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RESEARCH ARTICLE

Evaluation of Analgesics and Anti-Inflammatory Activiyt of *Sudard*, A Poly-Herbal Formulation

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ABSTRACT

Rheumatoid arthritis is a chronic multi-system disease of unknown cause. It affects the people in their prime of life, predominantly between the ages of 20-50 years with unpredictable course. Sudard is used in the ayurvedic system of medicine for the treatment of inflammation and pain associated with rheumatoid arthritis, osteo-arthritis, frozen shoulder, sciatica, ankylosing spondylitis and chronic backache. Our study was aimed to evaluate efficacy of sudard using different animal models such as formalin (2% v/v)-induced acute inflammation, carrageen (1% v/v)-induced polyarthritis, adjuvant-induced arthritis, effect on subacute inflammation by sponge implantation technique and analgesic activity by Eddy's hot plate method. The results indicate that the formulation sudard possesses good anti-inflammatory, anti-arthritic and analgesic activities in the experimental animal models.

Keywords: *Sudard; Anti-arthritic; Anti-inflammatory; Analgesic*

Rheumatoid arthritis is a chronic multi-system disease of unknown cause affecting people predominantly between the ages of 20-50 years with unpredictable course. If left unchecked, it leads to the destruction of the tissues within joints and consequent physical disability in the greater majority.

Although there are drugs that have been shown to improve signs and symptoms, alter the natural history of the disease and improve quality of life, but there is still no cure. In addition, these available therapies are associated with potential risks of death or irreversible organ damage. The challenge for society is to balance these known potential risks of therapy with acknowledged benefits despite the fact that these drugs do not lead to a cure.

'Sudard' is a poly-herbal formulation containing extracts of 11 medicinal plants. Each tablet contains Guggulu (*Commiphora mukul*) - 100 mg, Rasna (*Pluchea lanceolata*) – 50 mg, Gandha Prasarini (*Paederia foetida*) – 50 mg, Nirgundi (*Vitex negundo*) – 50 mg, Ginger (*Zingiber officinalis*) – 50 mg, Eranda mula (*Ricinus communis*) – 50 mg, Chandra sura (*Lepidium sativum*) – 30 mg, Suranjan (*Colchicum luteum*) – 30 mg, Dwipantra wacha (Smilax glabra) – 30 mg, Kupilu (*Strychnous nuxvomica*) – 10 mg and Shilajatu (*Mineral pitch*) – 50 mg. The anti-inflammatory, analgesic, anti-arthritis or anti-oxidant effects of these plants have been mentioned in the literature [1-11].

Sudard is used in the ayurvedic system of medicine for the treatment of inflammation and pain associated with rheumatoid arthritis, osteo arthritis, frozen shoulder, sciatica, ankylosing spondylitis and chronic back ache. The formulation was made and marketed by referring ancient ayurvedic literature that mentions that mixture of these herbs is beneficial for treatment of pain and fever. No scientific study has been carried out so far. Hence, the present study was carried out to evaluate analgesic, anti-inflammatory and anti-arthritic activity of *sudard* using different animal models.

MATERIALS AND METHODS

Experimental animals Male albino Wistar rats weighing between 200-250g and male Swiss albino mice weighing between 25-35g were used. Institutional Animal Ethics Committee approved the experimental protocol. Animals were maintained under standard conditions in an animal house approved by Committee for the Purpose of Control and Supervision on Experiments on Animals (CPCSEA).

Drug and dosage The poly-herbal formulation "*Sudard*" was manufactured and supplied by Anglo French Drugs and Industries Ltd, Bangalore, India. The formulation was administered at doses of 150 mg/kg *p.o* and 300 mg/kg *p.o* in the form of suspension prepared in

