Original Article

Anti-arthritic Activity of *Dashanga Ghana* (An Ayurvedic Compound Formulation) Against Freund's Adjuvant Induced Arthritis in Charles Foster Albino Rats

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ABSTRACT

Introduction: Arthritis is the most common cause of disability, limiting the activities of adults throughout the world. Apart from the conventional treatment strategies using non-steroidal anti-inflammatory drugs, disease-modifying anti-rheumatic drugs, and glucocorticoids, newer and safer drugs are continuously being searched, as long-term usage of these drugs have resulted in adverse effects. Besides this, currently a number of medicinal plants are under scientific evaluation to develop a promising remedy in these cases. There is a need to investigate the complete therapeutic potential of these herbals for providing newer and safer treatment options with minimum side effects. Considering this, a polyherbal Ayurvedic compound formulation (*Dashanga Ghana*) has been studied in experimental animals to evaluate anti-arthritic activity. **Materials and Methods:** *Dashanga Ghana* has been prepared in the laboratory by following standard guidelines. Charles Foster albino rats were used to evaluate the activity through Freund's adjuvant induced arthritis model. **Results and Conclusions:** *Dashanga Ghana* is found to possess significant anti-arthritic activity. Further studies are required to identify and characterize exact active phyto-constituents and to elucidate the exact mechanism of action, which is responsible for the observed pharmacological profile.

Key words: Arthritis, Ayurveda, *Dashanga Ghana*, Freund's adjuvant, paw oedema

INTRODUCTION

Arthritis is the most common cause of disability, limiting the activities of adults throughout the world. The current treatment of arthritis is intended to minimize pain and inflammation with non-steroidal anti-inflammatory drugs, as well as to decelerate the progress of the disease by using disease modifying anti-rheumatic drugs. Because of the limitations and risks of conventional therapy, adverse reactions, chronic nature of the disease, and advanced age, people are exploring alternative measures to treat this

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condition. Alternative treatments, which are relatively safe and free of side effects, have been used both as adjunct and an alternative to conventional therapies in many countries.[1] Herbal therapies occupy a large section of alternative therapy. Ayurveda, the Indian traditional system of medicine, which is popular throughout the globe, has a large number of remedies that may be beneficial in cases of arthritis. Many such formulations are in common use even today.

Ayurvedic treasure house has a number of remedies, which can be beneficial in conditions of arthritis. The combination selected for this study (*Dashanga Ghana*) is based on the version available in Sharangadhara Samhita, which is said to be significant in combating various types of inflammation.[2] Considering this, this study was planned to revalidate anti-arthritic activity of the compound in complete Freund's adjuvant induced arthritis in Charles Foster albino rats.

MATERIALS AND METHODS

Test formulation

The formulation composition (*Dashanga Ghana*) is a combination of 10 herbal ingredients of equal parts [Table 1]. Drugs such as *Yashtimadhu*, *Tagara*, *Sukshmaila*, *Jatamansi*, *Haridra*, *Daruharidra*, *Shirisha,* and *Kushta* were procured from pharmacy, Gujarat Ayurved University, Jamnagar, Gujarat, India. *Raktachandana* was procured from Moodbidri, Karnataka, India and *Hribera* from Palakkad, Kerala, India. Random samples of the collected drugs were subjected to pharmacognostical studies with an intention to check their identity and genuineness at Pharmacognosy Laboratory, Institute for Post Graduate Teaching and Research in Ayurveda (IPGT and RA), Gujarat Ayurved University, Jamnagar, Gujarat, India. After establishing proper identity; dried individual ingredients were converted in to coarse powder (sieve 10). All the powders were mixed together thoroughly in specified proportions to prepare a homogenous blend, which was shifted to a stainless steel container, added with specified amounts (16 parts) of water and kept aside undisturbed throughout the night. On the following day, the contents were subjected to mild heat maintaining temperature in between 95 and 100°C. The contents were stirred constantly, in order to avoid the possibilities of settling down of the contents and their charring. When the volume was reduced to one quarter; the contents were filtered through a clean cotton cloth in to a stainless steel container to obtain decoction. The decoction was further subjected to the process of reboiling with continuous stirring till the contents become semi solid. The semi-solid contents were shifted in to a tray and subjected to drying maintaining the temperature around 50°C. The dried contents thus received (*Dashanga Ghana*) were collected carefully with the help of scraper and used in the study.

