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Analgesic and Anti-inflammatory properties of RD-A and RD-B extracted from the leaves of *Rhododendron dauricum* Linn.

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ABSTRACT

To further understand the purpose of the traditional processing method of the leaves of *Rhododendron dauricum* Linn. (Ericaceae) as well as analgesic and anti-inflammatory activities of RD-A and RD-B extracted from this medicinal plant, various pain and inflammatory models were employed in the present study to investigate their pharmacological profiles. Both RD-A and RD-B revealed significant protective effects against thermal and chemical stimuli in hot plate and writhing test. However, on different phases, they exhibited analgesic activities in formalin test. RD-B showed stronger inhibitory effect than RD-A in carrageenan induced rat-paw oedema, both of them, significantly inhibited the release of prostaglandin E_2 in inflammatory tissue, reduced acetic acid-induced vascular permeability and the content of 6-Keto $PGF_{1\alpha}$ in Freund's complete adjuvant (FCA) induced arthritis rat's blood plasma, while increase the content of 5-hydroxytryindole-3-acetic acid (5-HIAA), accordingly. These results suggest that central and peripheral mechanism are involved in the pain modulation and anti-inflammatory effects of RD-A and RD-B, biochemical mechanisms of RD-A and RD-B are different.

KEY WORDS: Analgesic, Anti-inflammatory, COX, *Rhododendron dauricum*, RD-A, RD-B.

INTRODUCTION

Rhododendron dauricum Linn. is an evergreen shrub, belonging to the family *Ericaceae* and is widely distributed in India, China, Korea and Japan. Its decoction is used in inflammation and bronchitis in folk medicine & Ayurveda. Earlier, we observed that the aqueous extract of leaves of *Rhododendron dauricum* Linn. exhibited inhibition of carrageenan-induced rat paw oedema and analgesic activity (1). Further, the possible role of TNF-α has been elucidated in the anti-inflammatory activity of the plant (2). In this study, the two compounds isolated from the plant, RD-A and RD-B have been evaluated for their analgesic and anti-inflammatory activities and their possible mechanism.

MATERIALS AND METHODS

The leaves were collected from Dalhousie, District Chamba, Himachal Pradesh, India.

The plant samples were identified and authenticated in the Herbarium, Department of Botanical Sciences, Guru Nank Dev University, Amritsar. A voucher specimen RD-11 was deposited in the Department of Pharmacognosy, Sri Sai Institute of Pharmaceutical Edu. and Research, Badhani, Pathankot. RD-A and RD-B were isolated from the dried leaves of *Rhododendron dauricum* Linn. and confirmed by UV, IR, NMR & Mass spectroscopic methods. Carrageenan and Acetyl salicylic acid (Sigma), Acetic Acid and Formalin (Merck), Pethidine and Indomethacin (APL Ltd., Amritsar) and chemicals used in High Performance Liquid Chromatography (HPLC) (Sigma and Fluka) were used.

Animals

Sprague – Dawley (SD) rats weighing 150-200g and Swiss mice weighing 18-22g, of both sexes were used for the experiment. They were bred and housed in standard cages (five rats or mice per cage) at a temperature of 22 ± 2°C and maintained with pellet diet and tap water *ad libitum*, before the experiment, and kept in 12 hour light / dark cycle. Procedures involving animals and their care were conducted in conformity with Committee for the Purpose of Control and Supervision of Experiments on Animals (Regd. No.911/ac/95/CPCSEA).

Statistical Analysis

The results were expressed as mean ± S. E. M. The statistical analysis involving two groups was performed by means of Student's t-test, whereas Anova, followed by Dunnet's multiple comparison tests, was used in order to compare more than two groups. $P \leq 0.05$ was considered significant. Regression analysis was used to calculate ED_{50} and 95% confidence limit of tested chemicals.

