Journal of Traditional and Complementary Medicine 6 (2016) 389–394

Contents lists available at ScienceDirect

Journal of Traditional and Complementary Medicine

journal homepage: http://www.elsevier.com/locate/jtcme

Original article

Anti-arthritic activity of a classical Ayurvedic formulation *Vatari Guggulu* in rats

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ARTICLE INFO

Article history:
Received 17 April 2015
Received in revised form
13 July 2015
Accepted 10 August 2015
Available online 4 September 2015

Keywords:
Vatari Guggulu
Formaldehyde
Adjuvant induced arthritis
ESR
Haemoglobin
Cholesterol

ABSTRACT

In India, *Vatari Guggulu* has been traditionally used in the Ayurvedic system of medicine to treat rheumatoid arthritis (RA). The current study was undertaken to evaluate anti-arthritic activity of alcoholic extract of *Vatari Guggulu* in rats. Arthritis was induced by administration of formaldehyde (2%v/v) or Complete Freund's Adjuvant (CFA) into the sub-plantar surface of left hind paw of the animals. The extract was administered to the rats by oral gavages in different doses. Joint swelling was measured in formaldehyde induced arthritis. Various physical, biochemical and histopathological parameters were determined in CFA induced arthritis. *Vatari Guggulu* extract (VGE) produced significant ($P < 0.05$) inhibition of joint swelling in both formaldehyde and CFA induced arthritis. The treatment also brought to normalcy the increased white blood cell (WBC) count, rheumatoid factor (RF), erythrocyte sedimentation rate (ESR), cholesterol, triglycerides and LDL with an enhancement of haemoglobin (Hb) levels and red blood cell (RBC) count. These effects were found to be dose dependent. These effects were comparable with standard drug indomethacin. Histo-pathological studies of the ankles of VGE treated animals exhibited significant improvements. VGE did not show any toxic symptoms even at a dose of 2000 mg/kg in acute toxicity studies on rats. Thus, *Vatari Guggulu*, a classical Ayurvedic formulation of the Indian System of Medicine, exhibited significant anti-arthritic activity in formaldehyde and CFA induced arthritis in rats. This study corroborates the claims of *Ayurveda* on *Vatari Guggulu*.

1. Introduction

Rheumatoid arthritis (RA) is a chronic, inflammatory condition of unknown aetiology, affecting approximately 1% of the general population. It is a progressive, disabling, chronic multisystem disease that is characterized by pain, swelling and stiffness of the synovial joints.[1] Although spontaneous remission can occur, it often progresses to a chronic state associated with significant functional disability and substantial morbidity.[2]

Treatment for RA is aimed at relieving pain, reducing joint swelling, slowing or preventing joint damage and improving physical function and wellbeing. In certain cases people suffering from arthritis have used Complimentary and Alternative Medicines (CAM) involving predominantly herbal therapies,[3] *Vatari Guggulu*, a classical Ayurvedic formulation. It consists of a mixture of fine powders of dried pericarps of *Haritaki* (*Terminalia chebula* Retz. F.- Combretaceae), Bibhitaka (*Terminalia belerica* Roxb. F.- Combretaceae), and *Amalaki* (*Phyllanthus emblica* Linn., F.- Euphorbiaceae), oleo-gum resin of *Guggulu-suddha* (*Commiphora mukul* Engl., F.- Burseraceae), fine powder of Gandhak-suddha (purified sulphur), and *Vatari Taila* (Castor oil) in equal quantities.

It is recommended in rheumatism, lower backache, sciatica, gout, paraplegia and deformed knee in the Ayurvedic system of medicine.[4] However, no scientific study is available on anti-arthritic activity of this formulation. Hence, the present study was undertaken with an objective to investigate the anti-arthritic activity of *Vatari Guggulu* and to correlate the changes in lipid and blood profiles that occur during the disease in albino rats.

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Peer review under responsibility of The Center for Food and Biomolecules, National Taiwan University.

http://dx.doi.org/10.1016/j.jtcme.2015.08.007

2. Materials and methods

2.1. Plant materials

Oleoresin *of Guggulu*, fruits of *Haritaki*, *Bibhitaka* and *Amalaki* were procured from a reputed herbal drug supplier, M/s. Yucca Enterprises, Mumbai, India and were authenticated by a botanist through morphological and microscopic studies. The samples were also compared with the herbarium specimens deposited in the authors' department. After removing the seeds, pericarps of *Haritaki*, *Bibhitaka* and *Amalaki* were dried thoroughly and powdered. The powders were passed through 80 # sieve and stored. Pure sulphur (*Gandhak*) and *Vatari Taila* (Castor oil) were of Pharmacopoeial grade. As per the method mentioned in *Ayurveda*, *Guggulu* was purified using cow's urine; *Gandhak* was purified by cow's milk and ghee.[5] After purification, they were dried properly and powdered for further use. For the preparation of *Vatari Guggulu* formulation, *Guggulu* was first homogenized in castor oil; powders of other ingredients including purified *Gandhak* were added and mixed well.

