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Toxicity and Efficacy Study of Novel Herbal Formulation of Hadjoint® Tablets

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ABSTRACT: Osteoarthritis, one of the most common forms of arthritis is a cause of concern especially in women and the elderly age group. Alternative modes of treatment like herbal medicines are preferred, since conventional methods of treatment do not treat the underlying cause. HADJOINT® tablet, a novel herbal formulation was prepared by combining different plant extracts like *Cissus quadrangularis, Moringa oleifera, Emblica officinalis* and others. The aim of the study was to determine the toxicity and efficacy of the herbal formulation in adjuvant induced osteoarthritis in Wistar rats. The study found HADJOINT® to be non-toxic till 2000 mg/kg body weight. It also showed lowered levels of erythema, edema and limping indicating reduced arthritis. The absence of clinical signs of mortality or toxicity, changes in hematology and biochemical parameters on treatment implies safety and efficacy potential comparable to Diclofenac.

Keywords: Edema, Erythema, Osteoarthritis, Toxicity

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INTRODUCTION

Osteoarthritis is the second most common rheumatologic problem and the most frequently occurring joint disease with a prevalence of 22% to 39% in India (Pal et al., 2016). Osteoarthritis is the clinical and pathological outcome of a range of disorders that results in structural and functional failure of synovial joints. It involves the entire joint organ including the subchondral bone, menisci, ligaments, periarticular muscle, capsule and synovium. Traditionally, it had been considered a disease of articular cartilage and most cases of osteoarthritis have no known cause. Primary osteoarthritis unlike secondary osteoarthritis is mostly related to aging presented as localized, generalized or as erosive osteoarthritis. The clinical symptoms of pain are particularly predominant after prolonged activity and weight-bearing, whereas stiffness is experienced after inactivity. The prevalence of osteoarthritis increases dramatically with age and is found more commonly in women than men (Hunter and Felson, 2006; Pal et al., 2016).

Currently, the cure for osteoarthritis remains elusive. Non-pharmacological management includes physical therapy, aerobic exercises, muscle strengthening, weight reduction, walking aids, knee braces, footwear and insoles, electromagnets, thermal modalities and acupuncture. Pharmacological management of osteoarthritis targets symptoms of the disease rather than the underlying cause with analgesics and non-steroidal anti-inflammatory drugs (NSAIDs) representing the mainstay of treatment (Altman, 2009). These drugs are the most widely prescribed drugs for osteoarthritis therapy generally to improve function and decrease pain and stiffness, although, the beneficial effects to the underlying use of these drugs have not been demonstrated (Abramson, 2003). Other treatment options of selective cyclooxygenase 2 (COX-2) inhibitors (rofecoxib) for pain management is reported to be associated with gastrointestinal and cardiovascular adverse events (Akhtar and Haqqi, 2012). Intra-articular therapies like glucocorticoid and hyaluronan injections used for pain relief have shown recent observations to suggest accelerated cartilage breakdown (Gonzalez-Fuentes et al., 2010). Glucosamine and chondroitin sulfate are most commonly used dietary supplements for treatment and prevention of osteoarthritis (Akhtar and Haqqi, 2012). However, evidence is conflicting regarding the efficacy of glucosamine and chondroitin for osteoarthritis (Sawitzke et al., 2010).

Long-term use of available pharmacological agents to relieve osteoarthritis symptoms is associated with serious adverse events and highlights the importance of developing safer alternatives and prevention strategies. Nutraceuticals and dietary supplements derived from herbs have long been used in traditional medicine with considerable evidence towards inflammation and joint destruction in osteoarthritis (Akhtar and Haqqi, 2012). Nutraceuticals and medicinal plants have become the focus of current medical research in the treatment or prevention of diseases (Pandey

et al., 2011). Some plants like *Cissus quadrangularis, Moringa oleifera, Emblica officinalis, Withania somnifera, Zingiber officinale* and *Commiphora mukul* have been reported by various studies to have anti-inflammatory effect while *Terminalia arjuna* has been found to improve calcium and hardness of lumbar vertebrae in ovariectomized female Sprague Dawley model rats. The present study aims at determining the toxicity and efficacy of a novel herbal formulation HADJOINT® tablets comprised of different medicinal plants in osteoarthritis.

