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BIOLOGICAL ACTIVITIES OF *RUBUS FRUTICOSUS* L. COLLECTED FROM DIR (L), PAKISTAN

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Abstract

Rubus fruticosus L. (Rosaceae) is an important medicinal plant used by the indigenous communities of Dir valley to treat various disorders. The present study was designed to rationalize the traditional uses of extract of this plant for analgesic and anti-inflammatory capacity. Formalin induced inflammation in mice and Carrageenan induced inflammation in rats were used to evaluate anti-inflammatory activity. Dose dependent anti-inflammatory effects were observed for both fruit and leaves extract of *R. fruticosus*. Leaves extract exhibited different mode of inhibition however the extract of fruit exhibited the same inhibition in both phases of Formalin test. The root and stem extracts did not exhibit significant anti-inflammatory effect. The pain-reduction capacity in paw edema test by *R. fruticosus* various extracts followed the order; leaves > fruit > root > stem. Leaves and fruit exhibited higher analgesic effect compared to root and stem extracts of *R. fruticosus* in hot plate, tail flick and writhing test. Our results support the traditional use of this plant as analgesic and anti-inflammatory remedy.

Keywords: *Rubus fruticosus,* Analgesic, Anti-inflammatory, Medicinal plant

Introduction

Medicinal plants are factories of secondary metabolites that may be used as decoction, powder, crude extract or isolated form to treat different disorders of animals, humans and plants. The world population using medicinal plants for the treatment of various diseases is about 80 percent. Dir (L) is one of the important districts of Khyber Pukhtunkwa, Pakistan, that is rich of medicinal plants, and people of the rural locality are dependent on these plants for their primary healthcare and diseases.

Rubus fruticosus L. (*Rosaceae*) is commonly found in Pakistan (Chitral, Dir, Mansehra, Malakand and Kotli), Europe, Asia, UK and America. It is famous for its fruit, called blackberry, which is sold worldwide due to its luscious taste, agreeable flavour. The people of Pakistani community named it locally as *Karwara*, *Ach*, *Akhara* and *Baganrra*, the plant is famous for various traditional uses like dysentery, depurative, astringent, tonic, vulnerary, diuretic, diarrhoea, asthma, cystitis and haemorrhoids (Chiej, 1984; Hummer & Janick, 2007; Murad *et al*., 2011; Riaz *et al*., 2011; Zia-Ul-Haq *et al*., 2014a). Chemically it is reported for various flavonoids, anthocyanins, phenolic acids, vitamins, carotenoids, tocols, tocotrinols and various elements (Zia-Ul-Haq *et al*., 2014a; Korkmaz & Karakus, 2015).

To the best of our knowledge there is no such kind of studies in which various parts of the *R. fruticosus* L. from Dir lower are evaluated systematically for analgesic and anti-inflammatory potential. The in-vivo experimental studies on mice have been designed as per our continuous group research (Riaz *et al*., 2013a,b; Riaz *et al*., 2014) to explore cost effective plant base medicine from Pakistani medicinal plants.

Materials and Methods

Plant parts were collected from District Dir lower, Khyber Pukhtun Khwa, Pakistan in 2008. The plants *Rubus fruticosus* L. was identified by Dr. Mansoor Ahmad, Department of Pharmacognosy, University of Karachi and voucher specimen RIPS-200812, were deposited in the same Institute.

The plant parts were cut and dried in shade. Dried plant parts were awash in methanol for 15 days. The methanol extract were filtered and evaporated under vacuum to concentrated thick mass. These extracts were used for biological evaluation.

Experimental animals: Albino mice and rats of both sexes were acquired from HEJ Research Institute of Chemical and Biological Sciences, Karachi and were kept under standard laboratory conditions. They were allowed free access to laboratory diet and water *ad libitum*.

Drugs and reagents: Carrageenan, was purchased from the Sigma Chemicals Co., St. Louis, MO, USA and aspirin was obtained from the Reckitt & Colman, Pakistan, sodium chloride from BDH Laboratory supplies, Poole, England. All chemicals used were of highest grade available, and were solubilized in distilled water/saline while carrageenan was used as suspension with acacia.

