EFFECT OF POLYHERBAL FORMULATION RUMALAYA FORTE ON ADJUVANT INDUCED ARTHRITIS IN RATS

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ABSTRACT

Anti-arthritic activity of Rumalaya Forte (RF), a polyherbal formulation was evaluated by *in vivo* Complete Freund's Adjuvant induced arthritis animal model in rats. RF 80 mg/kg and 160 mg/kg offered significant anti-inflammatory activity by inhibiting primary lesion on day 5, RF 160 mg/kg body weight has profound anti-arthritic activity than low dose RF 80 mg/kg with significant reduction in mononuclear infiltration, pannus formation and bone erosion in as observed in histological studies. Dexamethasone as standard exhibited a greater reduction in body weight compared to other groups while arthritic score was comparably significant with that of RF 160 mg/kg concentration.

Keywords: Arthritis, Complete Freund's Adjuvant, Dexamethasone, Rumalaya Forte.

INTRODUCTION

Today's medicine is based on traditional medicine. Traditional medicines exist in every continent of the globe and in every cultural area of the world. The most famous ones are traditional Ayurvedic medicine in India, Chinese medicine in East Asia and formerly Galenic medicine in Europe. Herbal medicine is the oldest form of healthcare known to mankind, much of medicinal use of plants have been developed through observation of wild animals, and by trial and error method [1]. The use of traditional medicine is wide spread and plants still present a large source that might serve as leads for development of novel drugs. Rheumatoid arthritis (RA) is a chronic, systemic autoimmune disorder that causes the immune system to attack the joints, causing inflammation (arthritis). Pharmacological treatment of RA can be divided into disease-modifying anti-rheumatic drugs (DMARDs), anti-inflammatory agents and analgesics, immunomodulators and biological agents [2].

The DMARDs cover a wide variety of drugs with different chemical groups and with different modes of action, but none of them is able to halt the long-term progression of the disease rather developing adverse effects. Anti-inflammatory and analgesics improve pain and stiffness but do not prevent joint damage or slow the disease progression and their undesirable effects on long term usage are again a limitation. Immunomodulators and biological agents may be effective when other DMARDs fail to achieve adequate responses, but they are considerably more expensive to use and has increased risk for infection [3].

In the advent of internationally accepted protocol for evaluation of a drug and the established understanding of the plants available in the literature of Ayurveda better utilization may be achieved. Rumalaya Forte is one such poly herbal formulation used by ayurvedic practitioners in rheumatoid arthritis for years, several clinical studies have been undertaken with Rumalaya Forte. However experimental studies to understand the mechanism are negligible. This study is focused on evaluation of anti-arthritic activity of Rumalaya Forte on complete Freund's adjuvant (CFA) induced arthritis in Wistar

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strain albino rats. The choice of the animal strain has been found to be very important for the performance of this test. Wistar-Lewis rats have been proven to be very suitable in contrast to other sub strains[4]. CFA induced arthritis in rats is a model of chronic arthritis with features that resemble rheumatoid arthritis in humans. CFA contains *Mycobacterium tuberculosis*, induction of adjuvant arthritis is attributed to a mycobacterial cell wall component peptidoglycan and muramyl dipeptide[5]. Rat adjuvant arthritis is believed to be the result of a sequence of immunopathologic events involving sensitization of antigen, proliferation of immunocompetent cells, cellular hypersensitivity and mediator release[6].

MATERIALS AND METHODS

Drugs, Chemicals and Reagents

Dexamethasone as standard (Dexona, Cadila Healthcare Ltd., India), Rumalaya Forte as test drug (Himalaya Healthcare Ltd., India) brought from medical college & hospital pharmacy, carboxymethyl cellulose as a suspending agent. Complete Freund's adjuvant was obtained from Sigma Aldrich (Saint Louis, Missouri, USA).

