Clinical evaluation of *Dashangalepa* formula for pain and swelling of joint diseases with *in-vitro* anti-inflammatory activity

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Abstract

Joint diseases are categorized into different types based on their symptoms in Ayurveda, predominant symptoms with pain and swelling, loss of activity, present deformities. Among them *Sandigatha vata* (osteoarthritis) and *Amavata* (Rheumatoid arthritis) are very common conditions. *Dashangalepa* is used as topical application to manage acute and chronic painful inflammatory musculoskeletal conditions. Aim of this study to evaluate the efficacy of reducing pain and swelling with anti- inflammatory activity. Volunteer Sixty (60) patients with *Sandigatha vata* or *Amavata* were selected and divided into two groups (n=30). Group I and II apply the test drug *Dashangalepa* and reference drug Rumalaya gel respectively. Pain was evaluated using universal pain assessment tool and swellings of the joints were taken by using tape measurements, in every 14 days for 8 weeks. Biochemical investigations were estimated for ESR, RH-factor, C-reactive proteins, SGOT, SGPT. Statistically significant reduction ($P< 0.05$) was observed in symptoms treated with test drug. Biochemical parameters not showed any alterations in pre and post treatment stages, which confirmed its safer efficacy. In vitro anti- inflammatory activity of *Dashangalepa* aqueous acidic preparation and Rumalaya gel were assessed separately using Human Red Blood Cell membrane stabilizing activity method, results revealed activity of both test and reference drug are similar and activity is increased by increasing the concentrations. Results can be concluded that test drug is similar herbal formula to previously proven reference drug, with safer effective anti-inflammatory pain and swelling reducing herbal formula for treat joint diseases.

Keywords: Joint diseases, pain and swelling, anti-inflammatory, topical application

Introduction

Joint diseases are described in different aspects in Ayurveda medicine, as per the concepts vitiated doshas comes to the joint and caused pain and swelling of that joint, that causes displace the joint and disturbed the activity of that particular joint. In Ayurveda system of medicine joint diseases are categorized into different types according to their symptoms[1]. They are pain predominant diseases (*Shoola pradhana*), swelling predominant diseases (*Shotha pradhana*), pain and swelling predominant diseases (*Shoola, Shotha pradhana*), diseases predominant loss of activity (*Kriya hani pradhana*) and deformities predominant diseases (*Vikurthi pradhana*)[2]. Among the several joint diseases *Sandigatha vata* (osteoarthritis) and *Amavata* (Rheumatoid arthritis) are very common conditions symptomatically present pain and swelling in patients. Causative factors for these diseases are age factors, structural changes due to the inflammation, free radical activity, and behavioral changes as well as climatic conditions.

About 1% of the world population is afflicted by rheumatoid arthritis, women are affected 3 times than man, rheumatoid arthritis occurred throughout

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the world and all the ethnic groups, climates, altitude and geography[3]. Osteoarthritis (OA) is more generalized and more serve in older women. It is estimated that approximately 50% of the world population over 65 years of age is affected by osteoarthritis[4]. Osteoarthritis is characterized by joint pain and tenderness, limitation of movements, crepitus, occasional effusion and variable degree of inflammation without systemic effects[5].

Inflammation is considered as a primary physiological defense mechanism that helps body to protect itself against infection, burn, toxic chemicals, allergens or other noxious stimuli[6]. Although it is a defense mechanism, the complex events and mediators involved the inflammatory reaction can induce, maintain or aggravate many diseases[7]. The drugs used in the treatment of rheumatoid arthritis act rapidly, relieve pain and control inflammation, and help to improve and maintain joint function and to prevent deformities.

Dashangalepa (DL) is a polyherbal drug preparation which is used for edematous conditions as topical application to manage acute and chronic painful inflammatory musculoskeletal conditions in Ayurveda system of medicine[8,9]. Furthermore, topical applications offer short and long term safety from adverse events such as burning, stinging and erythema because topical applications are mainly limited to the site of application[10].Therefore it is important to develop and evaluate the efficacy of topical applications practice in Ayurveda clinical practices. Clinical studies have proven the benefits of topical analgesics, in the management of certain acute and chronic painful inflammatory musculoskeletal conditions[11]. Currently used anti-inflammatory drugs are associated with some severe side effects. Therefore, the development of potent anti- inflammatory drugs with fewer side effects is necessary. Therefore, this attempt has been taken to evaluate the efficacy of *Dashngalepa* formula with its anti-inflammatory effect.

