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Evaluation of Marketed Herbal Formulations on Chronic Inflammatory Muscle Hyperalgesia

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Abstract: In the present study two marketed herbal formulations widely used in Satara region for musculoskeletal disorders, viz. Rumalya Forte tablets (Himalaya drug company, Bangalore) and Repare AR (Discovery, Mankind), were chosen for evaluation for their pharmacological activity in a preclinical model of inflammatory muscle hyperalgesia. Wistar Rats were injected with 3% carrageenan unilaterally in gastroenemius muscle and hyperalgesia to heat was measured as decrease in Paw withdrawal latency. Heat hyperalgesia was assessed before and at varying times after injection, i.e. after 24 hours for acute inflammatory hyperalgesia and after two weeks for chronic inflammatory hyperalgesia. Histopathological studies were carried to evidence the testing hyperalgesia. The present study revealed that the formulation treated groups showed decrease in the chronic inflammatory hyperalgesia comprehensively as compared with the acute one. The histopathological studies reveal that Rumalya Forte and Repare AR were effective in inhibiting chronic responses as minimal macrophage response was observed with no muscle necrosis and absence of mast cells. The study supports the fact that herbal products are need of the hour as better therapeutic options comparable with their allopathic counterparts when toxicity and side effects are concerned in long term therapy for chronic diseases.
Keywords: Formulations, Carrageenan, Hyperalgesia and Histopathology.

INTRODUCTION:

Chronic musculoskeletal pain is a major clinical problem.[1] Based on empirical experience, herbal therapies have been found to be effective in the treatment of musculoskeletal diseases, especially in case of chronic disorders and where the usual OTC drugs have prohibiting toxicity or side effects.[2] Recently interest have been revived in the use of indigenous drugs based formulations such as Rumalaya and Repare AR in the management of chronic disorders most importantly osteoarthritis (OA) and rheumatoid arthritis (RA). Rumalaya forte and Repare AR are polyherbal formulations which are frequently recommended for the management of OA and RA.

Current study aims to examine the anti-hyperalgesic effects of polyherbal formulations using a validated preclinical model of inflammatory muscle hyperalgesia.

MATERIALS AND METHODS:

1. **Animals** Wistar rats of either sex (125-160g) were maintained at ambient temperature of 25-30^{0}c with food and water ad libitum. Behavioral tests were done between 9 a.m. and 2 p.m. except for the 8 h testing after inflammation, which was done before 5 p.m. All experiments were approved by the institutional ethical committee and were carried out according to the institutional ethical guidelines.

2. **Drugs** Carrageenan (Sd. fine Chemicals) Normal saline (Claris NS NaCL IP 0.9% w/v), Each film coated tablet of **Rumalaya forte** containing Boswellia / Shallaki (*Boswellia serrata*) 240mg, Indian Bedellium / Guggul (*Commiphora wightii*) 200mg, Rasna (*Alpinia galanga*) 70mg, Licorice / Yashti madhu (*Glycyrrhiza glabra*) 70mg, Small caltrops / Gokshura (*Tribulus terrestris*) 60mg, Tinospora Gulancha / Guduchi (*Tinospora cordifolia*) 60mg. Each coated tablet of **Repare AR** containing Glucosamine sulphate-750mg, *Boswellia serrata* extract- 230mg, *Curcuma longa* extract- 20mg, *Zingiber officinale* extract- 20mg.

3. **Preparation of Drug solutions: -**
   Individual drugs were weighed on a electronic balance and were dissolved in dimethly sulphoxide (DMSO). The stock solution of each drug was adjusted such that each 0.2 ml contain the specific dose for the individual class of drug treatment groups. The stock solutions were stored in amber colored containers at ambient temperature.

4. **Induction and assessment of carrageenan-induced inflammatory nociception (hyperalgesia)[3]**
   Induction Inflammation was induced in the left gastrocenemius muscle belly of Wistar rats by injecting 100µl of freshly prepared solution of 3 % carrageenan, in normal saline under light ether anesthesia.
   Heat testing / assessment[4]
   Test animal's were tested for behavioral responses to Thermal stimuli by Hot plate method[14]. On 6, 10, 12, 14 and 16 days after respective injection at 55^{0}c ±2^{0}c. They were first placed in glass chambers of a thermal Analgesiometer and were allowed to acclimate for at least 2 minutes and basal reaction time was taken for each group. Baseline latency to paw withdrawal from thermal source was established thrice, 5 min apart, and average of the three readings was taken. A cut-off time of 15 s was imposed to avoid any injury to the paw. A decrease in withdrawal latency is interpreted as heat hyperalgesia for the purpose of this study.

