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An Evaluation of the Activity of Vanda Roxburghi and Glycyrrhiza Glabra Linn in Animal Model of Arthritis an Experimental Study

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ABSTRACT

Introduction: Arthritis a "great cripler "and exact etiopathology is not clear, inflammatory reaction underlying the genesis of rheumatoid arthritis. Presently prolonged therapy with steroids and NSAIDs associated with number of adverse effect. Since ancient time indigenous plants are being used for arthritis, but not properly screened and evaluated. Therefore we planned to study of these indigenous plants i.e. Glycyrrhiza glabra linn (G.Glabra) and Vanda roxburghi (V.roxburghi).

Study design: A prospective study was designed for acute, subacute and chronic inflammatory arthritis and anti-pyretic models were produced in albino rats. They were treated orally with graded doses of these drugs and their outcomes were observed and compared with standard drugs i. e. phenylbutazone and aspirin. Statistical analysis was performed by student's 't' test and 'P' value was calculated referring to the appropriate table..

Results: LD50 was found 2399 mg for both the herbs on oral administration. Both the drugs in a dose of 400 mg/kg, were found to be significant ($p<0.05$) anti-inflammatory activity against caragennin and histamine, but significance increase in a dose of 800 mg/kg & 1600 mg/kg ($p<0.01$). G.Glabra was highly significant anti-inflammatory agent against formalin induced odema in comparison to V.roxburghi in a dose of 1600 mg/kg, after 11/2 hour observation ($p<0.001$), but both were equally effective in this dose after 4 and 24 hour observation. Both the drugs contain anti-pyretic activity but V.roxburghi has quicker onset of action.

Conclusion: Both the drugs were found to be highly significant in reducing the inflammation of acute, sub-acute and chronic animal model of arthritis, it was also observed that both the compound possess significant anti-pyretic property.

KEYWORDS Glycyrrhiza glabra linn, Vanda roxburghi, rheumatoid arthritis, phenylbutazone

Introduction:

Arthritis is one of the known disease to be the great "cripler" and "the king of human miseries" affecting all age groups. The exact cause of arthritis is not known. Inflammatory response is closely intervened in this repair. The inflammatory reactions underlie the genesis of rheumatoid arthritis but the exact pathogenesis is not yet clear. Majority of the anti-arthritic drugs includes synthetic compound i.e. steroids and NSAIDs. Steroids are well known anti-inflammatory agent but their prolonged medication is associated with large number of adverse effects. [1].

Most of the NSAIDs are also associated with major toxicity on gastrointestinal tract leading to development of peptic ulcer, renal toxicity causing renal papillary necrosis and skin rashes are more serious side effects. Oedema, goiter, aplastic anaemia, hepatotoxicity and acute anaphylactic reaction may also develop. [2]

A large number of indigenous plants have been tried by people in rural as well as urban areas for their anti-inflammatory properties and have not been screened scientifically for their anti-inflammatory activity. In this study we examined the LD50, anti-inflammatory and anti-pyretic properties of indigenous plants i.e. *Glycyrrhiza glabra linn* (G.glabra) and *Vanda roxburghi* (V. roxburghi).

Material & methods

Roots & stems of V. roxburghi. & G.glabra was collected dried in shade, powdered separately. Alcoholic extracts were prepared by maceration process & the water soluble portions of these extracts were used for the study. [3] Institutional animal ethical committee permission was obtained before conducting this study.

For LD50, albino rats were observed for any abnormal behavior or mortality initially for two hours continuously, then half hourly for further 4 hours. Finally the overnight mortality was recorded. LD50 was derived by method of Paget & Barnes. [4]

To determine the anti-inflammatory activity: both non-immunological and immunological methods have been used.

