



१८७२ से आयुर्वेद सेवा

TO EVALUATE THE CHONDROPROTECTIVE EFFECT OF AN AYURVEDIC FORMULATION SWARNA MAHAYOGA IN EXPERIMENTAL MODEL OF OSTEOARTHRITIS IN RATS

Principal Investigator

Dr. Mangala Lahkar

Head of the Department & Chief Academic Coordinator

Department of Pharmacology

National Institute of Pharmaceutical Education & Research (NIPER) Guwahati, Assam

Co-investigator

Dr. Mukesh B. Chawda

Senior Manager - Medical Services

Solumiks Herbaceutical Limited

Mr. Nilakash A. Selkar

In-Charge Biomedical Services

Shree Dhootapapeshwar Ayurveda Research Foundation (SDARF)

Study Co-ordinator

Mr. Lingesh Allakonda

MS (Pharm) Pharmacology and toxicology

National Institute of Pharmaceutical Education & Research (NIPER)

Guwahati, Assam

Date of commencement of study : Dec 2010 | Date of Completion of study : Feb 2011

Introduction

Swarna Mahayoga is a proprietary polyherbal formulation containing Suvarna Bhasma manufactured by Shree Dhootapapeshwar Ltd. Swarna Mahayoga is mainly recommended for vata disorders specially sandhigata vata, osteoarthritis, Rheumatoid arthritis, cervical spondylitis frozen shoulder, gout, lumbago, etc.. The recommended dose of Swarna Mahayoga is one tablet twice or thrice a day.

Table: 1 Composition of the Swarna Mahayoga

#	Ingredients	Botanical/Common Name	Qty/Tab
1	Suvarna Bhasma	Gold	1.00mg
2	Shuddha Hingu	Processed Ferula narthex	1.078 mg
3	Shunthi	Zingiber officinale	1.078 mg
4	Pippali	Piper longum	1.078 mg
5	Pippalimoola	Piper longum	1.078 mg
6	Chavya	Piper chaba	1.078 mg
7	Chitraka	Plumbago zeylanica	1.078 mg
8	Jeeraka (Krishna)	Carum carvi	1.078 mg
9	Ajamoda	Apium leptophyllum	1.078 mg
10	Sarshapa	Brassica campestris	1.078 mg
11	Jeeraka (Shveta)	Cuminum cyminum	1.078 mg
12	Renuka beej	Vitex negundo	1.078 mg
13	Patha	Cissampelos pareira	1.078 mg
14	Indrayava	Holarrhena antidysenterica	1.078 mg
15	Vidanga	Embelia ribes	1.078 mg
16	Katuka	Picrorhiza kurroa	1.078 mg
17	Gajapippali	Scindapsus officinalis	1.078 mg
18	Ativisha	Aconitum heterophyllum	1.078 mg
19	Bharangi	Clerodendrum serratum	1.078 mg
20	Vacha	Acorus calamus	1.078 mg
21	Murva	Marsdenia tenacissima	1.078 mg
22	Haritaki	Terminalia chebula	14.01 mg
23	Bibhitaka	Terminalia belerica	14.01 mg
24	Amalaki	Emblica officinalis	14.01 mg
25	Shuddha Guggulu (Dashamoola shodhit)	Processed Commiphora wightii	64.681 mg
26	Mandoor Bhaa		17.247mg
27	Rajat Bhaa		17.247mg
28	Vang Bhaa		17.247mg
29	Naga Bhaa		17.247mg
30	Abhrak Bhaa		17.247mg
31	Rasasindoor		17.247mg

Owing to the indicated therapeutic uses of the drug, the present study was conducted to evaluate the chondroprotective effect of the formulation on monosodium iodoacetate induced arthritis in rats.

Selection of Animals:

Healthy young wistar rats between 2-3months of age (male and females) weighing 150-200gm was randomly selected to the control and treatment groups. The females were non-pregnant and kept in the cages for five days prior to the start of the study to allow acclimatization.

Housing:

The temperature in the experimental animal room was maintained at 22°C with relative humidity between 50-60%. Artificial lighting was provided which include 12 hours light, 12 hours dark. All the animals were given standard pelleted feed and drinking water was supplied ad-libitum.

Induction of osteoarthritis:

The rats were anesthetised with Ketamine hydrochloride and osteoarthritis was induced in by giving a single intra-articular injection of 1 mg Mono sodium iodoacetate [MIA] (Crystal Powder M=185.96 g/mol, Germany, Sigma). MIA was dissolved in physiologic saline and administered in a volume of 50µl using a 30-gauge needle through the intra patellar ligament of the left knee.

Design of experiment:

Three groups' viz normal group, control group and a test group were used to study the chondroprotective effect of Swarna Mahayoga in MIA induced osteoarthritis in rats. Each group had eight animals of either sex. Four animals from each treatment group were sacrificed and examined for the histopathological examinations on 14th day of treatment and remaining on the 28th day of treatment. The dose for rats was calculated extrapolating the human therapeutic dose (HTD) using the following formula:

$$\text{Dose in rats/200g of body weight} = \text{HTD} \times 0.018$$

Human Therapeutic Dose (HTD) = 2 tablet thrice a day.
(Avg. wt. of tab = 304 mg)

Table: 2 Design of the Experiment

No.	Groups	No. of Groups	Therapeutic Dose (mg/200gm of body wt)	No. of animals (8 animal per group)
1	Normal Control (NC)	1	Saline water	8
2	Osteoarthritic Control (OC)	1	1% CMC	8
4	Swarna Mahayoga (SM)	1	33mg	8

Administration of doses: The test drug (Swarna Mahayoga) was suspended in 1% CMC solution in distilled water and administered using oral gavage. The control and normal group given 1% CMC solution in distilled water as mentioned in the above table for 28 days starting from day one post the MIA injection.