MATERIALS AND METHODS

Oral acute toxicity and efficacy studies were carried out using standard operating procedures of Liveon Biolabs Pvt. Ltd., Tumkur, Karnataka, India along with study plan mutually agreed and approved with the sponsor.

Ethics committee approval

The two studies were carried out in accordance with the recommendation of the Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA) guidelines for laboratory animal facility and protocols approved by Institutional Animal Ethics Committee (IAEC); OECD Guidelines for Testing of Chemicals (No. 423) on conduct of "Acute Oral Toxicity – Acute Toxic Class Method" (Adopted: 17th December 2001).

Acute oral toxicity study

The fixed dose procedure was employed with 12 female in-house bred Wistar rats, 8-12 weeks of age with a body weight range of 141.12 g – 167.11g. They were acclimatized for duration of 5 days to laboratory conditions, fed *ad libitum* and observed for clinical signs daily. The fixed dose levels of 300 and 2000 mg/kg body weight were selected as per the sequential dose selection followed in the OECD guidelines and administered orally in a single dose. The animals were fasted overnight before the test item administration and observed for clinical signs of toxicity or mortality for a period of 24 hours. The dosage volume of 10 ml/kg body weight administered to each rat was adjusted according to the body weight recorded on the day of dosing. The formulation was freshly prepared using distilled water and administered within 30 minutes of preparation. Food was offered 3 hours after the dosing and the total duration of the study was 25 days. The animals were observed for clinical signs of toxicity and mortality during the 14 day observation period. All the animals were sacrificed on day 15 using carbon dioxide exposure method, subjected to necropsy and detailed gross pathological examination.

Efficacy study

In-house bred male Wistar rats 9-10 weeks of age, with a body weight range of 280 g-300 g were divided into 5 groups of 6 animals each (Table 1). The animals were acclimatized for 13 days to laboratory conditions, fed *ad libitum* and observed for clinical signs daily. The dosage volume of the test item and vehicle was maintained at 10 ml/kg body weight for all the animals. The total duration of the study was 28 days including 13 days of acclimatization period and 15 days of study duration. The test items were administered daily after induction of osteoarthritis with FCA (Freund's Complete Adjuvant). Individual animal body weights were recorded and observed for clinical signs of toxicity, mortality and morbidity. Edema in the right ankle of all the animals was measured before the injection of FCA and prior to the initiation of treatment. Measurements were made and therapeutic effects of test item were observed after starting the treatment process. Scoring for erythema, edema and limping of all the animals were performed and recorded.

Table 1. Study design for efficacy study of HADJOINT® (coded GP006) had animals divided into groups as follows.

Group	Treatment	Dose	Route of administration
G1	Vehicle control	0	Oral
G2	Positive Control (FCA)	100 µl	Sub plantar region
G3	FCA	100 µl	Sub plantar region
	Diclofenac	30 mg/kg	Oral
G4	FCA	100 µl	Sub plantar region
	GP006	150 mg/kg	Oral
G5	FCA	100 µl	Sub plantar region
	GP006	300 mg/kg	Oral

All animals were anesthetized using isoflurane 24 hours after the last treatment on the 16th day; blood was collected into tubes with and without anticoagulant to evaluate hematology and biochemistry parameters, respectively. Biochemistry parameters of calcium, phosphorous, ALT (Alanine aminotransaminase), AST (Aspartate aminotransaminase), ALP (Alkaline Phosphatase), bilirubin and total proteins while hematology parameters of total RBC (Erythrocytes), WBC (Leucocytes) and platelets were analyzed and recorded. The animals were subjected to external and internal gross pathological examinations. After the completion of the

study period, all the animals in each group were humanely sacrificed by carbon dioxide asphyxiation and subjected to external and internal gross necropsy. Knee joint with femur and tibia were collected and preserved for future histopathological evaluations.