2.2. Preparation of Vatari Guggulu extract (VGE)

Vatari Guggulu (100 g) was extracted exhaustively with ethyl alcohol (1000 ml) by cold maceration at room temperature (3×24 h). The extract (VGE) was initially concentrated at 50 °C on a rotary evaporator (Equitronrotevar, Medical Instrument Manufacturing Company. Mumbai, India) and then dried in a vacuum desiccators till free from solvent.

2.3. Animals

Male Wistar albino rats (200–250 g) maintained in the institute's animal house, were used for the experiments. The animals were maintained at a temperature of 25 ± 2 °C, relative humidity of $75 \pm 5\%$ and 12 h dark–light cycle. Standard pellet diet (M/s. Hindustan Lever Ltd., Bangalore, India) and water were given *ad libitum*. The study protocol was approved by the Institutional Animal Ethical Committee (IAEC) for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA), India and the experiments were conducted in accordance with the principles prescribed for laboratory animal use (letter Nos. PIPH 06/12 and PIPH 36/13).

2.4. Acute toxicity study

Acute toxicity study was carried out for the VGE as mentioned in OECD Guidelines (2001).[6] VGE (2000 mg/kg body weight), suspended in water with 0.5% gum acacia, was orally administered to overnight-fasted, healthy rats ($n = 6$). The animals were observed continuously for 24 h for mortality and then for the next 14 days.

2.5. Animal grouping and dosing

In both formaldehyde and CFA induced arthritis models, animals were divided into six groups of six animals each. Group I served as a normal control receiving 0.5% gum acacia in water as a vehicle. group II served as arthritic disease control, group III was administered standard indomethacin (10 mg/kg p.o.),[7] groups IV, V and VI were given 135 mg/kg, 270 mg/kg and 540 mg/kg (p.o.) VGE (reconstituted in water with 0.5% gum acacia) respectively. All the test drugs and vehicle were given orally through oral gavages.

2.6. Formaldehyde induced arthritis

The test samples were given for a period of 10 days. Thirty minutes after administration of the vehicle/drug, arthritis was induced by subplantar administration of 0.1 ml formaldehyde (2%v/v) into the left hind paw of all the animals on day 1 and 3.[8] Increase in joint diameter was measured on days 0, 2, 4, 6, 8 and 10 as paw volume.

2.7. Paw volume in formaldehyde induced arthritis

The left hind paw volumes of all animals were measured on day 0, 2, 4, 6, 8 and 10 using digital plethysmometer (Orchid Scientific& Innovative India Private Limited, Bombay, India). The change in paw volume was measured as the difference between the final and initial paw volumes.[9,10]

2.8. Complete Freund's adjuvant (CFA) induced arthritis

Arthritis was induced by a single injection of 0.1 ml CFA, (Sigma Aldrich, USA) into the sub-plantar surface of the left hind paw. Indomethacin and VGE were administered each day starting from 14th day after adjuvant injection till the 28th day.[11]

Paw volume and body weight changes were measured during the experiment. On the 28th day, blood was collected from animals and the animals were sacrificed by cervical decapitation for histopathology study of ankle joint.

2.9. Paw volume and body weight in CFA induced arthritis

The left hind paw volumes of all animals were measured just before CFA injection on day 0 and thereafter at different time intervals till day 28 using Digital Plethysmometer. Body weights of animals were measured by animal laboratory balance used for weighing small animals.

2.10. Blood analysis in CFA induced arthritis

The haematological parameters like haemoglobin (Hb), RBC, WBC, PCV, platelet and ESR were measured by Hematology Cell Counter (HEMA 2062, Analytical Technologies Limited, India). Rheumatoid factor was determined by using diagnostic kit (RF Turbilatex Tl020 from Chemelex, S.A, Barcelona). Estimation of serum total cholesterol (TC) by Cholesterol-LS kit (Piramal Enterprises Limited, Navi Mumbai, India), triglyceride (TG) by triglycerides-LS kit (Piramal Enterprises Ltd.), high density lipoprotein-cholesterol (HDL) by HDL direct kit (ErbaLachema S.R.O., Karasek, CZ.), low density lipoprotein-cholesterol (LDL) and very low density lipoprotein-cholesterol (VLDL) were done by using diagnostic kit with semi-auto analyzer (Photometer 5010, Piramal Healthcare V5.1, Mumbai, India).