Antinociceptive Test

Hotplate Test

Experiments were carried out according to method described by Adzu et al (3). Mice were divided into 10 groups, 4 groups for RD-A, 4 groups for RD-B, 2 groups for negative and positive controls, respectively; 10 animals were included for each group. Animals were habituated twice to the hot plate in advance, 24 hours before the test (1min.), and again 20 minutes before test (1min.). For testing, mice were placed on hot plate maintained at 55 ± 5ºC. The time that elapsed until occurrence of either a hind paw licking or a jump off the surface was recorded as the hot plate latency. Mice with baseline latencies of <5 or >30s were eliminated from the study. After the determination of baseline response latencies, hot plate latencies were re-determined at 15, 30, 60, 120 and 240 min after intraperitoneal administration of RD-A, RD-B and 0.9% NaCl solution. RD-A was tested at doses of 30, 20, 14 and 10mg /kg, RD-B was tested at doses of 200, 140, 100 and 70mg /kg, pethidine (i.p.) at a dose of 40mg/kg was used as a reference drug. For each group, the percentages of pain inhibition were calculated according to the following formula;

Pain inhibition percentage

$$P/P = \left(\frac{(T_1 - T_0)}{T_0} \right) \times 100$$

Where T_1 in latency post-drug

T_0 in latency pre-drug

Differences in pre and post-drug latencies were analysed by Student's t-test; ED_{50}, 95% confidence limit of antinociceptive effects of drugs were calculated by using Carmines method (4).

Writhing Test

Intraperitoneal injection of acetic acid produced a writhing or stretching syndrome characterized by a wave of contraction of the abdominal musculature followed by extension of the hind limbs within 3 to 10 minutes (5). In this test, mice of both sexes (equal numbers) were divided into 10 groups, 4 groups for RD-A, 4 groups for RD-B, 2 groups for positive and negative control, respectively; each group consisted of 10 animals. RD-A was tested at doses of 30, 15, 7.5 and 3.75mg /kg, RD-B was tested at doses of 200, 100, 50, and 25mg /kg. Acetylsalicylic acid, a reference peripheral analgesic compound, was used at a dose of 70mg /kg. Drug and reference substance were injected (i.p.) 20 min. before administration of 0.6% acetic acid (i.p.) in a volume of 0.2 ml/10g. Control animals received 0.9% NaCl solution under the same experimental-conditions (0.2ml /10g, i.p.) The number of constrictions of each animal within 25 min. after acetic acid injection was cumulatively counted immediately and the percentage protection was calculated using the following ratio,

$$Percentage \quad of \quad protection = \frac{Controlmean - Treatedmean}{Controlmean} \times 100$$

Formalin test

SD rats of both sexes were divided into 8 groups, 3 groups for RD-A, 3 groups for RD-B and 2 groups for positive and negative controls, respectively. A 20 µl of 2.5% formalin was injected subcutaneously, under the plantar surface of the hind paw of the rats. RD-A with does of 30, 15 and 7.5 mg/ kg and RD-B with doses of 200, 100 and 50 mg/ kg were injected (i.p.) into animals 30 minutes before formalin injection (5). Negative control animals received 10 mg/ kg saline, while 50 mg/ kg of indomethacin was injected (i.p.) into animals in the positive control group. The time, the rats spent licking or biting the injected paw or leg was recorded. On the basis of the response pattern described by Tjolsen et al (6), two distinct periods of intensive licking activity were identified and scored separately, the first period (early phase) was recorded 0-5 min. after the injection of formalin, while the second phase (late phase) was recorded 20-30 min. after injection. The percentage inhibition of licking was calculated based on the following ratio.

(Mean vehicle treated group time − Mean drug treated group time/Mean vehicle treated group time) x 100

$$\frac{Mean\ vehicle\ treated\ group\ time - Mean\ drug\ treated\ grouptime}{Mean\ vehicle\ treated\ group\ time} \times 100$$

Anti-inflammatory Activity

Carrageenan- induced rat paw oedema

SD rats of both sexes were divided into 6 groups and each group consisted of 10 animals; 2 groups for RD-A (30 and 15 mg/ kg) and 2 groups for RD-B (200 and 100 mg/ kg), 1 group for indomethcin (80 mg/ kg), 1 group for saline. The rats were injected subcutaneously with 0.1 ml. of 1% carragenan solution in 0.9% w/v Nacl. into the sub-plantar region of the left hind paw (7). The swelling of the carrageenan and its contra-lateral saline-injected feet was measured before and 1, 2, 3, and 4 hours after injection of carrageenan. Saline solution was used for control group while indomethacin served as reference drug. Vehicle, RD-A, RD-B and indomethacin (i.p.) were administered 30 minutes before the injection of carrageenan. The difference in footpad thickness between the right and left foot was measured with digital gauge callipers. Percent inhibitory effects were estimated according to the following formula;