Statistical analysis

The raw data obtained from the study was subjected to statistical analysis. The data was subjected to one-way ANOVA (Analysis of Variance) with Dunnett's post test. All the analysis and comparisons were evaluated at the 95% level of confidence ($P<0.05$).

RESULTS AND DISCUSSION

Toxicity study

No significant treatment related changes in body weight and percent body weight, clinical signs of toxicity and mortality or gross pathological changes were reported over the study period in all the doses tested. In the present toxicity study, tablets of HADJOINT® were found to be safe and non toxic as no clinical signs of toxicity and mortality were observed till the dose concentration of 2000 mg/kg bodyweight. Single acute oral administration of polyherbal formulation Zigbir® did not cause any mortality in female Sprague-Dawley rats and the median lethal dose was found to be more than 2000 mg/kg b.w. (Allan et al, 2012). In a previous study, whole plant aqueous extract of *Cissus quadrangularis* was found to be non-toxic at 3000 mg/ kg body weight in Swiss albino mice evaluated as per OECD guideline (Andugula et al., 2018). The LD_{50} of *Withania somnifera* root extract through acute and chronic toxicity was found to be more than 1000 mg/ kg in female albino rat (Sahni et al., 2018).

Acute toxicity studies are commonly used to determine LD_{50} of drugs or chemicals and assess toxicity data required to predict the safety before use (Chanda et al., 2015). Observations in behavioral changes, hematological and biochemical parameters, body weight changes and mortality during acute toxicity studies indicated ayurvedic formulation to be safe similar to the present. Incomplete knowledge about the toxicity profile of a putative drug will entail certain amount of risk to the recipient study (Kotecha et al., 2013). Herbal preparations need to be authenticated by scientifically validated tests for toxicological properties before being introduced for widespread consumption (Allan et al, 2012).

Efficacy study

In the present study no clinical signs of toxicity or mortality were observed except for ankle edema caused by administration of FCA. There were no significant changes in body weight and body weight gain. Treatment with HADJOINT® showed non statistically significant changes in hematological (Figure 1) and biochemical parameters (Figure 2) when compared with the control. The effects of the dose of 150 mg/kg body weight of the formulation HADJOINT® was comparable to Diclofenac. These findings are similar to the toxicological, biochemical and histopathological evaluation of Tridham, a siddha medicine evaluated in Wistar albino rats (Jaganathan et al., 2012). Studies on *Moringa oleifera* rich in calcium could be a contributing factor as found in previous study (Abbas et al., 2018).

The reduction in limping was observed with treated groups showing decrease in arthritis index comparable to Diclofenac and when compared to the untreated group. There was significant reduction in erythema similar to Diclofenac (Figure 3) and decrease in edema was better than Diclofenac in the present study (Figure 4). No external and internal gross pathological changes were found in the present study similar to the observations made in a prior study (Jaganathan et al., 2012). Reduced inflammation by carrageenan induced acute rat paw edema method and chronic Rexin pellet granuloma method have reported anti-inflammatory activity of *E. officinalis* in both acute as well as chronic model of inflammation comparable to Diclofenac (Santoshkumar et al., 2013). A poly herbal formulation (Aller-7) containing *Phyllanthus emblica* had powerful anti-inflammatory activity against compound 48/80-induced paw edema in both Balb/c mice and Swiss Albino mice as well as carrageenan-induced paw edema in Wistar albino rats (Pratibha et al., 2004). *W. somnifera* extracts caused a significant reduction in both paw swelling and bony degenerative changes in Freund's adjuvant induced arthritis as observed by radiological examination (Begum and Sadique, 1988). A study on herbal formulation found potential therapeutic property in protecting articular cartilage against progression of osteoarthritis (Kim et al., 2011). Significant inhibition of both maximal and total carrageenan-induced rat paw edema was attributed to Gugulipid present in *Commiphora mukul* (Duwiejua et al., 1993).