Anti-inflammatory activity

Formalin test: The groups of Swiss albino mice (25-30gm) were made as below

Groups	Right hind paw ventral surface	Orally (30 minutes before formalin injection)
Group 1	injected with 20 µl of 2% Formalin	20 µl of normal saline
Group 2	-do-	100 mg e/kbw
Group 3	-do-	300 mg e/kbw
Group 4	-do-	500 mg e/kbw
Group 5	-do-	Standard drug

e/kbw = extract /kg body weight

After formalin injection, licking and biting of right hind paw were observed within 10 minutes (early phase) and late phase that last from 10 to 30 minutes (Hunskaar & Hole, 1987).

Rat paw edema test: Similar scheme of grouping was used as in formalin induced inflammation, however 5th group was treated with aspirin 300 mg/kg. A mark was made on both the hind paws just below the tibiotarsal junction. Paw volume was recorded by vernier callipar in mm up to 4 hours after carrageenan induced inflammation. Percent edema inhibition is calculated by:

$$\% = \frac{Vc - Vt}{Vc} \times 100$$

where Vt and Vc are the mean relative changes in the paw volume of the test and control respectively (Liu *et al*., 2005; Vishnukanta, 2008).

Analgesic activity

Hot plate test: Twenty five mice (25-30 gm) were divided equally (n=5) into Group-I for control, Group-II and Group-III and Group IV for 100 mg/kg, 300 mg/kg and 500 mg/kg oral doses of crude extract respectively, and Group-V for standard (Aspirin as 300 mg/kg). The *R. fruticosus* extract and the aspirin were diluted in distilled water, and equal volume of saline was administered orally to control group (Dharmasiri *et al*., 2003; Ibrar *et al*., 2015).

Acetic acid induce writhing test: Koster *et al*., method was used to evaluate the extract for analgesic activity using acetic acid (Koster *et al.,* 1959). Twenty five mice (25-30 gm) were divided equally (n=5) into Group-I for control, Group-II and Group-III and Group IV for 100 mg/kg, 300 mg/kg and 500 mg/kg oral doses of *R. fruticosus* extract respectively, and Group-V for standard (Aspirin as 300 mg/kg). Writhes were induced by intra peritonial (IP) injection of acetic acid solution 10 ml/kg. Mice were treated with the extract 30 minutes prior IP injection of acetic acid and number of writhes was counted for 30 minutes.

Statistical analysis: All Results are written as mean values ± S.E.M. Results were considered significant probability of $p<0.05$ using One-way ANOVA test followed by Dunnet's multiple comparison post-test.

Results

In formalin induced inflammation (Fig. 1), *R. fruticosus* (100, 300 and 500 mg/kg) caused a dose dependent inhibition. The fruit extract showed significant inhibitions at $p\leq0.05$ were 35±1.39 and 35±0.73; 60.67% and 48.52 % at a dose of 500 mg/kg, for first and second phase respectively. Leaves extract (55%, 55.88%), root extract (21.34%, 19.11%) and stem extract (10%, 11.76%) showed inhibitions at 500 mg/kg for first and second phase, respectively. The inhibitory activity of *R. fruticosus* extracts against Carrageenan induced paw edema are given in Table 1. Aspirin, at dose of 300 mg/kg markedly reduced the edema (25.59%; 24%; 33.46% respectively). In hot plate test, *R. fruticosus* extract showed significant response for all higher doses (Table 2).

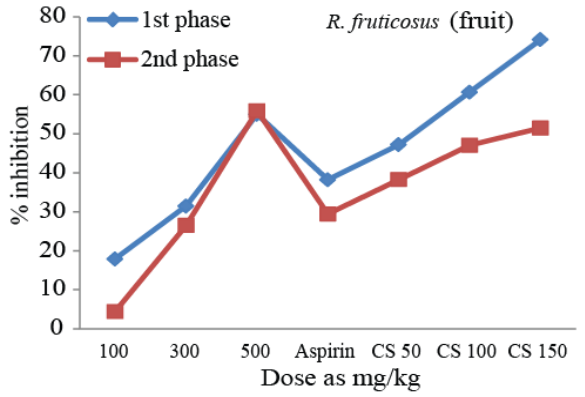
R. fruticosus fruit extract showed 61.06% (first phase) and 63.26% (second phase) inhibition at 500 mg/kg dose, leaves extract showed about 79.73% and 77.55% inhibition at both 1st and 2nd phase respectively at 500 mg/kg dose, root extract 60.93%, 48.57% while stem extract 21.86%, 19.97% at 500 mg/kg dose for first and second phase respectively in acetic acid induced writhing test (Table 3).

