

A STUDY ON THE ACTIVITY OF SWERTIA CHIRATA AND OCIMUM SANCTUM IN ANIMAL MODEL OF ARTHRITIS.

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INTRODUCTION: Arthritis a "great cripler" and exact etiopathology is not clear, inflammatory reaction underlay the genesis of rheumatoid arthritis. Presently prolonged therapy with steroids and non-steroidal anti-inflammatory drugs 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. *Swertia chirata* (S. chirata) and *Ocimum sanctum* (O. sanctum). **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 2239 mg for S. chirata and was 4505 mg/kg for O. sanctum on oral administration. Both the drugs were found to be effective in the reduction of carrageenan induced inflammation but S. chirata produced statistically significant effect at 200 mg/kg, 400mg/kg, 800 mg kg ($p < 0.01$), and highly significant on 1600 mg/kg ($p < 0.001$). Histamine induced inflammation was prevented by S.chirata in all doses significantly in a dose dependent manner, but not significant with O. sanctum. Formalin induced oedema and cotton pellet granuloma was significantly prevented by S.chirata in all doses, in a dose dependent manner, but not significant with O. sanctum. Both the drugs contain anti-pyretic activity but O. sanctum has quicker onset of action.

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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 posses significant anti-pyretic property.

INTRODUCTION: Arthritis especially rheumatoid arthritis makes a person distressing and disabled. The exact etiopathogenesis is not crystal clear. The inflammatory reactions are associated with process of repair. Inflammatory reactions underlie the genesis of rheumatoid arthritis, life threatening, hypersensitivity reactions and some forms of fatal renal diseases. [1]

Majority of the anti-arthritic drugs includes synthetic compound i.e. steroids and non-steroidal anti-inflammatory drugs. Steroids are well known anti-inflammatory agent but their prolonged medication is associated with large number of adverse effects. [2].

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. [3]

Available anti-inflammatory drugs limit, control or modify the normal inflammatory reaction. Ideal drug which enhances salutary effects of inflammation and also controls its destructive and harmful sequelae but devoid of undesirable side effects is yet to be developed. 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. *Swertia chirata* (*S. chirata*) and *Ocimum sanctum* linn (*O. sanctum*).

MATERIAL & METHODS: Leaves and stems of these plants i.e. *S. chirata* & *O. sanctum* were 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. [4] Institutional animal ethical committee approval was taken before conducting this study.

Acute toxicity study done in albino rats of either sexes and 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. [5]

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 carrageenan, formalin and histamine. [6,7,8] Paw volume was measured by plethysmometric method.[9] Sub-acute inflammation was produced by cotton pellets granuloma[10] and chronic inflammatory arthritis was induced by formaldehyde.[11]

Immunological method: for this tuberculin sensitivity test was performed. This was performed on the fourteenth 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.[12]

Anti-pyretic activity was tested by inducing pyrexia with Brewer's yeast.[13]

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STUDY DESIGN: Albino rats of either sexes (weighing 100 gm-250gm) were taken in this study. For acute toxicity study, determination of LD50, 120 animals were taken. Out of this 60 were treated orally with *S. chirata* in graded dose i.e. 200mg/kg, 400mg/kg, 800mg/kg, 1600mg/kg, 3200mg/kg, 6400mg/kg. Remaining animals were treated similarly with *O. Sanctum*. The animals were allowed to take water and food ad libitum and observed continuously for 2 hours and then half hourly for upto 4 hours and finally overnight mortality was recorded.

For screening the anti-inflammatory activity of *S. chirata* & *O. sanctum* 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 \pm 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, on oral administration, LD50 was found to be 2239 mg/kg for *S. chirata* whereas it was found 4505 mg/kg for *O. sanctum*. (Table-1). On autopsy no cause of death could be determined.

NON-IMMUNOLOGICAL ANTI-INFLAMMATORY ACTIVITY: ACUTE STUDY: In group (i), treated with normal saline, carrageenan increased hind paw volume by 74.00 ± 8.78 % after 3 hour. Both the drugs were found to be effective in the reduction of carrageenan induced inflammation but *S. chirata* produced statistically significant effect at 200 mg/kg, 400 mg/kg, 800 mg/kg ($p < 0.01$) and highly significant on 1600 mg/kg ($p < 0.001$).

The inflammation induced by histamine injection into the hind paw of rat was reduced by both *S. chirata* & *O. sanctum* but *S. chirata* was found to be significant at 200 mg/kg ($p < 0.05$), more significant at 400 mg/kg ($p < 0.01$) and highly significant at 800 mg/kg and 1600 mg/kg. ($p < 0.001$). There was no statistical significant result found with *O. sanctum* in all tested doses.