5. **Experimental Protocol**
   The animals were divided into the following groups where n=6 for each group.
   - Group1:- Receiving DMSO 0.2 ml intra - peritoneally daily as control.
   - Group2:- Receiving of Rumalaya forte (7.2 mg/kg) intraperitoneally daily.
   - Group3:- Receiving Repare AR (7.2 mg/kg) intraperitoneally daily.

6. **Calculation of % Antihyperalgesia[5]**
   % Antihyperalgesia was calculated as described in our previous study to assess the effect of the formulation for the heat stimuli i.e. thermal hyperalgesia and the extent to which they normalized the exaggerated pain behavior in the current study.
   % Antihyperalgesia =

$$\frac{\text{Test latency} - \text{Mean basal PWL}}{15 - \text{Mean basal PWL}} \times 100$$

7. **Histopathological studies**
   Two animals of each group were sacrificed at 24 h. and 2 weeks, respectively, after the injection of carrageenan in control and drug treatments. Left gastrocenemius muscles were dissected and fixed in 10% formalin. The dissected muscle was embedded in paraffin, and sections of all tissues were stained with hematoxylin and eosin and examined by light microscopy using compound microscope under 40x magnification power. Analysis of histological findings was descriptive and performed in a blinded fashion by a pathologist.

8. **Statistical analysis**
   All the Statistical calculation were performed using Graphpad instat software version 3.05,32 bit for win 95/NT © 1999-2000. All data were expressed as mean ± standard error of mean (SEM) and was analyzed by using one way one way analysis of variance (ANOVA) using Dunnett's multiple comparison test as post hoc test. P values < 0.05 were considered significant as compared to control.

RESULTS:

I - Effect of carrageenan injection in the gastrocnemius muscle

- **Mean paw withdrawal latency**
  The Total Mean basal paw withdrawal latency was calculated in the normal (uninflammed) rats for all experimental groups (n = 18) to calculate %

antihyperalgesia, which was similar to approximately at 7.776 ± 0.105.

- **Spontaneous pain behavior**
  The Spontaneous pain behavior signs were observed in animals by guarding the injected paw and weight bearing on the contralateral paw during the first 2 days after injection of carrageenan. After 3 days, no sign of spontaneous pain were observed but the animals exhibited curling of the paw ipsliaterally uptill 2 weeks.

II - Effects of Rumalya Forte and Repare AR on heat hyperalgesia

The intraperitoneal administration of Rumalya Forte and Repare AR caused a rapid reduction in hyperalgesia which later returned to near normal values within 6-7 days in the chronic inflammatory muscle hyperalgesia conditions when compared with the control (Tables 1). The animals did not show exaggerated response to heat stimuli and also the spontaneous pain behavior was not observed to heat stimuli. The effect was maximum after the chronic administration of Rumalya Forte and Repare AR uptill 10 days and the effect started sustaining thereafter.

III - Antihyperalgesic Effects of Repare AR and Rumalaya forte against heat hyperalgesia

Maximal inhibition of hyperalgesia was achieved 12, 14 after 16 day after the continual dosing of the two tested formulations. While on contrary the analgesic effect persisted throughout the study period. The control group showed marked decrease in % antihyperalgesia. While the antihyperalgesic effect of Repare AR and Rumalya on inflammatory muscle hyperalgesia ranged from 3.18 -97.69 %. The Repare AR treated group showed maximal effect of 74.64 ± 0.1032 % which was closely matched by Rumalya which showed 77.129 ± 0.23. The results are as shown in Figure no. 1.

