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Research paper

Anti-inflammatory effect of hydroalcoholic extract of hibiscus rosa on acute and chronic inflammation

Pankaj Verma *, Swati Sharma, Vikas Kumar, Hema Chaudhary

P.D. Memorial College of Pharmacy, Bahadurgarh, India
**Corresponding author E-mail: vermapankaj1614@gmail.com*

Abstract

Background: The present study was carried out to explore the efficiency of Indian herbal source from Hibiscus rosa against a chronic inflammatory disease. Hibiscus rosa belongs to Malvaceae, acts by suppression of inflammation mediators.
Methods: Hydroalcoholic extract form Hibiscus rosa is prepared through soxhlet extraction and Diclofenac is used as the standard. Carrageenan and formaldehyde are administered to induce acute and chronic inflammation. Animals are divided into 6 groups with 6 animals each including Normal group, inflammatory control group, Diclofenac treated group and Hibiscus rosa treated group at different doses of 250 mg/kg, 500 mg/kg and 1000 mg/kg.
Results: Different concentrations of Hibiscus rosa treated groups i.e. 250 mg/kg ($p<0.05$), 500 mg/kg ($p<0.05$) and 1000 mg/kg ($p<0.01$) showed significant reduction in Paw edema as compared to controls. Significant reduction in Body weight was also observed in Hibiscus rosa treated groups. Hematological profiles of Hibiscus rosa treated group are satisfying and significant.
Conclusion: Results showed significant anti-inflammatory effect of Hydroalcoholic extract of Hibiscus rosa and justifying its therapeutic role in inflammatory condition..

Keywords: *Hibiscus Rosa; Hematological Parameters; Diclofenac; Inflammation; Paw Edema.*

1. Introduction

Inflammation is considered immunological defense mechanism that is elicited in response to mechanical injuries, burns, microbial infections, allergens and other noxious stimulus and enables the immune system to efficiently remove the injurious stimuli and initiate the healing process (Tripathi et al., 2010). Inflammatory process involves synthesis and release of local inflammatory mediators such as prostaglandins (PGs), leukotrienes (LTs) and platelet activating factor (PAF) induced by phospholipase A2 (PLA2), cyclo-oxygenase (COXs) and lipo-oxygenase (LOXs), chemokines, cytokines, vasoactive amines, kinins, nitric oxide (NO) are also released during inflammatory process (Coleman et al., 2007). Inflammation leads to rheumatoid arthritis, osteoarthritis, inflammatory bowel disease, multiple sclerosis, psoriasis, asthma, atopic dermatitis etc. (Caussens et al., 2002). This disease is 2 to 3 times more common in women than in men and can start at any age but most commonly starts in middle adult life (Alamanos et al., 2002). Various drugs have been discovered which are used as anti-inflammatory drugs. However, these drugs results in side effects such as infections (Ruyssen-Witrand et al., 2010), gastrointestinal distress (Musumba et al., 2012), osteoporosis (Lekamwasam et al., 2012), diabetes (Kim et al., 2011), cardio-toxicities (McKenna, 2011). However, there is utmost need growing on research of potent natural sources having anti-inflammatory activity.
Thus, the present study was focused on herbal plant for research work and on the basis of vast literature survey Hibiscus rosa (Malvaceae) hydroalcoholic leaves extract is explored for acute and chronic anti-inflammatory property. Hibiscus rosa leaves contains various chemical constituents such as flavones ,quercetin - 3,7-diglucoside, alkaloid, cyaniding chloride, ascorbic acid, riboflavin,-xylosylglucoside,thiamine, β sit taraxeryl acetate, cyclic acids sterulic , malvalic acid (Gurib-fakim et al., 2006; Rossia et al., 2008) which helps in decrease in inflammation. Extract of Hibiscuss rosa significantly suppress swelling of paws and decreases paw volume in both acute and chronic phase which may be due to the suppression of inflammation mediators. It is suggested that the mechanism of action of the hydroalcoholic extract of Hibiscus rosa may be related to histamine and prostaglandin synthesis inhibition.
The present study was carry out to see the efficiency of Indian herbal source against a chronic inflammatory diseases i.e. arthritis. In the present study rats were selected to induced arthritis as they develop a chronic swelling in joints due to accumulation of inflammatory cells, erosion of joint cartilage and bone destruction. The Hibiscus rosa extract also showed significant effect on various hematological and serum parameters.

