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Case study

Evaluation of Swarna Guggulu in management of arthritis basis *in vitro* anti-inflammatory efficacy and ingredient based benefits

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Abstract

Arthritis and osteoarthritis are common joint problems that eventually become significant enough to impact daily activities of those afflicted, often due to severe pain caused by joint inflammation and at times; due to their serious complications. Swarna Guggulu is an Ayurvedic herbomineral formulation, comprising ingredients traditionally known for maintaining the health of joints. We investigated the benefits of Swarna Guggulu in arthritis basis *in vitro* anti-inflammatory efficacy by quantitation of modulation in the production of proinflammatory chemokine and cytokines such as MIP-1-α, TNF-α and IL-1-β secreted by Swarna Guggulu treated dendritic cells as compared to LPS stimulated dendritic cells alone. At non-cytotoxic concentrations, treatment of LPS stimulated DCs with Swarna Guggulu in the concentration range of 0.1µg/ml-50µg/ml, resulted in considerable inhibition of production of LPS induced proinflammatory markers-MIP-1-α, TNF-α, and IL-1-β. Down regulation in levels of LPS induced TNF-α (56.2-71.2 %), MIP-1-α (32.3 – 41.7 %) and IL-1-β (34.4-45.2 %) compared to LPS treated DCs alone, suggested potential anti-inflammatory activity of Swarna Guggulu. Results of *in vitro* study were in sync with the documented benefits of its ingredient in arthritis.

Key words: Anti-inflammatory, arthritis, herbomineral, *in vitro*, Swarna Guggulu, ingredient based benefits

1. Introduction

Arthritis is a general term for conditions that affect the joints and surrounding tissues. Of these, osteoarthritis (OA) is the most common joint condition, accounting for more than 85% of the arthritic cases (Pandey and Mishra, 2011). Most consider OA to be a problem, owing to severe pain caused by joint inflammation that eventually restricts their movements significantly, impacting their daily activities. At times, there are more serious complications of osteoarthritis including chondrolysis, osteonecrosis, hair line crack in the bone, infection in the joint and deterioration or rupture of the tendons and ligaments around the joint, leading to loss of stability, and so on. In modern medical science, arthritis can be managed with analgesics and nonsteroidal anti-inflammatory drugs (NSAIDs), joint replacement surgery, *etc.*(van Laar *et al*., 2012). Recent studies on NSAIDs report that these drugs may increase the risk of chronic renal disease and may have adverse effects such as gastric ulcers, liver toxicity, aseptic meningitis and renal failure. Also, surgery may not give long-lasting results in many patients (Woolf and Pfleger, 2003). As such, the need to explore safe, effective and lasting treatment options for arthritis and osteoarthritis has arisen.

In Ayurveda, arthritis and osteoarthritis are covered under the aegis of Vataroga. The term encompasses a variety of conditions like Sandhigatavata (Osteoarthritis), Vatarakta (Gout) and Amavata (Rheumatoid arthritis) (Sharma *et al*., 2013) that result in the process of gradual degeneration of the structural units of body like bones and joints (CCRAS, 2016). Swarna Guggulu (Mfd: Dabur India Limited) is an Ayurvedic herbomineral formulation, comprising ingredients like Swarnabhasma, Kumkuma, Ashvagandha and Mahayogaraj Guggulu processed in decoctions of Eranda Mool and Rasna (Sen, 2012), which have been used traditionally for their beneficial effects in joint health and management of arthritis. The present study was carried out to evaluate the efficacy of Swarna Guggulu for the treatment of arthritis basis *in vitro* anti-inflammatory activity.

2. Material and Methods

2.1 Study product

Swarna Guggulu (DRDC/AY/8049) is an Ayurvedic herbomineral formulation. The composition details of Swarna Guggulu are given in Table 1. All the ingredients of Swarna Guggulu have traditionally been used since long and are reported to be safe.