Non-immunological method: Acute inflammation was induced in hind-paw by carragenanan, formalin and histamine. [5,6,7] Paw volume was measured by plathysmometric method.[8] Sub-acute inflammation was produced by cotton pellets granuloma[9] and chronic inflammatory arthritis was induced by formaldehyde.[10]

Immunological method: for this tuberculin sensitivity test was performed. This was performed on the fourteen day after injecting mycobacterial adjuvant. Purified tuberculin (PPD) was

injected intradermally (0.01 ml of 1:10 dilution) into the flank of the albino rats which were previously depilated. The diameter of the tuberculin reaction was measured 24 hour and 48 hour after injection. The drugs were administered 3 hour before injecting PPD.[11]

Anti-pyretic activity was tested by inducing pyrexia with Brewer's yeast.[12]

Study design:
In this study albino rats (weighing 100 gm-250gm) were taken.

For determination of LD50, 120 animals were taken. Out of this 60 were treated by V. roxburghi orally, in graded dose i.e. 200mg/kg, 400mg/kg, 800mg/kg, 1600mg/kg, 3200mg/kg, 6400mg/kg. Remaining animals were treated similarly with G.glabra.

For screening the anti-inflammatory activity of G. glabra & V. roxburghi in experimentally induced acute, sub-acute and chronic arthritis in albino rats were divided into six groups consisting of six animals in each group. The group (i) was kept as control and treated with saline. Group (ii) was treated with standard anti-inflammatory drug i.e. phenyl butazone (50mg/kg). Group (iii, iv, v, vi) were fed with graded doses of water soluble portion of alcoholic extracts the each drug in the doses of 200mg/kg, 400mg/kg, 800mg/kg and 1600mg/kg respectively.

Anti-pyretic activity was determined and compared with standard drug i.e. aspirin in a similar design as laid down for anti-inflammatory activity.

Statistical analyses: Statistical analyses were performed by the SPSS program, version 10.05 (SPSS, Inc., Chicago, IL, USA). Data were expressed as mean ± SEM. All the result were calculated by student's 't' test of significance, fisher exact probability test and P values were calculated referring to the appropriate table.

Results:
From the Probit log dose response curve, LD50 was found to be 2399 for both herbs on oral administration (Table-1).

Non-immunological anti-inflammatory activity:
Acute study:
In group (i), treated with normal saline, carrageenan increased hind paw volume by 74.00±8.79 % after 3 hour. In a dose of 200 mg/kg G. glabra & V. roxburghi were ineffective in reducing inflammation but both drugs were found to be effective in dose of 400 mg/kg, 800 mg/kg and 1600 mg/kg ($p< 0.05$ to 0.01). The inflammation induced by histamine injection into the hind paw of rat was significantly ($p< 0.05$ to 0.01) prevented by both G. glabra & V. rox burghi in all the doses tested. Formalin induced oedema- was significantly prevented by both the drugs at $1_{1/2}$, 4 and 24 hour in 400, 800 and 1600 mg/kg doses in dose dependent manner. The 200 mg/kg dose however suppressed the oedema only at 24 hour. (Table-2 & 3)

Subacute study:
Cotton pellet granuloma- Both G. glabra & V. rox burghi significantly ($p<0.001$) reduced granulation tissue formation in 800 mg and 1600 mg/kg doses, in addition V. rox burghi reduced granulation tissue formation significantly in 400 mg/kg doses also. (Table- 4 & 5).

Chronic study:
In the 400 mg/kg G. glabra & V. roxburghi reduced the inflammation significantly ($p<0.05$) only on the second and third day. The 800 mg/kg dose of both the drugs prevented the rise in formaldehyde induced hind paw volume from 2nd to 10th day. This prevention was significant ($p<0.05$) on 2nd day and the significance value increased with the passage of time and showed very high significant value ($p< 0.001$) on the 10th day. In a dose of 1600 mg/kg , significant protection on 2nd and 3rd was observed with G. glabra but all the animals treated with this drug died after that period. V. roxburghi prevented the inflammation highly significantly from 2nd to 5th day, after that all the rats died treated with this drug. (Table-6 & 7)

Immunological anti-inflammatory activity:
Tuberculin sensitivity test: Marked tuberculin reaction developed 24 hour after intradermal injection of PPD in the control group. A significant reduction in tuberculin reaction was noted by both the drugs in 800 mg/kg ($P<0.05$) and 1600 mg/kg ($P<0.01$) dose at 24 hour and 48 hour respectively. (Table- 8 & 9).