Table 1: Formulation composition of *Dashanga Ghana*

Drug	Botanical name	Part used	Quantity
Shirisha	*Albizia lebbeck* (L.) Benth.	Dried St. Bk.	1 part
Yashtimadhu	*Glycyrrhiza glabra* Linn.	Dried St.	1 part
Tahara	*Valeriana wallichii* DC.	Dried Rz.	1 part
Raktachandana	*Pterocarpus santalinus* Linn.	Dried Ht. Wd.	1 part
Sukshmaila	*Elettaria cardamomum* (Linn.) Maton	Dried Sd.	1 part
Jatamansi	*Nardostachys grandiflora* DC.	Dried Rz.	1 part
Haridra	*Curcuma longa* Linn.	Dried Rz.	1 part
Daruharidra	*Berberis aristata* DC.	Dried St.	1 part
Kushta	*Saussurea lappa* CB. Clarke	Dried Rt.	1 part
Hribera	*Coleus vettiveroides* KC. Jacob	Dried Wh. Pl.	1 part

Animals

Charles Foster albino rats weighing 200 ± 30 g of either sex were obtained from the animal house attached to the Pharmacology Laboratory, IPGT and RA, Gujarat Ayurved University, Jamnagar, Gujarat, India. The animals were maintained on "Amrut" brand animal pellet feed of Pranav Agro Industries and tap water was given *ad libitum*. The temperature and humidity were kept at optimum and animals were exposed to natural day and night cycles. The experiments were carried out in conformity with the guidelines of the Institutional Animal Ethics Committee after obtaining its permission (IAEC03/08-11/PhD/02) and care was taken as per the Committee for the Purpose of Control and Supervision of Experiments on Animals guidelines.

Acute toxicity study

Acute oral toxicity of *Dashanga Ghana* formulation was carried out in female Charles Foster albino rats as per the OECD 425 guidelines. The formulation was tested at the limit dose of 2000 mg/kg body weight. The result showed that *Dashanga Ghana* did not produce any changes in observed parameters and there was no mortality even at the limit dose. The animal dose was extrapolated from human therapeutic dose mentioned in classical literature.

Dose fixation

Dose of the test formulation was fixed by extrapolating the human dose to laboratory animals on body surface area ratio.[3] The adult human dose (500 mg) was converted to animal dose. On this basis the rat dose was found to be 45 mg/kg. The test drug was suspended in distilled water by making uniform suspension with 0.5% carboxy methyl cellulose with suitable concentration depending up on body weight of animals and administered orally with the help of gastric catheter sleeved to syringe. The drug was administered to overnight fasted animals.

Anti-arthritic activity

The selected animals were grouped into four groups of 6 rats each. To the first group (normal control) tap water was administered. Second group (arthritic control) was also administered with tap water orally and injected with Freund's adjuvant. Third group (test drug group) was administered with *Dashanga Ghana* in the dose of 45 mg/kg. The fourth group (reference standard [RS]) was administered with the standard drug dexomethasone (100 µg/kg). The test drug and RS were administered for 30 consecutive days. On day 1, the complete Freund's adjuvant was made into fine emulsion with the help of a syringe and 0.1 ml of it was injected beneath the plantar aponeurosis in the left hind paw and 0.05 ml subcutaneously into the root of the tail. The volumes of both the hind paws were measured with the help of plethysmometer just before the adjuvant injection (initial). The paw volume of left hind limb was measured at 2nd, 3rd, 5th, 10th, and 15th day, while of right hind limb on 15th, 20th, 25th, and 30th day. Paw volume of the 0 (initial) days were taken as the reference value for determining the increase in paw volume on the subsequent days. On 31st day, animals were weighed and sacrificed by over dose of ether anesthesia. Blood was collected from neck blood vessels and serum was separated for the estimation of biochemical parameters.[4] Parameters such as blood urea,[5] serum creatinine,[6] serum glutamate oxaloacetate transaminase,[7] serum glutamate pyruvate transaminase,[8] and serum alkaline phosphatase[9] were estimated by feeding requisite quantity of serum to the auto analyzer (Fully Automated Biochemical Random Access Analyzer, BS-200; Lilac Medicare Pvt. Ltd., Mumbai, Maharashtra, India) which was automatically drawn in to the instrument for estimating different parameters. References given in the kit literature mentioning the basis of the methods on which test procedures was carried out. Further both right and left synovial joints were dissected out and the histopathological slides were prepared by referring to standard procedure.[10] The slides were viewed under trinocular research Carl-Zeiss's microscope at various magnifications to note down the changes in the microscopic features.

