Journal of Scientific and Innovative Research 2013; 2 (6): 1034-1039
Available online at: www.jsirjournal.com

Research Article

ISSN 2320-4818
JSIR 2013; 2(6): 1034-1039

Received: 13-10-2013
Accepted: 28-12-2013

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Rasapushpa-Effect on acute and sub acute inflammation

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Abstract

Inflammation is one of the basic pathological processes in almost every infective, allergic and traumatic disease. In Modern science a wide range of anti inflammatory drugs is available to serve mankind but none of the drug is able to combat every inflammatory process. In Ayurveda also number of herbal and mineral preparations such as guggul, dashmula varga is used in practice for the above purpose but still a more potent preparation with desired properties needs to be explored and proved. Keeping in view the above objective, a study was conducted to evaluate the anti-inflammatory activity of *Rasapuspa* on albino rats in multiple study groups along with standard. The group which received RP in a dose of 12mg/kg showed highly significant anti-inflammatory activity in acute inflammation against standard group which received diclofenac. Effect of RP was found to be dose dependent. In sub-acute inflammation, decrease in weight of granuloma by RP proved its significant proliferative activity also. Microscopic study also showed granulomatous tissue with predominantly collagen deposition that again confirms the anti-inflammatory activity of *Rasapuspa*. The above findings are highly promoting and enthusiastic. The further research on various formulations of *Rasapuspa* should be studied and preparation of new compounds comprising of *Rasapuspa* is recommended.

Keywords: *Rasapuspa* (RP), Acute inflammation, Chronic inflammation, Ayurveda.

Introduction

Inflammation is part of the complex biological response of vascular tissues to harmful stimuli, such as pathogens, damaged cells, or irritants. Inflammation is a protective attempt by the organism to remove the injurious stimuli and to initiate the healing process. The term inflammation is considered in Ayurveda as svayathu, shothaa, shofa. According to Achaarya Sushruta"Tvagraktamaansamedansipradushyasthisamaashrita, doshaahshofamshairghoramjaanyantyuchhritahbhrasham" i.e. If inflammation persist Progressive destruction of the tissue would compromise the survival of the organism.[1]

However, chronic inflammation can also lead to a host of diseases. The inflammatory response must be actively terminated when no longer needed to prevent unnecessary "bystander" damage to tissues. Failure to do so results in inflammation, and cellular destruction. Resolution of inflammation occurs by different mechanisms in different tissues. RP has been claimed efficacious in the management of vraṇadoṣa (complications of wound healing), phiranga (syphilis), etc. consequently anti-inflammatory property of RP was evaluated.

Material and Methods

Pharmaceutical study

Preparation of RP was carried out by kupipakva method as per Text *Rastarangini* (6/29-31).[2, 3] *Kajjali* was made from 100g purified mercury, 100g purified *kaasis* (ferrous sulphate) and 100g *saindhav* (rock salt) triturating together in mortar and pestle for 20 hrs, resulting grey colour powder without shining of mercury, on visible inspection. *kajjali* was filled in *kaanchkuppi* (glass bottle wrapped with seven layers of clay smeared cotton cloth) and kuppi was placed in *balukayantra* (sand bath) for 20 hrs. *Underkramaagni* (standard heating pattern in ascending array followed by self-cooling) keeping starting temperature 36°C and maximum temperature 296°C. After completion of process pearly white 32g RP was obtained

Experimental study

Animal experiments were performed on Wister strain rats of either sex; weight 180-200 gm. Temperature was maintained at 23 ± 1°C with 12 hrs light and dark cycle and rats were individually housed and maintained on standard laboratory diet with water ad-libitum. Rats were deprived of food but free access to water was allowed for 24 hrs prior to the experiment conducted.

RP is water insoluble so dilution and oral dose was made in form of suspension. The suspension of RP and Diclofenac was made with 0.25% of carboxymethyle cellulose sodium (CMC) by w/v. According to Text, dose of RP is 65-125 mg/day.[3, 4]

Dose of drug in rats

Dose in rats (per kg body weight) = human dose x 0.018 x 5

The doses of RP calculated in rats were 6 mg/kg & 12 mg/kg body weight.[5, 6] Suspension was prepared for 6 mg/ml/kg by 60 mg RP added in 10 ml of 0.25% of CMC and for 12 mg/ml/kg by 120 mg RP added in 10 ml 0.25% of CMC, drug was administered by tuberculin syringe.