Antipyretic effect: The Brewer's yeast induced pyrexia was significantly suppressed by both the drugs in 400 mg/kg and 800 mg.kg doses. The effect was more marked as compared to the standard drug 'aspirin' in 800 mg/kg doses. In case of G. glabra, the onset of antipyretic effect was at 90 min. and remained up to 120 min., but in case of V. roxburghi the onset was at 30 min and lasted up to 60 min. in 400 mg/kg dose and up to 120 min. in 800 mg/kg dose. (Table-10).

Discussion:
Since ancient time, the followers of Ayurvedic and Unani system of medicine have been using extracts of G. glabra and V. roxburghi alone or in combination for the chronic arthritis, pyrexia and sciatica.[13] It is not clear what portion of these plants caries such activity. So the active principle of these plants were extracted in alcohol and subject the water soluble fraction of this alcoholic extract to ascertain its qualitative and quantitative aspect with regard to such activity.

The inflammatory response is a polyphasic tissue reaction in which increase in vascular permeability, cellular infiltration and proliferation occurs. It is of prime importance that an anti-inflammatory compound should be assessed in acute, sub-acute and chronic inflammation along with immunological response which covers the entire process of inflammation.

Present study establishes the anti-inflammatory activity of the water soluble portion of alcoholic extract of G. glabra & V. roxburghi in a number of animal models representing different phase of inflammation. This indicates that the drugs somehow affect the vascular permeability, as the paw oedema produced by carragenannan, histamine and formalin results from vasodilatation and increase vascular permeability. G. glabra & V. roxburghi act by inhibiting these inflammatory medicators.[14] (Di Rosa et al, 1971,Bolam et al 1974). Both the herbs G. glabra & V. roxburghi was found to inhibit this significantly. The results were compared with the standard drugs. Phenylbutazone was used as standard drug for acute inflammation; dexamethasone was used as drug in case of subacute, chronic and immunologically induced inflammation.

These finding corroborate with the studies carried on similar herbs. G. glabra, R. communis, S. chirata and V. roxburghi.[13, 15,]

However suppression of rat hind paw oedema does not give a particularly valid assessment of clinical anti-inflammatory properties of drugs in current use because hind paw oedema seems to depend to an unusual extant upon the local release of 5-HT. In sub-acute model of cotton pellet test, test drugs were found to be efficacious. This test represents a proliferative phase of inflammatory response in connective tissue mediated by prostaglandins. [16] Our study drug seems to prevent the development of inflammatory response in proliferative phase, either by inhibiting the synthesis of prostaglandin or its release.

Formaldehyde induced chronic inflammation is representative of secondary inflammation and differs from sub-acute inflammation in migration and accumulation of fibroblast at the site of inflammation.[10]. In present study, test drug affected the cellular infiltration only with higher doses.

The tuberculin induced wheal formation is a result of anti-

gen-antibody reaction. [17] These drugs significantly affect the development of immunological induced inflammation.

Anti-pyretic effect of these drugs was found to be significant. Notable feature was that V. roxburgi showed quicker onset of action. This difference might be due to its different pharmacokinetic profile.

Possession of both anti-inflammatory and anti-pyretic activities of these compounds reflects to their closeness to NSAIDs. As the results are comparable to the classical NSAIDs i.e. aspirin and phenylbutazone, it seems that they might be acting through the same mechanism as NSAIDs. But it is not clear that this inhibition of prostaglandin synthesis is due to inhibition of cyclo-oxygenase or the inhibition of phospholipase A2. Since these compounds affect the immunologically induced inflammation also they seem to be act like corticosteroids which inhibit phospholipase A_2.

Further studies are needed to elucidate their exact mechanism of action.