Formalin induced oedema- was significantly reduced by *S. chirata* at $1_{1/2}$, 4 and 24 hour in 400 mg/kg, 800 mg/kg and 1600 mg/kg doses in dose dependant manner whereas *O. sanctum* significantly reduced oedema at a dose of 800 and 1600 mg/kg. *S. chirata* was found to be more effective in comparison to *O. sanctum* against formalin induced oedema. (Table-2 & 3).

Subacute study: Cotton pellet induced granuloma- *S. chirata* was found to be more effective and highly significant as compared to *O. sanctum*. Significant effect with *O. sanctum* obtained only at a dose of 1600 mg/kg. (Table - 4 & 5).

Chronic study: *S. chirata* produced more significant effects as compared to *O. sanctum*. In a dose of 800 mg/kg 1600 mg/kg *S. chirata* produced statistically significant effect on all days tested but in a dose of 400 mg/kg, statistically significant effect was found only upto 5th day, after that day

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no statistically significant effect observed. *S. chirata* in a dose of 1600 mg/kg produce mortality to all animals after 5th day. Statistically significant effect was obtained with *O. sanctum* in a dose of 1600 mg/kg only. (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 *S. chirata* in 400 mg/kg ($P < 0.05$) and 800 mg/kg ($P < 0.01$) at 24 hour and 48 hour respectively whereas *O. sanctum* was effective only in a dose of 800 mg/kg ($P < 0.05$). (Table- 8 & 9).

Antipyretic effect: The Brewer's yeast induced pyrexia was significantly suppressed by both the drugs when used in a dose of 400 mg/kg and 800 mg.kg doses. Only difference was that *O. sanctum* showed quicker onset of action(60 vs 90) and statistically more significant ($p < 0.01$) anti-pyretic action. (Table -10).

DISCUSSION: Since ancient time, the followers of ayurvedic and unani system of medicine have been using extracts of *S. chirata* & *O. sanctum* alone or in combination for the chronic arthritis, pyrexia and sciatica.^[14] It is not clear what portion of these plants exhibits 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 *S. chirata* & *O. sanctum* 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 carrageenan, histamine and formalin results from vasodilatation and increase vascular permeability. This drug act by inhibiting these inflammatory mediators.^[15] The extract of *O. sanctum* was effective only against formalin induced oedema & not for carrageenin or histamine induced oedema. However as the effect was seen against only formalin induced oedema it can be thought that this extract might be somehow preventing the formalin to induce the subsequent vascular sequence which results in oedema.

Singh et.al. showed anti-inflammatory activity in *Ocimum sanctum* as well which is due to the fact that they used fixed oil preparation for assessing these activities and not the water-soluble fraction ^[16]. These observations revealed a positive correlation between the anti-oedematous and vascular permeability decreasing property of anti-inflammatory drugs suggesting their protective effect against the inflammation and oedema produced by different phlogistic agents. 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. Rox burghi*.^[14, 17]

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

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depend to an unusual extent 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. [18] 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. [11]. In present study, test drug affected the cellular infiltration only with higher doses.

The tuberculin induced wheal formation is a result of antigen-antibody reaction. [19] *S. chirata* was found to affect more significantly in the development of immunological induced inflammation as compared to *O. sanctum*.

Anti-pyretic effect of these drugs was found to be significant. Notable feature was that *O. sanctum* 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 A₂. Further studies are needed to elucidate their exact mechanism of action.

TABLE-1 Oral LD50 of water soluble portion of alcoholic extract of *Ocimum sanctum* and *Swertia chirata* albino rats (n= 6).

Dose mg/kg/ day/oral	Ocimum sanctum		Swertia chirata	
	Corrected %	Probit	Corrected %	Probit
200	4.16	3.25	4.16	3.25
400	4.16	3.25	4.16	3.25
800	4.16	3.25	16.6	4.05
1600	4.16	3.25	33.33	4.56
3200	4.16	3.25	50.00	5.00
6400	4.16	3.25	50.00	5.00

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TABLE-2 Effect of water soluble portion of the alcoholic extract of leaves and stems of *Swertia chirata* on hind paw volume (% increase) induced by the phlogistic agents viz. carrageenin, histamine and formalin (n= 6).