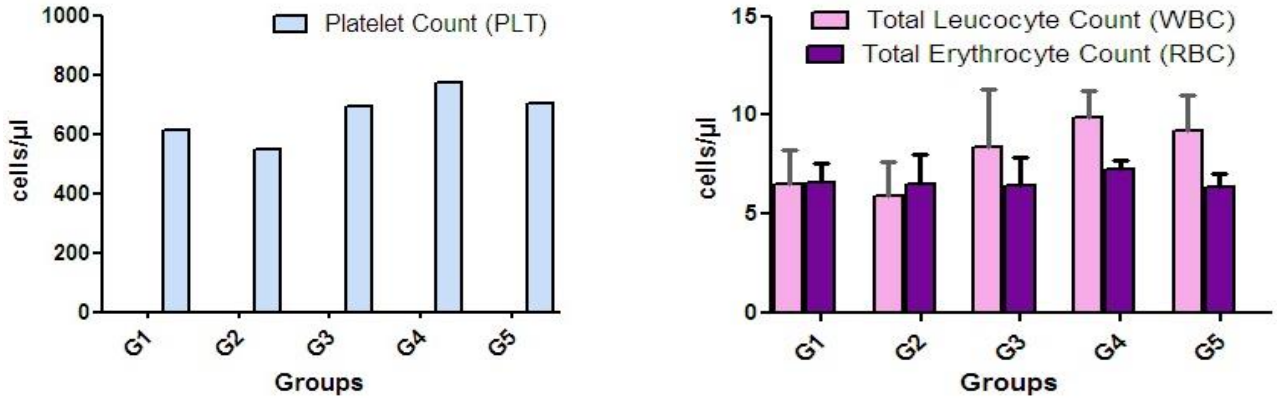

Figure 1. Effect of HADJOINT® on hematology parameters in FCA induced osteoarthritis in rats.

