

ANTI-INFLAMMATORY AND ANALGESIC ACTIVITIES OF
OLEANOLIC ACID 3-O-GLUCOSIDE (RDG-1) FROM
RANDIA DUMETORUM (RUBIACEAE)

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Abstract

Oleanolic acid 3-O-Glucoside (RDG-1) was isolated from the seeds of the plant *Randia dumetorum* (Rubiaceae). The compound showed significant anti-inflammatory activity in the exudative and proliferative phases of inflammation in the doses of 25 and 100 mg/kg orally. Significant analgesia was observed only on thermal stimulus. It did not show any antipyretic activity against Brewer's yeast induced pyrexia in rats. The approximate oral LD_{50} were found to be 3600 mg/kg and 1500 mg/kg in mice and rats respectively.

Key Phrases

Randia dumetorum Anti-inflammatory Analgesic Antipyretic
activities

Introduction

Randia dumetorum Lam. (Sanskrit : *Madana* : Tam : *Marrukkarai* : Hindi : *Mainphal* plant is a deciduous thorny shrub growing throughout India upto an altitude of 1,350 meters in the hills. The drug marrukkarai is one of the very important drugs in the Siddha system of medicine in Tamilnadu.

The fruit of the drug is said to be emetic, expectorant, diaphoretic, nauseant, anthelmintic, abortifacient

and antispasmodic. The bark is astringent and is given in cases of diarrhoea and dysentery (Chopra, Nayar and Chopra, 1950). It is administered internally and applied externally in the form of paste in rheumatism and to relieve pain of bruises and bone-aches during fevers and to disperse abscesses (Nadkarni, 1976). The aqueous extract of the root bark of the tree is used as an active insecticidal (Dastur, 1962).

From the alcoholic extract of the powdered seed of *Randia dumetorum*,

*Part of the paper was presented in the XIV Annual Conference of Indian Pharmacological Society held at Bombay in December, 1981.

a pentacyclic triterpene acid glycoside, identified and designated as Oleanolic acid 3-/3-Glucoside (RDG-1) has been isolated in the form of an amorphous powder. The compound has been reported to possess anti-fertility activity in albino rats (Pillai, Alam and Purushothaman, 1977). The present paper communicates the other activities of the compound. The structure of RDG-I is shown in Fig. 1.

C are the means of test and control group respectively. Students 't' test was applied for statistical analysis.

Anti-inflammatory studies-Rats (King Instt. Strain) of either sex (90-1 50g) were divided into several groups for each test procedure.

Carrageenin-induced edema-The method of Winter, Risely and Nuss (1962) was employed. 0.1 ml of 1%

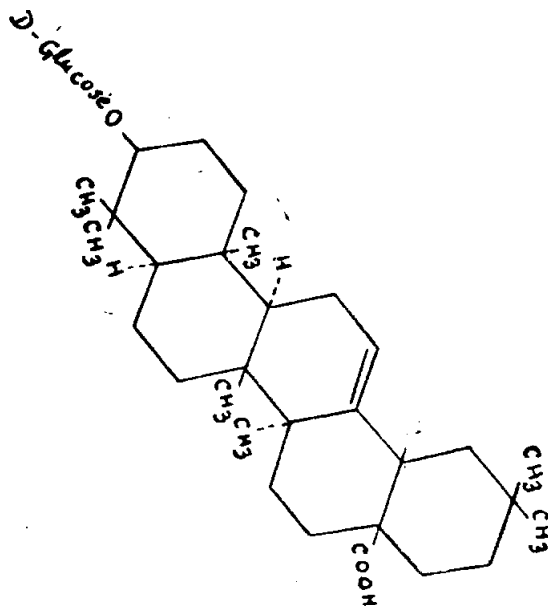


Fig. 1-Chemical structure of RDG- 1

Materials and Methods

The test compound was dissolved freshly in distilled water and used in the doses of 25, 100 and 500 mg/kg orally. The percent activity, when ever required was calculated by the formula $(1-T/C) \times 100$ where T and

suspension of Carrageenin in 0.5% Carboxy methyl cellulose (C.M.C.) was injected in the planter aponeurosis of right hind paw of rats. Paw volume was measured by a plethysmograph (Buttle, D'Arcy, Howard and Kellet, 1957).

Cotton Pellet granuloma.-The procedure as described by Seth, Dadkar and Kamat (1972) was followed. All the administrations were made daily once for seven days. Two groups received Phenylbutazone in the dose of 100 mg/kg orally and distilled water and served as standard and control groups respectively. On the 8th day, the pellets were taken out, dried till weighed constant.

Granuloma pouch.-The method of Selye (1953) was followed. Phenylbutazone in a dose of 100 mg/kg orally like above experiments served as standard.