Statistical analysis

The obtained data have been presented as a mean ± standard error of the mean, difference between the groups was statistically determined by Student's *t*-test for both paired and unpaired data for the treated group with the level of significance set at $P < 0.05$.

RESULTS

A normal progressive gain in body weight was observed in normal control group when the final values were compared with initial body weight. In arthritic control group significant decrease in body weight was seen in comparison to both initial values, as well as values of control group [Table 2]. Treatment with *Dashanga Ghana* showed gain in body weight similar to that of normal control group. In contrast an apparent and statistically significant decrease in body weight was found in dexomethasone treated group.

An apparent suppression in paw volume was observed in *Dashanga Ghana* treated group and also in dexamethazone treated group at different time intervals. The observed suppression in paw oedema in *Dashanga Ghana* treated group at 2nd, 5th, and 10th day was statistically significant in comparison to arthritis control group. In dexomethasone (RS) administered group the suppression of oedema observed at all the time intervals is found to be statistically significant [Table 3].

Treatment with *Dashanga Ghana* apparently inhibited the secondary oedema at all the time intervals, however only the inhibition observed on 30th day was found to be statistically significant in comparison to arthritic control group. In dexomethasone treated group inhibition of secondary oedema at all-time intervals in comparison to arthritic control group was highly significant ($P < 0.001$) [Table 4].

Statistically highly significant increase in blood urea was observed in arthritic control group in comparison to normal control group ($P < 0.001$) [Table 5]. Treatment with *Dashanga Ghana* significantly attenuated blood urea level. In dexamethsone treated group also significant attenuation of blood urea was observed. The other serum biochemical parameters are not altered to significant extent in arthritic control group and also in *Dashanga Ghana* treated group.

Microscopic examination of both the joints [Table 6] showed normal intact joint cytoarchitecture in normal control rats [Figure 1a]. In Freund's adjuvant arthritis control rats, remarkable degenerative changes in the form of cartilage erosion, synovial membrane proliferation and hyperplasia, and pannus formation were observed in both the joints [Figure 1b]. These changes were found to be very much decreased in *Dashanga Ghana* treated group [Figure 1c] and dexamethsone treated group [Figure 1d].

Table 2: Effect of the test drugs on body weight

Groups	Initial body weight (g)	Final body weight (g)	Actual change in body weight (g)	Percentage change in comparison to initial
Control	202.20±5.50	221.50±4.91	16.00±4.40	9.54↑
Arthritic control	192.00±22.40	179.66±14.84	−21.60±09.54###	06.42↓
Dashanga Ghana	177.50±5.80	199.25±5.52	21.75±10.55	12.42↑
Dexomethasone	224.50±9.17	158.50±10.53	−66.0±4.96###	29.48↓

Data: Mean±SEM, ###P<0.001 (comparison to initial body weight, paired *t*-test). ↑ = Increase, ↓ = Decrease, SEM = Standard error of the mean

Table 3: Effect of the test drugs on primary paw oedema (oedema of left hind paw)

Groups	Percentage increase in paw oedema when compared to initial paw volume				
	2nd day	3rd day	5th day	10th day	15th day
Arthritic control	59.37±4.33	55.65±3.27	61.20±5.10	60.50±7.80	56.19±6.47
Dashanga Ghana	41.73±4.55*	48.42±4.02	40.22±2.37**	40.41±2.10**	44.93±4.49
Dexomethasone	38.99±2.57**	32.38±2.18***	23.51±5.55**	22.55±6.44**	27.26±4.37*

Data: Mean±SEM. *P<0.05, **P<0.01, ***P<0.001 (comparison to arthritic control group, unpaired t-test). SEM = Standard error of the mean

Table 4: Effect of the test drugs on secondary paw oedema (oedema of right hind paw)

