

ACUTE TOXICITY AND PHARMACOLOGICAL ACTIONS OF ROSOLIC ACID

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ABSTRACT

Acute toxicity and pharmacological actions of rosolic acid were studied. Results: (1) LD₅₀s of rosolic acid on mice were 82 (78-87) mg/kg s.c. and 49 (41-58) mg/kg i.p. The animals survived after the oral administration. (2) Significant irritation on the mucosa was not observed. (3) Rosolic acid produced a rise in the blood pressure and a transient depression of respiration in the rabbit. (4) Rosolic acid increased the contractile force and heart rate in the isolated atria of the guinea pig. (5) Rosolic acid caused a contraction followed by a relaxation in the isolated rabbit and guinea pig ileum.

INTRODUCTION

The irrigation smear method introduced by Davis¹⁾ is supposed to be a useful cytologic screening procedure for cervical cancer detection. Procuring the specimen is performed without special technique using a plastic pipette containing an irrigating and cell preservative solution. Diagnostic accuracy of more than 95 per cent was reported by this method²⁾³⁾⁴⁾. Kawashima *et al.*⁵⁾⁶⁾ employed rosolic acid as a pH indicator in the irrigating solution*. The present study was designed to examine acute toxicity and some pharmacological actions of rosolic acid.

MATERIALS AND METHODS

1) *Acute toxicity*

Acute toxicities were determined by the intraperitoneal, subcutaneous and oral routes in male and female mice weighing 20-24 g (SMA strain) in groups of eight. The animals were kept in an ambient temperature of 20°C and observed for 24 hours. Each group was administered with one of a logarithmic series of doses of rosolic acid, and from the mortalities observed the LD₅₀s and limits of error were calculated. 0.25% and 0.5% solutions of rosolic acid in 0.65% saline containing 0.2% NaOH were injected to the mice. For the

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* SSP solution®

oral administration and the preliminary tests 1-10% suspensions of rosolic acid in 0.9% saline containing 1% polysorbate 80 were also employed.

2) *Eye irritation*

0.1 ml of 0.05% and 0.005% rosolic acid in saline was instilled into the conjunctival sac of the rabbit to examine the irritation on the mucosa. SSP solution which contains 0.005% rosolic acid and 20% ethyl alcohol was also tested.

3) *Histological observation*

Isolated rabbit ileum and vagina were soaked in 0.05% and 0.005% rosolic acid solution for 2 hours. The tissues were fixed in formalin and stained with hematoxylin and eosin and examined microscopically.

Experiments on the vaginal mucosa of the rabbit *in vivo* were performed by applying SSP solution in the vagina several times using a plastic pipette. After 2 hours the tissues were isolated and examined microscopically as mentioned above.

4) *Effects on blood pressure, respiration and ECG*

Rabbits weighing 2.5-3.0 kg were anesthetized with urethane and the blood pressure of the carotid artery was recorded electrically. Lead II was used for ECG studies. Respiration was recorded on smoked paper through a tambour. Drugs were administered from the femoral vein.

5) *Effects on isolated guinea pig atria*

Isolated guinea pig atria were suspended in Ringer-Locke solution, and the contractile force and heart rate were recorded on an ink-writing oscillograph by means of a semiconductor strain gauge and a tachometer, respectively. Bath temperature was 30°C and the volume of the bath fluid was 50 ml.

6) *Effects on isolated guinea pig and rabbit ileum*

The preparations of isolated guinea pig and rabbit ileum were made by the Magnus method. Bath temperature was kept at 35°C and the volume was 50 ml. The contractions were recorded on smoked paper through a lever.

RESULTS

1) *Acute toxicity*

By the oral administration of less than 0.5 ml of 0.5% solution and 1-10% suspensions of rosolic acid, no death was observed and the body weight did not decrease significantly. Most of the applied drug seemed to be excreted in faeces without absorption.

Results of the subcutaneous and intraperitoneal administration were shown in Table 1. The calculated LD 50s were 82 (78-87) mg/kg and 49 (41-58) mg/kg, respectively. Equivalent quantities of the solvent alone were injected

TABLE 1. Mortality (acute toxicity of rosolic acid on mice)

Route of administration	Subcutaneous				Intraperitoneal					
Dose (mg/kg)	130	100	77	59	100	77	59	46	35	27
Mortality	8/8	7/8	3/8	0/8	8/8	7/8	6/8	3/8	2/8	0/8

to the control groups, but no death was observed. Administration of the suspension by the subcutaneous route did not cause death.