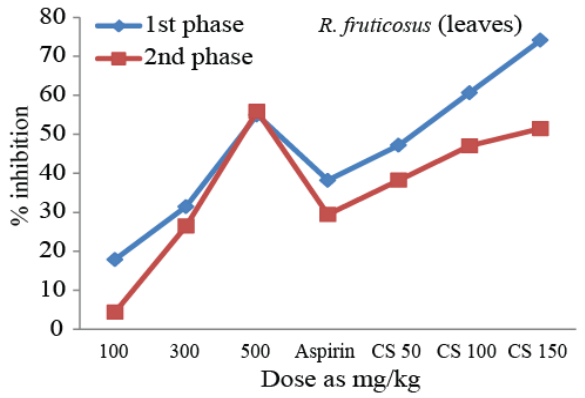
Table 1. Assessment of (carrageenan induced) anti-inflammatory activity.

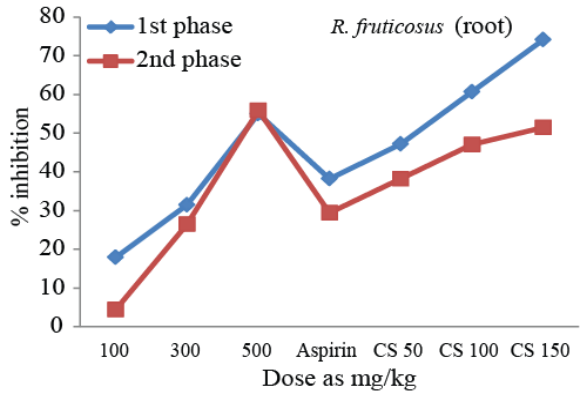
Treatment	Dose mg/kg orally	Mean diameter of rat paw in mm ± S.E.M			% of inhibition		
		1hr	2hr	3hr	1hr	2hr	3hr
Control	0.5 ml Saline	16.8 ±1.67	20 ± 2.37	24.5 ±1.97	-	-	-
R. f fruit	100 mg/kg	11.8±1.32	12.1±1.31	20.3 ±1.12	29.76	39.5	17.14
	300 mg/kg	11.6 ±1.29	11.9 ± 2.23*	18.7 ±1.13	30.95	40.5	23.67
	500 mg/kg	11.1±1.24*	11.5 ± 0.81	17.2 ±1.89	33.92	42.5	29.79
R. f leaves	100 mg/kg	12.2±1.49	14.5 ±1.15	19.3±1.25	27.38	27.5	21.22
	300 mg/kg	12± 1.14	12.7 ±1.72	18.3±1.23*	28.57	36.5	25.30
	500 mg/kg	10.5±0.83**	11.4±1.42**	15.3±0.73*	37.54	43	37.55
R. f root	100 mg/kg	16.1±1.13	17.3 ±1.43	22.8 ±1.73	4.16	13.5	6.93
	300 mg/kg	15.4±1.71	16.8 ±1.42	20.9±0.43*	8.33	16	14.69
	500 mg/kg	14.3 ±1.22	16.2±1.28	19.6±1.52	14.88	19	20
R. f stem	100 mg/kg	15.3±1.42	16.8 ±1.32	23.2 ±1.31	8.92	16	5.30
	300 mg/kg	14.2 ±1.49	16.4 ±1.38	22.1 ±1.99	15.47	18	9.79
	500 mg/kg	14 ±1.79	14.3±1.47	19.9±1.89	16.66	28.5	18.77
Aspirin	300 mg/kg	12.5± 1.34*	15.2± 0.84	16.3±0.72*	25.59	24.0	33.46

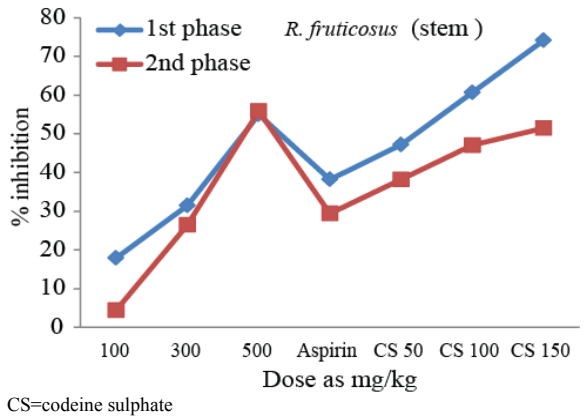
Mean ± S.E.M; N = 5; Significance with respect to control.