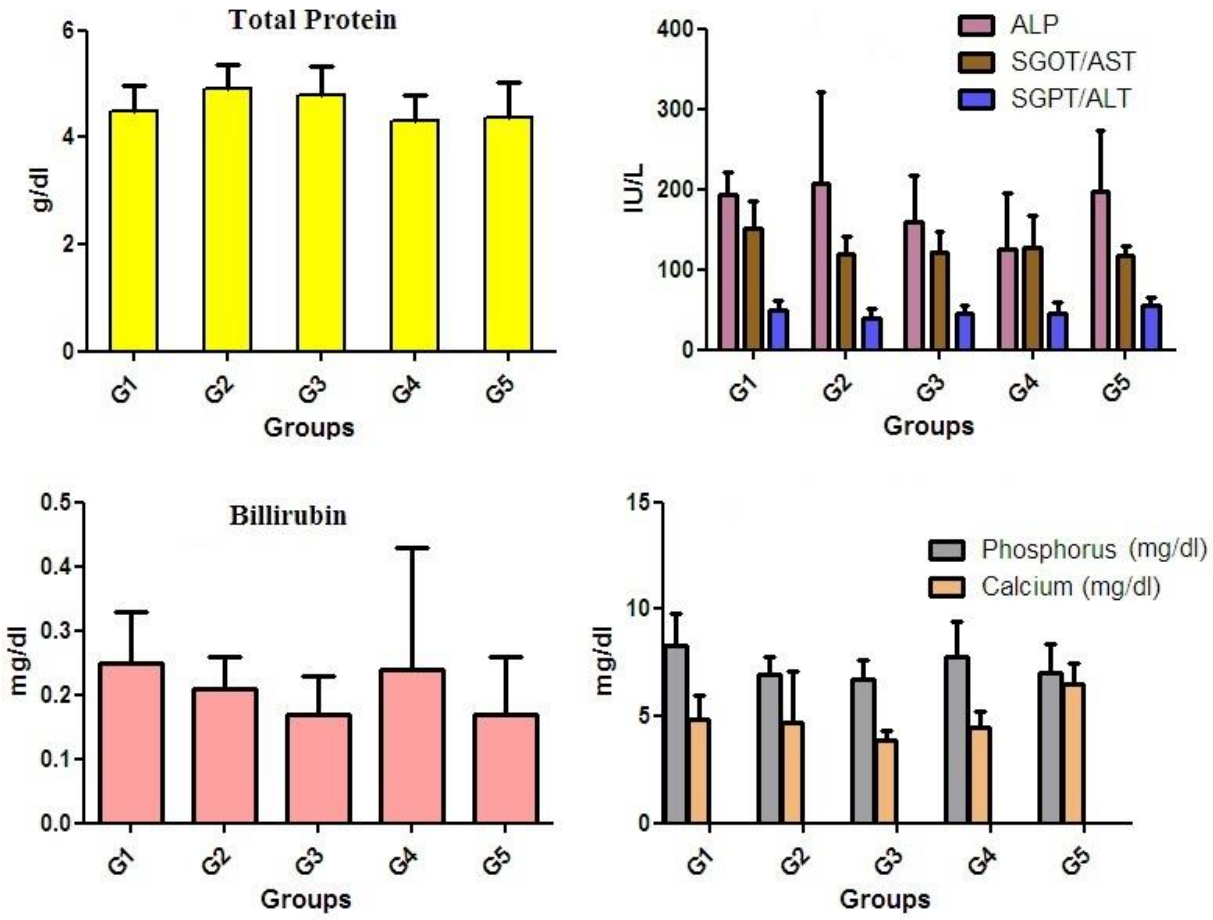

Figure 2. Effect of HADJOINT® on biochemical parameters in FCA induced osteoarthritis in rats.

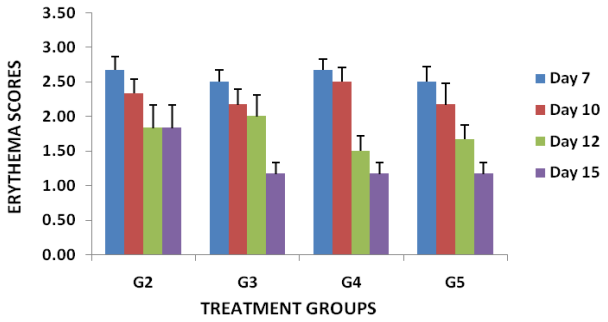

Figure 3. The effect of HADJOINT® on erythema scores in FCA induced osteoarthritis.

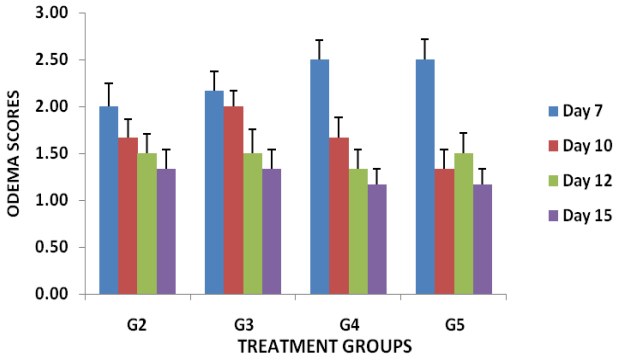

Figure 4. The effect of HADJOINT® on edema scores in FCA induced osteoarthritis

CONCLUSION

The results of the study concluded that HADJOINT® is non toxic up to 2000 mg/kg body weight and showed significant reduction in erythema, edema, limping and lowered arthritic index against FCA induced osteoarthritis. Thus, HADJOINT® tablets can be considered safe and efficacious for osteoarthritis.

DECLARATIONS

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Competing interests

The author declares to have no competing interests.

REFERENCES

Abbas R, Elsharbasy F and Fadlelmula A (2018). Nutritional Values of Moringa oleifera, Total Protein, Amino Acid, Vitamins, Minerals, Carbohydrates, Total Fat and Crude Fiber, under the Semi-Arid Conditions of Sudan. Journal of Microbial and Biochemical Technology, 10(2): 56-58.

Abramson S (2003). The role of NSAIDs in the treatment of osteoarthritis. In Brandt, K.D., Doherty, M. and Lohmander, L.S. (eds). Osteoarthritis. Oxford: Oxford University Press, pp. 251–258.

Akhtar N and Haqqi T (2012). Current nutraceuticals in the management of osteoarthritis: a review. Therapeutic Advances in Musculoskeletal Disease, 4(3): 181–207.