$$\%\ Inhibition = [(Na - Nb) / Na\ ] \times 100$$

where Na was the average difference in the thickness between the left and right hind paw of control group and Nb was that of drug-treated group. The measurement of PGE_2 content released from inflammatory tissue was according to the method described (8). All groups of rats were killed 4 hours after carrageenan administration; the hind paws injected with carrageenan were cut along the boundary between the long and short hairs of the feet. The paw was immersed in 7 ml NaCl solution for 1 hour after its surface skin was torn open and weight recorded. The solution was centrifuged at 3000 rpm for 10 min and 2 ml of 0.5 NaOH was added into the supernatant. The mixture was incubated at 50ºC for 20 minutes. After addition of 5 ml methanol, the solution was read with a Beckman DU-600 nuclear protein analyzer at 280 nm. The final concentration of PGE_2 contained in the inflamed hind paw was expressed as OD/ tissue (g).

Acetic Acid – induced vascular permeability

Based on the method of Kweifo-Okai (9) mice of both sexes were divided into 6 groups, each group consisted of 10 animals, 2 groups received RDA-A (20 and 15 mg/kg), 2 groups received RD-B (200 and 100mg/kg), 1 group received saline and 1 group received indomethacin (80mg/kg). The mice were injected (i.p.) with 0.25ml of 0.6% acetic acid solution 1h after administration of RD-A or RD-B (i.p.) while indomethacin served as the reference drug and animals in control group received 0.2ml saline at a dose if 10ml/kg. Immediately after administration, 10ml/kg of 10% (v/v) Evan's Blue was injected intravenously through the tail vein and the mice were killed at 30 min. after injection. The viscera was exposed and irrigated with distilled water over a petridish. The elution was filtered and constituted to 10ml, the dye leaking out into the peritoneal cavity was measured by spectrophotometer (Perkin-Elmer Lamda 3) using visible spectra at 610nm.

Adjuvant-induced arthritis

Induction of adjuvant-induced arthritis

SD rats of both sexes were divided into7 groups; 2groups received RD-A (30 and 15mg/kg), 2 groups received RD-B (200 and 100mg/kg) and 2groups received acetylsalicylic and (70mg/kg) and saline, respectively, the last group served as blank control (injected liquids paraffin). Rats were injected subcutaneously with 0.1ml of FCA (Freund's complete adjuvant, namely heat-killed Mycobacterium tuberculosis suspended in liquid paraffin) into the plantar surface of right hind paw (10). Rats in this group reproduced the inflammed control group; non-inflammed control group consisted of rats injected with 0.1ml liquid paraffin. The test group consisted of FCA-injected rats challenged with either RD-A or RD-B 1h prior to the injection of FCA. Acetylsalicylic acid (i.p.) served as a reference drug, while the animals in the control group received saline at a dose of 10ml/kg.The drug administration was continued at the same time of the day until day 20.

Effects of RD-A and RD-B on the contents of TxB_2 and 6-keto-PGF_{1a} in adjuvant-induced arthritis rat's blood

Apparatus

The HPLC system (Shimadzu) consisted of a LC-10AD pump, a SIL-10A auto-injector using glassy Carbon electrode, a stainless ODS-C18 reversed-phase column, DY-89-1 glass homogenizer, SZ-93 distilled water generator and a KQ-500B ultrasonic cleaner.

Chromatography

The mobile phase was a mixture of 0.1m sodium acetate, 0.1 citric acid, 0.5 mm sodium octyl sulfate, 0.15mM EDTA, 1mm dibutyl amine and 10% methanol v/v, pH-3.70. This mobile phase was passed through 0.22µm filter (GVWPO4700, Millipore) and degassed under vacuum by means of ultrasonic agitation. All separations were performed at a flow rate of 1.0 ml/min. at room temperature. The detector potential was normally maintained at 0.8 mV usual an Ag/ AgCl reference electrode (11, 12).

Standards and Calculations

The stock solutions of standards (5-HT and 5- HIAA) were prepared in mobile phase and stored at –80ºC. for the purpose of quantification and examination of the linear range between sample loading and detector signal, a set of solutions, which contained 5-HT from 706.8 to 44.2 ng/ml, 5-HIAA from 250 to 7.5 ng/ml were sequentially diluted from stock solution at a ratio of 1:2. Each 20µl of these dilutions were injected into the HPLC system. The plasma levels of the substances were calculated by comparing the height of the peaks in the sample with the heights of the peaks in the standard solution. The final concentration was expressed as ng/ml.