2.2 Chemical and reagents

DMSO (Merck), ELISA kit IL-1-β, ELISA kit MIP-1-α, ELISA kit TNF- α (R&D systems), FBS (Tissue Culture Biologics), HBSS (SIGMA), LPS (SIGMA), MTT (ACROS Organics), PBS (Oxford Biochemicals), Penicillin/Streptomycin(Krishgen), rmGMCSF (*E.coli*) (R & D systems), RPMI-1640 (Lonza), Swarna Guggulu (Dabur India Limited).

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Table 1: Composition details of Swarna Guggulu*

Contents (Each tablet of 125 mg)	Quantity (mg)
Swarnabhasma (Calcined gold)	2
Kumkuma (*Crocus sativus*, Stl. /Stg.)	3
Ashwagandha (*Withania somnifera*, Rt.)	20
Mahayograj Guggulu (Each 100 mg of Mahayograj Guggulu is prepared from powder of the following:Shunthi (*Zingiber officinale*, Rz.), Pippali (*Piper longum*, Fr.), Chavya (*Piper retrofractum*, St.), Pippallmula (*Piper longum*, Rt.), Chitraka (*Plumbago zeylanlca*, Rt.), Suddha Hingu '(*Ferula foetida*, Exd.), Ajamoda (*Apium leptophyllum*, Fr.), Sarshapa (*Brasslca campesteris*, Sd.), Shvetajiraka (*Cuminum cyminum*, Fr.), Krshnajiraka (*Carum carvi*, Fr.), Renuka (*Vitex negundo-official substitute*, Fr.), lndrayava (*Holarrhena antldysenterica*, Sd.), Patha (*Cissampelos pareira*, Rt.), Vidanga (*Embelia ribes*, Fr.), Gajaplppali (*Scindapsus officinalis* Fr.), Katuka (*Picrorrhiza kurroa*, Rz.), Ativisha (*Aconitum heterophyllum*, Rt. Tr.), Bharangi (*Clerodendrum serratum*, Rt.), Vacha (*Acorus calamus*, Rt.), Murva (*Marsdenia tenacissirna*, Rt.) - each 0.43 mg Amalaki (*Emblica officinalis*, P.), Haritaki (*Terminalia chebula*, P.), Bibhitaka (*Terrninalla belerica*, P.)-each 4 mg, Suddha Guggulu (*Comml phora wightii*, Exd.)-25.86 mg, Vanga Bhasma, Rajat Bhasma, Naga Bhasma, Lauh Bhasma, Abhraka Bhasma, Mandura Bhasma, Ras Slndura-each 6.9 mg	100

***** Part used: Bk. = Bark, Dr. Fr.= Dried fruit, Fl.= Flower, Fl. Bd,= Flower bud, , Fr.= Fruit, Gl. Gall of Insect, Ht. Wd. = Heart wood, Rt. = root, Lf.= Leaf, P = Pericarp; Pl.= Plant, Rt. = Root, Rt. Tr. =Root tuber, Rz. = Rhizome, Sd.=Seed, St = Stem, St. Bk. = Stem bark, Stmn. = Stamen; Wd. = Wood

2.3 Experimental design

The study was carried out in the Pharmacology Division of Althea Lifesciences Ltd, New Delhi - 110029, India. Anti-inflammatory potential of Swarna Guggulu was investigated, using dendritic cells (DCs), generated from murine bone marrow precursors.

Non-cytotoxic concentrations (leading to >80 % viability) of Swarna Guggulu were determined for DCs by cytotoxicity assay. At selected non-cytotoxic concentrations, anti-inflammatory activity of Swarna Guggulu was determined by quantitation of levels of pro-inflammatory cytokines and chemokine secreted by LPS stimulated DCs. Any modulation in the production of pro-inflammatory chemokine and cytokines such as MIP-1-α, TNF-α and IL-1-β secreted by Swarna Guggulu treated DCs as compared to LPS stimulated DCs alone to evaluate the anti-inflammatory activity.