Allan J, Bhide R and Agarwal A (2012). Safety Assessment of Zigbir: A Polyherbal Formulation in Sprague-Dawley Rats. Journal of Toxicology, Article ID 589520.

Altman R (2009). Practical considerations for the pharmacologic management of osteoarthritis. The American Journal of Managed Care, 15 (8 Suppl.): S236–S243.

Andugula K, Babu A, Nadendla R and Chandra Sekhar G (2018). Acute Toxicity Study Of *Cissus Quadrangularis* In Swiss Albino Mice. Panacea Journal of Pharmacy and Pharmaceutical Sciences, 7(1): 748-756.

Begum V and Sadique J (1988). Long term effect of herbal drug *Withania somnifera* on adjuvant induced arthritis in rats. Indian Journal of Experimental Biology, 26:877-882.

Chanda S, Parekh J, Vaghasiya Y, Dave R, Baravalia Y and Nair R (2015). Medicinal plants-from traditional use to toxicity assessment: a review. International Journal of Pharmaceutical Sciences and Research, 6(7):2652-2670.

Duwiejua M, Zeitlin I, Waterman P, Chapman J, Mhango G and Provan G (1993). Anti-inflammatory activity of resins from some species of the plant family Burseraceae. Planta Medica, 59:12-16.

Gonzalez-Fuentes A, Green D, Rossen R and Ng B (2010). Intra-articular hyaluronic acid increases cartilage breakdown biomarker in patients with knee osteoarthritis. Clinical Rheumatology, 29: 619–624.

Hunter D and Felson D (2006). Osteoarthritis. British Medical Journal, 332(7542): 639–642.

Jaganathan R, Ravinayagam V, Panchanadham S and Palanivelu S (2012). Toxicological, biochemical and histopathological evaluation of Tridham, a siddha medicine in Wistar albino rats. Journal of Biochemical Technology, 4(1):541-548.

Kim J, Park S, Kang J, Kim Y, Lee S, Shin J, et al. (2012). Effect of GCSB-5, a Herbal Formulation, on Monosodium

Iodoacetate-Induced Osteoarthritis in Rats. Evidence-Based Complementary and Alternative Medicine, Article ID 730907.

Kotecha K, Kotecha B, Shukla V, Prajapati P and Ravishankar B. (2013). Acute toxicity study of Vasaguduchyadi Kwatha*:* A compound Ayurvedic formulation. Ayu, 34(3): 327–330.

Pal C, Singh P, Chathurvedi S, Pruthi K and Vij A. (2016). Epidemiology of knee osteoarthritis in India and related factors. Indian Journal of Orthopaedics, 50(5): 518–522.

Pandey N, Meena R, Rai S and Paey-Rai S (2011). Medicinal Plants Derived Nutraceuticals : A Re-Emerging Health Aid. International Journal of Pharma and Bio Sciences, 2(4): 420-441.

Pratibha N, Saxena V, Amit A, D'Souza P, Bagchi M and Bagchi D (2004). Anti-inflammatory activities of Aller-7, a novel polyherbal formulation for allergic rhinitis. International journal of tissue reactions, 26:43-51.

Sahni Y, Sharma M and Pandey G (2014). Studies on Phytochemistry And Toxicity Of *Withania Somnifera*. International Journal of Animal, Veterinary, Fishery and Allied Sciences, 1(1):12-16.

Santoshkumar J, Devarmani M, Sajjanar M, Pranavakumar MS, and Dass P (2013). A study of Anti-inflammatory activity of fruit of *Emblica officinalis* (Amla) in Albino rats. Medica Innovatica, 2(1):17- 26.

Sawitzke A, Shi H, Finco M, Dunlop D, Harris C, Singer N, et al. (2010). Clinical Efficacy and Safety of Glucosamine, Chondroitin sulfate, their combination, celecoxib, or placebo taken to treat osteoarthritis of the knee: 2 year results from GAIT. Annals of the Rheumatic Diseases, 69(8):1459-64.