Animals

Twenty four healthy adult Wistar albino rats of either sex (average wt. 150-200 g) were obtained from institute's central animal facility for toxicology and developmental research (CAFT, SRU). The animals were housed in polypropylene cages at 25 $\pm$ 2^0 with relative humidity of 45-55% under 12-h light:12-h dark cycles. They were fed with standard laboratory pellet diet feed with free access to water throughout the study. The experimental protocol was subjected to the scrutiny of the institutional animal ethical committee and was approved (IAEC-XII/ SRU/74/2008), this study compiled with current ethical regulations on animal research of this institute (SRU). The animals used in experiment received humane care and handled as per guidelines of animal care. The studies were conducted from Feb 2008 to Aug 2008.

METHOD

Freund's Complete Adjuvant induced Arthritic Model

Twenty four Wistar albino rats were selected and marked with picric acid, (head, body, tail, head & body, head & tail and colorless) for identification. They were randomly divided into 4 groups, further subdivided into 6 animals in each group (n=6) and acclimatized for one week. Experimental arthritis was induced according to the method of Sarkar D. *et al*[7]. Briefly, 0.1 mL of Freund's adjuvant was injected intradermally into the plantar aspect of the hind paw of each animal in all group stat.

Animals were divided into four groups, consisting of six animals each (Table I).

Animals were administered Rumalaya Forte in both low dose 80 mg/kg, b. wt., bid, orally and as high dose 160 mg/kg, b. wt., bid, orally and dexamethasone 0.1 mg/kg, b. wt., od, orally, as a standard reference for initial 13 days and with a pretreatment period for 3 days[7]. The lower and higher dose of polyherbal formulation was calculated according to daily human dose using conversion factor based on body surface area. Doses of the drugs were calculated for each animal based on the body weight and respective volumes were administered orally twice daily with the help of tuberculin syringe (1 mL) and sterile oral gavage needle the respective volume of drugs were administered orally to the animals.

The degree of inflammation was measured by a water plethysmograph (Ugo Basile) accordingly; edema formation and the percentage of inhibition on days 1, 3, 5, 9, 13 and 21 were calculated as follows.

$$\frac{E_c - E_t}{E_c} \times 100$$

Where E_c is the edema volume of the control group and E_t is the edema volume of the treated group.

Primary and Secondary Lesions

Primary lesion refers to the edema formation in the injected hind paw that peaks 3-5 days after injection of the phlogistic agent and is measured on day 5 by calculating percent inhibition of the edema volume of the injected paw using the formula described above.

Secondary lesions are immunologically mediated changes characterized by inflammation of the non-injected sites (hind leg, forepaws, ears, nose and tail), decrease in weight that occur after a delay of 11-12 days. % Reduction in body weight is calculated using the formula :

$$\frac{B_0 - B_{21}}{B_0} \times 100$$

Where B_0 is body weight recorded on day 0 and B_{21} is body weight recorded on day 21.

Accordingly secondary lesions were evaluated by calculating the percent inhibition of paw volume of the non-injected right paw over control on day 21 as follows[4].

Arthritic Index

An arthritic index is calculated as the sum of the scores as indicated below for each animal.

Lesion site	Nature of lesion	Score
Ears:	Absence of nodules and redness	 0
	Presence of nodules and redness	 1
Nose:	No swelling of connective tissue	 0
	Intensive swelling of connective tissue..	1
Tail:	Absence of nodules	 0
	Presence of nodules	 1
Forepaws:	Absence of inflammation	 0
	Inflammation of at least 1 joint	1
Hind paws:	Absence of inflammation	 0
	Slight inflammation	 1
	Moderate inflammation	 2
	Marked inflammation	 3

An arthritic index is calculated as the sum of the scores as indicated above for each animal. The average of treated animals is compared with the control group[4].

Histological Assessment

After euthanasia at the end of 21st day, the hind paws of all rats were amputated above the knee joint and were fixed in 7.4% formalin solution. The paws were then decalcified using 10% nitric acid, embedded in paraffin and sectioned in a mid-sagittal plane. The sections of articulation of the tarsal joints were stained with haematoxylin and eosin and were examined microscopically for any bony destruction, presence of mononuclear infiltration and pannus formation[9, 10].