Table-1
Oral LD50 of water soluble portion of alcoholic extract of Glycyrrhizia glabra and Vanda roxburghi in albino rats (n= 6).

Dose mg/kg/ day/oral	G.glabra		V. roxburghi	
	Corrected %	Probit	Corrected %	Probit
200	4.14	3.25	4.16	3.25
400	4.14	3.25	4.16	3.25
800	16.6	4.05	16.6	4.05
1600	16.6	4.05	16.6	4.05
3200	16.6	4.05	33.32	4.56
6400	50.00	5.00	50.01	5.00

Table-2
Effect of water soluble portion of the alcoholic extract of the roots of Gycyrrhiza glabra linn on hind paw volume (% increase) induced by the phlogestic agents viz. carrageenin, histamine and formalin (n= 6).

Drug	Dose mg/kg/oral	% increase in hind Paw Volume ± SEM				
		Carrageenin	Histamine	Formalin		
		At 3 hrs.	At 1hr.	At 1½ hrs.	At 4hrs.	At 24hrs.
Saline	5.00 ml	74.00 ± 8.79	55.40 ± 6.0	94.62 ± 15.37	126.15 ±19.65	113.57 ±15.36
Phenylbutazone	50.00	13.16 ± 2.68***	11.40 +5.95***	30.98 ±4.63***	51.38 ±6.55***	23.86 ±4.41***
G.glabra	200.00	54.62 ±4.85	37.12± 6.80**	60.84 ±11.24	87.46 ±9.32	69.26 ±5.02*
G.glabra	400.00	43.94 ±9.52*	22.44+ 7.8**	47.22 ±4.04*	61.54 ±3.02**	46 ±8.22**
G.glabra	800.00	38.57 ±2.159**	20.46 ±8.2**	23.82 ±8.86**	50.52 ±9.02**	36.23 ±3.86***
G.glabra	1600.00	33.84 ±4.25**	18.22 ±6.64**	19.04 ±5.84***	30.42 ±4.62***	30.82 ±4.06***

*****P <0.05; ******P<0.01; *******P<0.001 in relation to saline group.

Table-3
Effect of water soluble portion of the alcoholic extract of whole plant of Vanda roxburghi on hind paw volume (% increase) induced by the phlogestic agents viz. carrageenin, histamine and formalin (n= 6).

Drug	Dose mg/kg/oral	% increase in hind Paw Volume ± SEM				
		Carrageenin	Histamine	Formalin		
		At 3 hr.	At 1hr.	At 1½ hr.	At 4hr.	At 24hr.
Saline	5.00 ml 50	74.00 ± 8.75	55.40 ± 6.0	94.62 ± 15.37	126.15±19.66	113.54±15.36
Phenyl butazone	200	13.17±2.68***	11.40±5.93***	30.98±4.63***	51.36±6.55***	23.85±4.41***
V.roxburghi	400	64.67±3.196	35.12±6.32*	63.24±10.86	89.53±9.67	72.21±5.20*
V.roxburghi	800	37.58±7.17**	21.2±8.20**	46.44±3.05*	72.54±3.54*	48.0±7.02**
V.roxburghi	1600	40.52±5.97**	18.74±6.02**	26.86±8.86**	56.56±8.72**	40.00±3.02***
V.roxburghi		35.84±1.248**	14.76 + 5.64***	24.84±9.01**	32.82±5.02***	30.16±4.65***

*****P <0.05; ******P<0.01; *******P<0.001 in relation to saline group.

Table-4
Effect of water soluble fraction of alcoholic extract of the roots of Glycyrrhiza glabra linn and dexamethasone on cotton pellet implantation in albino rats (n= 6).