Materials and methods

Selection of patients

Volunteer Sixty (60) patients with clinical symptoms of pain and swelling in *Sandigatha vata* (osteoarthritis) or *Amavata* (rheumatoid arthritis) were included from selected Ayurveda hospitals of Sri Lanka. Ayurveda Teaching Hospital Borella, Bandaranayake Memorial Ayurveda Research Institute, Navinna, in western province and Ayurveda base Hospitals of Ratnapura, Kegalla and Embilipitiya in Sabaragamuwa province of Sri Lanka after the approval of the Ethical Review Committee of Institute of Indigenous Medicine, University of Colombo, (ERC 12/01) 2012. The patient whose age was between 20-80 years of both sexes and the patients who were having signs and symptoms of pain and swelling were included to the study and patients with renal, hepatic or cardiac problems, bone fractures, pregnant and nursing mothers were excluded from the study. A detailed clinical examination and relevant laboratory investigations were conducted and all required information were documented in a specially prepared performa with written consent of the patients.

Preparation of drug

Dashangalepa herbal formula was prepared according to the *Lepa* (external applications) preparation method of Ayurveda Pharmacopeia. Shade dried ten ingredients of *Dashangalepa Albizzia lebbeck* (stem bark), *Glycerhizzia glabra* (stem parts), *Valeriana wallchi* (whole plant), *Pterocarpus santalinus* (heart wood), *Elataria cardamomum* (seeds), *Nadostachus jatamansi* (root), *Curcuma longa* (rhizome), *Cosinium fenestratum* (stem parts), *Saussurea lappa* (roots) and *Plectrathus zeylanicus* (whole plant) were finely powdered separately, using a mechanical grinder (Disk Mill, SHC 23, China). Equal amounts (10g) of powder of ten ingredients of the *Dashangalepa* topical application were mixed together and prepared the powder form of *Lepa* (paste).

Selection of reference drug

Rumalaya gel (R) polyherbal formula was taken as the standard reference drug for the clinical study. Rumalaya gel has analgesic, anti-inflammatory, antioxidant, counterirritant, glycosaminoglycan building and cartilage healing properties. It also has vasodilatation of cutaneous vasculature, which increases blood circulation and produces a feeling of warmth. Consequently, cutaneous receptors are stimulated for thermal sensations, which serve to distract deep seated pain sensations[12].

Ingredients of Rumalaya gel are *Menthaavensis (Pudina*), *Gaulthera fragrantissima (Gandhapura taila), Pinusroxburghii (Sarala), Cinnamomum zeylanicum (Thvak), Cedrus deodara (Badradaru), Vitex negundo (Nirgundi), Boswella serrata (Shallaki)* and *Zingiber oficinale (Shunti).*

Administration of drug

The patients were divided into two groups (n=30), test drug DL (group I) and R (group II) were distributed among them. Test drug of 100g packets were given and advised them to convert the powder into a paste according to the following instructions. Put the sufficient amount of powder into a clay pot and mixed with fresh juice of leaves of *Tamarindus indicus* until cover the amount of powder. Then convert it into a semi solid paste by application of mild heat. Apply the paste over the swollen joints with pain and swelling once day, and kept it for eight hours, for 14 days. Patients were instructed to apply Rumalaya gel amount need to cover the area of swelling from the 30gram of tube as a topical application once a day and kept it until dries up, for 14 days. Symptomatically improvement of pain and swelling of each group was recorded.

Assessment criteria

Symptomatically improvement of pain and swelling of each group was recorded using clinical performa once in every 14 days for 8 weeks. Biochemical investigations included ESR, RH-factor, C-reactive proteins, SGOT, SGPT were performed before and after the treatment. Joint pain was evaluated according to visual analogue scale using universal pain assessment tool13,14,15 as described here [(0-no pain), (1-mild pain= can be ignored), (2-moderate pain=interferes with tasks/sleep),(3-severe pain= interferes with basic needs)]. Evaluation of swelling of the joints were done by using tape measurements16. Follow up was done after 8 weeks of treatment period for two months of duration in every 14 days.

Statistical analysis

The reduction in pain and swelling score were evaluated comparing to standard reference drug. Efficacy of DL preparation comparatively with the reference drug was evaluated by using swelling measurements and pain reduction scale. Paired sample t- test was used with 95% confidence interval to analyze the efficacy of this preparation.