2. Materials and methods

All the chemicals procured were of analytical grade or higher grade and used as such without purification.

2.1. Plant material

Plant was collected randomly from college garden (PDM) Bhadurgarh and authenticated by Dr. E.Roshani Nayer , Principal scientist , National Herbarium Of Cultivated Plant, National Bureau Of Plant Genetic Resources New Delhi with voucher no. NHCP/NBPGR/2013-50. Plant materials were dried in the shade at room temperature. Digital plethysmometer (Medicaid, Digital volume meter, India) was used to determine the volume of edema.

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2.2. Extraction and isolation

The powdered Hibiscus rosa leaves were successively extracted by Soxhlet extraction with 70% methanol and 30% water solution .The solvents were removed under reduced pressure in rotary evaporator until they become completely dry. The percentage yield of extract was determined to be 24% (Kokate et al., 2005; Harbone et al., 1998).This was administered orally to the rats by oral feeding cannula during the experimental period.

2.3. Experimental animals

Male Wistar rats of either sex weighing around 150-200 grams were purchased from disease free animal house of V.B Patel Chest Institute, New Delhi. Animals were housed separately in groups of 6 per cage (Polycarbonate cage size: 29x22x14cm) under laboratory conditions with alternating light and dark cycle of 12 hr each. The animals had free access to food & water. Inflammation was induced by carrageenan and formaldehyde in acute and chronic studies respectively in rats.
The rats were divided into six groups comprising of six rats in each groups as follow:
Group 1: Normal group
Group 2: Inflammatory control rats
Group 3: Diclofenac, 10 mg/kg (Standard Drug)
Group 4: Hibiscus Rosa, 250 mg/kg (H. rosa Low dose)
Group 5: Hibiscus Rosa, 500 mg/kg (H. rosa Medium dose)
Group 6: Hibiscus Rosa, 1000 mg/kg (H. rosa High dose)

2.4. Acute toxicity studies

Oral acute toxicity studies were carried out with wistar rats weighing 150-200gm.The extracts were administered as per the staircase method .The rats were fed with hydroalcoholic extracts of Hibiscus rosa at dose 500, 1000, 1500, 2000, 2500, 5000mg/kg body weight. The animals were observed continuously for 2 hours for the gross behavioural changes & then intermittently once in every 2 hours and finally at the end of 24 and 72 hours to note for any signs of toxicity including death. From the acute toxic study, the extract were found to be safe up to 5000mg/kg body weight so $1/10^{th}$ of this dose i.e. 500mg/kg was medium dose , 250mg/kg as a low dose and 1000mg/kg as high dose (Winter et al., 1974).

2.5. Carrageenan-induced paw edema

Anti-inflammatory activity was evaluated on the basis of inhibition of carrageenan induced rats hind paw edema. Rats were divided into 6 groups (n=6 animals in each group) .A mark was made on the hind paw just below the tibio-torsal junction. 30 minutes after the oral administration of the test substances, edema was induced in the left hind paw with a dose of 0.1 ml of 1% w/v carrageenan in normal saline was injected into the sub plantar surface of the left hind paw. The paw volume of rats was measured by digital plethysmometer (Medicaid system, Chandigarh). The relative increase in paw volume was measured in all groups at 1, 2,3,4,5 hrs after carrageenan injection .The increase in paw volume in group 2, 3, 4,5,6 was compared with group 1.The increase in the paw volume over the initial reading was calculated. The increase in paw volume was calculated according to the following formula:

Paw volume change = Final paw volume – initial paw volume

Where, final paw volume is the volume after injecting carrageenan.
Initial paw volume is the volume before injecting carrageenan.