Formaldehyde induced arthritis.-Formaldehyde (0.1 ml of 2% V/V) was injected subcutaneously under the plantar aponeurosis, in each foot of the rats on first and third day (Brownlee, 1950). Prednisolone (Wysolone-Wyeth) in a dose of 10 mg/kg orally served as standard group. Assessment was made by measuring daily the linear cross-section immediately below the ankle joint with micrometer screwgauge.

Analgesic studies.-Analgesic activity was tested in mice (King Instt. Strain) weighing between 20-250 with six number of animals in each group.

Acetic acid induced writhing response.-The method of Turner (1965a) was employed. The stretching episodes of individual mice

were counted for 30 min post-administration of acetic acid. Analgin in a dose of 500 mg/kg orally served as standard,

Hot-plate response in mice.-The test was carried out using the method described by Turner (1965b). Analgin in the dose of 500 mg/kg orally served as standard. At every 30 min the response time was recorded on the hot-plate (55°-55.5°C).

Anti-pyretic study.-The rats were made pyretic by a sub-cutaneous injection of 20% suspension of dried Brewer's yeast in normal saline in a dose of 1 ml/100 g. The rectal temperature **was** recorded by introducing a clinical thermometer about 2 cm deep into the rectum for a duration of one min before and 4 h after yeast injection. Animals showing pyrexia were Selected and the drugs were administered. The rectal temperature was recorded at hourly intervals for 5 h.

Acute toxicity studies.-Fasted (for six hours) albino mice of either sex weighing between 20-25 g were grouped, ten animals in each. The test compound was administered orally in the doses of 25, 50, 100, 250, 00, 1000 and with an increase of 1000 mg upto 6000 mg/kg.

Similar procedure was adopted for rats weighing between 100-150 g. The gradation of doses employed were the same as in mice.

Table 1
Effect of RDG-1 on carrageenin induced edema in Hind paw of rats
 (Values are Mean \pm S.E., Number of observations in parenthesis)

Groups	Dose mg/kg oral	Increase in paw volume in ml	% inhibitory activity	P-Value
Control (11)	Distt. water	0.60 ± 0.04	—	—
Phenylbutazone (8)	103	0.24 \$0.01	60.12	<0.001
RDG-I (6)	25	0.20 f0.02	66.22	<0.001
RDG-1 (6)	100	0.34 ± 0.04	42.41	<0.001
RDG-1 (6)	500	0.33 ± 0.04	43.79	<0.001

Table 2
Effect of RDG-1 on cotton pellet granuloma in rats
 (Values are Mean \pm S. E., Number of observations in parenthesis)

Groups	Dose in mg/kg oral	Dry weight (mg) of cotton pellet	% inhibitory activity	P-Value
Control (16)	Distt. water	32.88 \pm 1.01	—	—
Phenylbutazone (16)	100	26.29 + 0.77	20.05	(0.001)
RDG-I (16)	50	29.40 \pm 1.64	10.59	N.S.
RDG-I (16)	100	25.99 \pm 1.22	20.98	<0.001
RDG-I (16)	500	23.14 \pm 1.19	27.81	<0.001

N.S. - Not significant.

Table 3
Effect of RDG- I on inflammatory exudate of granuloma pouch in rats
 (Values are Mean \pm S. E., number of observations in parenthesis)

Groups	Dose in mg/kg oral	Exudate in ml.	% inhibitory activity	P-value
Control (6)	Distt. water	5.95 \pm 0.37	—	—
Phenyl- butazone (6)	100	1.20 \pm 0.11	79.84	<0.001
RDG-1 (6)	2.5	4.65 \pm 0.33	21.85	<0.05
RDG-I (6)	100	4.03 \pm 0.46	32.27	<0.01
RDG-1 (6).	500	5.10 \pm 0.30	14.29	N.S.

N.S.-Not significant.

Table 4

Effect of RDG-1 on formaldehyde induced arthritis in rats(Values are mean \pm S. E. Number of observations in parenthesis)

Groups	Dose in mg/kg oral	Initial Diam. in mm.	Ten day Diam. in mm.	% inhibitory activity	P-value
Control (6) Distt. water		2.46 ± 0.13	5.09 ± 0.23	—	
Prednisalone (6)	10	3.13 ± 0.23	4.26 ± 0.14	16.31.	<0.01
RDG-1 (6)	25	3.12 ± 0.29	4.56 10.20	10.42	N. S.
RDG-1 (6)	100	2.22 ± 0.11	4.20 ± 0.09	17.49	<0.01
RDG-1 (5)	500	2.16 ± 0.16	5.05 ± 0.24	0.74	N. S.