2) Eye irritation

Treatment with 0.05% and 0.005% solutions of rosolic acid did not cause any change on the conjunctival sac of the rabbit. SSP solution produced irritation characterized by mild hyperaemia and lachrymation. It was recovered within 30 to 60 minutes, and no secondary irritation was observed for 24 hours.

3) Histological observation

There were no significant pathological changes both in the isolated rabbit ileum and vagina soaked in rosolic acid solution and the rabbit vagina *in vivo* treated with SSP solution.

4) Effects on blood pressure, respiration and ECG

Administration of 5-10 ml of the solvent containing 0.2% NaOH caused a slight decrease in the blood pressure and depression in the respiration. Whereas, injection of 2-5 ml of 0.5% rosolic acid solution produced a rise of 20-40 mmHg in the blood pressure. Fig. 1 illustrates a more prolonged increase induced by 10 ml of the solution. The R-R interval was prolonged but no other changes in ECG were observed by a single administration. The respiration almost ceased temporarily, which was followed by a gradual recovery (Fig. 2). Repeated administration of rosolic acid finally produced a decrease in the blood pressure and depression of ST level and decrease in height of R wave in ECG.

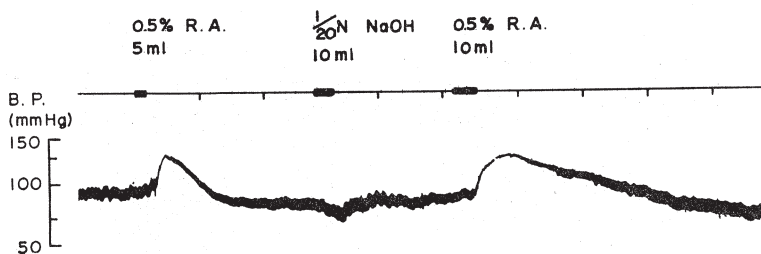


FIG. 1. Effects of rosolic acid on blood pressure of the rabbit. R.A.: rosolic acid, 1/20 N NaOH; the solvent, Time marks; 2 minutes.

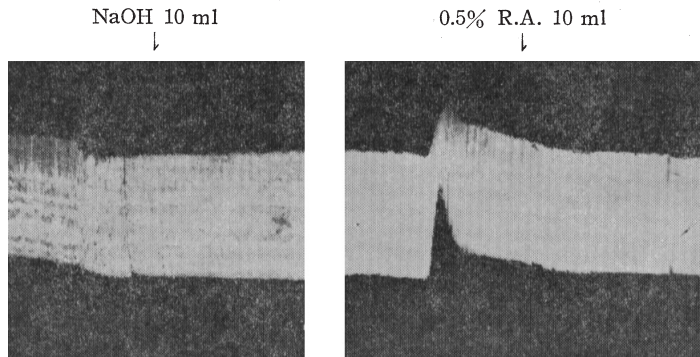


FIG. 2. Effects of rosolic acid on respiration of the rabbit.

5) *Effects on isolated guinea pig atria*

As shown in Fig. 3, administration of 0.5 ml of 0.5% rosolic acid produced an increase in the contractile force and heart rate. The solvent alone did not produce any changes.

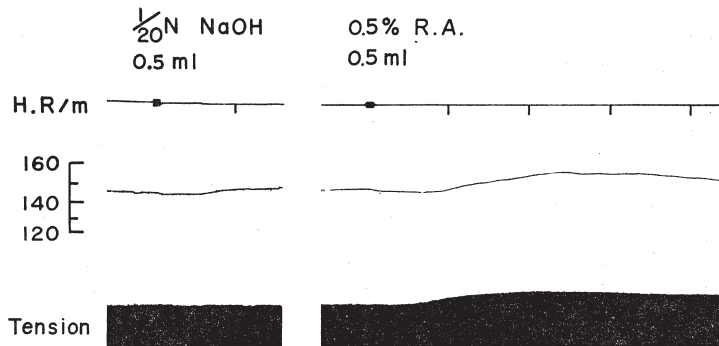


FIG. 3. Effects of rosolic acid on heart rate (upper tracing) and contractile force (lower tracing) of the isolated guinea pig atria.
R.A.: rosolic acid, 1/20 N NaOH: the solvent, Time marks: 2 minutes.