Experimental evaluation of *Dashangalepa* formula

Preparation of acidic aqueous extract of *Dashangalepa* for anti-inflammatory activity

The powdered preparation of *Dashangalepa* (100 g) mixture was added to 400 mL of fresh juice of Tamarindus indicus (*Siyabala)* leaves and kept on the shaker for six hours. The mixture was filtered and the filtrate was freeze dried using a freeze dryer (Labconco, cat. 01, Missouri). The weight of the extract was recorded.

Investigation of in vitro anti-inflammatory activity of aqueous extract of Dashangalepa and Rumalaya gel

In vitro anti- inflammatory activity of DL aqueous acidic preparation and Rumalaya gel were assessed separately using HRBCMS (Human Red Blood Cell membrane stabilizing activity) method as described previous research17. From the test samples of the drug preparation (DL) and (R) (1 mg / mL) were dissolved in 0.2 mL of DMSO and diluted to 5 mL by adding appropriate amount of normal saline. The reaction mixture was prepared with 5 mL of test solution and 0.5 mL of 10 % RBC suspension. The negative control was prepared using equal amount of DMSO and normal saline (Figure 1). All the centrifuge tubes containing reaction mixtures were incubated in a water bath at 56^{o}C for 30 min and the

tubes were cooled under running tap water (Figure 2). The reaction mixtures were centrifuged at 3000 rpm for 10 min and the absorbance of the supernatants were taken at 560 nm using UV-Visible spectrophotometer (Aqua Mate 8000, Singapore). The test sample was performed in triplicates and the percentage stability was calculated using the equation (1) mentioned below.

$$***\%\ Stability\ = \frac{A_{control} - A_{sample}}{A_{control}} \text{--------} (1)$$

Figure 1 and 2 shows the HRBCM stability assay conducted for anti-inflammatory activity

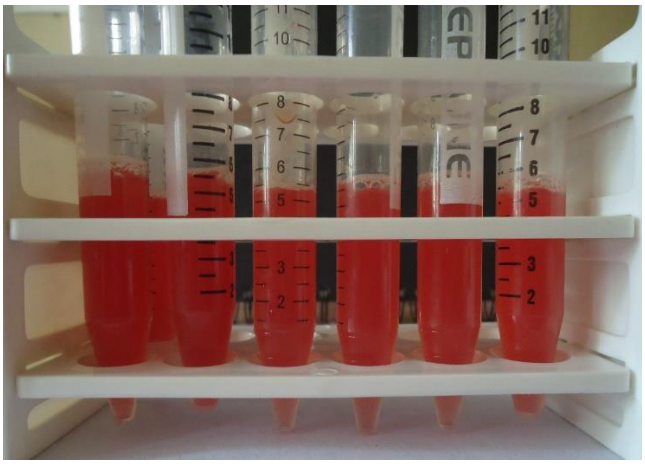


Fig. 1: With different concentrations of DL extract before incubation

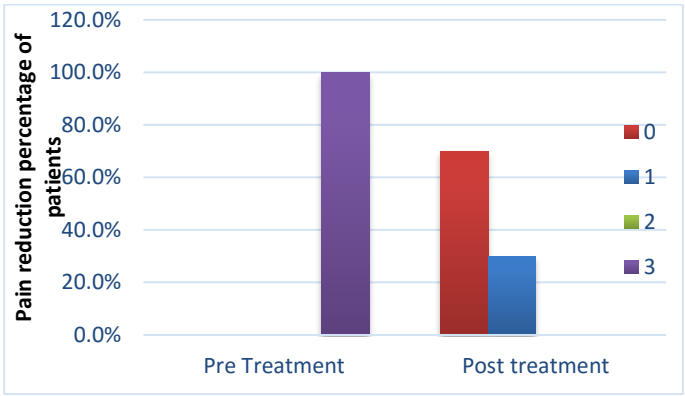
Fig. 2: With different concentrations of DL extract after incubation

The percentage stability versus test concentrations were plotted in order to compare the anti-inflammatory activity with the standard drug separately. The IC50 of the samples were calculated using Probit analysis (MINITAB® Release 14.2 Minitab Inc. 2003 statistical software).

Results

Observations of the clinical study

Total sixty patients were completed this trial, among them age incidence of the cases registered for this study belongs to the age group between twenty to eighty years. Hundred percent (100%) of patients in pretreated stage included to severe pain category of the group treated with the DL preparation. The observations revealed pain reduction as a percentage was reduced up to 65% (no pain), 35% (mild pain) are shown in Figure 3 at the stage of post treatment. There was no any patient remain in severe or moderate pain categories. These results revealed DL is an effective preparation. Pain reduction of post treated stage in group of DL preparation demonstrated significant change at the level of 95% confidence interval of the difference (P< 0.05).