2.3.2 *Preparation of stock solutions*

285.4 mg of Swarna Guggulu was weighed and dissolved in 1.427 ml of DMSO to obtain a stock solution of 200 mg/ml. This stock solution was used for preparation of subsequent dilutions in serum free RPMI-1640 to achieve final concentrations in the range of 0.1 µg/ml - 500 µg/ml. (0.1,1,10, 20, 50, 80, 100, 200, 300, 500 µg/ml). This broad concentration range was taken to inlcude the minimum and maximum possible testing concentations for this assay.

2.3.3 *Generation of primary dendritic cell cultures*

Femurs were removed from 7 weeks old C57BL/6 mice and separated from the surrounding muscle tissue. Bone marrow was collected by gently flushing the femur with HBSS using a 23-gauge needle. At Day 0, 2 X 106 bone marrow precursor cells were seeded in 90 mm petridishes containing 10 ml of culture medium with GMCSF (10 ng/ml). At Day 3, another 10 ml of culture medium was added to culture petridishes for replenishment and cultures were incubated at 37°C. Immature DCs were harvested by gentle pipetting on day-06 and used for the assays.

2.3.4 *Determination of non-cytotoxic concentrations of Swarna Guggulu*

Immature DCs were seeded at a density of 5 X 10^4 cells/ well in 96-well culture plates and treated with Swarna Guggulu corresponding to final concentrations, ranging from 0.1 µg/ml-500 µg/ml in triplicates. The cytotoxic effect of Swarna Guggulu on DCs was determined after 24 h by addition of MTT (0.5 mg/ml). After 3 h, plates were centrifuged at 250 g for 8 min. Supernatants were removed and cell pellets were resuspended in 150 µl of DMSO. Absorbance of the samples was measured at 490 nm. Concentrations of Swarna Guggulu that resulted in >80 % viability of DCs were determined and selected for subsequent cytokine analysis studies (Mosmann, 1983, and Madaan *et al*., 2013).

The percentage viability of DCs at respective concentrations was calculated using the following formula:

$$\% \text{ Viability} = [100 - (\% \text{ Cytotoxicity of DCs})]$$

where % cytotoxicity of DCs = [1 – {O.D.490 nm of DCs treated with Swarna Guggulu / O.D.490 nm of DCs treated with 0.25% DMSO}]*100

2.3.5 *Evaluation of anti-inflammatory activity*

Immature DCs were seeded at a density of 16 X 10^4 cells in 24-well plates and stimulated with LPS (10 ng/ml) for 30 min at 37°C. DCs were then treated with Swarna Guggulu corresponding to achieve final concentrations of 0.1 µg/ml - 500 µg/ml in duplicates. The plates were kept in CO_2 incubator at 37°C for 24 h. Culture supernatants were collected from all the wells and stored at – 20°C for subsequent estimation of cytokine levels using ELISA. DCs stimulated with LPS (*E. coli*) were used as control at 10 ng/ml studies (Kumar *et al*., 2009; Madaan *et al*., 2013).

2.3.6 *Estimation of cytokines/chemokines using* ELISA

Levels of MIP-1-α, TNF-α and IL-l-β were estimated at 5 non-cytotoxic concentrations of Swarna Guggulu selected on the basis of cytotoxicity assay using ELISA as per manufacturer's instructions. These assays employ the quantitative sandwich enzyme immunoassay technique. Commercially available polyclonal antibody, specific for mouse cytokine (precoated onto a microplate) were used.

Concentrations of cytokine/chemokine were then determined from the standard curve. Percentage change in levels of cytokine/ chemokine in culture supernatants was determined using the following formula:

$$\{(B-A)/A\}*100,$$

where, B = Concentration of cytokine/chemokine (pg/ml) secreted by LPS-stimulated DCs treated with Swarna Guggulu; A= Concentration of cytokine / chemokine (pg/ml) secreted by LPS-stimulated DCs alone.