Statistical Analysis

Statistical analysis is performed using software Graph pad, USA. Data are expressed as mean ± SEM and statistically assessed using one-way ANOVA followed by Tukey test; $P < 0.05$ was considered significant. Probability values were denoted as $^{*}P < 0.05$ and $^{**}P < 0.01$ and $^{***}P < 0.001$.

RESULTS

The percentage inhibition on day 5 in all the three groups is significant with Rumalaya Forte low

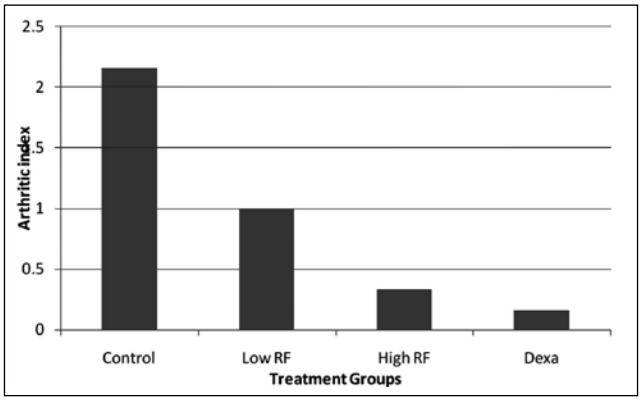

Fig. 1: Average mean arthritic index of groups

*Arthritic score was measured as explained in materials and methods. Data is expressed as average mean arthritic index. n = 6 in each group. *P<0.05;**P<0.01;***P<0.005 as compared to control by one way ANOVA followed by Tukey test*