Drug	Dose mg/kg/oral	% increase in hind Paw Volume \pm SEM				
		Carrageenin	Histamine	Formalin		
		3 hr.	1hr.	1½ hr.	4hr.	24hr.
Saline	5.00 ml	74.00 \pm 8.78	55.40 \pm 6.0	94.62 \pm 15.37	126.15 \pm 19.65	113.57 \pm 15.36
Phenylbutazone	50.00	13.17 \pm 2.68***	11.40 \pm 5.95***	30.98 \pm 4.63***	51.38 \pm 6.55***	23.86 \pm 4.41***
S. chirata	200.00	49.69 \pm 0.30**	33.20 \pm 6.20*	52.24 \pm 11.46	72.42 \pm 6.25*	60.22 \pm 4.40**
S. chirata	400.00	43.5 \pm 1.216**	21.64 \pm 7.20**	46.42 \pm 4.02*	61.43 \pm 4.6**	30.42 \pm 6.42**
S. chirata	800.00	38.21 \pm 2.116**	14.62 \pm 6.10***	30.32 \pm 7.24**	54.54 \pm 6.84**	28.14 \pm 4.24***
S. chirata	1600.00	29.13 \pm 5.935***	12.32 \pm 6.4***	12.26 \pm 3.42***	40.80 \pm 8.86**	26.22 \pm 5.22***

*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 the whole plant of *Ocimum sanctum* linn on hind paw volume (% increase) induced by the phlogistic agents viz. carrageenin, histamine and formalin (n= 6).

Drug	Dose mg/kg/oral	% increase in hind Paw Volume \pm SEM				
		Carrageenin	Histamine	Formalin		
		3 hr.	1hr.	1½ hr.	4hr.	24hr.
Saline	5.00 ml	74.00 \pm 8.78	55.40 \pm 6.0	94.62 \pm 15.37	126.15 \pm 19.65	113.54 \pm 15.36
Phenylbutazone	50.00	13.17 \pm 2.68***	11.40 \pm 5.95***	30.98 \pm 4.63***	51.38 \pm 6.55***	23.86 \pm 4.41***
O. Sanctum	200.00	69.16 \pm 12.66	44.40 \pm 6.34	72.42 \pm 11.46	92.8.42	81 \pm 9.34
O. Sanctum	400.00	62.00 \pm 2.78	36.2 \pm 6.20	61.2 \pm 6.04	80.24 \pm 8.20	64.20 \pm 4.20*
O. Sanctum	800.00	58.66 \pm 3.20	28.2 \pm 8.2	53.46 \pm 7.37*	66.34 \pm 5.10*	52.22 \pm 10.26**
O. Sanctum	1600.00	56.57 \pm 2.089	20.2 \pm 7.6	47.23 \pm 5.02*	54.46 \pm 11.4*	36.46 \pm 8.2**

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

Table-4 Effect of water soluble portion of the alcoholic extract of leaves and stems of *Swertia chirata* 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***
<i>S. chirata</i>	200	74.46 ± 2.86	186.15 ± 7.15
<i>S. chirata</i>	400	63.24 ± 3.06*	158.1 ± 7.68*
<i>S. chirata</i>	800	56.42 ± 4.12**	141.05 ± 10.3**
<i>S. chirata</i>	1600	46.24 ± 3.26***	115.6 ± 8.15***

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 portion of the alcoholic extract of whole plant of *Ocimum sanctum* 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 ± 17.0
Dexamethasone	0.5	45.17 ± 3.49***	112.92 ± 5.20***
<i>O. sanctum</i>	200	84.40 ± 1.26	211 ± 3.15
<i>O. sanctum</i>	400	76.46 ± 3.26	191.15 ± 8
<i>O. sanctum</i>	800	68.68 ± 6.36	171.7 ± 159
<i>O. sanctum</i>	1600	63.46 ± 3.24*	156.65 ± 81*

Initial weight of Pellets = 40 mg

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

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Table-6 Effect of water soluble Portion of alcoholic extract leaves and stems of *Swertia chirata* and dexamethasone on formaldehyde induced arthritis in hind paw of albino rats (n= 6).

Drugs	Dose mg/kg/ day/oral	% Increase in Paw Volume \pm SEM on Day								
		2	3	4	5	6	7	8	9	10
Saline	5 ml	113.54 \pm 15.36	96.40 \pm 24.08	140.52 \pm 17.17	137.54 \pm 16.39	133.38 \pm 16.39	131.60 \pm 16.24	125.82 \pm 17.92	112.92 \pm 19.05	103.43 \pm 17.74
Dexamethasone	.5	23.86 \pm 4.41***	14.47 \pm 2.82**	39.49 \pm 4.64***	34.25 \pm 5.48***	32.93 \pm 6.10***	25.60 \pm 4.52***	24.80 \pm 4.07***	19.02 \pm 3.47***	10.49 \pm 2.32***
S.chirata	400	59.26 \pm 4.36**	38.26 \pm 3.60**	86.0 \pm 8.8*	84.16 \pm 14.24*		104.40 \pm 12.8	110.28 \pm 16.36	116.8 \pm 18.68	104.26 \pm 16.28
S.chirata	800	54.26 \pm 4.02**	19.24 \pm 2.42**	67.95 \pm 3.92**	44.64 \pm 5.20***	92.20 \pm 12.46	44.46 \pm 3.80***	37.60 \pm 4.40***	24.26 \pm 3.20***	17.08 \pm 3.40***
S.chirata	1600	24.23 \pm 8.86***	16.36 \pm 2.82***	40.40 \pm 6.62***	43.24 \pm 4.62***	42.32 \pm 4.32***				
							Animal Dead			

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

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Table-7 Effect of water soluble Portion of the alcoholic extract of whole plant of *Ocimum sanctum* linn and dexamethasone on formaldehyde induced arthritis in hind paw of albino rats (n= 6).