Anti-inflammatory activity

Two models were employed to evaluate anti-inflammatory effect. For acute inflammation, Carrageenan induced paw oedema technique was applied while for sub-acute inflammations foreign body (Grass piths and cotton pellets) induced granuloma method was adopted.

Acute inflammation- Carrageenan induced paws Oedema

Four group of animals contain six rats of either sex in each group were taken and starved over night with free access to water. Groups I was served as control, Group II was treated with Diclofenac sodium (standard), and Group III & Group IV was treated with prepared compound RP in two different doses. The group I was served as control and orally administered 1 ml/kg CMC. Group II was orally administered Diclofenac sodium (Standard) at the dose of 10 mg/kg and group III & group IV was orally administered RP at the dose of 6 mg/kg and 12 mg/kg respectively.

Just above the tibio-tarsal joint of hind paw was marked to ensure uniform dipping every time. Diclofenac sodium and RP compound were administered 1 hr prior to carrageenan injection. Oedema was developed according to the method described by Winter et.al. (1962)7 by sub-planter injection 0.1 ml of 1% freshly prepared suspension of carrageenan. Immediately after injection, paw oedema volume was measured by plethysmograph by mercury displacement to note initial volume. Similar procedure was repeated at the interval of 0.5, 1, 2, 3, 4 and 5 hrs. The difference between the initial and subsequent reading at given time was the actual volume. This oedema volume in control (Vc) and in-group treated with drugs (Vt) were calculated. Mean increase in paw volume was measured and percentage of inhibition was calculated.[7]

$$\% \text{ of inhibition} = 100\,[1-(Vt/Vc)]$$

Sub-acute inflammations– Foreign body (Grass piths and cotton pellets) induced granuloma

Modified version of technique described by winteret.al.[8] was used to study the influence of RP compound on granuloma formation. In this study 3 groups containing 6 rats in each group of either sex were selected. Each animal was implanted dried sterilize cotton pellets (50±1 mg) sub cutaneous bilaterally in axilla and sterilized grass piths (2.5 x 0.2 cm) bilaterally in groin region through a small incision. The procedure was carried out under ether anesthesia with semi aseptic measures. Group I was served as control and given CMC 1 ml/kg. Group II was treated with Diclofenac sodium, 10 mg/kg orally. Group III was treated with compound R.P. 12 mg/kg orally.

All these animals were given treatment once daily for 10 days. On the 11th day the cotton pellets and grass piths were dissected out. The pellets were dried at 60°C for 24 hrs and dry weight were recorded. The actual weight of cotton pellets was subtracted from the weight of dried granuloma pellets. The mean dry granuloma weight was

calculated. The dissected grass piths with granuloma tissues were immediately preserved in 10% neutral buffered formalin. Sections of 10-µ thickness were stained with haematoxylin and eosin (H&E) for histopathological examinations.[8]

Statistical analysis

Student "t" test has been used for analyzing the data during study. The Values of drug treated group were compared with control group. P value < 0.05 is considered as statistically significant

Results and discussion

In acute inflammation models, the results indicated that the R.P. in dose of6 mg/kg shows less anti- inflammatory activity. The inhibition of oedema was 40% at 0.5 hr., 51% at 1 hr., 47% at 2 hr., 44% at 3 hrs. , 29.3% at 4 hr. and 30.1% at 5 hr. Paw oedema volume measured by plethysmograph. But when increased dose of RP i.e. 12 mg/kg was given, showed good anti-inflammatory activity at various intervals; the inhibition of oedema was 74% at 0.5 hr, 79% at 1 hr, 71% at 2 hr, 70 % at 3 hr., 67% at 4 hrs, and 69 % at 5 hrs. Which showed that up to the two hrs it possess more anti-inflammatory than Diclofenac sodium (standard) and Diclofenac sodium showed the anti-inflammatory activity is 35% at 0.5 hr, 38% at 1 hrs, 41 % at 2 hr, 44 % at 3 hr, 41% at 4 hr& 35% at 5 hr, which showed the less anti-inflammatory activity in compression to compound R.P. (given in the dose of 12 mg/kg).