Sample preparation

The pathological model of adjuvant induced arthritis and the treatment method of animals were kept the same as described earlier. At 20^{th} day, 5 ml of blood was drawn rapidly from the rats eye and mixed with 1/10 volume of 3.13% sodium citrate. The citrated blood was added with ascorbic acid to prevent 5-HT from oxidation and 250 µl was transferred in

polypropylene centrifuge tubes. Solution of 50µl of 1.5M ascorbic acid and 50µl of 3.4M perchloric acid were added into the tube and tube placed on ice for 15 minute. The supernatant was diluted 1:2 with mobile phase, passed through a 0.22µm filter (GVMP 01230, Millipore) and stored at -80ºC until assay; alternatively, a 20µl of the filtrate was directly applied into the HPLC analytical column to test (13).

RESULTS

Hot plate test

The results shows that RD-A significantly increased pain threshold of mice in a dose-dependent manner (Figure 2). The analgesic effect of RD-A with the highest dosage of 30 mg/kg peaked at 30min after drug administration and then slowly diminished. The highest pain inhibition intensity of RD-A is very close to that of pethidine with 40mg/kg at 15 minutes after administration. At 120 minutes after administration, the PIP of RD-A was still as high as 109% while pethidine was 48.3% ($p<0.05$ versus control), which suggests that RD-A exerted a more lasting analgesic effect than that of pethidine. The results (Figure 3) demonstrate that the analgesic effect or RD-B, to a great extent, is similar to that of RD-A with the exception that it has a weaker pain relieving effect. It reached a maximal analgesic effect (204%) at the dose of 200 mg/ kg, about 73.1% of the maximal effect of pethidine. Table 1 shows that the effective analgesic dose of RD-B is higher than that of RD-A, its ED_{50} being five to six times that of RD-A at 30 and 60 minutes after drug administration, respectively.

Writhing test

The results of dose-response effects of RD-A and RD-B on acetic acid-induced writhing in mice are given in Table 2. RD-A at doses 15 and 30mg/kg and RD-B at 50 and 200mg/kg significantly inhibited the writhing response of mice caused by the intraperitoneal administration of acetic acid. The maximal inhibition of the nociceptive response was 79.5% for RD-B at the dose of 200 mg/kg, which was very close to that of acetylsalicylic acid (84.6%), and 19.8% for RD-A at the dose of 30mg/kg. RD-B exerts its pain-relieving effect in a dose-dependent manner.

Formalin test

It can be seen in figure 4 that RD-A, with doses ranging from 7.5 to 30 mg/kg, caused significant inhibitory effects on both early and late phase of pain stimulus, but RD-B showed significant inhibition only on the late phase, while the analgesic intensity of RD-B (200 mg/kg), which is close to that of indomethacin, is stronger than RD-A on the late phase. Indomethacin did not show significant anti-nociceptive effect on the initial phase, but stronger effect on the late phase.

Carrageenan- induced rat paw-oedema

The effects of RD-A and RD-B on carrageenan induced rat paw oedema are given in Table 3. Statistical analysis demonstrated that both compounds showed significant anti-inflammatory effects but the anti-inflammatory effect of RD-B (200mg/kg) lasts longer than that of indomethacin. Both RD-A and RD-B exerted their maximal anti-inflammatory effects at 2hr after drug administration, 80.3% for RD-B at a dose of 200mg/kg and 46.1% for RD-A. RD-B showed a more potent activity than that of RD-A. The effects of RD-A and RD-B on the inhibition of PGE_2 content in the inflammatory tissue are given in Table 4. Both RD-A and RD-B showed a close effect in reducing the contents of PGE_2 in the rat's hind paw, however, neither RD-A nor RD-B had a stronger inhibitory effect when compared to that of indomethacin.

Acetic acid-induced vascular permeability

In Table 5, both RD-A and RD-B show reduced degrees of peritoneal inflammation produced by acetic acid in mice. The amount of dye leakage was significantly decreased at all doses of RD-A (30 and 15mg/kg) and RD-B (200 and 100mg/kg), the maximal inhibitory activities being 29.3% for (200mg/kg) and 26.0% for RD-A (30mg/kg) while the effect of indomethacin (80mg/kg) was the strongest.