2.6. Induction of chronic inflammatory model

Formaldehyde- induced arthritis: Animals were randomly divided into six groups of six animal each (n=6). Rats were injected with 0.1ml of 2% (v/v) of formaldehyde solution in the planter surface of the left foot on the first and third day of the test .Drug treatment was started from the initial day i.e. from the day of formaldehyde injection (0 day) and continued till 21st day. The rat paw volume was recorded daily by using following Digital Plethysmometer (Medicaid system Chandigarh) (Singh et al., 1996).

2.7. Physical examination

Paw edema in rats were measured by using digital plethysmometer at the time of acclimatization and during the dosing period. Paw diameter of all rats were measured by using vernier caliper at the time of acclimatization and during the dosing period. Mortality was checked daily for experimental rats throughout the experimental period. Body weights of all rats were weighed by using weighing balance at the time of acclimatization and the dosing period and calculate mean body weight.

2.8. Biochemical examinations

The blood collection from retro orbital plexus in eppendrof tube and then placed in micro-centrifuge tube and centrifuged at 4000 rpm for 15 min. The serum thus obtained was analyzed for SGOT, SGPT. They were analyzed by commercial diagnostic kits (Erba Mannheim, Mumbai). Others haematological parameters such as C - reactive protein, ESR, WBC count, RBC count, Platelet count, Hb were also measured.

2.9. Statistical analysis

The values were expressed as mean± SEM (n=6).The significance was assessed using one way analysis of variance (ANOVA) followed by Dunnet's test and $P<0.05$, $P<0.01$ and $P<0.001$ as compared with control were considered to be statistically significant.

3. Results

In the present study, anti-inflammatory effects of hydroalcoholic extract of Hibiscus rosa were investigated after sub-plantar injection of carrageenan-induced edema and formaldehyde induced arthritis in rats paw.

3.1. Effect of hibiscus Rosa extract in carrageenan- induced acute inflammation

Sub planter injection of carrageenan (0.1ml) showed increase in paw edema which reached peak in 4 hr and decreases slightly within 5hr in control group. However, diclofenac (10mg/kg) treated rats showed significantly suppressed paw edema after 2hr ($P<0.01$) and remains significant upto the 5th hrs. Moreover treated with hydroalcoholic extract of Hibiscus rosa (250mg/kg) showed significant inhibition in paw edema after 3hr ($P<0.05$) when compared with the control. Rats treated with hydroalcoholic extract of Hibiscus rosa (500mg/kg) showed significant inhibition in paw edema after 3hr ($P<0.05$). Also rats treated with hydroalcoholic extract of Hibiscus rosa (1000mg/kg) showed significant inhibition in paw edema after 2hr ($P<0.01$) as compared to control group.

Table 1: Effect of Hibiscus Rosa on Paw Edema

Group	1 hr	2 hr	3 hr	4 hr	5 hr
Normal	0.72+0.06	0.71±0.07	0.70+0.07	0.72+0.05	0.72±0.04
Control	0.98+0.15	1.57+0.17	1.81+0.23	2.01+0.34	1.76+0.18
Diclofenac (10mg/kg)	0.90+0.34	1.10+0.24[b]	1.01+0.11[b]	0.92+0.29[c]	0.85+0.13[c]
H.rosa low dose (250mg/kg)	0.97+0.08	1.43+0.33	1.62+0.22[a]	1.13+0.12[a]	1.07+0.23[b]
H.rosa med.dose (500mg/kg)	0.94+0.13	1.36+0.17	1.43+0.13[a]	1.01+0.19[a]	0.94+0.20[b]
H.rosa high dose (1000mg/kg)	0.92+0.06	1.19+0.16[b]	1.04+0.22[b]	0.94+0.13[c]	0.90+0.11[c]

Values are expressed as mean ± SEM (n=6). [a]P<0.05, [b]P<0.01, [c]P<0.001 as compared with control (One way ANOVA followed by Dunnet's- test).