VI - Histopathological studies

Histopathological examination of the tissues in the current study shows inhibition of inflammatory changes in the formulation treated groups when compared with the hyperalgesic control, that parallels the long lasting hyperalgesia (Figure 2A and 2B). In the hyperalgesic controls the chronic inflammation was severe, epimysial and perimysial accompanied by myonecrosis, with presence of macrophages and few scattered mast cells. The Rumalaya forte and Repare AR treated rats showed significant decrease in myonecrosis and also showed absence of macrophages and mast cells as compared to control group. Figure no. 2C and 2D.

Figure No. 1: Antihyperalgesic Effects of Repare AR and Rumalya on heat hyperalgesia.

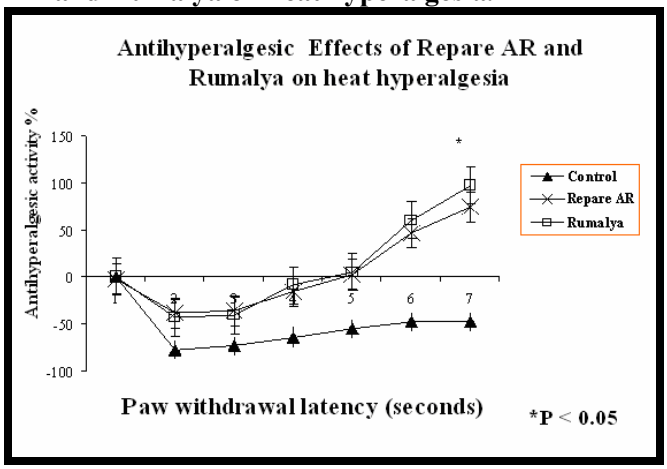
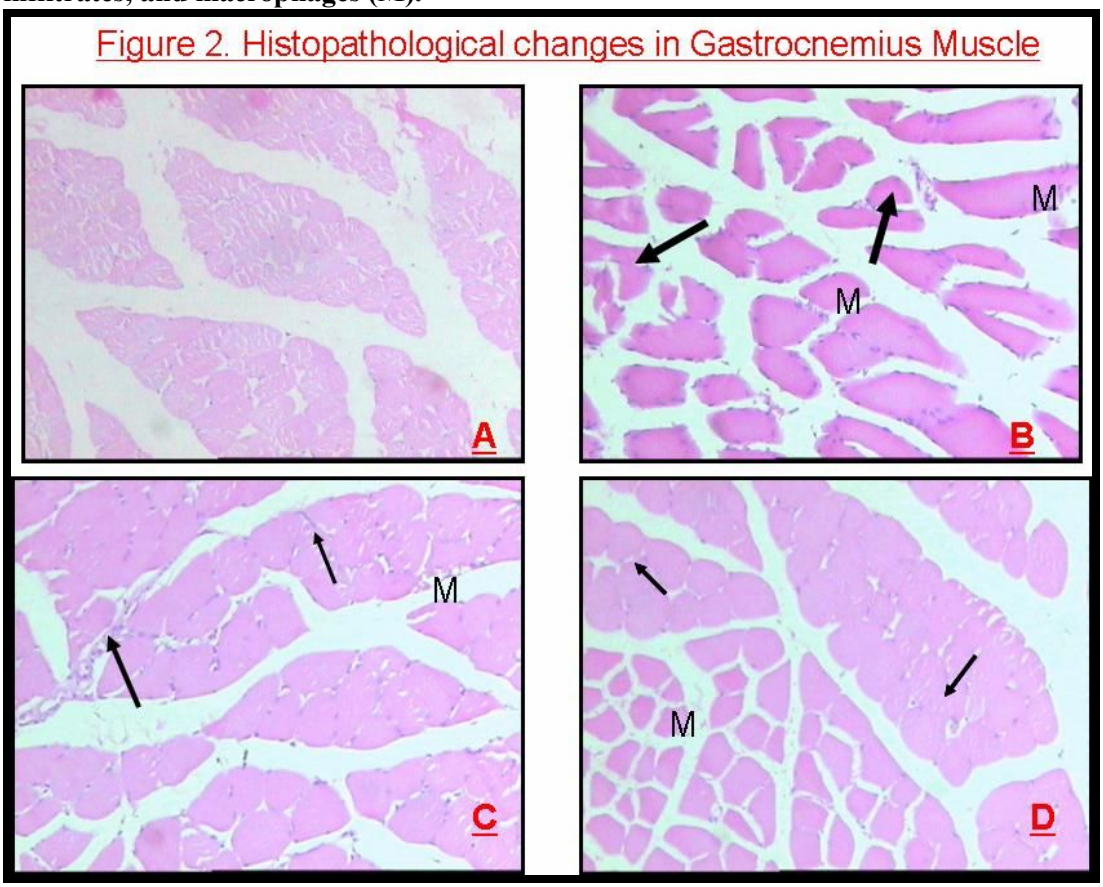