Adjuvant –induced arthritis

Effects of RD-A and RD-B on the contents of TXB_2 and 6-Keto PGF_{1a} in FCA-induced arthritis rat's blood

The result given in Table 6 show that neither RD-A nor RD-B at any dose significantly reduced the content of TXB2 in FCA- induced arthritis rat's blood plasma. In contrast, both RD-A and RD-B decreased the 6-Keto PGF1a contrast with maximal inhibitory effects of 25.5% for RD-B at 200 mg/kg and 15.0% for RD-A at 30mg/kg. All the test drugs exerted significant inhibitory effects against thickening of the inflammatory foot injected with FCA. In this case, RD-B showed a stronger effect when compared to RD-A while acetylsalicylic acid possessed the strongest effect.

Effects of RD-A and RD-B on the content of 5-HT and its metabolite 5-HIAA in adjuvant-induced arthritis rat's blood

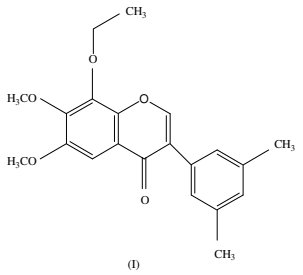
RD-A (30 and 15 mg/kg) and RD-B (200mg/kg) significantly decreased the content of 5-HT while increased the amount of 5-HIAA in rat's blood plasma. Acetylsalicylic acid did not show any effect on the con

-tents of 5-HT and 5-HIAA (Figure 5 & Figure 6).

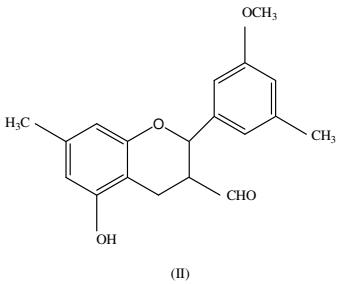
DISCUSSION AND CONCLUSIONS

Our previous studies have revealed the analgesic and anti-inflammatory properties of aqueous extract of *Rhododendron dauricum* Linn. (1). Further, the anti-inflammatory activity of the aqueous extract of leaves of *Rhododendron dauricum* Linn was evaluated in models which are mediated by tumour necrosis factor-alpha (TNFα) (2).

The present study involves the screening of analgesic and anti inflammatory activity of *Rhododendron dauricum* Linn. The result showed that these two compounds act by different mechanisms. The results of hot-plate test showed that both RD-A and RD-B provide significant protective effects on thermal pain stimuli. Such an effect is a character of the central analgesic effect, like morphine, while peripheral analgesic is known to be inactive on this kind of painful stimuli (14). In addition, both RD-A and RD-B showed long lasting analgesic effects as compared to pethidine. RD-B also showed a stronger analgesic activity than that of RD-A in acetic acid-induced writing test. The results led to the hypothesis that RD-A and RD-B play a role in the inhibition of prostaglandin synthesis, since the abdominal writhing in level by acetic acid involved the process or the release of arachidonic acid (AA), metabolite via cyclooxygenase (COX) and prostaglandin biosynthesis (5).



I → RD-A



II → RD-B

Fig 1.: Structural formular of RD-A & RD-B

Table 1. ED_{50} analgesic effects of RD-A and RD-B at 30 and 60 min after administration in hot plate test.

	ED (95% confidence limits)	
Drug	30 minutes	60 minutes
RD-A (mg/kg)	28.32 (13.76- 24.20)	33.05 (14.82-35.48)
RD-B (mg/kg)	159.59 (92.3-275.92)	250.65 (103.34-817.44)

Table 2. Analgesic effects of RD-A and RD-B on acetic acid induced writhing test.

Experimental groups	Dose (mg/ kg)	N[a]	Writhing times (means ± S. E. M.)	Percentage protection
Control	--	10	42.57± 7.23	--
Acetyl salicylic acid	70	10	6.57 ± 2.59 ***	84.6
RD-B-1	200	10	8.72 ± 3.21 ***	79.5
RD-B-2	100	10	15.64 ± 4.58 ***	63.2
RD-B-3	50	10	36.15 ± 6.75*	15.1
RD-B-4	25	10	40.16 ± 4.16	5.7
RD-A-1	30	10	34.18 ± 3.98**	19.8
RD-A-2	15	10	37.34 ± 4.58*	12.3
RD-A-3	7.5	10	38.42 ± 4.24	9.7
RD-A-4	3.75	10	42.16 ±4.05	0.1

[a]*Number of Mouse per group, *Statistical significance: p<0.05, (vs. control group), **Statistical significance; p<0.01, (vs. control group), ***Statistical significance; p< 0.001, (vs. control group).*