Table 1: Control Group I - Paw oedema volume measure by plethysmograph

No.	Wt. of Rat(gm)	Dose (ml)	0.00 hr	0.30 hr	1.00 hr	2.00 hr	3.00 hr	4.00 hr	5.00 hr
1	190	0.19	8.0	11.2	11.4	12.0	12.2	12.2	13.0
2	185	0.18	8.4	10.4	11.2	12.2	14.0	15.0	15.2
3	175	0.17	9.0	11.4	14.0	15.4	16.0	16.0	16.2
4	180	0.18	8.0	12.0	13.0	13.2	13.2	13.4	14.0
5	180	0.18	8.0	11.0	13.2	13.4	14.0	14.0	14.2
6	185	0.18	9.0	11.2	12.0	12.4	14.2	15.0	15.0

Table 2: (Diclofenac) Group-II - Paw oedema volume measure by plethysmograph

No.	Wt. of Rat(gm)	Dose (ml)	0.00 hr	0.30 hr	1.00 hr	2.00 hr	3.00 hr	4.00 hr	5.00 hr
1	180	0.18	7.0	9.0	9.4	9.4	10.4	10.4	10.4
2	190	0.19	8.0	10.0	10.0	10.0	10.0	10.2	11.0
3	185	0.18	8.0	10.0	10.6	10.2	11.0	12.0	13.0
4	185	0.18	8.0	9.0	11.0	11.4	11.4	12.0	12.0
5	180	0.18	8.0	10.0	11.0	11.0	11.0	11.4	12.0
6	180	0.18	7.2	9.2	9.4	11.0	11.0	11.0	12.0

Table 3: (RP 6 mg/kg) Group-III- Paw oedema volume measure by plethysmograph

No.	Wt. of Rat(gm)	Dose (ml)	0.00 hr	0.30 hr	1.00 hr	2.00 hr	3.00 hr	4.00 hr	5.00 hr
1	190	0.19	6.0	9.2	10.4	10.2	10.6	11.0	11.0
2	190	0.19	9.0	11.0	11.2	11.6	12.2	13.0	14.0
3	180	0.18	7.0	9.0	9.0	9.6	10.4	12.0	12.0
4	195	0.19	9.0	9.2	9.4	10.2	11.0	12.0	12.2
5	180	0.18	8.2	9.0	9.0	10.2	10.6	12.0	12.0
6	180	0.18	7.0	9.0	9.2	9.6	10.0	11.0	11.0

Table 4: (RP 12 mg/kg) Group-IV Paw oedema volume measure by plethysmograph

No.	Wt. of Rat(gm)	Dose (ml)	0.00 hr	0.30 hr	1.00 hr	2.00 hr	3.00 hr	4.00 hr	5.00 hr
1	185	0.18	9.0	10.0	10.0	10.2	10.4	11.0	11.0
2	180	0.18	9.0	9.2	9.2	10.0	10.0	10.2	10.2
3	180	0.18	8.0	9.0	9.0	9.2	9.4	10.0	9.4
4	190	0.19	9.0	9.2	9.4	10.4	11.0	11.0	11.0
5	195	0.19	9.0	10.0	10.4	11.0	11.0	11.2	11.4
6	190	0.19	8.0	9.0	9.2	9.4	10.2	10.2	10.4

In sub-acute inflammation models, the weight of the granuloma tissue formation was significantly (P< 0.001) reduced by compound R.P. (given in the dose of 12 mg/kg) and Diclofenac sodium (standard) (given in the dose of 10 mg/kg) the percentage of inhibition of pellets granuloma weight was 46% of RP and 49% of Diclofenac sodium the results of the study are summarized in table no.05. Microscopic study revealed reduced number of fibroblast increased collagens contents and fibrous tissue in compound RP and Diclofenac treated groups as compared to the control group.

Table 5: Effect of RP compound on sub-acute inflammatory model in rats

Group n=6	Dose	Wt. of Dry cotton pellet granuloma (mg) Mean ± SD	Percentage of inhibition
Control (CMC)	1 ml/kg	149.33±22.75	-
Diclofenac	10 mg/kg	76.16±15.85***	49%
RP-II	12 mg/kg	81.33±11.55***	46%

Values are expressed as mean ± SD; n=6 in each group, *** P<0.001 compared to control; NS- Statistically non significant.