2.11. Histological analysis in CFA induced arthritis

The animals were sacrificed on day 28 by cervical dislocation. Ankle joints were separated from the hind paw, immersed in 10% buffered formalin for 24 h and processed for microtome sectioning. Thin sections of 5 μm thickness were taken on a rotary microtome RM 2125 RTS (Leica Microsystem Incorporation, US). The sections were stained with haematoxylin and eosin and evaluated under light microscope for the presence of hyperplasia of synovium, pannus formation and destruction of joint space.[12]

2.12. Statistical analysis

The data was analysed by two-way ANOVA followed by Bonferroni test for multiple comparisons using Graph Pad Prism 5.03 software. Values with $P < 0.05$, $P < 0.01$ and $P < 0.001$ were considered significant.

3. Results

3.1. Acute toxicity test

Preliminary studies showed that there was no perceptible change in the autonomic and behavioural patterns of animals on the administration of VGE in the prescribed dose. There was no mortality in any rat even after 14 days. No signs of toxicity were observed in any rat.

3.2. Effect of VGE on paw volume and paw thickness in formaldehyde induced arthritis

Subplantar administration of formaldehyde into left hind paw showed an increase in joint diameter in all animals during the study. When compared to control, indomethacin and VGE treated groups showed a decrease in joint swelling in a dose dependent manner (Fig. 1).

Maximum inhibition of joint swelling was observed in group V (VGE 270 mg/kg) and in group VI (VGE 540 mg/kg).

3.3. Effect of VGE on paw volume and body weight in CFA induced arthritis

There was a significant increase in paw volume in all adjuvant treated groups compared to normal control group. The maximum increase in paw volume was observed on day 14. On treatment with VGE (135 mg/kg, 270 mg/kg and 540 mg/kg) from day 14 onwards, significant dose dependent inhibition was observed as compared with arthritic control animal group (Fig. 2).

On day 28, percent inhibitions in joint swelling observed in group V (VGE 270 mg/kg) and group VI (VGE 540 mg/kg) were 96% and 98.5% respectively (% Inhibition was calculated by dividing difference of paw volume on 28th day and 0 day with difference of paw volume on 14th day and 0 day of the same group and subsequently subtracting it from 100). No significant changes were observed in body weights of animals.

3.4. Effect of VGE on blood parameters in CFA induced arthritis

Marked changes in the haematological parameters were observed with induction of arthritis. Hb, RBC and packed cell volume (PCV) decreased in arthritic rats while there was an increase in WBC, platelet count, ESR and rheumatoid factor (RF). Significant decrease in HDL, increase in total cholesterol (TC), triglyceride (TG), LDL and VLDL were observed in arthritic rats. Such marked changes in biochemical and haematological parameters were not observed in rats treated with VGE or indomethacin (Table 1).

3.5. Effect of VGE on histology of ankle joint

The joint tissue sections of CFA injected arthritic control rats revealed major pathological changes when compared with joints of normal rats. In CFA induced arthritic control, oedematous inflammation, synovial hyperplasia, increased vascularity owing to vasodilation, marked mononuclear inflammatory cell infiltration with formation of lymphoid follicle at places were seen. Treatment with VGE (540 mg/kg and VGE 270 mg/kg) showed clear reduction in histological injury and changes as compared to arthritic control (Fig. 3).

4. Discussion

Guggulu, one of the ingredients of the formulation under study, has been studied in inflammation and pain in joints, bones and muscles.[13–16] Guggulu contains active phytoconstituent known as guggulsterones. Activation of NF-kappa B has been linked with inflammatory diseases affected by guggulusterones. Guggulusterones were found to suppress DNA binding of NF-kappa B induced by TNF- α and IL-1 which has been attributed for its anti-inflammatory activities.[17] *Triphala* (contains *Haritaki*, *Bibhitaka* and *Amalaki*) was found to suppress inflammatory processes by reducing the production of TNF- α in adjuvant induced arthritic mice and effective in rheumatoid arthritis.[18]

Vatari Guggulu was found to be safe in the preliminary toxicological studies.