Groups	Percentage increase in paw oedema when compared to initial paw volume			
	15th day	20th day	25th day	30th day
Arthritic control	15.59±1.62	21.33±2.27	23.27±1.22	22.80±0.98
Dashanga Ghana	13.13±2.05	16.20±1.14	16.46±5.79	11.63±1.45*
Dexomethasone	10.36±0.98*	11.35±0.57**	6.68±0.91***	5.06±1.17***

Data: Mean±SEM. *P<0.05, **P<0.01, ***P<0.001 (comparison to arthritic control group, unpaired t-test). SEM = Standard error of the mean

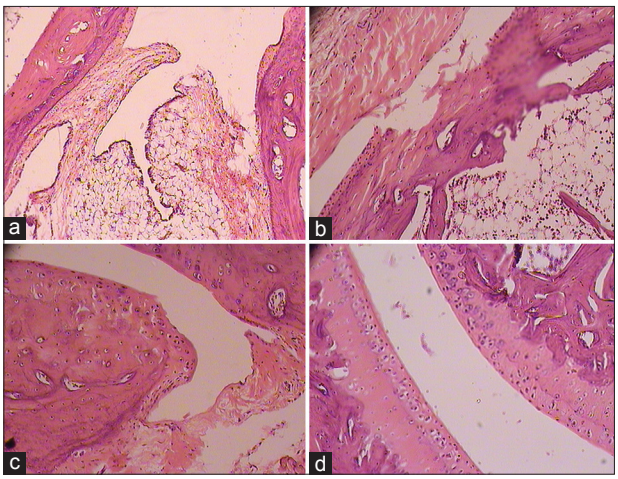

Figure 1: (a) Photomicrographs of joint from control group (×100). (b) Photomicrographs of joint from Freund's adjuvant arthritis control group (×100). (c) Photomicrographs of joint from *Dashanga Ghana* treated group (×100). (d) Photomicrographs of joint from dexamethsone treated group (×100)

DISCUSSION

The Freund's adjuvant induced arthritis model in rats is the most common model used by several scientists to evaluate anti-arthritic activity of new drugs. This model closely resembles clinical arthritis and is the most widely used model of experimental arthritis which has been used for screening purposes in the disease produced in the rat by injection of complete Freund's adjuvant into certain dermal and tissue sites.[11] Therefore, this model is used with a relatively high degree of validity for evaluating agents with potential anti-arthritic activity. Researchers distinguished four phases of arthritis on the basis of biochemical markers of arthritis viz., days 1–4 with acute local inflammation and systemic effects, days 7–12 with remission of acute inflammation and periarthritis, days 12–28 with chronic inflammation, periarthritis, and osteogenic activity and day 35 onward with permanent articular deformity and minimal inflammation.[12] This model has been used as a model of subchronic or chronic inflammation in rats and is considerable relevance for the study of patho-physiology and pharmacological control of inflammatory processes.

Body weight was considered as an indirect index of health status and recovery from disease. A change in body weight of rats was also measured as one of the parameter to assess the course of the disease and the response to therapy of anti-inflammatory and anti-arthritic drugs. As the incidence and severity of arthritis is increased, a decrease in body weights of the rats also occurred during the course of the experimental period and this observation was supported by the findings of previous study on alterations in the metabolic activities of diseased rats.[13] It has been suggested that, the decrease in body weight during inflammation is due to deficient absorption of nutrients through the intestine and that treatment with anti-inflammatory drugs normalizes the process of absorption.[14] The evident restoration of the body weight of rats in the *Dashanga Ghana* treated group may involve improvement of intestinal absorption of the nutrients and a reduction in the distress caused by the severity of the arthritis.

The determination of paw swelling is apparently simple, sensitive, and quick procedure for evaluating the degree of inflammation and assessing of therapeutic effects of drugs. In this study, the rat was selected as an animal model since they develop a chronic swelling in multiple joints with an influence of inflammatory cells and followed by erosion of cartilage in joints and destruction of bones. Paw volumes of both hind limbs were recorded on the day of adjuvant injection and again measured at 2nd, 3rd, 5th, 10th, and 15th day, and 15th, 20th, 25th, and 30th day for primary and secondary lesions, respectively. The 15th day measurement is indicative of primary lesions and its onward measurement will aid in estimating secondary lesions. On 21st day, the secondary phase of rheumatoid arthritis becomes more evident and inflammatory changes spread systemically and become observable in the limb not injected with Freund's adjuvant. This is because of the manifestation of cell-mediated immunity.[15]