* = Significant results, ** = highly significant results. *R.f = Rubus fruticosus*









CS=codeine sulphate

Fig. 1. Anti-inflammatory activity (formalin induced inflammation)

Table 2. Effect of crude extract of on Hot plate Analgesiometer in mice.

Variation in flicking time with ± SEM (Time in sec at 55 ± 1°C)

Group	0hr	0.5hr	1hr	1.5hrs	2hrs	2.5hrs	3hrs	3.5hrs	4hrs	4.5hrs
Control	11.2± 1.19	11.4± 1.14	11.4± 1.83	10.4± 1.49	12.2± 1.72	12.2± 1.16	11.9± 1.12	12.3± 1.11	10.6± 1.21	11.2± 1.02
R. f fruit 100 mg/kg	11.2± 0.95	15.2± 0.86	17.3± 1.05	20.3± 0.82	22.4± 0.62	25.3± 0.79	25.3± 0.59	22± 1.24	20.5± 2.11	17± 1.13
R. f fruit 300 mg/kg	12.8± 0.92	24± 1.24	28.3± 1.25	26.8± 1.33	33.2*± 1.05	35.4± 0.89	25± 1.29	20.6± 1.54	18.8± 2.18	15.6± 1.43
R. f fruit 500 mg/kg	14.2± 1.79	27.6± 0.70	24± 1.19	27.2± 1.66	34.4*± 1.69	29.6± 1.39	27.8± 1.12	26.2± 1.03	19.2± 1.83	16± 2.18
R. f leaves 100 mg/kg	12.5± 0.76	13.7 ± 1.17	16.2 ± 1.18	16.3± 1.16	16.2± 1.70	16.3± 1.71	14.4± 1.17	14.2± 1.99	14.1± 1.83	13±1.28
R. f leaves 300 mg/kg	13.1± 0.71	13.9± 0.82	17.7± 0.86	20.2± 1.17	22.2± 0.80	16.8± 1.59	16.2± 0.86	12.2± 2.13	12.2± 2.13	11.8± 1.28
R. f leaves 500 mg/kg	10.4± 0.90	18± 0.71	25.4± 0.92	28± 0.79	35.8*± 0.82	24.8± 1.70	22.8± 0.62	18.2± 2.07	15.4± 1.17	14.4± 1.24
R. f root 100 mg/kg	10.6± 0.92	13 ± 0.71	15.2 ± 1.12	17.3± 0.89	19.2 ± 1.19	20.5± 0.96	18.3± 1.14	16.2 ± 1.24	14.2± 1.05	12.2± 1.55
R. f root 300 mg/kg	11.7± 1.12	15.2 ± 1.09	18 ± 1.16	22± 0.49	25.3± 0.82	25.8 ± 1.67	22.3± 0.79	20.5 ± 1.76	15.6± 1.43	13.3± 1.82
R. f root 500 mg/kg	10.6± 0.92	13 ± 0.71	15.2 ± 1.12	17.3± 0.89	19.2 ± 1.19	20.5± 0.96	18.3± 1.14	16.2 ± 1.24	14.2±1.05	12.2± 1.55
R. f stem 100 mg/kg	10.2±1.07	12.5±0.92	13±1.07	13.5±0.70	14±0.80	14.5±1.24	14.3± 1.93	14.3± 1.02	13.8±1.28	12.6± 1.13
R. f stem 300 mg/kg	11± 0.79	13± 0.60	15± 1.70	15.5± 0.70	17± 0.99	18± 1.02	17.4± 0.72	16± 0.84	14.2±0.61	12.5± 1.11
R. f stem 500 mg/kg	9.4± 0.29	10.5± 0.79	17± 0.59	17± 1.02	18.2± 1.84	15.8± 1.17	13.8± 1.90	13.8± 1.19	12.1±1.41	11.7± 1.02
Aspirin 300 mg/kg	13 ± 1.23	36* ± 0.26	36* ± 0.72	40* ± 0.32	42* ± 1.31	46**± 1.16	35 ± 0.71	24 ± 0.61	24±0.11	20± 0.81

Values represent the mean ± SEM. Statistically significant from control and standard drug.
* Significant, ** Highly significant: *R.f = Rubus fruticosus*

Table 3. Assessment of analgesic activity (Acetic acid induced writhing).