Drugs	Dose mg/k g/ day/ oral	% Increase in Paw Volume \pm SEM on Day								
		2	3	4	5	6	7	8	9	10
Saline	5 ml	113.5 4 \pm 15.36	96.40 \pm 24.08	140.52 \pm 17.17	137.54 \pm 16.39	133.38 \pm 16.27	131.60 \pm 16.24	125.82 \pm 17.92	112.92 \pm 19.05	103.43 \pm 17.74
Dexamethasone	.5	23.86 \pm 4.41** *	14.47 \pm 2.82** *	39.49 \pm 4.64***	34.25 \pm 5.48***	32.93 \pm 6.10***	28.60 \pm 4.52***	24.80 \pm 4.07***	19.02 \pm 3.47***	10.49 \pm 2.32***
O sanctum	400	79.75 \pm 6.36	60.24 \pm 6.24	130.28 \pm 16.24	140.28 \pm 14.48	130.86 \pm 12.42	126.8 \pm 8.64	120.8 \pm 12.34	110.86 \pm 16.46	106.84 \pm 18.34
O sanctum	800	78.75 \pm 8.24	60.24 \pm 6.24	124.24 \pm 14.34	128.24 \pm 12.46	120.20 \pm 10.24	118.4 \pm 7.64	110.8 \pm 10.48	112.86 \pm 316.36	96.42 \pm 16.36
O sanctum	1600	64.24 \pm 3.86*	26.62 \pm 6.36*	79.74 \pm 10.46*	86.68 \pm 5.32*	100.24 \pm 15.20	96.00 \pm 14.36	90.40 \pm 14.36	86.04 \pm 14.00	78.12 \pm 16.02

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Table-8 Effect of water soluble portion of the alcoholic extract of leaves and stems of *Swertia chirata* and dexamethasone on tuberculin sensitivity test in albino rats (n= 6).

Drug	Dose mg/kg/oral	Diameter of Wheal mm ± SEM	
		24 hr	48 hr
Saline	5 ml	12.67 ± 1.58	11.67 ± 1.76
Dexamethasone	0.5	3.67 ± 0.92***	3.33 ± 0.62***
S.chirata	200	10.24 ± 1.8	10.12 ± 1.4
S.chirata	400	8.24 ± .96*	7.32 ± .82*
S.chirata	800	6.36 ± .98**	6.50 ± 1.08*

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

Table-9 Effect of water soluble portion of the alcoholic extract of whole plant of *Ocimum santum* linn and dexamethasone on tuberculin sensitivity test in albino rats (n= 6).

Drug	Dose mg/kg/oral	Diameter of Wheal mm ± SEM	
		24 hr	48 hr
Saline	5 ml	12.67 ± 1.58	11.67 ± 1.76
Dexamethasone	0.5	3.67 ± 0.92***	3.33 ± 0.62**
O.santum	200	11.67 ± 1.56	10.62 ± 1.52
O.santum	400	10.64 ± 2.34	9.42 ± 1.96
O.santum	800	8.34 ± 1.06*	7.02 ± 1.9*

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

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Table-10 Effect of water soluble portion of the alcoholic extract of the whole plant of whole plant of *Ocimum sanctum*, leaves and stems of *Swertia chirata* and aspirin on brewer's yeast induced pyrexia in albino rats (n= 6).

Drug	Dose mg/kg/oral	Initial	Temperature °C ± SEM				
			Pyretic	30 Minutes	60 Minutes	90 Minutes	120 Minutes
Saline	5 ml	37.02 ± 0.25	38.70 ± 0.16	38.93 ± 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*	38.03 ± .30*	37.93 ± .15**	38.53 ± .031
O. sanctum	400	37.1 ± .18	38.4 ± .23	38.02 ± .21	37.6 ± .28**	37.4 ± .27**	37.6 ± .2**
O. sanctum	800	37.2 ± .13	38.8 ± .31	38.6 ± .28	37.8 ± .22**	37.4 ± .24**	37.4 ± .22**
S. chirata	400	37.2 ± .21	38.6 ± .22	38.4 ± .22	38.2 ± .22	38.00 ± .30*	38.2 ± .32*
S. chirata	800	37.4 ± .24	39.1 ± .26	38.8 ± .22	38.8 ± .24	38.2 ± .29*	38 ± .27*

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