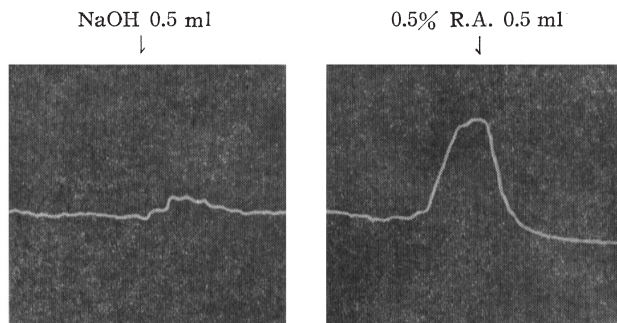


FIG. 4. Effects of rosolic acid on the isolated guinea pig ileum.

6) *Effects on isolated guinea pig and rabbit ileum*

Administration of 0.25–0.5 ml of 0.5% rosolic acid solution usually caused marked contractions in isolated ileum, followed by a gradual relaxation and decrease of spontaneous movement (Fig. 4). After the preparations were washed out, the second administration of rosolic acid did not produce contraction. In some preparations rosolic acid did not exhibit an increase of contraction, but only induced a gradual decrease after the administration.

DISCUSSION

As rosolic acid is practically insoluble in water (0.12%) and the administration of solution and suspension of rosolic acid in saline did not cause death in mice, the experiments of acute toxicity were performed using 0.25–0.5% solutions in 0.65% saline containing 0.2% NaOH referring to the final concentration. LD 50s were calculated as 82 (78–87) mg/kg by the subcutaneous route and as 49 (41–58) mg/kg by the intraperitoneal one.

SSP solution, which is used for cervical cancer detection, contains rosolic acid as a pH indicator. Therefore, irritation tests and histological observation were also performed on the rabbit mucosa. Rosolic acid in a low concentration as is contained in SSP solution caused no significant pathological changes on mucosal surface. SSP solution produced mild primary changes on the conjunctival mucosa, which were recovered within 60 minutes. This irritating action is due to ethyl alcohol included in SSP solution.

The quantity of SSP solution for one practical use is about 5 ml, in which 0.25 mg of rosolic acid is included. It is much less than the lethal dose calculated in mice. Moreover, as most of the solution squirted into the vagina are withdrawn by the pipette, the quantity of rosolic acid in SSP solution is not considered to cause toxic effects on the body.

In the pharmacological studies, rosolic acid induced a rise in the rabbit blood pressure and an increase in the contraction of isolated ileum of the rabbit and guinea pig. The occurrence of the acute toxicity may be caused by the contraction and the following relaxation of the smooth muscles. Rosolic acid increased the contractile force and heart rate in the isolated guinea pig atria, whereas heart rate of the rabbit *in vivo* was decreased. This effect is supposed to be due to the reflex mechanism induced by a rise in the blood pressure.

REFERENCES

- 1) Davis, H. J., The irrigation smear—A cytologic method for mass population screening by mail, *Amer. J. Obstet. Gynec.*, **84**, 1017, 1962.
- 2) Davis, H. J., The irrigation smear: accuracy in detection of cervical cancer, *Acta Cytol. (Balt)*, **6**, 459, 1962.
- 3) Ishizuka, N. and Kawashima, Y., Early detection for uterine cancer, *Obstet. Gynec.*

- Ther. (Tokyo)*, **12**, 525, 1966 (in Japanese).
- 4) Kawashima, Y., Ino, S., Tashiro, K., Tachibana, M. and Masukawa, T., Mass cervical cancer screening program by irrigation smear in Japan, *Obstet. Gynec.*, **32**, 17, 1968.
 - 5) Kawashima, Y., Ino, S., Tashiro, K. and Tachibana, M., Study on the self-irrigation smear technique, *Jap. J. Obstet. Gynec. Soc. (Tokyo)*, **18**, 931, 1966 (in Japanese).
 - 6) Kawashima, Y., Ino, S., Tashiro, K., Tachibana, M., Inagaki, M., Hatta, M. and Takabe, N., Mass population screening for cervical cancer by irrigation smear technique, *Obstet. Gynec. Ther. (Tokyo)*, **14**, 354, 1967 (in Japanese).