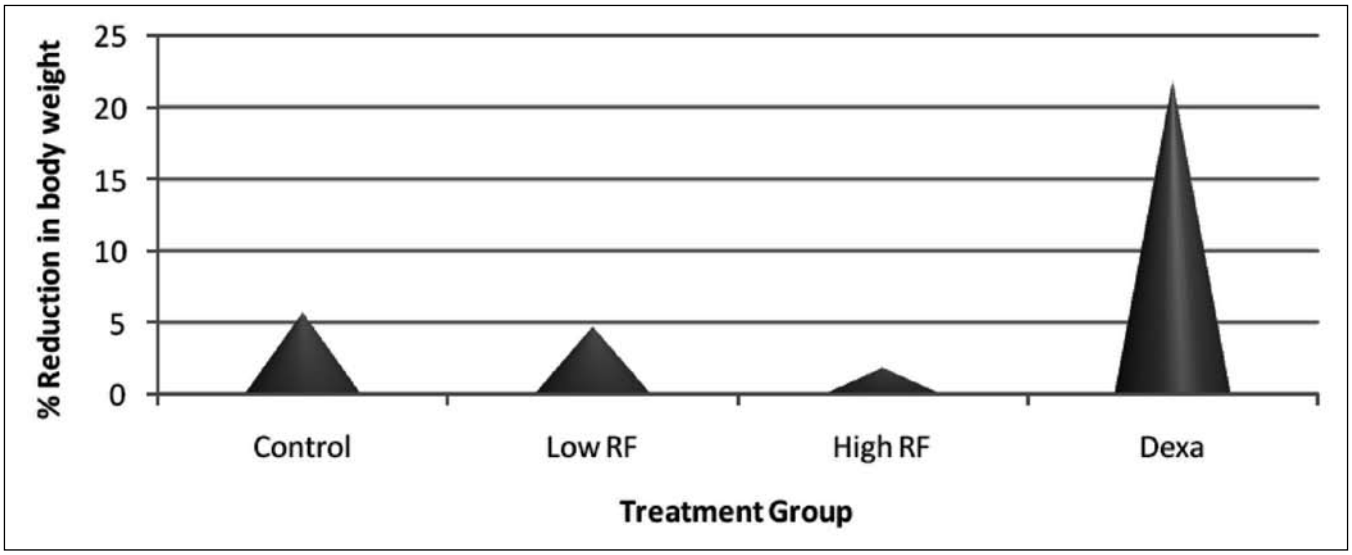

Fig. 2: Percentage reduction in body weight

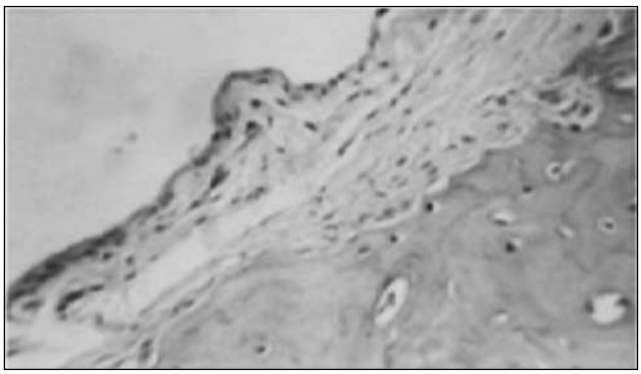

Fig. 3: Transverse section of knee joint control

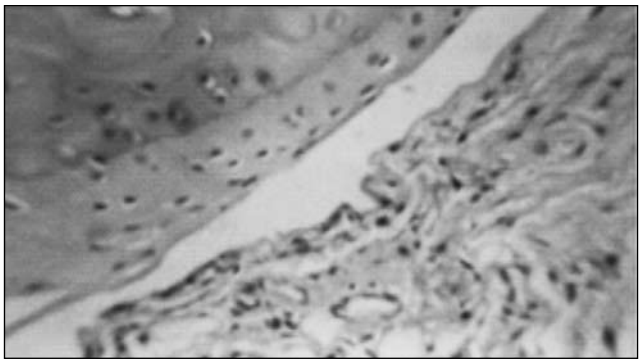

Fig. 5: TS of knee joint of dexamethasone treated rat

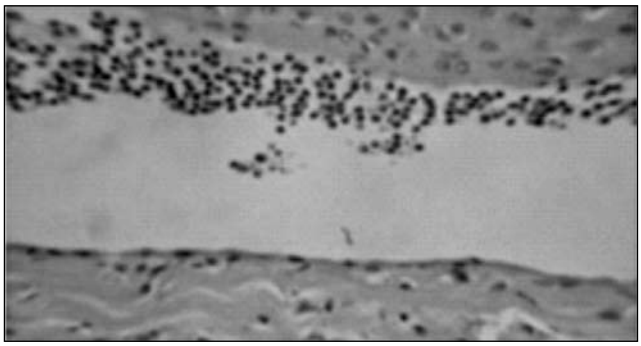

Fig. 4: TS of knee joint of arthritic rat showing mononuclear infiltration & bone erosion

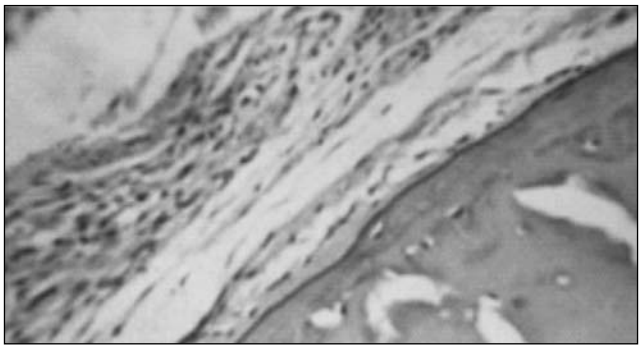

Fig. 6: TS of knee joint of RF 80 mg/kg treated rat

dose 80 mg/kg is 33.33%, P < 0.005, high dose RF 160 mg/kg is 50.66%, P < 0.005 and dexamethasone 63.39%, P < 0.005 as compared to controls. On the 21st day, a significant decrease in edema volume was observed in the injected paw of low RF (45%, P < 0.005), high RF (63.17%, P < 0.005) and the dexamethasone treated group (66.39%, P < 0.005) as compared to the control.