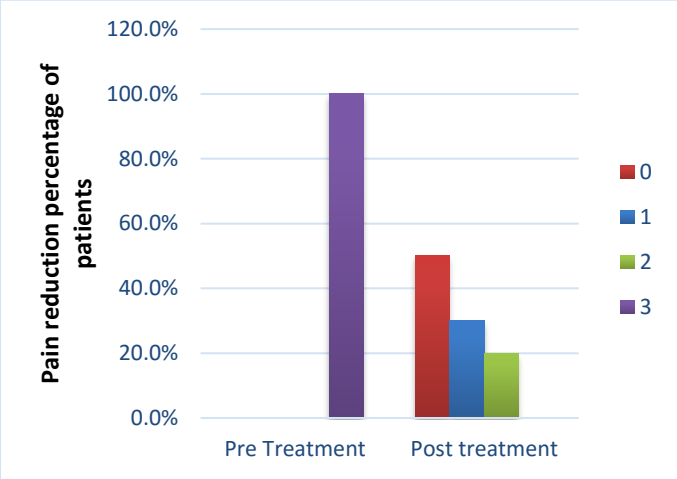


0 - no pain 1 – mild pain 2 – moderate pain 3 – severe pain

Fig. 3: Effectiveness of *Dashangalepa* on pain of the arthritis patients (n = 30)

Hundred percent (100%) of patients in pretreated group of Rumalaya Gel included severe pain category, shown at post treatment stage shown the percentage number of patients in that group reduced to 50%, 30%, and 20% no pain, mild pain, moderate pain respectively are demonstrated in Figure 4.

There were no any patient remain in severe pain category. Pain reduction of post treated group of (R) observed significant change at the level of 95% confidence interval of the difference (P< 0.05).



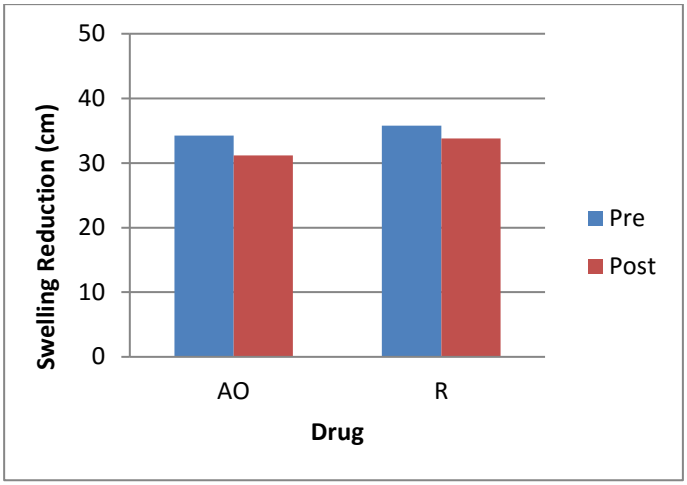
0 - no pain 1 – mild pain 2 – moderate pain 3 – severe pain

Fig. 4: Effectiveness of Rumalaya gel on pain of the arthritis patients (n = 30)

Reduction of swelling

Swelling reduction among DL compares to the standard drug Rumalaya gel in pre-treatment and post treatment stages were recorded considering the tape measurements. Pre-treatment and post treatment mean values of swelling measurements of standard drug Rumalaya gel are 36cm and 34cm respectively. The swelling reduction between pre and post treatment 2cm is shown in Figure 5.

Test drug DL mean value of swelling reduction of pre and post treatment was 34 cm and 31 cm respectively and the difference of swelling reduction between pre and post treatment is 3 cm demonstrated in Figure 5.



DL – Dashangalepa R- Rumalayagel Pre – Pre-treatment Post – Post treatment

Fig. 5: Effectiveness of DL and Rumalaya gel on swelling of the arthritis patients (n = 60)

Evaluation of biochemical parameters

Evaluation of biochemical parameters ESR, Rheumatoid Factor, C– reactive Protein, SGOT and SGPT no significant alterations were seen and they were demonstrated in Tables 1 and 2.

Results of bio chemical parameters; ESR, Rh factor, CRP, SGOT and SGPT not shown any alterations in pre and post treatment, it was not significant change in biochemical parameters.