Treatment	Dose mg/kg orally	Mean no. of writhes + S.E.M		Inhibition (%)	
		1st phase	2nd phase	1st phase	2nd phase
Control	0.5 ml Saline	75+ 4.36	49 + 2.81	-	-
R.f fruit extract	100 mg/kg	49.67±0.90	39.7±2.21	33.77	18.97
	300 mg/kg	37.5+ 1.22	30.39+ 0.92	50.00	37.97
	500 mg/kg	29.2±1.72*	18±0.73*	61.06	63.26
R.f leaves extract	100 mg/kg	41±1.32	29.2±1.24	45.33	40.40
	300 mg/kg	28.6 + 1.394	24.7 + 0.43	61.86	49.59
	500 mg/kg	15.2 + 1.34**	11 + 0.89**	79.73	77.55
R.f root extract	100 mg/kg	42.4±1.23	32.7±0.93	43.46	33.26
	300 mg/kg	31.2±1.71*	22.6±1.62*	58.4	53.87
	500 mg/kg	29.3±1.38*	25.2±1.52	60.93	48.57
R.f stem extract	100 mg/kg	70±1.21	49±1.49	06.66	0
	300 mg/kg	61.3±0.48	48.2 +0.79	18.26	1.63
	500 mg/kg	58.6±0.77	39.21±0.69	21.86	19.97
Aspirin	300 mg/kg	44.4+ 1.05	20+ 0.45*	40.80	59.18

Mean ± S.E.M; N = 5; Significance with respect to control

(* = Significant results, ** = highly significant results) *R.f = Rubus fruticosus*

Discussion

Anti-inflammatory activity: Inflammation is a non specific immune response of the body to harmful stimuli that may be chemicals, pathogens or injury (Ferrero-Miliani *et al*., 2007). Different signalling pathways are activated regularly that produces pro-inflammatory and anti-inflammatory mediators in continuous passion (Lawrence *et al*., 2001; Ahmed *et al*., 2015). Understanding these signalling networks gives the insight into new targets to control upsetting diseases, histamines, serotonin and bradykinins are released in first phase of the two pathways during inflammation while prostaglandins are released in second phase (Khanra *et al*., 2015; Vyas *et al*., 2008). Carrageenan and formalin introduction into the rat or mice paw rouse a local acute inflammatory reaction (Zia-Ul-Haq *et al*., 2014b). Currently various synthetic anti-inflammatory and analgesics are available to treat various type of inflammations but they have various adverse effects. Targeting new natural moiety inflammation in present work *R. fruticosus* various parts at 100, 300 and 500 mg/kg per oral dose were evaluated. Significant reduction in inflammation was observed for fruit extract of the plant almost same in both phases. Leaves extract of the plant was found significant at a dose of 500 mg/kg, for both phases. Our studies agree with the behaviours of analgesics and anti-inflammatory drugs in formalin biphasic response test (Tjølsen *et al*., 1992). Fruit extract was observed for maximum inhibition of second phase while leaves extracts showed maximum response in first phase compared to the rest of *R. fruticosus* extracts applied. Stem extracts were found to be least inhibitors for both phases. Our results for fruit and leaves extract at dose of 500 mg are comparable with standard drug aspirin in first phase showing aspirin like mechanism for anti-inflammatory effect and so further isolation studies are advised to new lead anti-inflammatory from *R. fruticosus*. In case of carrageenan induced inflammation, dose dependent inhibition was observed. Leaves and fruit extracts were found to have highest percent inhibition similar to formalin test, however the effect was much pronounced for leaves extracts.

Analgesic activity: "An unpleasant sensory and emotional experience associated with actual or potential tissue damage" is termed as pain or analgesia by the International Association for the Study of Pain (Hartrick, 2007; Sinatra, 2010). Thermal, mechanical or chemical tissue injuries activate nociceptors that are peripheral endings of A-delta and C sensory fibres for pain detection that ultimately led to complex physiological process of analgesia (Sinatra, 2010). In our experimental models we used two types of stimuli that are thermal (hot plate and tail flick) and chemical (writhing test) to evaluate the analgesic effect of *R. fruticosus*. The dose dependant pain reduction was observed. Fruit and leaves extract exhibited significant analgesic effect in all three tests, however comparatively less pain reduction was observed for root and stem extracts of the plant. It is well-known that alcoholic leaves extracts of *R. fruticosus* may follow the pathway of inhibiting NFκB factor analgesic effect (Kaur *et al*., 2009). This literature corroborate our studies so we recommend this plant for further screening of analgesic activity and possibly useful analgesic drug may be discovered with less side effects.

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