Table 6: Effect of RP compound on carrageenan induced rat paw oedema

Group n=6	Dose (Unit/Kg)	Oedema volume (mean ± SD) at the interval after					
		0.5 h	1h	2h	3h	4h	5 h
Control (O.25%CMC)	1 ml/kg	2.8±0.75	4.06±1.11	4.7±1.15	5.53±0.93	5.86±0.98	6.18±0.75
Diclofenac	10mg /kg	1.83±0.40* (35)	2.53±0.41* (38)	2.8±0.71** (41)	3.1±0.62*** (44)	3.46±0.67** (41)	4.03±0.77** (35)
RP-I	6 mg/kg	1.7 ± 1.06^{NS} (40)	2.00±1.4* (51)	2.53±0.98* (47)	3.1±0.90*** (44)	4.13±0.76** (30)	4.33±0.77** (30)
RP-II	12 mg/kg	0.73±0.41*** (74)	0.86±0.47*** (79)	1.37±0.34*** (71)	1.66±0.47*** (70)	1.93±0.37*** (67)	1.9±0.50*** (69)

*P< 0.05, **P< 0.01, ***P< 0.001, compared to control; NS – Statistically Non Significant, figures in parentheses indicate the % of anti-inflammatory activity.

Figure 1: Animal Experimental work on Albino rats

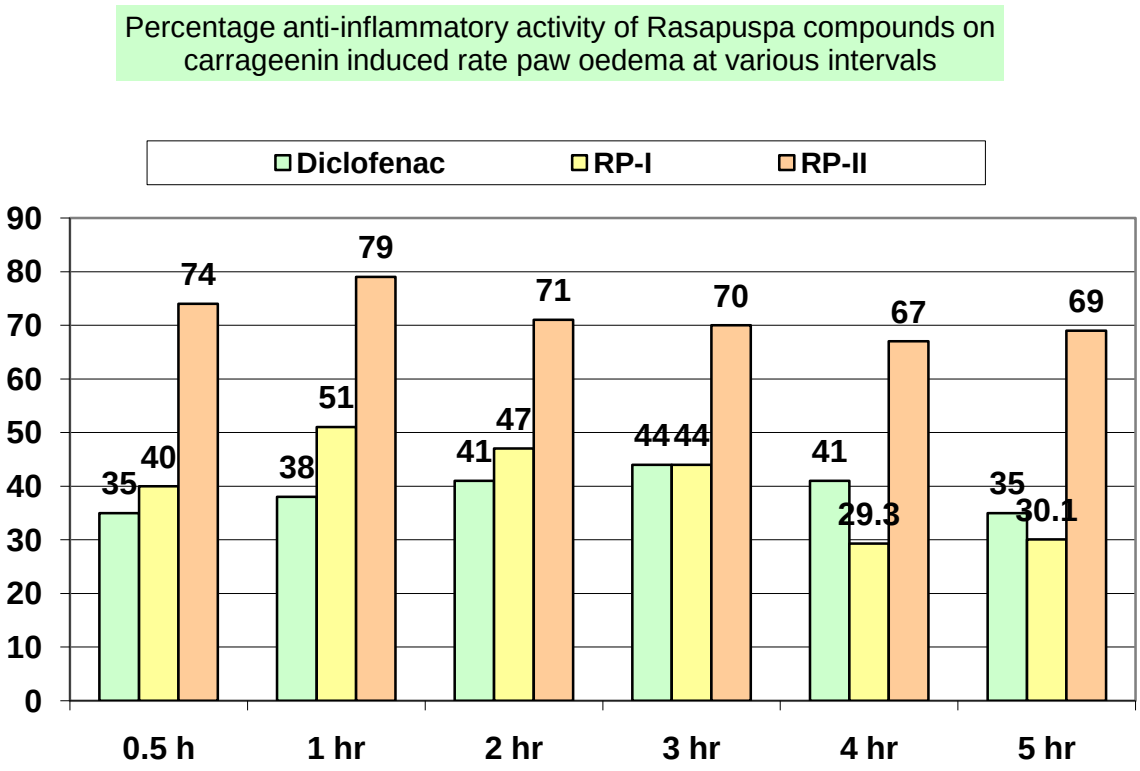

Figure 2: Percentage anti inflammatory activity of *Rasapuspa*

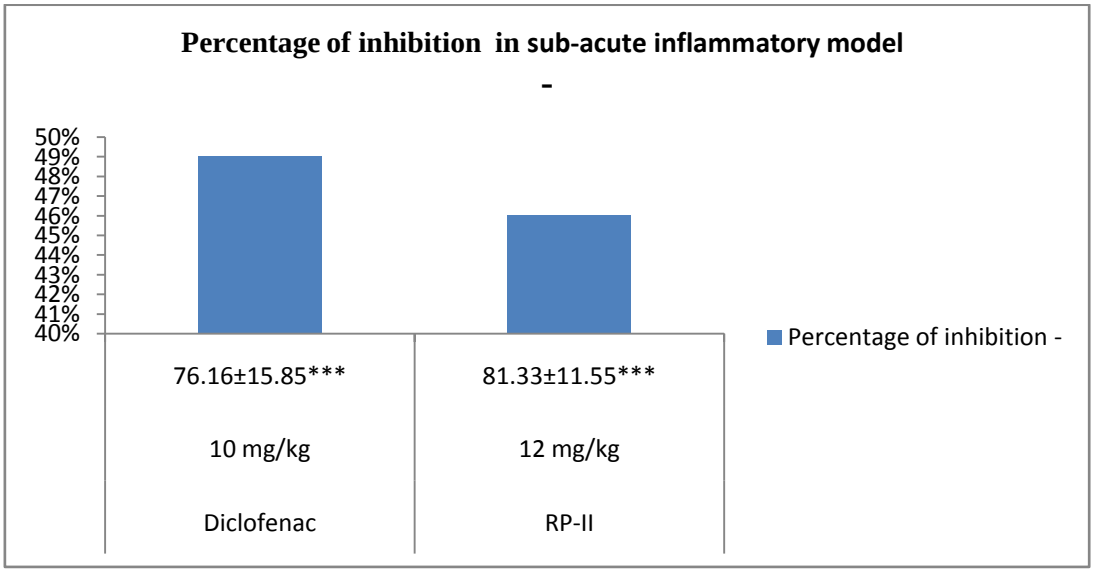

Figure 3: Percent inhibition of anti inflammatory activity

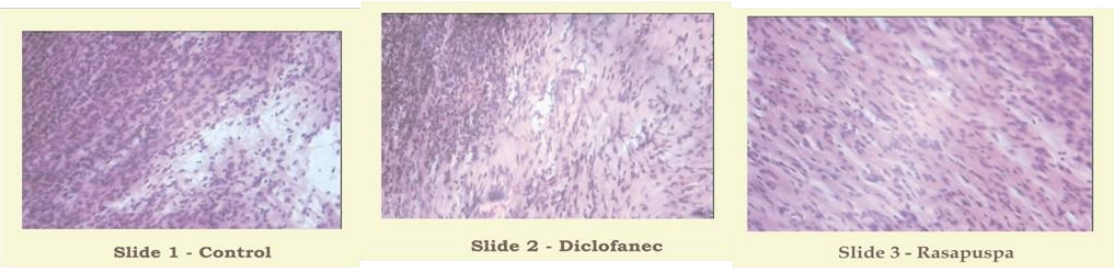

Figure 4: Histo-pathological slide of granuloma tissue

The chief goal of present study was to evaluate the anti inflammatory action of RP. For that carrageenan paw oedema method and foreign body (grass piths and cotton pellets) induced granuloma method were selected for acute and sub acute inflammatory condition respectively. The probable mechanism of action of carrageenan-induced oedema is biphasic. The first phase is attributed to the release of histamine, 5 H. T. and Kinin in the first hours while the second phase is related to the release of prostaglandin like substance in 2 to 3 hr.