Evaluation of Anti- inflammatory activity of Dashangalepa preparation

Results of Red Blood Cell Membrane stabilizing activity of *Dashangalepa* formula and standard drug R are presented in Figure 5. The results indicate that anti-inflammatory effect of DL preparation was similar to that of Rumalaya gel.

According to the results of membrane stabilizing activities of RBCM, it was shown that the activity is increased by increasing the concentration of *Dashangalepa* preparation and Rumalaya gel.

Results of bio chemical parameters

ESR, Rh factor, CRP, SGOT and SGPT not shown any alterations in pre and post treatment, it was not significant change in biochemical parameters.

Table 1: Biochemical Investigations of DL_4 treated group (n = 30)

Parameter	Pre-Treatment	Post Treatment	P value	Significance
ESR	49.06 ± 28.6	40.46 ± 29.5	0.000	NS
Rh Factor	1.37 ± 0.5	1.27 ± 0.5	0.184	NS
CRP	7.38 ± 12.8	7.79 ± 13.8	0.545	NS
SGOT (IU/L)	15.18 ± 6.4	15.57 ± 7.2	0.368	NS
SGPT (IU/L)	15.07 ± 7.6	14.74 ± 6.5	0.469	NS

Table 2: Biochemical Investigations of R treated group (n = 30)

Parameters	Pre-Treatment	Post Treatment	P value	Significance
ESR	37.7 ± 18.4	31.13 ± 17.41	0.001	NS
Rh Factor	1.17 ± 0.3	1.13 ± 0.3	0.326	NS
CRP	3.6 ± 4.6	2.8 ± 1.9	0.272	NS
SGOT (IU/L)	14.75 ± 6.1	14.94 ± 6.2	0.469	NS
SGPT (IU/L)	14.16 ± 5.1	14.96 ± 5.3	0.024	NS

Evaluation of Anti- inflammatory activity of Dashangalepa preparation

Results of Red Blood Cell Membrane stabilizing activity of *Dashangalepa* formula and standard drug R are presented in Figure 6. The results indicate that anti-inflammatory effect of DL preparation was similar to that of Rumalaya gel.

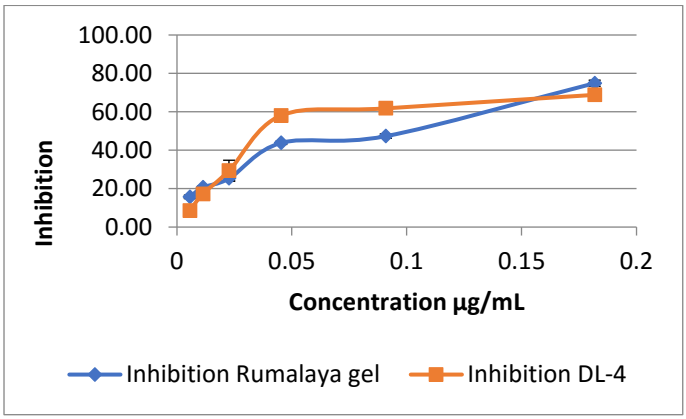


Fig. 6: Red Blood Cell membrane stabilizing activity of Dashangalepa preparation and Rumalaya gel. Each value is expressed as mean ± SD (n=3).

According to the results of membrane stabilizing activities of RBCM, it was shown that the activity is increased by increasing the concentration of *Dashangalepa* preparation and Rumalaya gel.

Erythrocyte membrane is structurally analogous to the lysosomal membrane. The IC_{50} (50 % Inhibition concentration) values obtained for anti-inflammatory assays are used for the interpretation of the results that is defined as the concentration of the test sample required to stabilize 50 % the red blood cell membrane where the response is reduced by half. Stabilization of lysosomal membrane is important in limiting the inflammatory response of reactive species[18]. Therefore, the use of erythrocyte membrane is a good model to study the protective effect of herbal products for evaluate Anti-inflammatory activity as an in-vitro evaluation method[19].

EC_{50} values of the tested preparations DL and R were estimated using Probit analysis (MINITAB® Release 14.2 Minitab Inc. 2003 statistical software) the results were given evidence of that similar EC_{50} values of and DL and Rumalaya gel 0.13 ± 0.21, 0.11 ± 0.02 (µg /mL) respectively indicate the effects of these preparations were similar to each other. The lower EC_{50} values demonstrated the higher anti– inflammatory activities that give beneficial for this product.