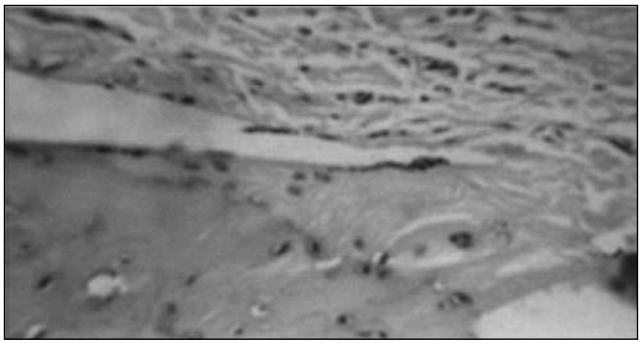

Fig. 7: TS of knee joint of RF 160 mg/kg treated rat showing reduced mononuclear infiltration & bone erosion

With regard to mean arthritic index, the average arthritic score of low RF was 1.00 whereas that of control is 2.16 which indicate low RF having mild anti-arthritic activity. In case of high RF and dexamethasone treated group it showed a profound anti-arthritic activity with the average arthritic score of 0.33 and 0.10 respectively, thereby these two groups decreased the arthritic index almost 10 fold when compared to that of control score being 2.16.

And finally there was a significant decrease in body weight in dexamethasone treated group with more than 20% and less than 5% in both 80 mg/kg and 160 mg/kg RF treated group.

Table I: Study Design

Group I (n=6) Positive control	**Group II** (n=6) Standard control Dexamethasone	**Group III** (n=6) Test drug RF Low dose (80 mg/kg)[8]	**Group IV** (n=6) Test drug RF High dose (160 mg/kg)[8]
0.1 mL of CFA i.d. on day 1 to right hind paw	0.1 mL of CFA i.d. on day 1 to right hind paw	0.1 mL of CFA i.d. on day 1 to right hind paw	0.1 mL of CFA i.d. on day 1 to right hind paw
Day 1 to day 13th 1% CMC only	Day 1 to day 13th Dexamethasone 0.1 mg/kg, od, orally	Day 1 to day 13th Rumalaya Forte test drug (80 mg/kg, bid., orally)	Day 1 to day 13th Rumalaya Forte test drug p.o (160 mg/kg, bid., orally)

Animals – Wistar strain albino rats (150 – 200 g), i.d. – intra dermal, RF-Rumalaya Forte, CMC – carboxy methyl cellulose, CFA – Complete Freund's Adjuvant, od-once daily, bid-twice daily

Table II: Effect of rumalaya forte (RF) in an adjuvant arthritis model (% inhibition)

Group	D3 (%)	D5 (%)	D9 (%)	D13 (%)	D21 (%)
Low RF	25.95	33.33	23.09	35.24	45.43
High RF	44.26	50.66	50.26	56.16	63.17
Dexa	60.38	63.73	61.11	67.04	66.39

D-day. Data is expressed as % inhibition of paw edema volume. Inhibition was measured on days 3, 5, 9, 13 and 21. Values are expressed on each day for all experimental groups in each column (n = 6 in each group)

Histopathological Report

Histological studies on transverse section of knee joint reports confirm that there is severe bone erosion with presence of neutrophil infiltration and pannus formation in control group (Fig. 3,4). Both treatment groups RF 80 mg/kg (Fig. 6) and 160 mg/kg revealed reduction in pannus formation and bone resorption with reduced neutrophil infiltration. Among the two 160 mg/kg proved a dose dependent action that is comparable to that of standard dexamethasone group[9] (Fig. 5).

DISCUSSION

The purpose of the study is to screen and evaluate anti-arthritic activity using modern scientific, internationally approved standard experimental procedure. Evaluation of anti-arthritic activity of Rumalaya Forte was studied on Complete Freund's Adjuvant (CFA) induced arthritis in Wistar strain albino rats. The choice of the animal strain has been found to be very important for the performance of this test. Wistar-Lewis rats have been proven to be very suitable in contrast to other sub strains[10].

To characterize the pharmacological profile of the test compound this experiment provides to screen for anti-inflammatory activity which is characterized by primary lesion on day 5 and anti-arthritic activity which is characterized by secondary lesion on day 21[11]. Thus test compounds having just anti-inflammatory activity do not inhibit secondary lesion, which are prevented or diminished by immunosuppressive agents.