Dashanga Ghana produced significant effect on primary oedema and moderate effect on secondary edema. In

Table 5: Effect of the test drugs on serum biochemical parameters

Parameters	Control	Arthritic control	*Dashanga Ghana*	Dexomethasone
Blood urea (mg/dl)	32.25±3.19	79.75±4.94^{aaa}	62.00±4.16*	44.50±3.47***
Creatinine (mg/dl)	00.60±0.04	00.63±0.06	00.59±0.02	00.65±0.09
SGPT (IU/L)	59.00±7.78	68.00±12.96	72.75±11.15	64.25±17.13
SGOT (IU/L)	167.50±18.55	196.22±16.74	170.50±63.49	182.50±25.61
Alkaline phosphates (IU/L)	256.75±31.82	238.00±77.33	209.25±19.80	232.50±54.34

Data: Mean±SEM, $^{aaa}P<0.001$(comparison to normal control group, unpaired *t*-test), *$P<0.05$, ***$P<0.001$ (comparison to arthritic control group, unpaired *t*-test). SEM = Standard error of the mean, SGPT = Serum glutamate pyruvate transaminase, SGOT = Serum glutamate oxaloacetate transaminase

Table 6: Effect of the test drugs in synovial joints (histopathological observations)

Organs	Arthritic control	*Dashanga Ghana*	Dexomethasone
Left synovial joint	Bone and cartilage erosion, synovial hyperplasia, pannus formation	Almost normal joint structure	Almost normal joint structure
Right synovial joint	Bone and cartilage erosion, synovial hyperplasia, pannus formation	Almost normal joint structure	Almost normal joint structure

contrast, dexomethasone administered group showed significant suppression of both primary and secondary oedema. This indicates presence of moderate to significant anti-arthritic activity in the test formulation and significant anti-arthritic activity in RS. This observation was further evidenced by histopathological study where joints from both test formulation and RS treated animals showed remarkable protection against Freund's adjuvant induced degenerative changes in the form of cartilage erosion, synovial membrane proliferation, hyperplasia, and pannus formation in both the joints.

Among the five serum biochemical parameters studied only one parameter was affected to significant extent by injection of Freund's adjuvant. Statistically significant increase in blood urea was observed in arthritic control group in comparison to normal control group. Increased blood urea level was reported in arthritic rats and it was hypothesized that substantial fraction of blood urea in arthritic rats comes from arginine synthesized in the kidney.[16] The test formulation significantly attenuated blood urea level. In dexamethsone treated group also significant attenuation of blood urea was observed.

Dashanga Ghana is a polyherbal compound formulation. Previously we have reported that this formulation have significant anti-inflammatory and analgesic activities.[17] Further, different fractions of the individual ingredients have been studied for analgesic, anti-inflammatory and anti-arthritic activities. *Sirisha* (*Albizia lebbeck*) has anti-inflammatory, analgesic activities.[18] *Yastimadhu* (*Glycyrrhiza glabra*) is anti-inflammatory, anti-arthritic and antioxidant.[19-21] *Tagara* (*Valeriana wallichii*) is analgesic.[22] *Raktachandana* (*Pterocarpus santalinus*) is anti-inflammatory, analgesic, and antioxidant.[23,24] *Jatamansi* (*Nardostachys grandiflora*) is antioxidant.[25] *Haridra* (*Curcuma longa*) is anti-inflammatory, antirheumatic, analgesic, and antioxidant.[26-30] *Daruharidra* (*Berberis aristata*) is anti-inflammatory.[20] *Kushta* (*Saussurea lappa*) is anti-inflammatory, anti-arthritic, and antioxidant.[31,32] These properties of individual drugs will contribute in expression of observed activity profile of the compound formulation.

CONCLUSIONS

Dashanga Ghana possesses significant anti-arthritic activity and can be used in treating different forms of arthritis. Thus this study provides unequivocal evidence of traditional use of this formulation in the management of different inflammatory conditions including arthritis. However, further studies are required to identify and characterize exact active phytoconstituents and to elucidate the exact mechanism of action, which is responsible for the observed pharmacological profile with an intension to provide newer and safer treatment options with minimum side effects.

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Conflicts of interest

There are no conflicts of interest.

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