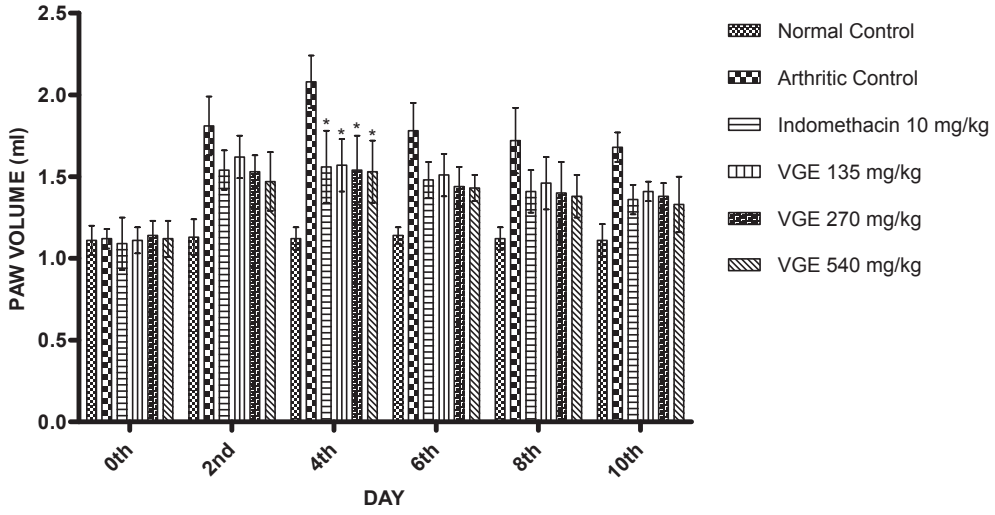

Fig. 1. Effect of *Vatari Guggulu* extract (VGE) on paw volume of formaldehyde treated animals. Values are expressed as mean ± S.E.M. of 6 animals. *P < 0.05 compared to arthritic control.

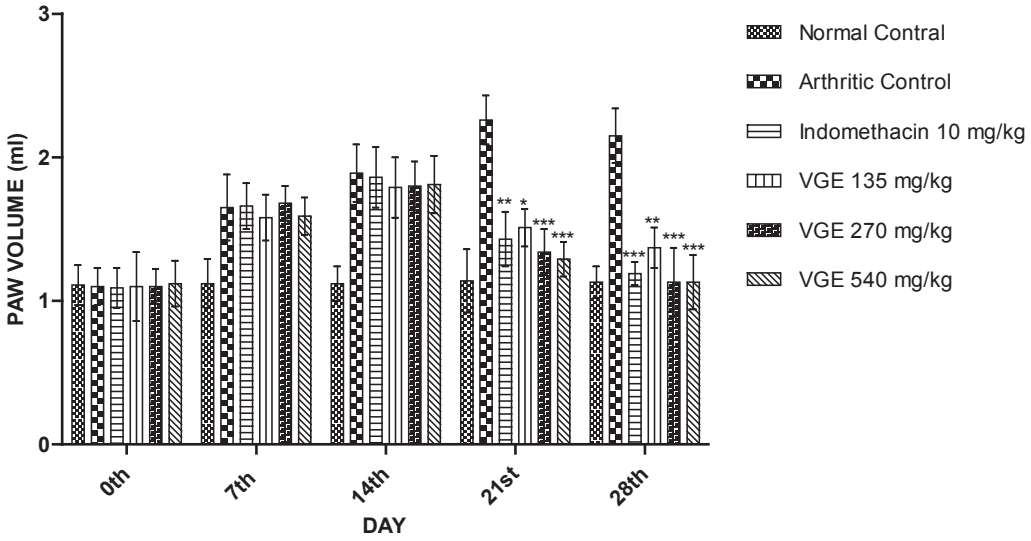

Fig. 2. Effect of *Vatari Guggulu* extract (VGE) on paw volume of CFA treated animals. Values are expressed as mean ± S.E.M. of six animals. *P < 0.05, **P < 0.01 and ***P < 0.001 compared to arthritic control.