Drug	Dose mg/kg/oral	Increase in weight of Pellets	
		mg ± SEM	% ± SEM
Saline	5 ml	85.33 ± 7.13	213.33 ± 17.09
Dexamethasone	0.5	45.17 ± 3.49***	112.92 ± 5.20***
G. glabra	200	80.40 ± 2.4	201.0 ± 6.0
G. glabra	400	76.24 ± 3	190.6 ± 7.5
G. glabra	800	57.40 ± 2.36**	143.4 ± 5.9**
G. glabra	1600	55.40 ± 3.36**	138.5 ± 8.4**

Initial weight of pellets 40 mg
*****P <0.05; ******P<0.01; *******P<0.001 in relation to saline group.

Table-5 Effect of water soluble fraction of alcoholic extract of the whole plant of Vanda roxburghi and dexamethasone on cotton pellet implantation in albino rats (n= 6).

Drug	**Dose mg/kg/oral**	**Increase in weight of Pellets**	
		mg ± SEM	**% ± SEM**
Saline	5 ml	85.33 ± 7.13	213.33 ± 17.09
Dexamethasone	0.5	45.17 ± 3.49***	112.92 ± 5.20***
V. roxburghi	200	84.60 ± .90	211.5 ± 2.25
V. roxburghi	400	56.50 ± 1.12**	141.25 ± 2.80**
V. roxburghi	800	55.51 ± 2.10**	138.73 ± 5.21**
V. roxburghi	1600	52.24 ± 3.06**	130.61 ± 7.65**

Initial weight of pellets 40 mg
*****P <0.05; ******P<0.01; *******P<0.001 in relation to saline group.

Table-6

Effect of water soluble Portion of alcoholic extract of the roots of Glycyrrhiza glabra and dexamethasone on, formaldehyde induced arthritis in hind paw of albino rats (n= 6).

Drugs	Dose mg/kg/ day/oral	**% Increase in Paw Volume** ± SEM on Day								
		2	3	4	5	6	7	8	9	10
Saline	5 ml	113.51 ± 15.36	96.40 ± 24.08	140.52 ± 17.17	137.55 ± 16.38	133.34± 16.39	131.60 ± 16.24	125.82 ± 17.92	112.92 ± 19.05	103.43 ± 17.74
Dexamethasone	5	23.86 ± 4.41***	14.47 ± 2.82**	39.49 ± 4.64***	34.25 ± 5.48***	32.93 ± 6.10***	25.60 ± 4.52***	24.80 ± 4.07***	19.02 ± 3.47***	10.49 ± 2.32***
G. glabra	400	64.24 ± 4.36*	40.12 ± 3.60*	94.58 ± 9.86	110.33 ± 14.32	130.20 ± 16.20	128.42 ± 12.86	122.36 ± 16.86	118.36 ± 18.26	
G. glabra	800	56.26 ± 3.98**	38.21 ± 3.40*	75.80 ± 6.80**	52.50 ± 5.64***	45.70 ± 6.23***	41.38 ± 4.42***	38.60 ± 4.60***	26.24 ± 4.12***	106.36 ± 16.23
G. glabra	1600	26.08 ± 9.36***	17.87 ± 2.52**	24.22 ± 3.42***						
		Animal Dead								

*****P <0.05; ******P<0.01; *******P<0.001 in relation to saline group

Table-7

Effect of water soluble portion of alcoholic extract of whole plant of Vabda roxburghi and dexamethasone on formaldehyde induced arthritis in hind paw of albino rats (n= 6).

Drugs	Dose mg/kg/ day/oral	% Increase in Paw Volume ± SEM on Day								
		2	3	4	5	6	7	8	9	10
Saline	5 ml	113.54 ± 15.36	96.41 ± 24.08	140.52 ± 17.17	137.55 ± 16.39	133.38 ± 15.28	131.61 ± 16.24	125.82 ± 17.92	112.92 ± 19.05	103.43 ± 17.74
Dexamethasone	5	23.86 ± 4.41***	14.47 ± 2.82**	39.49 ± 4.64***	34.25 ± 5.48***	32.93 ± 6.10***	25.60 ± 4.52***	24.51 ± 4.07***	19.02 ± 3.47***	10.49 ± 2.32***
V.roxburghi	400	67.27 ± 4.70*	40.24 ± 4.20*	136.82 ± 18.72	143.28 ± 18.77	140.05 ± 18.48	130.17 ± 19.03	124.18 ± 18.24	119.56 ± 18.30	106.11 ± 18.20
V.roxburghi	800	60.24 ± 4.24**	25.52 ± 5.42*	64.36 ± 6.23**	63.26 ± 5.34**	40.26 ± 4.24***	37.34 ± 4.26***	36.54 ± 3.98***	22.96 ± .61***	16.08 ± 3.24***
V.roxburghi	1600	24.04 ± 8.86***	22.28 ± 9.06*	42.40 ± 8.64***	41.89 ± 5.26***					
		Animal Dead								