3. Results and Discussion

3.1 Anti-inflammatory efficacy of Swarna Guggulu

Effect of Swarna Guggulu on viability of DCs was determined at concentrations ranging from 0.1 - 500 µg/ml. The concentrations selected for cytokine/chemokine analysis, which led to > 80 % viability of DCs were 0.1 µg/ml, 1 µg/ml, 10 µg/ml, 20 µg/ml and 50 µg/ml. These were selected non cytotoxic concentrations used for further evaluation of anti-inflammatory activity (Figure 1).

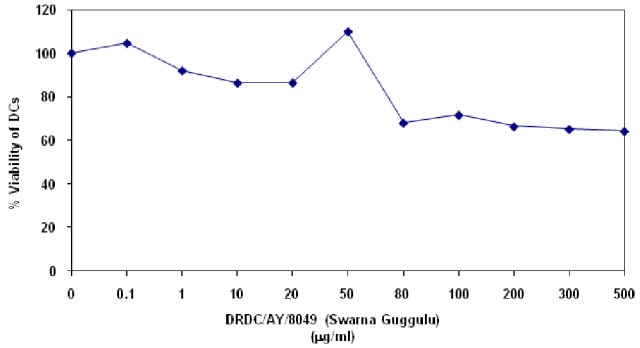

Figure 1: Effect of Swarna Guggulu on viability of DCs after 24 h of treatment

Treatment of LPS stimulated DCs with Swarna Guggulu in the concentration range of 0.1µg/ml-50 µg/ml resulted in considerable inhibition of production of LPS induced proinflammatory markers; TNF-α, MIP-1-α and IL-1-β. Down regulation in levels of LPS induced TNF-α (56.2-71.2 %), MIP-1-α (32.3-41.7 %) and IL-1-β (34.4-45.2 %) compared to LPS treated DCs alone which suggested potential anti-inflammatory activity of Swarna Guggulu (Figures 2-4).

Inflammation is a physiological response of a host to any stimuli including infections and tissue injury and is executed to protect it from these inflammatory stimuli. However, excessive or persistent inflammation causes a variety of pathological conditions such as bacterial sepsis, rheumatoid arthritis and skin inflammation (Longmore *et al*., 2004 ; Agarwal and Malviya, 2005).

Dendritic cells (DCs) are professional antigen-presenting cells that play pivotal roles in the induction of protective immunity as initiators of T cells responses against microbial pathogens, tumors and inflammation, sepsis, rheumatoid arthritis and skin inflammation (Banchereau *et al*., 2000; Lebre and Tak, 2008). By presenting antigens to naive T cells, DCs initiate adaptive immunity. Upon maturation by stimuli such as lipopolysaccharide (LPS), DCs produce excess proinflammatory chemokines and cytokines; such as macrophage inflammatory protein (MIP-1-α) (Cook, 1996; Kumar *et. al*., 2014), tumor necrosis factor (TNF-α) (Feldmann and Maine, 2003) and interleukin-1 beta (IL-1-β) (Dinarello, 1991) into extracellular environment and mimic inflammatory scenario. Excess secretion and increased accumulation of various proinflammatory cytokines and chemokines is a hallmark feature of inflammatory disorders. An inhibitory effect on secretion of these proinflammatory cytokines secreted by DCs indicates promising anti-inflammatory properties of the test compound. DCs represent an ideal target cell population to evaluate the anti-inflammatory action of a test agent and, hence, these cells were selected to evaluate the anti-inflammatory action of Swarna Guggulu.

Anti-inflammatory activity of Swarna Guggulu was assessed at concentrations up to a maximum of 50 µg/ml. Within the concentration range of 0.1-50 µg/ml, a considerable inhibition of key proinflammatory markers secreted by DCs (TNF-α, MIP-1-α and IL-l-β) was exhibited by Swarna Guggulu, which was indicative of its anti-inflammatory potential.