Table 2: Effect of Hibiscus Rosa on Paw Diameter

Group	1hr	2hr	3hr	4hr	5hr
Normal	4.4+0.13	4.5+0.14	4.5+0.13	4.4+0.10	4.5+0.12
Control	9.0+0.39	11.1+0.26	13.2+0.08	15.5+0.49	14.9+0.38
Diclofenac (10mg/Kg)	6.7+0.42	7.8+0.24[b]	7.4+0.28[b]	6.3+0.29[c]	5.8+0.41[c]
H.Rosa Low Dose (250mg/Kg)	8.4+0.59	9.5+0.26	11.7+0.17[a]	10.3+0.74[a]	9.5+0.42[b]
H.Rosa Med.Dose (500mg/Kg)	8.3+0.49	9.0+0.15	10.2+0.10[a]	9.7+0.27[a]	8.1+0.61[b]
H.Rosa High Dose (1000mg/Kg)	7.9+0.42	8.8+0.16[b]	8.3+0.19[b]	7.6+0.13[c]	6.6+0.34[c]

Values are expressed as mean ± SEM (n=6). [a]P<0.05, [b]P<0.01, [c]P<0.001 as compared with control (One way ANOVA followed by Dunnet's- test).

Table 3: Effect of Hibiscus Rosa on Paw Edema in Chronic Inflammation

Group	Day 1	Day 3	Day 6	Day 10	Day14	Day 21
Normal	0.86±0.09	0.87±0.07	0.86±0.08	0.88+0.08	0.86 ± 0.09	0.87+0.07
Control	1.02±0.12	1.73±0.18	1.85+0.22	2.15+0.28	2.42+0.13	1.74+0.23
Diclofenac (10mg/kg)	0.92±0.06	1.19+0.12	1.35+0.28	1.08+0.45[b]	0.94+0.38[c]	0.81+0.21[c]
H.rosa low dose (250mg/kg)	0.99±0.23	1.47+0.13	1.66+0.33	1.89+0.41	2.05+0.19[a]	1.10+0.34[c]
H.rosa med.dose (500mg/kg)	0.96±0.16	1.30+0.20	1.51+0.48	1.78+0.67	2.01+0.10[a]	1.25+0.22[b]
H.rosa high dose (1000mg/kg)	0.94±0.14	1.25+0.20	1.38+0.13	1.16+0.10[b]	0.99+0.16[c]	0.94+0.36[c]

Values are expressed as mean ± SEM (n=6). [a]P<0.05, [b]P<0.01, [c]P<0.001 as compared with control (One way ANOVA followed by Dunnet's- test)

Table 4: Effect of Hibiscus Rosa on Paw Diameter in Chronic Inflammation

Group	Day 1	Day 3	Day 6	Day 10	Day 14	Day 21
Normal	5.6+2.42	5.5+2.32	5.4+2.41	5.5±2.39	5.6±2.31	5.7±2.22
Control	11.0+ 0.10	14.0+0.23	16.6+0.32	19.9+0.09	22.0+0.50	20.8+0.52
Diclofenac (10mg/kg)	7.3+0.13	9.0+0.36	10.4+0.11	8.0+0.23[b]	7.2+0.21[c]	6.6+0.19[c]
H.rosa low dose (250mg/kg)	9.40+0.14	11.7+0.44	15.4+0.66	17.5+0.09	19.0+0.33[a]	12.3+0.36[b]
H.rosa med.dose (500mg/kg)	9.05+0.19	11.9+0.13	14.0+0.56	16.8+0.21	18.7+0.22[a]	10.3+0.24[b]
H.rosa high dose (1000mg/kg)	8.43+0.10	9.9+0.09	11.5±0.29	8.9±0.24[b]	7.5+0.74[c]	7.0+0.12[c]

Values are expressed as mean ± SEM (n=6). [a]P<0.05, [b]P<0.01, [c]P<0.001 as compared with control (One way ANOVA followed by Dunnet's- test).