*P <0.05; **P<0.01; ***P<0.001 in relation to saline group.

Table - 8

Effect of water soluble portion of alcoholic extract of the roots of G. glabra and dexamethasone on tuberculin sensitivity test in albino rats (n= 6).

*****P <0.05; ******P<0.01; *******P<0.001 in relation to saline group.

Drug	Dose mg/kg/oral	**Diameter of Wheal mm** ± SEM	
		At 24 hrs.	At 48 hrs.
Saline	5 ml	12.66 ± 1.58	11.68 ± 1.76
Dexamethasone	0.5	3.67 ± 0.92***	3.34 ± 0.62***
G. glabra	200	9.35 ± 1.26	9.32 ± 1.24
G. glabra	400	6.86 ± 1.09 *	6.05 ± 1.04*
G. glabra	800	6.03 ± 1.02**	5.32 ± 0.92**

Table - 9

Effect of water soluble portion of alcoholic extract of the whole plant of Vanda roxburghi and dexamethasone on tuberculin sensitivity test in albino rats (n= 6).

Drug	Dose **mg/kg/oral**	**Diameter of Wheal mm** ± SEM	
		At 24 hr	At 48 hr
Saline	5 ml	12.68 ± 1.58	11.66 ± 1.76
Dexamethasone	0.5	3.65 ± 0.92***	3.34 ± 0.62***
V. roxburghi	200	11.37 ± 1.42	10.8 ± 1.36
V. roxburghi	400	8.27 ± 1.1*	6.85 ± 1.24*
V. roxburghi	800	5.23 ± 1.36**	5.14 ± 1.22**

*****P <0.05; ******P<0.01; *******P<0.001 in relation to saline group.

Table -10

Effect of water soluble portion of the alcoholic extract of the whole plant of Vanda roxburghi,roots of Glycyrrhiza glabra, and aspirin on brewer's yeast induced pyrexia in albino rats (n= 6).

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Drug	Dose mg/kg/oral	Initial	**Temperature ^{o}C ± SEM**				
			Pyretic	30 Minutes	60 Minutes	90 Minutes	120 Minutes
Saline	5 ml	37.01 ± 0.25	38.70 ± 0.16	38.92 ± 0.16	39.25 ± 0.45	39.45 ± 0.16	39.50 ± .16
Aspirin	300	37.18 ± 0.27	39.15 ± 0.20	38.03 ± 0.30*	38.02 ± .30*	37.93 ± .15**	38.53 ± .0.31
V. roxburghi	400	37 ± 0.09	39.13 ± 0.40	38.05 ± 0.27*	37.7 ± .30*	38.64 ±0.25	38.73 ± 0.30
V. roxburghi	800	37.02 ± .14	38.9 ± .24	37.7 ± .23**	37.4 ± .28**	37.2 ± .36***	37.2 ± .16***
G.glabra	400	37.03 ± .16	38.8 ± .21	38.6 ± .22	38.2 ± .24	38.0 ± .32**	37.9 ± .34**
G.glabra	800	37.31 ± .2	39.2 ± .19	39.1 ± .24	38.8 ± .19	37.5 ± .18***	37.2 ± .16***

*P <0.05; **P<0.01; ***P<0.001 in relation to saline group.

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