N.S.-Not significant,

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Effect of RDG. 1 on acetic acid induced stretching episodes in mice
 (Values are Mean \pm S.E., Number of observations in parenthesis)

Groups	Dose in mg/kg oral	Stretching episodes for 30 min	% inhibitory activity	P-value
Control (6)	Distt. water	36.33 \pm 5.77	—	—
Analgin (6)	500	3.16 \pm 1.19	91.31	<0.001
RDG-1 (6)	25	40.16 \pm 8.19	—	N.S.
RDG-1 (6)	100	46.16 \pm 9.33		N.S.
RDG-1 (6)	500	26.00 \pm 4.46	28.44	N.S.

N. S.-Not significant.

Table 6

Analgesic Activity of RDG-1 on Hot plate in Mice

(Figures are Mean \pm S. E., Number of observations in parenthesis)

Groups/ Treatment	Dose (mg/kg) oral	Initial response time (Sec)	Response time in Seconds after					
			30 min,	60 mid,	90 mid,	120 min.	180 min.	
Analgain (6)	500	5.05 \pm 0.48	7.41 \pm 1.21 N. S.	14.46 \pm 1.83 P<0.001	18.55 \pm 1.24 P<0.001	19.14 \pm 1.09 P<0.001	14.63 f1.87 P<0.001	7.55 f0.88 P<0.05
RDG-1 (6)	25	5.01 f0.68	6.51 \pm 0.45 N. S.	8.80 \pm 1.20 P<0.02	7.18 \pm 0.29 P<0.02	6.86 \pm 1.07 N. S.	5.96 \pm 0.74 N. S.	6.51 \pm 0.64 N. S.
RDG-1 (6)	100	3.51 f0.32	7.20 f0.53 P<0.001	7.38 \pm 0.96 P<0.01	7.10 \pm 1.06 P<0.01	6.75 \pm 0.86 P<0.01	5.41 \pm 0.91 N. S.	6.20 \pm 0.94 P<0.05
RDG-1 (6)	500	5.21 f0.65	7.63 f0.98 N. S.	6.11 \pm 1.11 N. S.	7.75 \pm 1.06 N. S.	6.48 f0.73 N. S.	6.35 \pm 0.41 N. S.	5.43 \pm 0.55 N. S.

N. S. = Not significant,

Table 7
Effect of RDG-1 On Brewer's Yeast Induced Pyrexia in Rats
 (Values are Mean \pm S. E., Number of animals in parenthesis)

Groups with dosage	Body weight (in g)	Fasting rectal temp.	initial pyrexia	Mean rectal temperature in Fahrenheit at				
				1st h	2nd h	3rd h	4th h	5th h
Control (6)	131.25 ± 6.57	99.45 ± 0.05	102.75 ± 0.29	102.95 ± 0.13	103.10 ± 0.17	103.70 ± 0.33	103.85 ± 0.20	103.26 ± 0.27
Paracetamol 500 mg/kg (6)	163.75 ± 8.50	99.30 ± 0.15	102.35 ± 0.24	100.85* ± 0.11	99.70* ± 0.21	100.20** ± 0.63	101.25 $\pm 0.25^{**}$	101.80 ± 0.21 N.S.
RDG-I 25 mg/kg (6)	148.75 ± 13.90	99.30 ± 0.23	102.80 ± 0.25	102.50 ± 3.28 N.S.	102.00	102.85	103.85	102.25
RDG-I 100 mg/kg (6)	111.25 ± 9.43	99.05 ± 0.22	102.60 ± 0.29	103.00 ± 0.25 N.S.	± 0.42 , N.S.	102.00	103.05	103.60
RDG-I 500 mg/kg (6)	125.00 ± 8.89	98.90 ± 0.17	101.85 ± 0.62	102.50 ± 0.97 N.S.	102.20	101.95	102.50	101.10
					± 0.39 N.S.	± 1.01 N.S.	± 0.4 N.S.	± 2.03 N.S.

* = P<0.001 ;

**=p<0.01

N. S. = Not significant.

Result

Carrageenin induced oedema.- RDG-1 in the doses of 25, 100 and 500 mg/kg (Table 1) significantly inhibited carrageenin oedema.

Cotton pellet granuloma.-The compound showed dose-dependent inhibition of cotton pellet granuloma in rats in the dose range of 25 mg to 500 mg/kg (Table 2).

Granuloma pouch.-RDG-1 in the doses of 25 and 100 mg/kg significantly inhibited the exudate volume. Whereas, 500 mg/kg dose had no significant effect. (Table 3).

Formaldehyde induced arthritis.- Significant activity in reducing the arthritic swelling was observed only in the dose of 100 mg/kg. (Table 4).

Analgesic effect.-The compound did not show significant analgesia on stretching episodes induced by acetic acid in any dose (Table 5), whereas, it showed significant effects in the doses of 25 and 100 mg/kg on hot plate test. The effect with maximum dose was not significant (Table 6).