In formaldehyde induced arthritis, VGE at a dose of 540 mg/kg showed marked inhibition in joint swelling. CFA induced arthritis is one of the most widely used models as it has been shown to share a number of clinical and immunological features with human arthritis.[11] Changes in haematological parameters were observed due to arthritic condition.[19] Pro-inflammatory cytokines such as TNF α, IL-1 and IL-6, generated in the synovial tissue, are released into the systemic circulation. These circulating cytokines tend to alter the function of distant organs and generate a spectrum of pro-atherogenic changes that include endothelial dysfunction, insulin resistance, dyslipidemia, prothrombotic effects, and pro-oxidative stress.[20]

The decrease in Hb, RBC reflects is a common extra articular manifestation in RA.[21] Anemia of chronic disease is immune driven; cytokines and cells of the reticulo-endothelial system induce changes in iron homoeostasis, proliferation of erythroid progenitor cells, production of erythropoietin, and life span of red cells, all of which contribute to the pathogenesis of anemia.[22] Increase in RF, WBC and platelet count might be due to stimulation of immune system against invading pathogenic microorganisms.[23] Increase in ESR reflects the chronicity of the disease.

The increased level of cholesterol in arthritic rats might be due to the presence of pro-inflammatory cytokines TNF-α and IL-I. TNF-α increases serum TG level by stimulating LDL production.[24] In our study, VGE significantly controlled the levels of Hb, RBC, PCV, WBC, platelet count, ESR and RF in a dose dependent manner as compared with arthritic control and indomethacin treated groups. Treatment with VGE re-established lipids to normal levels in arthritic rats during the treatment (Table 1).

Histological studies showed noticeable increase in synocytes number and decrease in number of inflammatory cells when compared with standard indomethacin treated group. Treatment with VGE 135 mg/kg showed moderate healing of joint injury when compared with standard treated group. VGE in doses of 270 mg/kg and 540 mg/kg demonstrated distinct reduction in histological injury (Fig. 3).

The present study illustrates the beneficial effect of VGE in adjuvant induced arthritic rat model with respect to its anti-arthritic activity. The purification process of guggul, sulphur and the addition of castor oil in the formulation may be responsible in regulating various cytokines and disease modifying processes thereby resulting in a comprehensive improvement in arthritis, lipid profile and haematological parameters. Guggul is also known to have lipid lowering activity whereas triphala is a powerful anti-oxidant that minimizes the cellular damage. This can be attributed to the ingredients of *Vatari Guggul* and the specific purification processes prescribed in the classical texts. Based on our study, further studies involving quantification of certain inflammatory

Table 1
Effect of *Vatari Guggulu* extract on CFA induced arthritic rats.

Parameters	Normal control[a]	Arthritic control[a]	Indomethacin[a] 10 mg/kg	VGE[a] 135 mg/kg	VGE[a] 270 mg/kg	VGE[a] 540 mg/kg
TC (mg/dl)	96.85 ± 1.33	145.05 ± 0.88	113.85 ± 1.53*	93.27 ± 1.53*	85.58 ± 2.00*	83.76 ± 1.18*
TG (mg/dl)	71.69 ± 1.06	96.60 ± 1.20	78.02 ± 0.88*	82.52 ± 1.02*	74.13 ± 0.58*	73.21 ± 0.97*
HDL (mg/dl)	29.40 ± 0.33	16.13 ± 0.58	24.67 ± 0.58*	27.33 ± 0.42*	31.96 ± 0.88*	32.09 ± 0.57*
LDL (mg/dl)	53.11 ± 1.73	109.60 ± 1.16	73.57 ± 1.20*	49.43 ± 1.13*	38.79 ± 1.12*	37.03 ± 1.31*
VLDL(mg/dl)	14.34 ± 0.37	19.32 ± 0.87	15.60 ± 0.48*	16.51 ± 0.37*	14.83 ± 0.67*	14.64 ± 0.52*
Hb (gm/dl)	13.92 ± 1.27	9.97 ± 1.03	13.23 ± 1.43*	12.76 ± 1.12*	14.67 ± 1.57*	14.97 ± 1.19*
RBC (millions/mm^3)	7.32 ± 0.87	6.58 ± 0.74	7.31 ± 0.91	7.28 ± 0.86	7.42 ± 0.83	7.45 ± 0.92
WBC (thousands/mm^3)	8.22 ± 0.67	11.42 ± 0.79	8.89 ± 0.74*	9.86 ± 1.01*	8.00 ± 0.81*	7.89 ± 0.96*
Platelet (lakhs/ml)	2.46 ± 0.23	4.15 ± 0.41	2.86 ± 0.33*	2.59 ± 0.32*	2.35 ± 0.16*	2.26 ± 0.26*
PCV (%)	44.04 ± 1.21	30.56 ± 1.43	36.5 ± 1.50*	34.33 ± 1.89*	42.62 ± 1.61*	44.16 ± 1.91*
ESR (60 min)	3.76 ± 0.31	8.03 ± 0.42	3.95 ± 0.47*	5.47 ± 0.62*	3.81 ± 0.62*	3.52 ± 0.52*
RF (IU/ml)	7.17 ± 0.13	26.77 ± 1.46	10.78 ± 0.77*	9.23 ± 0.48*	8.12 ± 0.67*	9.06 ± 0.28*

[a] Mean ± S.E.M. (n = 6) *P < 0.001.