Table 3. Effects of RD-A and RD-B on carrageenan–induced paw-oedema in rats[a]

Experimental groups	Dose (mg/ kg)	N[b]	Inhibition of paw oedema (%)			
			1h	2h	3h	4h
Indomethacin	80	10	96.3***	85.3**	31.4*	10.3
RD-B-1	200	10	10.2	80.3**	71.2**	64.4**
RD-B-2	100	10	5.4	54.1*	53.6*	59.1*
RD-A-1	30	10	4.1	46.1*	26.5	1.3
RD-A-2	15	10	6.9	32.4*	8.4	3.6

*[a]Drugs were given 30 min. before carrageenan injection; [b]Nature of animals; *Statistical significance p<0.05, student's t-test vs. control; **Statistical significance; p<0.01, student's t-test vs. control; ***Statistical significance p< 0.001, student's t-test vs. control.*

Table 4. Effects of RD-A and RD-B on the content of PGE_2 in inflammatory tissue of carragenan- induced paw oedema in rats[a]

Experimental groups	Dose mg/ kg	OD/ tissue (g)
Control	--	0.35 ± 0.16
RD-B-1	200	0.20± 0.08**
RD-B-2	100	0.24± 0.18*
RD-A-1	30	0.22 ± 0.12*
RD-A-2	15	0.25 ± 0.11
Indomethacin	80	0.18 ± 0.05***

*[a]OD were measured at 4 h after carragenan injection, n=10, each value are expressed as the mean ± S. E. M. of 10 animals; Statistical significance; p<0.05, student's t-test vs. control; **Statistical significance; p<0.01, student's t-test vs. control; ***Statistical significance; p< 0.001, student's t-test vs. control.*

Table 5. Effects of RD-A and RD-B on acetic acid-induced vascular permeability in mice.

Experimental groups	Dose mg/ kg	Amount of dye leakage (µg)[a]	Inhibition %
Control	--	64.3 ± 13.2	--
RD-B-1	200	45.4 ± 16.3 **	29.3
RD-B-2	100	46.5 ± 18.7 *	27.7
RD-A-1	30	47.6 ± 14.9**	26.0
RD-A-2	15	50.2 ± 13.7*	21.9
Indomethacrin	80	32.6 ± 13.8***	49.3

*[a]Each value was expressed as the mean ± S. E. M. of 10 mice; *Statistical significance: p<0.05, student's t-test vs. control; **Statistical significance; p<0.01, student's t-test vs. control; ***Statistical significance; p< 0.001, student's t-test vs. control.*

Table 6. Effects of RD-A and RD-B on the contents of TXB_2 and 6-Keto-PGF_{1a} in FCA- induced artistries rat's blood.

Experimental groups	Dose (mg/ Kg)	TXB_2 (pg/ ml)	6-keto PGF_{1a} (pg/ml)	Inhibition of thinkening (%)
Acetyl salicylic acid	70	1.78 ± 0.26 ***	200.3 ± 32.2*** (27.3)[b]	64.4*
RD-B-1	200	2.43± 0.38	205.2 ± 46.3*** (25.5)[b]	58.5*
RD-B-2	100	2.51 ± 0.46	233.2 ± 38.7** (15.4)[b]	39.5*
RD-A-1	30	2.44 ± 0.36	234.3 ±40.9** (15.0)[b]	45.4*
RD-A-2	15	2.16 ± 0.42	249.5 ± 36.7* (9.0)[b]	29.8*
Control	10	2.72 ± 0.35	275.5± 28.9	--

*[a]Each value was expressed as the mean ± S. E. M. of 10 mice; [b]Percentage inhibition; *Statistical significance: p<0.05, student's t-test vs. control; **Statistical significance; p<0.01, student's t-test vs. control; ***Statistical significance; p< 0.001, student's t-test vs. control.*

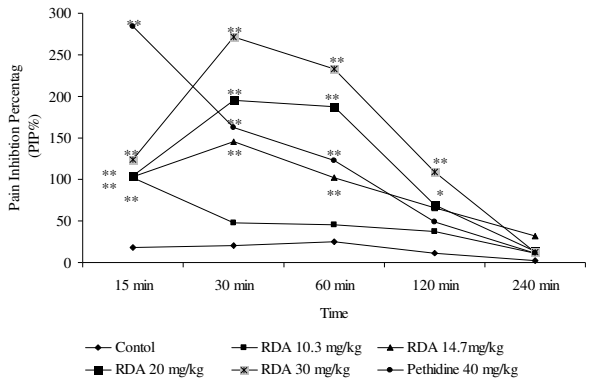


Figure 2. Effects of RDA on hot plate test at 15, 30, 60, 120 & 240 min after administration. ***p<0.001, **p<0.01, *p<0.05 (vs. control)