Discussion

According to the pharmacological properties describe in Ayurveda *Dravyaguna Vignana* this formula contained best *Vedana sthapana* (pain reliever) and *Shotha hara* (eradicated edema) drugs *Albizzia lebbeck, Glycerhizzia glabra, Valeriana wallchi, Pterocarpus santalinus, Nadostachus jatamansi, Curcuma longa, Cosinium fenestratum, Saussurea lappa* as ingredients[20].

Both diseases investigated in this study are pain (*Vedana /Shoola*) and swelling (*Shotha*) predominant diseases. In Ayurveda texts they have stated *A. lebbeck* (AL) is *Vedana sthapana* (pain reducing) and *Shotha hara* (swelling reducing) drug material[21]. Rareness of AL many other substitutes were used for this DL formula instead of (AL), therefore This clinical study with experimental part designed to evaluate the efficacy of DL prepared with *Albizzia lebbeck* original plant in Sri Lanka to reduce the pain and swelling of joint diseases that main symptoms complain by the patients.

Erythrocyte membrane is structurally analogous to the lysosomal membrane. Stabilization of lysosomal membrane is important in limiting the inflammatory response of reactive species. Therefore, the use of erythrocyte membrane is a good model to study the protective effect of medicinal plant extracts and the herbal products. The ingredient of this formula *Albizzia lebbeck* shown its solvent and aqueous extracts already proven for Anti - inflammatory activity[22].

When considering the causative factors for this type of degenerative changes of the bony structures defense mechanism such as anti –oxidants are very important for drug evaluation with their discoveries. Many research papers have been published on the antioxidant properties of *Albizia* species[23]. reported the antioxidant activity of *A. julibrissin* and in their study it was reported that the bark extract contained quercetin derivative, hyperoside and quarcitrin which contributes to the higher antioxidant activity. As AL is included to the same genus it also has the trend to constitute with similar chemical compounds that may help to show the certain efficacious results. Antioxidants are the health protective compounds that responsible to neutralize the free radical formation in the body which destruct the body tissues same way effect on the bony structures regarding to the bone and joint diseases. Antioxidant activity of the bark extract of *A. lebbeck* has been studied by using DPPH and reducing power already proven its anti-oxidant power[24].

This study confirmed swelling reduction efficacy of drug preparation effective than the standard reference drug Rumalaya gel for reducing swelling in arthritis patients. Certain other ingredients of the preparation such as *Curcuma longa* and *Glycyrrhizaglabra* which were known as anti-inflammatory herbs[25,26].

Earlier research proved plant extracts exerted maximum membrane stabilizing activity to standard anti- inflammatory drugs. The mode of action of the extract of the formula and standard anti-inflammatory drugs could be connected with binding to the erythrocyte membranes with subsequent alteration of the surface charges of the cells. This might have prevented physical interaction with aggregating agents or promote dispersal by mutual like repulsion charges which are involved in the haemolysis of red blood cells. Research reports were proven that methanol and aqueous extracts of plants presence of saponins and tannins aided to exhibit the anti-inflammatory activity [27]. Earlier research reported ingredient AL was positive for saponin[28] and also reported significant anti-inflammatory effect can be induced due to the presence of glycosides or steroids[29]. Present study also exhibited freeze dried aqueous extract of DL have red blood cell membrane stabilizing activity was demonstrated well. These findings confirmed that this preparation can be developed as anti-inflammatory value-added product to treat arthritis patients in Ayurveda system of medicine.

Earlier research reported Rumalaya gel has the beneficial effect for heal arthritis patients due to the synergistic actions of its ingredients. Rumalya gel has analgesic, anti- inflammatory, antioxidant, counterirritant, glycoseaminoglycan- building and cartilage healing properties. Rumalaya gel induces vasodilation of cutaneous vasculature, which

increases blood circulation and produces a feeling of warmth. Consequently, cutaneous receptors are stimulated for thermal sensations, which serve to distract deep-seated pain sensations. Present study shown that comparable efficacy of DL to the standard drug Rumalaya gel due to the presence of chemical constituents which are responsible for defend inflammatory process, same manner DL also act as the reference drug R gel.

Conclusion

In this study there was an excellent relief of pain and reduction of swelling at the end of the therapy. Also it is comparable in activity to the already proven reference drug Rumalaya gel. Biochemical analysis gives evidence in safety of this formula that not shown any significant increase of bio chemicals. Results of this study provide a good working base for future workers, especially for those who work along the lines of drug actions and their active chemical constituents. Anti-inflammatory effect of this formula is good outcome of this study to develop novel herbal topical applications for further research with value addition to the conventional *Lepa* formulations.

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