Table 1. Anti-inflammatory activity on formalin induced rat paw edema

Treatment	Increase in paw volume (ml)					
	1 hr	2 hr	3 hr	4 hr	5 hr	24 hr
Control, Normal saline 5ml/kg, *p.o.*	0.300 ± 0.028	0.358 ± 0.027	0.383 ± 0.027	0.308 ± 0.015	0.225 ± 0.021	0.200 ± 0.022
Diclofenac sodium 10mg/kg, *p.o*	0.208 ± 0.015*	0.225 ± 0.030*	0.258 ± 0.035*	0.216 ± 0.027*	0.141 ± 0.015*	0.108 ± 0.015*
Sudard 150mg/kg, *p.o*	0.208 ± 0.023*	0.233 ± 0.030*	0.283 ± 0.030*	0.222 ± 0.021*	0.141 ± 0.020*	0.091 ± 0.008**
Sudard 300mg/kg, *p.o*	0.200 ± 0.025*	0.216 ± 0.030**	0.250 ± 0.018**	0.208 ± 0.023*	0.133 ± 0.016**	0.083 ± 0.010**

n=6, * $p<0.05$, ** $p<0.01$ as compared to control group

Table 2. Anti-inflammatory effect on carrageen induced paw edema in rats

Treatment	Edema volume(ml)									
	1 day	2 day	3 day	4 day	5 day	6 day	7 day	8 day	9 day	10 day
Control, normal saline 5ml/kg, *p.o.*	0.366 ± 0.076	0.475 ± 0.025	0.316 ± 0.147	0.308 ± 0.070	0.300 ± 0.057	0.258 ± 0.008	0.241 ± 0.059	0.241 ± 0.023	0.225 ± 0.025	0.15 ± 0.018
Diclofenac sodium 10mg/kg, *p.o.*	0.153 ± 0.019*	0.341 ± 0.087	0.216 ± 0.033	0.140 ± 0.027*	0.133 ± 0.021**	0.100 ± 0.012**	0.091 ± 0.015*	0.083 ± 0.025**	0.075 ± 0.011**	0.066 ± 0.010**
Sudard 150mg/kg, *p.o*	0.164 ± 0.031*	0.383 ± 0.144	0.241 ± 0.047	0.150 ± 0.018*	0.141 ± 0.015**	0.116 ± 0.010**	0.108 ± 0.015*	0.091 ± 0.025**	0.083 ± 0.010**	0.075 ± 0.011**
Sudard 300mg/kg, *p.o*	0.157 ± 0.017*	0.290 ± 0.060	0.225 ± 0.046	0.141 ± 0.024*	0.133 ± 0.010**	0.116 ± 0.010**	0.091 ± 0.015*	0.083 ± 0.010**	0.075 ± 0.010**	0.066 ± 0.010**

n=6, * $p<0.05$, ** $p<0.01$ as compared to control group

water. The doses were selected based on the human dose mentioned in the ayurvedic literature.

Formalin and carrageen were obtained from SD Fine Chemicals, Mumbai, India and Freund's adjuvant was procured from Sigma-Aldrich, USA.

Treatment Albino Wistar rats/Swiss albino mice were divided into four groups of 6 animals each. Group I served as control (normal saline 5ml/kg body weight orally) Group II was given diclofenac sodium (10mg/kg, *p.o*) Group III served with test drug (150 mg/kg, *p.o*) and Group IV served with test drug (300 mg/kg, *p.o*).

Experimental Models

Formalin (2% v/v) induced acute inflammatory model [12] The volume of the hind paw of the animals was measured initially using plethysmograph. After taking the initial reading, 0.1 ml of formalin (2% v/v in water) was injected into sub-plantar aponeurosis of the left hind foot. The paw volume was measured at 1, 2, 3, 4, 5, and 24 h after injection. Drugs were given orally 1 hr before formalin injection.

Carrageen (1% v/v) induced polyarthritis [13, 14] The rats were injected with 0.1 ml of carrageen (1% w/v in water) into the sub-plantar area of right hind paw. The drugs were given orally one hour prior to carrageen injection and treatment continued for 10 consecutive days. The volume of rat paw was measured daily using plethysmograph during treatment period.

Adjuvant induced arthritis (Immunological model) [15, 16] The animals were injected with 0.5 ml of Freund's adjuvant into the sub-plantar surface of right hind paw. Drugs were administered orally once a day commenced on the day of injection of adjuvant and continued for 28 days. The assessment of the change in the inflammatory reaction was made by measuring the paw volume plethysmographically on 1st, 4th, 8th, 12th, 16th, 20th, 24th, and 28th day after injection of Freund's adjuvant.