Table 5: Effect of Hibiscus Rosa on Body Weight

Group	Day 1	Day 3	Day 6	Day 10	Day 14	Day 21
Normal	179±0.6	179±0.8	179±1.1	181±1.3	181±1.1	183±1.5
Control	174±1.6	172±1.2	170±2.1	169±2.2	164±2.4	167±0.9
Diclofenac (10mg/kg)	178±1.7	176±2.4	174±3.3	177±1.3[a]	179±2.5[b]	180±1.7[b]
H.rosa low dose (250mg/kg)	177±2.7	175±1.3	173±1.8	172±1.2	174±3.2[a]	176±2.4[a]
H.rosa med.dose (500mg/kg)	176±1.5	175±1.7	174±1.2	173±1.8	175±3.4[a]	177±3.3[a]
H.rosa high dose (1000mg/kg)	177±1.1	174±1.3	172±2.1	175±2.4[a]	177±2.6[b]	179±2.9[b]

Values are expressed as mean ± SEM (n=6). [a]P<0.05, [b]P<0.01, [c]P<0.001 as compared with control (One way ANOVA followed by Dunnet's- test).

Table 6: Effect of Hibiscus Rosa on Serum Parameters

Group	ESR (mm/hr)	Hb (gm %)	CRP (mgm/L)	Platelet Count (103/µL)	RBC Count (106/µL)	WBC Count (103/µL)	SGPT (IU/L)	SGOT (IU/L)
Normal	3.85±0.11	13.8±0.29	4.22±0.21	6.10±0.19	5.14±1.65	3.13±0.07	41.12±0.69	30.51±0.51
Control	11.5 +6.42	7.8+0.91	7.41±0.08	9.42±0.14	3.91±1.44	6.24±0.06	99.28+13.8	78.56+10.3
Diclofenac (10mg/kg)	5.44+7.08	11.70+1.04	5.28±0.11	6.90±0.31	5.71±0.24	3.79±1.12	50.44+6.15	46.02+2.10
H.rosa low dose (250mg/kg)	10.89+6.89[a]	10.67+1.04[a]	6.79±0.27[b]	7.71±0.07[b]	4.79±0.06[b]	5.27±1.03[b]	74+8.32a	59.33+1.57[a]
H.rosa med. dose (500mg/kg)	8.7+8.34[a]	10.44+0.91[a]	6.18±0.21[b]	7.42±1.23[b]	4.98±0.18[b]	5.11±0.67[b]	70.23+8.21[a]	55.90+1.74[a]
H.rosa high dose (1000mg/kg)	6.4+9.02[b]	11.56+1.34[a]	5.97±0.15[b]	7.12±0.16[b]	5.92±0.29[b]	4.22±0.91[b]	67.98+4.90[b]	49.47+5.91[b]

Values are expressed as mean ± SEM (n=6). aP<0.05, bP<0.01, cP<0.001 as compared with control (One way ANOVA followed by Dunnet's- test).

3.2. Effect of hibiscus Rosa extract in formaldehyde-induced chronic inflammation

Paw edema was significantly decreased from 14th day onwards in 250mg/kg, 500mg/kg. Whereas from 10th day in 1000mg/kg treated rats. Diclofenac also showed decrease in paw edema in 10th day. Inhibition of paw edema in formaldehyde- induced arthritis may be due to the anti-arthritic potential of` Hibiscus rosa. Sub-planter injection of formaldehyde (0.1ml) showed increase in paw edema which reached peak on 14th day & after that it slightly decreased on day 21st.