Table 1: Effects of Repare AR and Rumalaya on heat hyperalgesia

Treatments	Paw withdrawal latency (seconds) at time (days)						
	0	1	4	7	10	14	16
Control	7.833 ± 0.3073	2.167 ± 0.3073	2.50 ± 0.2236	3.167 ± 0.3073	3.833 ± 0.3073	4.333 ± 0.333	4.333 ± 0.2108
Repare AR	7.667 ± 0.2108	5.0 ± 0.3651	5.167 ± 0.3073	6.667 ± 0.4216	8.00 ± 0.3557	*11.167 ± 0.3073	*13.167 ± 0.3073
Rumalaya	7.833 ± 0.3073	4.667 ± 0.210	4.833 ± 0.3073	7.167 ± 0.1667	8.167 ± 0.3073	*12.167 ± 0.4773	*14.833 ± 0.4773

* P values < 0.05

Figure No. 2: Histopathological changes in the gastrocnemius muscle of rats are shown A] Normal muscle apparatus in normal healthy rat, B] Hyperalgesic control after 16 days and C] Repare AR treated rats D] Rumalya treated rats after 16 days. Arrows show foci of muscle necrosis and group of inflammatory cell infiltrates, and macrophages (M).



DISSCUSION:

Rumalaya forte and Repare AR ingestion in the test animals significantly increased the reaction time of the animals to the painful stimuli suggesting its central analgesic activity, which may be due to inhibition of prostaglandin synthesis.

The common ingredients of Rumalaya forte and Repare AR is the *Boswellia serrata***,** *which* has been reported to be an potent inhibitor of leukotriene (LT) biosynthesis.[6,7] The active ingredients of *Boswellia serrata* are boswellic acids, monoterpenes, sesquiterpenes, diterpenes, and triterpene acids.[8] Boswellic acid, which is the principle ingredient has been shown to blocks the synthesis of pre-inflammatory chemomediators, like 5-lipooxygenase and also reduce the glycosaminoglycan degradation, essential to prevent articular damage.[9,10] It has also been reported to possses strong immunostimulant activity. Menon and Karr have reported potent sedative and analgesic effects of *Boswellia serrata*.[9]

Antihyperalgesic Effects of Repare AR are possibly may be due to various constituents of Repare AR like *Boswellia serrata, Zingiber officinale and Curcuma longa,* which are known to have analgesic and anti-inflammatory potential.

Glucosamine sulphate: Glucosamine (2-amino-2-deoxy-D-glucose), an amino derivative of glucose, is found abundantly in the human body, particularly in connective and cartilage tissue. It serves as a substrate for biosynthesis of mucopolysaccharides and biopolymers of the articulations and bones and has been reported to facilitate the hexosamine pathway of proteoglycan synthesis.[11]

***Curcuma longa*:** [5, 12] The chief active ingredients are curcumin and curcuminoids. Various views have been expressed about curcumin's anti-inflammatory activity, with its mechanism of action. Its anti-inflammatory activity is mainly due to the inhibition of arachidonic acid (AA) metabolism, cyclooxygenase (COX), cytokines [interleukins and tumor necrosis factor (TNF)] and neurotrophic factor (NF-κB). Also curcumin is reported to stabilize lysosomal membrane and causes the uncoupling of oxidative

phosphorylation, besides having strong oxygen radical scavenging activity.