Anti-inflammatory activity by sponge implantation technique [17] Polyurethane foam sheets were used as sponges for implantation (thickness 5 mm) in this model. Discs are punched out to a standard size and weight (10.0 ± 0.02 mg) using a 13 mm cork borer. The disc shape sponges were then soaked in 70% v/v ethanol for 30 min and rinsed four times in water and heated at 80 ^{0}C for 2 hr. The sponges were soaked in the sterile 0.9% v/v saline prior to implantation in the animal.

Sponges were implanted in female albino rats weighing between 200-250g under ketamine (100 mg/kg, *i.m*) anesthesia. A 20 mm dorsal incision was made and four sponge pellets were implanted per rat in the dorsal region and was sutured. The sponge implanted rats were treated for 21 days. Sponges were removed from rats by opening the original incision after 21 days of implantation. Each pellet and associated tissue was weighed wet and then dried to a constant weight at 60 ^{0}C and reweighed.

Analgesic activity by Eddy's hot plate method [18] Albino Swiss mice were placed on the hot plate and the time until either licking (or) jumping occurs was recorded by a stop-watch. A cutoff period of 15 sec was maintained to avoid damage to the paw. Animals that showed short reaction time were selected for the study.

Table 3. Anti-inflammatory effect against Freud's adjuvant induced paw edema in rats

Treatment	Increase in paw volume (ml)							
	1 day	4 day	8 day	12 day	16 day	20 day	24 day	28 day
Control, normal saline 5ml/kg *p.o.*	0.450 ± 0.020	0.458 ± 0.015	0.450 ± 0.025	0.383 ± 0.030	0.416 ± 0.024	0.408 ± 0.030	0.400 ± 0.031	0.383 ± 0.030
Diclofenac sodium 10mg/kg, *p.o.*	0.383 ± 0.380	0.325 ± 0.044	0.366 ± 0.030	0.341 ± 0.027	0.300 ± 0.028*	0.300 ± 0.018*	0.291 ± 0.021*	0.291 ± 0.020*
Sudard 150mg/kg, *p.o*	0.483 ± 0.010	0.383 ± 0.016	0.383 ± 0.030	0.333 ± 0.021	0.291 ± 0.023*	0.258 ± 0.023**	0.308 ± 0.008*	0.325 ± 0.028
Sudard 300mg/kg, *p.o*	0.500 ± 0.022	0.400 ± 0.028	0.391 ± 0.023	0.325 ± 0.075	0.300 ± 0.034*	0.22 ± 0.021**	0.300 ± 0.022*	0.291 ± 0.015*

n=6, * $p<0.05$, ** $p<0.01$ as compared to control group

Table 4. Effect on thermal stimulus-induced pain (hot plate test) in mice

Treatment	Mean Reaction Time (sec)						
	0 hr	½ hr	1 hr	2 hr	3 hr	4 hr	6 hr
Control, normal saline 5ml/kg, *p.o*	5.0 ± 0.577	5.555 ± 0.619	5.111 ± 0.609	5.500 ± 0.670	4.833 ± 0.477	6.000 ± 0.516	4.300 ± 0.494
Diclofenac sodium 10mg/kg, *p.o*	5.833 ± 0.401	9.333 ± 0.498**	9.666 ± 1.170**	9.888 ± 1.078**	9.830 ± 1.222**	9.600 ± 0.666**	6.600 ± 0.802**
Sudard 150mg/kg, *p.o*	5.333 ± 0.499	7.500 ± 1.232	9.600 ± 1.174**	7.333 ± 0.614**	9.300 ± 0.494**	8.000 ± 0.683*	5.100 ± 0.477
Sudard 300mg/kg, *p.o*	5.600 ± 0.802	9.333 ± 1.229**	9.666 ± 1.202**	8.555 ± 1.432**	9.555 ± 0.562**	9.000 ± 0.856**	5.600 ± 0.04**

n=6, * $p<0.05$, ** $p<0.01$ as compared to control group

The drugs or vehicle were administered orally and the reaction time was observed again at 0, ½, 1, 2, 3, 4 and 6 hr after drug administration.