The current experimental data reveals that there is a significant decrease in the primary lesion as evident on day 5 was observed in both Rumalaya Forte (low RF) at 80 mg/kg b.w., (33.33%, $P < 0.005$), Rumalaya Forte (high RF) at 160 mg/kg b.w., (50.66%, $P < 0.005$) and the dexamethasone treated group (63.39%, $P < 0.005$) (Table II) as compared to control. Thus the above data explains the dose dependent anti-inflammatory action of Rumalaya Forte.

With regards to the secondary lesion, it could only be partially evaluated as no edema formation was significant in the contra lateral hind paw of control animals. On the 21st day, a significant decrease in edema volume was observed in the injected paw of low RF (45%, $P < 0.005$), high RF (63.17%, $P < 0.005$) and the dexamethasone treated group (66.39%, $P < 0.005$) (Table II) as compared to the control. Here again exhibiting the dose dependent action of RF.

With regard to mean arthritic index, the average arthritic score of low RF was 1.00 whereas that of control is 2.16 (Fig. 1) which indicate low RF having mild anti-arthritic activity. In case of high RF and dexamethasone treated group it showed a profound anti-arthritic activity with the average arthritic score of 0.33 and 0.10 respectively (Fig. 1), thereby these two groups decreased the arthritic index almost 10 fold when compared to that of control score being 2.16.

And finally regarding body weight changes (Fig. 2) in each group, the control and low RF group showed decrease of an average weight of 11 g and 9 g respectively which does not make any difference when compared to control, whereas in high RF there was a decrease in an average weight of 3 g in group was seen which exhibits the tolerability of the drug during disease progression but in dexamethasone treated group there was a profound decrease on average body weight of 54 g in group which explains that corticosteroids though having good anti inflammatory and immunosuppressant activity, its adverse effects and toxicity in long term administration limits their use in rheumatoid arthritis. Thus all put together the *in vivo* experiments confirm that both treatment groups significantly reduced paw volume without affecting the body weight in rats. The inhibition of the increase in hind paw volume may be associated with inhibition of neutrophil infiltration, pannus formation and bone erosion, the above said is supported by the present study histopathological reports[12] (Fig. 7).

The other studies on individual ingredients of RF discussed below reveals that polyherbal formulation containing extracts of *Boswellia serrata*, *Alpinia galanga*, *Commiphora wightii*, *Glycyrrhiza glabra*, *Tinospora cordifolia* and *Tribulus terrestris*, are shown to have anti-inflammatory, anti-arthritic, immunomodulatory, muscle relaxant and analgesic activities.

Boswellia serrata is a potent inhibitor of leukotriene (LT) biosynthesis[13]. *Alpinia galanga* inhibits the release of pro-inflammatory cytokines (IL-1-β, TNF-α, COX-2 and NF̃kappa-β)[14]. *Tribulus terrestris* is a potent inhibitior of COX-2 activity[15]. *Tinospora cordifolia* reduces IL-1-β production and inhibits TNF-α[16]. *Vitex negundo* is a strong anti-inflammatory

agent[17]. *Commiphora wightii* has demonstrated down regulation of TNF-α and IL-1-β[18].

Thus the present experimental results indicate the efficiency of RF as a therapeutic agent in rheumatoid arthritis and provide a scientific basis to explain the significant anti-inflammatory and anti-arthritic action of various ingredients in RF acting synergistically and working in concert for overall anti arthritic activity.

CONCLUSION

Rumalaya Forte exhibited significant anti-arthritic activity on complete Freund's adjuvant induced arthritis model in rats. To conclude Rumalaya Forte both high (160 mg/kg b. wt.) and low dose (80 mg/kg b. wt.) showed significant anti-inflammatory activity. Whereas high dose showed profound anti-arthritic activity, than low dose which explains a dose dependent action of Rumalaya Forte. Further in depth research on individual medicinal plants may lead us to identify potential drug candidates for arthritis, as for Rumalaya Forte further clinical studies are needed to establish the efficiency in arthritic patients.