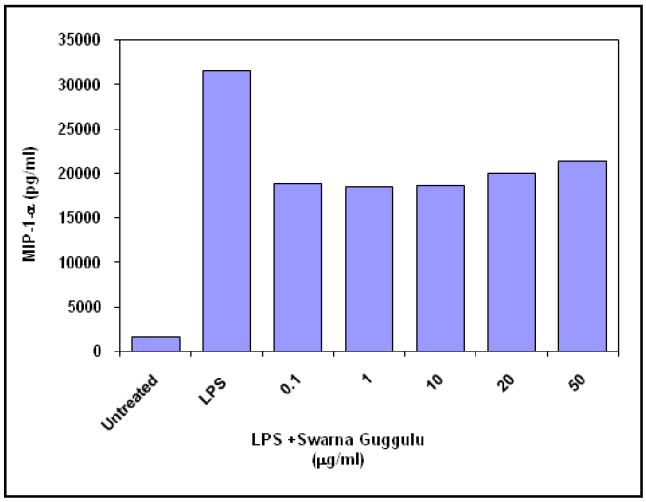

Figure 2: Effect of Swarna Guggulu on the expression levels of MIP-1-α secreted by LPS-stimulated DCs

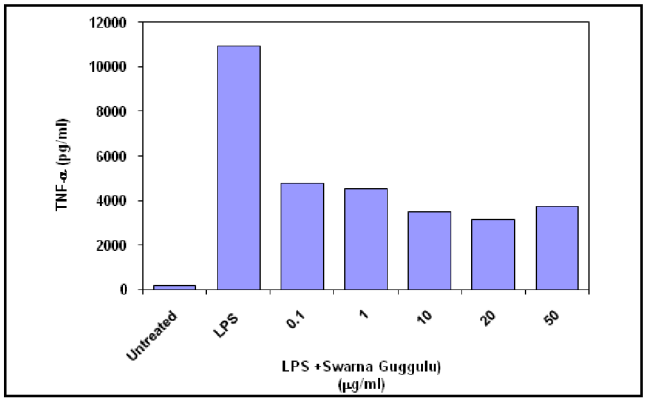

Figure 3: Effect of Swarna Guggulu on the expression levels of TNF-α secreted by LPS-stimulated DCs

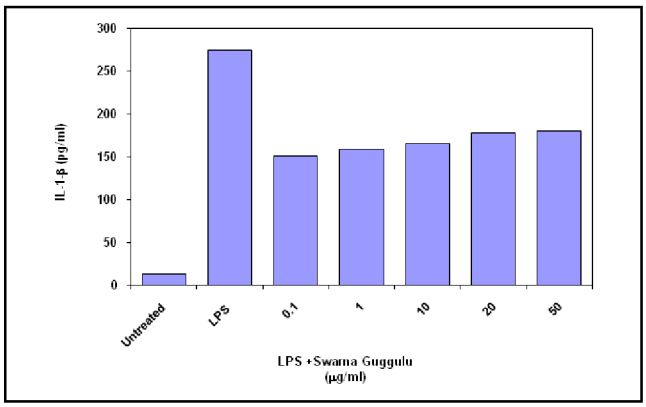

Figure 4: Effect of Swarna Guggulu on the expression levels of IL-1- β secreted by LPS-stimulated DCs

3.2 Ingredient based benefits

Swarnabhasma (Calcined gold) is a Rasayana as per Ayurveda texts. Modern pharmacology interprets Rasayana as "A product having properties of antioxidant, immunomodulatory, antistress, antiageing and provides promotion of health" (Govindarajan *et al.*, 2005). Swarnabhasma has been used by Ayurvedic physicians to treat arthritis and rheumatoid arthritis in its Ayurvedic ash form since ancient times, while modern medicine inadvertently discovered its use as disease-modifying antirheumatic drug (DMARD) in the last century (Chopra and Saluja, 2010). Swarnabhasma is reported to exhibit analgesic activity against four types of noxious stimuli-chemical, electrical, thermal and mechanical in preclinical studies (Bajaj and Vohra, 1998). Free radical scavenging, antioxidant and immunomo-dulatory properties of Swarnabhasma are also reported. Colloidal gold has been reported to be a potent and effective antiarthritic agent in suppressing different types of arthritis in rats (Mitra *et al.*, 2002; Pal and Sahu, 2014).

