Con-

* 2 α , 3 β , 23-Trihydroxy-12-ursen-28-oate of 1-[O- α -L-rhamnopyranosyl (1 $\rightarrow$ 4)-O- β -D-glucopyranosyl (1 $\rightarrow$ 6)]O- β -glucopyranose. Supplied to us through the courtesy of Laboratories Laroche Navarron, Paris, as undiluted Madecassol and as the 2% powder.

TABLE I. Change in Tensile Strength (g/cm) of Wounds in Rat After Treatment with Asiaticoside.

Treatment	Control		Test		% Diff.	P*
	No. rats	Mean $\pm$ 2 S.E.	No. rats	Mean $\pm$ 2 S.E.		
Undiluted Asiaticoside	10	101.0 $\pm$ 11.71	10	140.5 $\pm$ 9.22	+39	<.001
	9	118.7 $\pm$ 8.14	10	159.7 $\pm$ 19.64	+35	<.01
	10	100.8 $\pm$ 9.55	10	135.2 $\pm$ 10.74	+34	<.001
	10	109.3 $\pm$ 17.94	9	158.3 $\pm$ 13.09	+45	<.001
	10	91.2 $\pm$ 5.62	10	120.3 $\pm$ 20.64	+32	<.02
	10	88.9 $\pm$ 3.62	10	136.9 $\pm$ 12.37	+54	<.001
	10	109.3 $\pm$ 21.34	10	119.4 $\pm$ 18.72	+9	>.4
	10	107.6 $\pm$ 10.38	10	163.0 $\pm$ 26.47	+51	<.01
Composite	79	103.2 $\pm$ 4.68	79	141.5 $\pm$ 6.86	+37	<.001
50% Asiaticoside	10	113.4 $\pm$ 13.05	10	112.8 $\pm$ 14.85	-0.5	>.9
	10	108.9 $\pm$ 13.01	10	168.8 $\pm$ 28.73	+55	<.01
	10	102.1 $\pm$ 14.94	10	133.9 $\pm$ 17.92	+31	<.02
	10	99.5 $\pm$ 11.37	10	132.8 $\pm$ 12.45	+33	<.01
Composite	40	106.0 $\pm$ 6.55	40	137.1 $\pm$ 11.30	+29	<.001
25% Asiaticoside	10	109.3 $\pm$ 21.34	10	103.7 $\pm$ 15.00	-5	>.6
	10	107.6 $\pm$ 10.38	10	144.6 $\pm$ 16.06	+34	<.001
	10	104.9 $\pm$ 15.20	10	108.9 $\pm$ 12.30	+4	>.6
	10	132.9 $\pm$ 15.51	10	111.0 $\pm$ 13.48	-16	>.05
Composite	40	113.7 $\pm$ 8.49	40	117.1 $\pm$ 8.53	+3	>.5
10% Asiaticoside	10	91.2 $\pm$ 5.62	10	103.8 $\pm$ 9.35	+14	<.05
	10	88.9 $\pm$ 3.62	10	98.5 $\pm$ 12.79	+11	>.1
	10	113.4 $\pm$ 13.05	10	111.8 $\pm$ 10.10	-1	>.8
	10	92.2 $\pm$ 16.50	10	123.0 $\pm$ 13.02	+33	<.02
Composite	40	96.4 $\pm$ 6.14	40	109.3 $\pm$ 6.20	+13	<.01
2% Asiaticoside	10	111.0 $\pm$ 11.40	10	108.4 $\pm$ 18.90	-2	>.7
	10	103.9 $\pm$ 11.07	8	99.6 $\pm$ 21.38	-4	>.7
	10	102.7 $\pm$ 8.06	10	107.8 $\pm$ 16.50	+5	>.5
	10	108.9 $\pm$ 13.01	10	108.9 $\pm$ 9.89	0	>.9
Composite	40	106.6 $\pm$ 5.41	38	106.4 $\pm$ 8.13	0	>.9

* Student t test.

centrations below 50% showed less activity and were not as consistent as the higher concentrations. The 25% concentration produced a statistically significant increase of tensile strength in only 1 of 4 tests; the composite response of the 4 test groups did not differ significantly from the composite response of the 4 control groups. On the other hand, the 10% concentration gave statistically significant increases in 2 of 4 tests, and the mean of the 4 tests was significantly different from control at the $p < .05$ level, although the numerical increase in tensile strength was considerably less than with the higher concentrations. The 2% unadjusted powder gave no evidence of affecting tensile strength.

Summary. Asiaticoside, a triterpene glycoside from *Centella asiatica*, was tested by local application for its effect on the tensile strength of linear skin wounds in the rat.

Tensile strength was increased, and the results obtained with various concentrations indicated a concentration-response relationship.

1. Grillo, H. C., in *Research Methods in Surgery*, W. F. Ballinger, ed., Little, Brown & Co., Boston, Mass., 1964, p235.
2. Montagna, W., Billingham, R. E., ed., *Advances in Biology of the Skin*, Vol. 5, Wound Healing, Pergamon Press, Inc., Long Island City, N.Y., 1964.
3. DeVito, R. V., *Surg. Clin. North Am.*, 1965 v45, 441.
4. Prudden, J. F., *Arch. Surg.*, 1964, v89, 1046.
5. Prudden, J. F., Allen, J., *J. Am. Med. Assn.*, 1965, v192, 352.
6. Kiesswetter, H., *Wien Med. Wochschr.*, 1964, v114, 124.
7. Abou-Chaar, C. I., *Lebanese Pharm. J.*, 1963, v8, 14.
8. Fenton, H., West, C. B., *Brit. J. Pharmacol.*, 1963, v20, 507.

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