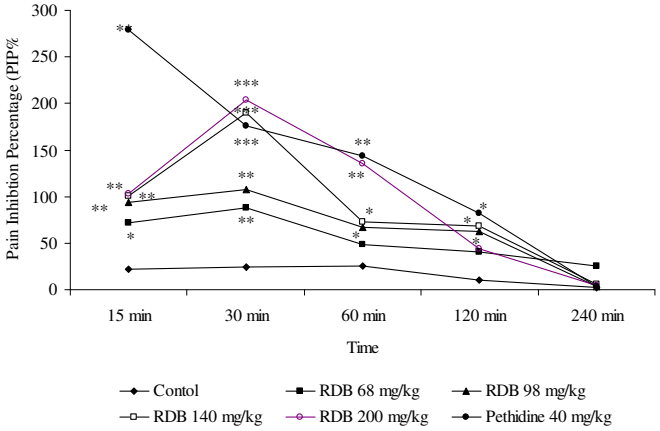


Figure 3. Effects of RDB on hot plate test at 15, 30, 60, 120 & 240 min after administration. ***p<0.001, **p<0.01, *p<0.05 (vs. control)

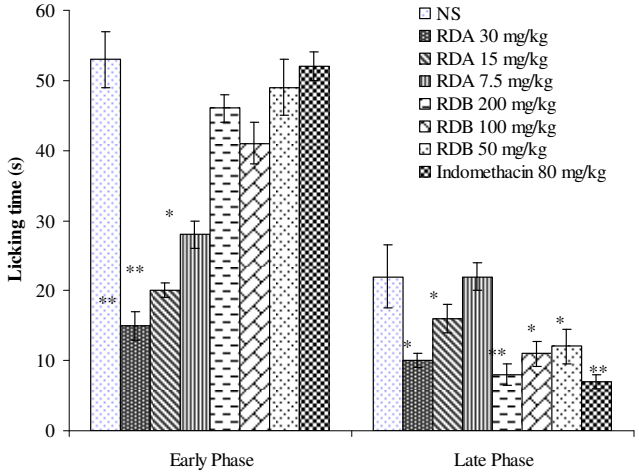


Figure 4. Antinociceptive effects of RDA &RDB in formalin test. Nociceptive behaviour in the early phase (0-5 min after the injection of formalin) and the late phase (20-30 min after the injection of formalin) were recorded with the amount of time (s) spent licking the injected paw or leg (mean ± S.E.M), n = 10. Significant differences between vehicle- and drug- treated groups indicated by **p<0.01 &*p<0.05 (Student's t-test).

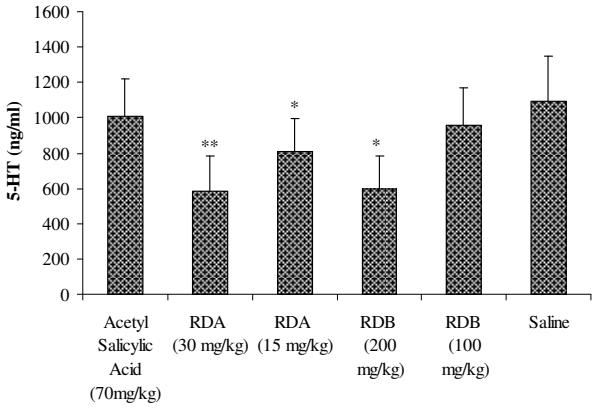


*Figure 5. Effects of RDA and RDB on the content of 5-HT in FCA-induced arthritis rat's blood. RDA was administered (i.p.) at doses of 30 and 15 mg/Kg; RDB was administered (i.p.) at doses of 200 and 100 mg/Kg; acetylsalicylic acid was administered at 70 mg/Kg. Each group consisted of 10 rats. **p<0.01, *p<0.05 compared with saline group (Student's t-test). Values given are mean ± S.E.M.*