Kumkuma or Saffron (*Crocus sativus*) is traditionally known to be beneficial in Vata disorders like Sandhivata (Osteoarthritis) and *Amavata* (Rheumatoid arthritis) (Chunekar, 2011a). Saffron stigma and petal extracts are reported to exhibit antinociceptive effects in chemically induced pain test as well as acute and/or chronic anti-inflammatory activity, due to presence of crocetin and carotenoids that are naturally present compounds in saffron (Srivastava *et al.*, 2010). Aqueous and ethanolic extracts of saffron petals exhibited radical scavenging as well as anti-inflammatory effects in xylene and formalin induced inflammation (Hosseinzadeh and Younesi, 2002). In another study, anti-inflammatory and antinociceptive activities for alkaloids of saffron - crocin and safranal was shown in carrageenan model of local inflammation and inflammatory pain (Rathore *et al.*, 2007; Tamaddonfard *et al.*, 2013).

Ashwagandha (*Withania somnifera*) is documented to be Rasayana and traditionally used to strengthen body tissues (Balya), is useful in general weakness and is beneficial in Sotha (Inflammations) and Ksaya (immuno suppressive /emaciating diseases) (Ayurvedic Pharmacopoeia of India, 1999; Chunekar, 2011b) *W. somnifera* root extract is reported to contain oestrogen-like withanolides that have shown antiosteoporotic activity (Nagareddi *et al.*, 2006; Singh *et al*, 2011). Aqueous extract of *Withania* root exhibited antioxidant and antiarthritic activity and reduced inflammation in rats and suggesting its potential use in the treatment of arthritis (Khan *et al.*, 2015). Antiulcerogenic effects of Aswagandha are also reported (Ayurveda Sara Samgraha, 2012).

Mahayogaraja Guggulu is a compound Ayurvedic formulation comprising powders of herbal ingredients processed with guggulu (oleoresin of *Commiphora wightii*). In Ayurveda, Mahayogaraja Guggulu is indicated in a variety Vataroga with different adjuvants (Basista *et al.*, 2012) and is traditionally used in management of rheumatoid arthritis (Aamavata). Anti-inflammatory activity of Mahayogaraj Guggulu is reported in preclinical studies (Bagul *et al.*, 2005; Lavekar *et al.*, 2010).

The findings of *in vitro* study suggest that Swarna Guggulu has anti-inflammatory activity and it may be useful in inflammatory conditions including arthritis. Results of *in vitro* study are in sync with the documented benefits of its ingredient.

5. Conclusion

Swarna Guggulu has been studied in preclinical studies, using dendritic cells which are professional antigen-presenting cells that play pivotal roles in the induction of protective immunity. Treatment of dendritic cells with Swarna Guggulu resulted in considerable inhibition of production of LPS induced pro-inflammatory markers, suggesting potential anti-inflammatory activity of Swarna Guggulu. Moreover, therapeutic efficacy of ingredients of Swarna Guggulu in Vataroga such as arthritis has been established basis their reported beneficial effects in classical Ayurvedic texts as well as published modern literature. Also, the ingredients of Swarna Guggulu have been traditionally used since long and are reported to be safe.

Therefore, basis *in vitro* studies on Swarna Guggulu and documented ingredient benefits, Swarna Guggulu may be considered one of the good options in the management of arthritis.

Conflict of interest

The authors (AG, RS, MS, JLNS, RKR) are currently employed with Dabur India Limited which manufactures / markets the study product Swarna Guggulu.

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