3.3. Effect of Hibiscus Rosa on body weight

Body weight was measured alternate days throughout the experimental period to evaluate the chronic anti-inflammatory potential of hydroalcoholic extract of Hibiscus rosa.

In arthritic control rats, decrease in body weight was recorded from 3rd day onwards which decreased further up to 14th day. Moreover the rats treated with diclofenac (10mg/kg) showed significant decrease on day 10th ($P<0.05$) and showed significantly increased on day 14th ($P<0.01$), when compared with control. However the group treated with hydroalcholic extract of Hibiscus rosa doses (250mg/kg) and (500mg/kg) showed decrease was observed from day 3rd and significantly increased after 14th day and 21st day ($P<0.05$). Further the rats treated with extract of Hibiscus rosa (1000 mg/kg) was observed significant decrease on day 10th ($P <0.05$) and found significant increased on day 14th ($P<0.01$) and day 21st ($P<0.01$).

3.4. Effect of Hibiscus Rosa extract on serum parameters

In the present study decrease in Hb and RBC count whereas increase in WBC count, Platelet count, ESR, CRP was observed in control group. Doses of 250mg/kg, 500mg/kg, 1000mg/kg showed significant increase in Hb and RBC count where as decrease in the levels of ESR, WBC count, CRP and Platelet count. The serum parameters such as SGOT, SGPT were also estimated for assessing the potential of Hibiscus rosa in chronic inflammation. In the present study, when the rats were challenged with formaldehyde it produced elevated serum in SGPT and SGOT. The extract of dose 250 mg/kg, 500 mg/kg and 1000 mg/kg has produced significant decrease in the levels of SGOT and SGPT as compared to the control group.

4. Discussion

The present study was carried out to see the efficiency of Indian herbal source against an acute & chronic inflammatory disease. The rats were selected to induce paw edema & arthritis swelling in joints occur due to accumulation of inflammatory cell, erosion of bone and cartilage & some destruction. Chronic inflammation involves the release of the number of mediators like cytokines (IL-1 and TNF-α), GM-CSF, interferon. These are responsible for destruction of cartilage that can lead to severe disability. However standard drug & hydroalcoholic extract of Hibiscus rosa significantly superseded the swelling of the paw & also decrease paw volume in both acute & chronic phases which may be due to suppression of inflammatory mediator released .Though actual mechanism of suppressing inflammation is not known but it can be correlated with the presence of alkaloids and flavonoids in suppressing the inflammation. As the incidence and severity of arthritis increase, the changes in the body weight of the rats also occurred during the course of experimental period.

The results of the present study also indicate that the methanolic extract of Hibiscus rosa leaves exhibits anti- arthritic effect in rats with formaldehyde- induced arthritis. The model of formaldehyde-induced arthritis in rats has been extensively used in the study of inflammatory processes .Shortly after the administration of formaldehyde into hind paw; pronounced swelling appears in the hind paw which persist for weeks (primary reaction). After few days, the contra-lateral paw as well as front paw also become swollen (delayed systemic response).

Hematological finding shows that the decrease in Hb and RBC levels in control group may be due to iron deficiency influenced by interleukin -1. Increase in WBC count in control group may be due to increased white blood cell production. In generally both the ESR and CRP measure the increase in inflammatory generated protein. Platelet count is elevated in control group. Elevated level of serum SGPT and SGOT in formaldehyde induced arthritic rats can be due to increase in the liver and bone fraction. This in turn implicates a localized bone loss in the form of bone erosion, as the enzyme is released into the circulation in the course of bone formation and resorption (Greenwald et al., 2001). This decreased enzyme levels by the extract treatment emphasis that there might be decrease in bone loss and organ protecting mechanism which may be due to the reduction in the release of chemical mediators of the inflammatory process.

5. Conclusion

The results of the current investigation concluded hydroalcoholic extract of Hibiscus rosa possess a significant anti-inflammatory activity and justifying its therapeutic role in inflammatory condition.

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