REFERENCES

1. Lester Packer, Choon Nan Ong, Barry Halliwell. "herbal and traditional medicine: molecular aspects of health", 1st edition. Newyork:Marcel Decker,2004.p.1-73.
2. O'Dell J.: Therapeutic strategies for rheumatoid arthritis, **N Engl J Med.** 2004, 350 (25): 2591-602.
3. Arthur A. Schuna Rheumatoid Arthritis. Pharmacotherapy a pathophysiological approach 6th edition.2005.Chapter 89, p 1671-1683.
4. Gerhard Vogel H.: Drug discovery and evaluation, pharmacological assays, Second edition 2002, p 802-803.
5. Williams R. O.: Rodent models of arthritis: relevance for human disease, **Clin Exp Immunol.** 1998,114, 330-332.
6. Carla Cicala *et al*: NO-naproxen modulates inflammation, nociception and downregulates T cell response in rat Freund's adjuvant arthritis, **Br J Pharmacol.** 2000,130 (6):1399-1405.
7. Sarkar D. *et al*: Effect of *Aloe vera* on nitric oxide production by macrophages during inflammation, **Indian J Pharmacol.** 2005, 37, 371-375
8. Guidelines for preclinical pharmacokinetic studies, United States, Food and Drug Act, http://www.fda.gov/cder/cancer/animalframe.htm accessed on 21st Feb. 2008. Accessed on 21st Feb. 2008.
9. Choi J., Yoon B. J., Han Y. N., Lee K. T. and Ha J. *et al*: Anti rheumatoid arthritis effect of *Rhus verniciflua* and of the active component, sulfuretin, **Planta Med.** 2003. 69: 899-904.
10. Michele M. B., Roffe E., Yokoro C. M., Tafuri W. L. and Souza D.G. *et al*: Anti-inflammatory and analgesic effects of atorvastatin in a rat model of adjuvant-induced arthritis, **Eur J Pharmacol.** 2005. 516: 282-289.
11. Murray M.T., Pizzorno J. E. *Glycyrrhiza glabra* In: Text book of Natural Medicine. Pizzorno, J.E. and Murray, M.T. (Eds.), Vol. 1, 2nd Edition, Churchill, Livingston, 1999, 767-773.
12. Abdul-Shakoor B., Tandan S. K., Kumar D., Krishna V. and Prakash V. R.: Interaction between inhibitors of inducible nitric oxide synthase and cyclooxygenase in adjuvant-induced arthritis in female albino rat: An isobolographic study, **Eur J Pharmacol.** 2007, 556: 190-199.
13. Shao Y. *et al*: Inhibitory activity of boswellic acids from *Boswellia serrata* against human leukemia HL-60 cells in culture, **Planta Med.** 1998, 64(4):328-231.
14. Yadav P. N., Liu Z. and Rafi M. M.: A diarylheptanoid from lesser *Alpinia galanga* inhibits pro-inflammatory mediators via inhibition of mitogen-activated protein kinase, p44/42 and transcription factor nuclear factor kappa B, **J Pharmacol Exp Ther.** 2003, 305(3):925-931.
15. Conrad J. *et al*: Novel furostanol saponin from *Tribulus terrestris* of Bulgarian origin, **Fitoterapia.** 2004, 75(2):117-122.
16. Subramanian M. *et al*: Anti-oxidant properties of a *Tinospora cordifolia* polysaccharide against iron-mediated lipid damage and gamma-ray induced protein damage, **Redox Rep.** 2002, 7(3):137-143.
17. Dharmasiri M. G., Jayakody J. R.: Anti-inflammatory and analgesic activities of mature fresh leaves of *Vitex negundo*, **J Ethnopharmacol.** 2003, 87(2-3): 199-206.
18. Raut A. A., Sarkar S., Sudha S., Pandita N. S., Vaidya A. D. B.: *Commiphora wightii* (guggulu) induced down regulation of macrophage released cytokines *in vitro*, **Indian Drugs.** 2007, 44(1): 39-42.