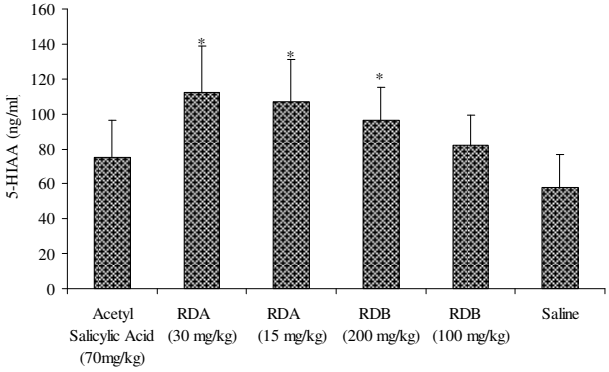


*Figure 6. Effects of RDA and RDB on the content of 5-HIAA in FCA-induced arthritis rat's blood. RDA was administered (i.p.) at doses of 30 and 15 mg/Kg; RDB was administered (i.p.) at doses of 200 and 100 mg/Kg; acetylsalicylic acid was administered at 70 mg/Kg. Each group consisted of 10 rats.*p<0.05 compared with saline group (Student's t-test). Values given are mean ± S.E.M.*

Result of studies have shown that the earlier phase of formalin- induced pain reflects the direct effect of formalin on nociceptors, whereas the late plase reflects inflammatory pain, which appears to be attributable to prostaglandin synthesis (15, 16). In this study RD-A inhibited both the phases of pain, whereas RD-B inhibited only the late phase, which suggested that RD-A is a more morphine like analgesic, whereas RD-B is a NSAID's- like drug. Carrageenan induced paw oedema as an *in vivo* model of inflammation has been frequently used to assess the anti edematous effect of natural products. It has been reported that various mediators are relaxed by carrageenan in the rat paw, thus, while the initial phase may be due to the release of histamine, the second phase is attributed to the release of PGs and in sensitive to most clinically effective anti- inflammatory changes (17). In the present study, RD-A and RD-B inhibited the second phase of the oedema, which differed from the effect of indomethacin on both initial and second phase. These results suggested that RD-A or RD-B has a selective effect on these mediations and their activities may be related to prostaglandin synthesis inhibition. Due to its chemical instability, PGE_2 is easily degraded and hard to detect in aqueous solution. However, under an alkaline condition, PGE_2 can be transformed into PGB_1, a more stable isomer of PGE_2, through a dehydration reaction and its subsequent

conformational change. Since PGB_1 has a maximal absorption at 280 nm, the content of PGE_2 may be indirectly estimated by spectrophotometer at 280 nm (18). In the present study, both RD-A and RD-B were shown to reduce the content of PGE_2 in carrageenan induced inflammatory paw of rats, which provides further support that the analgesic and anti-inflammatory activities of RD-A and RD-B are due to the inhibition of the release or synthesis of PGs.

Both RD-A and RD-B reduced the vascular permeability through the reduction of leakage into the peritoneum, which indicates that other mechanism, except the inhibition of PGs, could be involved in the pain modulation of these two compounds.

The anti-inflammatory effect of NSAID's is due to a reduction of prostaglandin synthesis through the inhibition of COX in AA metabolism (19, 20). In the present study, both RD-A and RD-B were shown to decrease the content of 6-Keto PGF_1a in blood plasma, but without any effect on TXB_2. This suggests that the mechanism of action of RD-A and RD-B was probably not completely similar to NSAIDS. The inhibition of COX might partly, but not solely be involved in the activities of RD-A and RD-B. It has been reported that 5-HT present in the inflammatory sites excite sensory neurons and pain may occur when 5-HT is released from platelets and mast calls during injury and inflammation (21). 5-HT is metabolized primarily by monoamine oxides (MAO) into 5-HIAA, then degrades; thus, the stimulation of MAO activity leads to the decrease of 5-HT, and the corresponding increase of 5-HIAA contents. In the present study, both RD-A and RD-B decreased the content of 5-HT and increased the content of 5-HIAA. The results suggest that RD-A or RD-B inhibit the release of 5-HT in inflammatory tissue, probably due to stimulation of MAO activity.

Taken together, RD-A is a more morphine like analgesic drug, while RD-B is a more NSAID's-like drug through the inhibition on the synthesis or release of PGs. The inhibition of COX and MAO activities might be involved in the antinociceptive and anti-inflammatory activities of RD-A and RD-B. RD-B showed a more potent anti-inflammatory activity compared to that of RD-A.

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