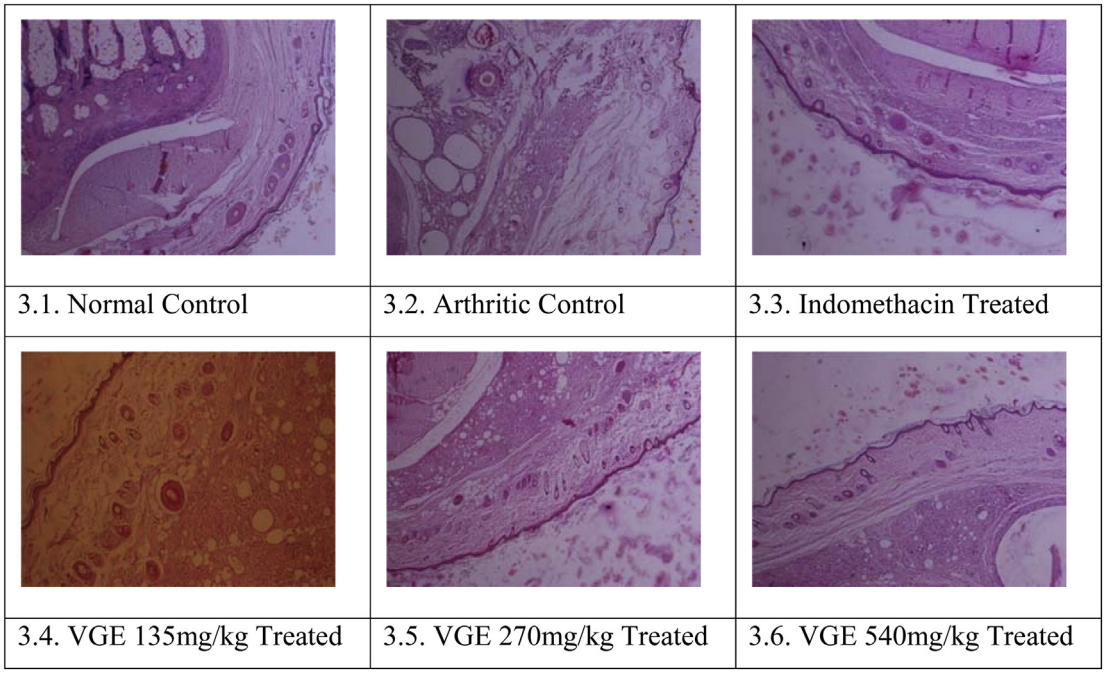

Fig. 3. Effect of *Vatari Guggulu* extract on histology of ankle joint in experimental rats in CFA induced arthritis. 3.1) Ankle joint synovial membrane structure in normal control rat; 3.2) Chronic inflammation involve synovial hyperplasia with increased vascularity, oedematous inflammation, inflammatory cell infiltrate (involve T-lymphocytes); 3.3) Synovial membrane structure re-establishing with the less oedema and inflammatory cell; 3.4) Moderate healing in the synovial membrane structure; 3.5) & 3.6) Decrease in inflammation with decrease in oedematous spaces, restructuring of synovial membrane with noticeable reduction of histological injury.

mediators might establish VGE as safer and potent option in the treatment of RA.

5. Conclusions

Vatari Guggulu not only reduced the inflammation and arthritis in rats but also improved a number of symptoms associated with the disease. This study clearly highlights the potential of *Vatari guggul* as a reliable treatment for arthritis substantiating the claims of the *Ayurvedic* system of medicine.

Conflict of interest

The authors report no conflicts of interest. The authors alone are responsible for the content and writing of this article.

Acknowledgements

The authors wish to thank a) The management of Parul Institute of Pharmacy, Limda and b) Dr. Manish D. Shah, Vice-President, Lok Jagruti Kendra Trust, Ahmedabad for providing the facilities to carry out the work.

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