Zingiber officinale: The active ingredients of *Zingiber officinale* are gingerols, diarylhepatanoids and oleoresins.[13] It has been reported to inhibit biotransformation of arachidonic acid comparable to indomethacin. *Zingiber officinale* has inhibitory effects on COX-2 enzymes and it attenuates COX-1 / thromboxane synthase enzymatic activity.[14] Cyclooxygenase-1 and -2 (regulated by the eukaryotic transcription factor nuclear factor kappa-b) has been recognized as a molecular target for actions of *Zingiber officinale*, and gingerol acts by interfering with the intracellular signaling cascades (those involving NF-kappa-b and mitogen-activated protein kinases).[15] *Zingiber officinale* has been reported to significantly inhibit prostaglandin-E-2 production. [16] *Zingiber officinale* has shown immunostimulation actions and raised the thymus index, spleen index, phagocytosis, and rate of alfa-naphthyl acetate esterase (a-ANAE+) and immunoglobulin M (IgM) titer, which indicates immunostimulation. [17]

Antihyperalgesic Effects of Rumalaya forte- These excellent antihyperalgesic activity of Rumalaya forte might be due to the synergistic actions of its ingredients, which have been previously reported.
Rumalaya forte also is known to posses potent antiinflammatory, antioxidant andimmunostimulant actions, which result in excellent symptomatic benefits and better clinical management of osteoarthritis. [18]

Commiphora wightii: *Commiphora wightii* has shown dose dependent anti-inflammatory activity and was also found to control inflammation and pain in osteoarthritis patients. [19,20] The main ingredients of *Commiphora wightii* are flavonoids which have potent anti-oxidant actions.[21] *Commiphora wightii* has been reported to inhibit nitric oxide formation [22] and also scavenges the effect of DPPH radicals. [23]

Alpinia galangal : *Alpinia galanga* has shown to inhibit lipid peroxidation.[24] The active constituents of Alpinia galanga are diarylheptanoids, phenylpropanoids and p-hydroxybenzaldehydes.[25,26] *Alpinia galangal* inhibits the release of pro-inflammatory cytokines (IL-1-b, TNF-a, COX-2, and NF-kappa-b). *Alpinia galanga* is also known to inhibit lipid peroxidation[24] and it inhibits the release of pro-inflammatory cytokines (IL-1b, TNF-a, COX-2 and NF-Kappa-b). *Alpinia galanga* induces biphasic activity in membrane stabilization, which is a contributory mechanism for its anti-inflammatory activity.[24]

Glycyrrhiza glabra: *Glycyrrhiza glabra* has demonstrated anti-inflammatory and anti-allergic activity,[27] possibly due to the presence of terpinoids, i.e. glycyrrhizin and glycyrrhetinic acids. The principle ingredients of *Glycyrrhiza glabra* are glabridin (pyranoisoflavan) and beta-glycyrrhetinic acid. [28]

Tribulus terrestris: The active ingredients of *Tribulus terrestris* are saponins (protodioscin) and a sitosterol glucoside. [29] which has potent inhibitiory activity on COX-2. [30]

Tinospora cordifolia: The active ingredient of *Tinospora cordifolia* is an arabinogalactan polysaccharide [31] which has been reported to reduces IL-1-b production, and inhibits TNF-a. [32]
It was known to reduce chemotactic activity of macrophages, and hence it protects against myelosuppression with an increase in WBCs and antibody titers. [33] *Tinospora cordifolia* has also shown a dose-dependent enhancement in compliment-mediated immunity. The anti-complementary and immunomodulatory activities of *Tinospora cordifolia* are due to inhibition of the C3-convertase of the classical complement pathway. [33] Tinospora cordifolia has shown to reverses chemically induced immunosuppression.[34] The activation of macrophages by *Tinospora cordifolia* has shown to increase in granulocyte-macrophage colony-stimulating factor (GM-CSF), which leads to leucocytosis and improves neutrophil function.[35] *Tinospora cordifolia* has also shown to normalize the phagocytic capacities of neutrophils. [36]

Clinical trials have proven the effectiveness and safety of Rumalaya forte and Repare AR. Both of these formulations exerts potent immunomodulatory, anti-inflammatory and analgesic actions, and thereby reduces swelling and relieves pain. The present investigations has demonstrated significant activity of Rumalaya forte and Repare AR in management of Chronic Inflammatory Muscle Hyperalgesia.

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