Anti pyretic effect.-There was no antipyretic effect observed in any of the doses employed (Table 7).

Acute toxicity.-The approximate oral LD₅₀ were found to be 3600 mg/kg and 1500 mg/kg in mice and rats respectively.

Discussion

RDG-1, a glycoside isolated from the seeds of *Randia dumetorum*, showed significant anti-inflammatory effect both in exudative and proliferative phases of inflammation (Table 1, 2, 3 and 4). In carrageenin induced edema, the effect produced by the dose of 25 mg/kg was little more than Phenybutazone in the dose of 100 mg/kg without any further increase in activity with higher doses. A dose responsive anti-inflammatory effect was observed in cotton pellet granuloma with comparable effect with Phenylbutazone in identical doses (Table 2). The maximum activity achieved (32.27%) in the dose of 100 mg/kg was less than half of the activity (79.84%) shown by Phenylbutazone. The only significant activity observed in the dose of 100 mg/kg against formaldehyde induced arthritis which was almost similar to the effect produced by Prednisolone in a dose of 10 mg/kg (Table 4).

The compound did not show significant analgesia in chemical writhing test (Table 5) whereas, on hot-plate, significant effect was observed in the doses of 25 and 100 mg/kg. The analgesia produced by the former doses was for a brief period (between 60 to 90 min) whereas, the effect produced by 100 mg/kg was more prolonged and with an earlier onset comparable to the effect produced by Analgin in a dose of 500 mg/kg (Table 6).

The compound is devoid of antipyretic effect in this model of experiment. The approximate LD_{50} levels in two species of animals do not signify that the compound is non-toxic, but its maximum anti-inflammatory and analgesic activities are almost around the dose of 100 mg/kg which certainly may make it interesting to study further in detail &fore it could be clinically tried.

Acknowledgment

The authors are grateful to the Director, Central Council for Research in Ayurveda and Siddha, New Delhi for permitting them to undertake the study. They also wish to thank Dr. K. K. Purushothaman, Asst. Director-in-charge, C.S.M.D. R.I., Madras-10 for providing them the compound. Thanks are also due to Dr. (Mrs.) Lalitha Kameswaran, Prof. and Director, Institute of Pharmacology, Madras Medical College, Madras for her constant guidance and encouragement. The technical assistance of all associated with this study is also acknowledged.

References

- BROWNLEE, G. (1950).** Effect of deoxycortone and ascorbic acid on formaldehyde induced arthritis in normal and adrenalectomised rats. *Lancet*, 1, 157-159.
- BUTTLE, G. A. H., D'ARCY, P. F., HOWARD, E. M., and KELLET, D. N. (1957).** Plethysmographic measurement of swelling in the feet of small laboratory animals. *Nature*, (London), 179, 629-632.
- CHOPRA, R. N., NAYAR, S. L. and CHOPRA, I. C. (1956).** In Glossary of *Indian Medicinal plants*, pp. 209. New Delhi, India. Council of Scientific and Industrial Research.
- DASTUR, J. F. (1962).** In *Medicinal Plants of India and Pakistan*, pp. 140. Bombay. D. B. Taraporevala Son's & Co., Pvt. Ltd.
- NADKARNI, K. M. (1976).** *Randia dumetorum*, Lamk. In *Indian Materia Medica*. Vol. 1, ed. Nadkarni, K. M. pp. 1047-1048, Bombay. Popular Prakashan Private Limited.
- PILLAI, N. R., ALAM MUZAFFER and PURUSHOTHAMAN, K. K. (1977).** Studies on the antifertility activity of Oleanolic acid 3-B-Glucoside (RDG-1). *J. Res. Ind. Med. Yoga & Homo*, 12, 3, 2628.
- SELYE, H. (1953).** on the mechanism through which hydrocortisone affects the resistance of tissues to injury. *J. Amer. Med. Assn.*, 152, 1207-1213.
- SHETH, U. K., DADKAR, N. K. and KAMAT, G., USHA (1972).** Miscellaneous topics. In *selected topics in experimental pharmacology*, pp. 194, Parel, Bombay The Kothari Book Depot.
- TURNER, R. A. (1965a).** Non-narotic analgesics. In *Screening Methods in Pharmacology*. Vol. 1, pp. 114-115, New York : Academic Press.
- TURNER, R. A. (1965b).** Thermal Stimulus. In *Screening methods in Pharmacology*, Vol. 1, pp. 104-105. New York : Academic Press.
- WINTER, C. A., RISELEY, E. A. and NUSS, G. W. (1962).** Carrageenin induced oedema in hind paw of the rats as an assay for anti-inflammatory drugs. *Proc. soc. Exp. Biol. Med.* (N. Y.) 111, 544-547.

Received 5-1-1983

Accepted 24-12-1983