Analgesic activity by acetic acid induced writing method [19] Albino mice of either sex were used for the study. Test animals were administered orally with the drugs 1 hour prior to acetic acid (0.7% v/v in water, 0.1 ml/10g, *i.p*) administration. The mice were placed individually in glass beakers 5 min after acetic acid injection and were then observed for 15 min and the number of writhing was recorded for each animal.

Statistical Analysis The statistical significance was assessed using one-way analysis of variance (ANOVA) followed by Dunnet's comparison test. The values are expressed as mean ± SEM and $p<0.05$ was considered significant.

RESULTS

Both doses of *sudard* (150 mg and 300 mg/kg, *p.o*) significantly inhibited the edema induced by formalin. The results were comparable to standard drug diclofenac sodium (Table 1).

In carrageen induced chronic model of inflammation, *sudard* at a dose of (150 mg and 300 mg/kg, *p.o*) significantly inhibited the rat paw edema in a dose dependent manner (Table 2).

Sudard when administered orally at doses of 150 mg and 300 mg/kg showed a marked inhibition of edema in the adjuvant-induced chronic arthritis model in rats. It was observed that *sudard* produced maximum effect on the 20th day. Diclofenac 10 mg/kg, *p.o* showed similar effect (Table 3).

There was a dose dependent reduction in granular tissue formation in *sudard* (150, 300 mg/kg) and diclofenac sodium (10 mg/kg) treated rats. The activity was found to be statistically significant for the dose ranges used (Fig 1).

The polyherbal formulation *sudard* showed good analgesic activity in both Eddy's hot plate and acetic acid induced writhing test. The duration of analgesic effect was higher in high dose treated animals compared to the low dose treated animals (Table 4).

Both *sudard* and aspirin caused an inhibition on the writhing response induced by acetic acid. *Sudard* produced 38.25% and 60.72% inhibition with low dose and high dose respectively, where as aspirin at dose of 50 mg/kg showed 31.26% inhibition of writhing compared to control (Fig 2).

DISCUSSION

The results of the present study show that the polyherbal formulation *sudard* possesses significant anti-inflammatory, anti-arthritic and analgesic activities in all the tested experimental models indicating inhibition of all phases of inflammation.

The development of edema in the paw of the rat after injection of formalin and carrageen is a biphasic event.

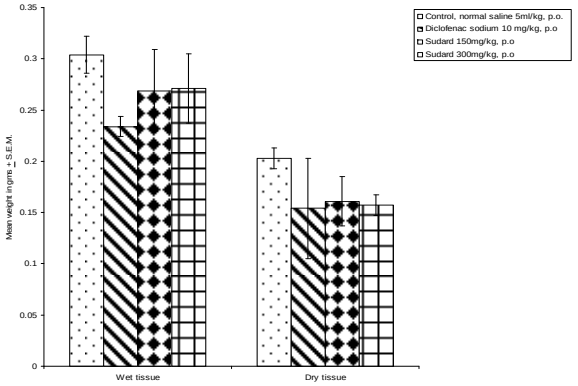

Fig 1. Effect of *sudard* on weight of wet tissue and dry tissue in sponge pellet induced granuloma in rats. n=6, ** $p<0.01$ as compared to control group

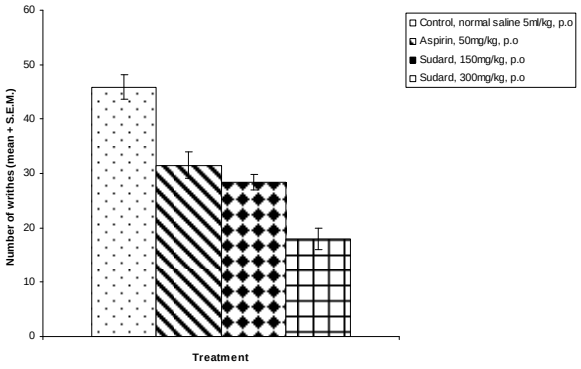

Fig 2. Effect of *sudard* on writhing response in mice ** $p<0.01$ as compared to control group

The initial phase of the edema is due to the release of histamine and serotonin and the edema is maintained during the plateau phase by kinin like substance [20] and the second accelerating phase of swelling due to the release of prostaglandin like substances. Inhibition of edema observed in formalin and carrageen models may be due to the ability of the *sudard* to inhibit these chemical mediators of inflammation.