[9] Effect of *Rasapuspa* compound was dose dependent and showed significant activity inhibition of carrageenan-induced oedema. It was observed that RP in dose of 6 mg/kg has little anti-inflammatory activity as labeled in table no- 03, on the other hand RP in 12 mg/kg showed good anti-inflammatory activity than standard drug (Diclofenac sodium). Study also indicates that RP was initially highly active for up to the period of 2 hrs but after two hrs the activity was slightly reduced whereas the activity of Diclofenac sodium was sustained to a reasonable level. In the sub-acute inflammation infiltration of macrophages neutrophils and proliferation of fibroblast is involved. Hence decrease in granuloma weight by RP compound indicates the antipoliferative activity of RP. This was further confirming by haematoxylin and eosin (H&E) stained to granuloma tissue sections in the RP treated group.

Conclusion

Study showed that anti-inflammatory effect of RP is dose dependent. RP showed highly significant anti-inflammatory activity in acute inflammation in the dose of 12mg/kg compared to Diclofenac (standard) but in dose of 6 mg/kg showed less significant anti-inflammation activity. In sub acute inflammation decrees in granuloma weight by RP indicate the significant proliferative activity. Microscopic study also showed granuloma tissue with predominantly collagen deposition that re-confirms the anti-inflammatory activity of RP.

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