Insertion of sponge pellet used for granuloma pouch, offer a model for exudation type of inflammation. *Sudard* showed potential inhibitory action on exudates formation. Kinin is said to be the main mediator of granuloma, as it both vasodilates and increase vascular permeability in the early stages of inflammation [21]. The effect of *sudard* on subacute inflammation confirmed that it inhibits the chemical mediators of inflammation.

The central analgesic activity of *sudard* was studied using hot plate method and peripheral activity in acetic acid induced writhing test. *Sudard* (150, 300 mg/kg) significantly increased the reaction time in hot-plate test and also reduced the writhing response in mice injected with acetic acid. Hence, it is speculated that apart from inhibition of chemical mediators of inflammation, *Sudard* may also modulate the pain response in the central nervous system.

As mentioned earlier, *Sudard* contains 11 different constituents and the formulation is described in the ancient ayurvedic literature. A survey on the activities of the constituents revealed that *Commiphora mukul*, *Mineral pitch*, *Colchicum luteum* and *Smilax glabra* are reported to be effective in experimental arthritis induced by mycobacterial adjuvant [1, 8, 9, 11]. *Commiphora mukul* contains mainly steroids, diterpenoid, carbohydrates and aliphatic esters [22]. The *Mineral patch* contains albuminoids, fatty acids and minerals [23]. *Colchicum luteum* contains alkaloids of which colchicine is the main constituent in addition to amino acids while the *Smilax glabra* has β-sitosterol and stigma sterol as its main constituents [24,25]

The other constituents of *sudard*; *Pluchea lanceolata*, *Paederia foetida*, *Vitex negundo*, *Zingiber officinalis*, *Strychnos nuxvomica* and *Ricinus communis* possess analgesic effect and also are effective in both acute and chronic inflammation [2-6,10]. *Pluchea lanceolata* is rich in volatile oil that contains methyl cinnamate, cineole, camphor and pinene [25]. *Paederia foetida* contains volatile oils alkaloids that include α-paederine and β-paedrine [22]. A number of constituents are known to be present in *Vitex negundo* which is known to contain alkaloids such as nishidine and hydrocotylene, glococononitol hydroxyl isopthalic acid, benzoic acid, tannic acid, aucubin, agnesside, casticin, oreintin, isooreintin and glucoside of tetrahydroxy monomethyl flavone [22]. *Zingiber officinalis*, commonly known as ginger contains zingerberol, borneol, linolool, gerariol, citral, gingerol, shogal, zingerone and resinous matter like starch mucilage [22]. *Strychnos nuxvomica* contains strychnine, brucin, strynic acid, vomicine and loganin [22]. *Ricinius communis* which is the common castor seeds, contains manly fixed oils which on hydrolysis yields ricinoleic acid. Other fatty acids present are isoricinoleic acid, stearic acid and iso-stearic acid [25].

The only constituent in *Sudard* that does not have any reported analgesic, anti-inflammatory or anti-arthritic effect is *Lepidium sativum*. However, this plant is reported to possess potent anti-oxidant effect [7]. The anti-oxidant action may indirectly help in treatment of inflammation by scavenging free radicals. *Lepidium sativum* contains volatile oil that has variable proportion of benzyl isothiocyanate and benzyl cyanide. It also has alkaloikds – glucotropucolin, sinapin and sinapic acid. Other constituents include protein, fat, carbohydrate and trace elements; iron, nickel, cobalt and iodine [23].

To conclude, the poly-herbal formulation '*sudard*' possess good analgesic, anti-inflammatory and anti-arthritic effects.

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