Parameters under study:

- 1. Body weight:** All animals were weighed once a week.
- 2. Morphology:** Morphology of the joint will be observed on 1st, 7th, 14th, 21st and 28th day and swelling will be calculated using Varner calliper scale using formula:

$$\text{Synovial fluid volume (mm}^3\text{)} = (a \times b^2) / 2,$$

Where, a = length in mm and b = width in mm

- 3. Histopathology:** Animals were sacrificed in two batches, four no. animals at 14th day and remaining four at 28th day by ether anesthesia at the time indicated. Soft tissue will be removed from the left (Osteoarthritic) legs and patella will be removed from each knee to facilitate thorough fixation of the joint. Tissue samples will be prepared for light microscopy using standard procedures. The microscopic observation will be done for chondrocytes damage area, chondrocytes necrosis, and inflammatory cells in synovial and synovial proliferation.

Results:

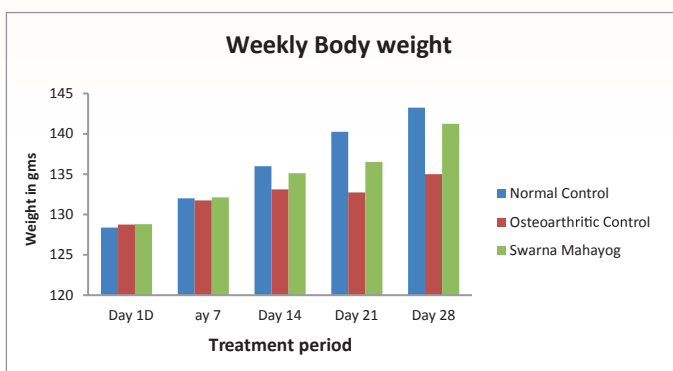
1. Weekly body Weight

The average body weight measured every week shows that after 14 day of the treatment the increase in the body weight of the OC group is lower than the weight of NC group animals whereas in the treated group the weight gain is equivalent to that of the normal rats as shown in table below.

Table: 3 weekly body weights of the animals with \pm S.E.M.

	Normal Control	Osteoarthritic Control	Swarna Mahayoga	No. of animals (8 animal per group)
Day 1	128.37 \pm 0.8	128.75 \pm 1.05	128.8 \pm 0.99	8
Day 7	132 \pm 0.9	131.75 \pm 1.36	132.12 \pm 1.07	8
Day 14	136 \pm 0.6	133.12 \pm 1.67	135.12 \pm 1.26	8
Day 21	140.25 \pm 0.73	132.75 \pm 2.52	136.5 \pm 1.65	
Day 28	143.25 \pm 1.1	135 \pm 2.85	141.25 \pm 1.8	

Fig. 1 Average body weights



2. Morphology:

Synovial fluid volume based on knee swelling: The knee swelling was calculated based on synovial fluid volume using Varner calliper scale. In the OC group the swelling was more as compared to that of the SM treated group during entire study period. In the SM treated group the increased swelling in the knee

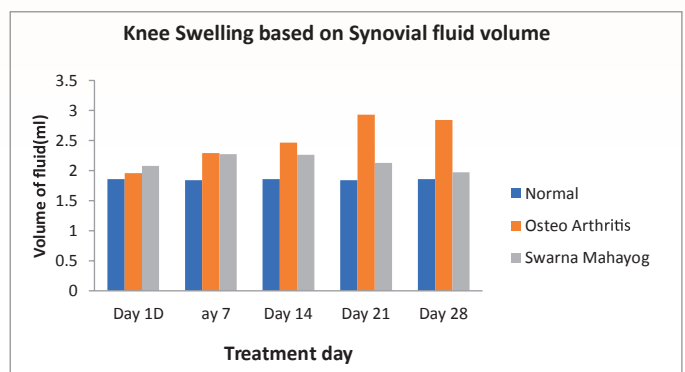
was observed till 14 day of the treatment which was gradually decrease to the normal levels equivalent to the volume of NC group animals on the 28th day of the study period where the swelling in the knee of the SM treated group was found significantly lower than the OC group at the significance level of $P < 0.5$ when compared using one way annova.

Table: 4. Synovial fluid volume based on knee swelling:

	Control	Osteoarthritis	Swarna Mahayog
Day 1	1.85 \pm 0.056	1.95 \pm 0.11	2.0 \pm 0.074
Day 7	1.84 \pm 0.07	2.29 \pm 0.16	2.2 \pm 0.046
Day 14	1.85 \pm 0.005	2.46 \pm 0.17	2.2 \pm 0.044
Day 21	1.84 \pm 0.054	2.92 \pm 0.05	2.1 \pm 0.43
Day 28	1.85 \pm 0.072	2.84 \pm 0.09	1.9* \pm 0.057

* $P < 0.5$ when compared with Osteoarthritic control

Fig.2. Synovial fluid volume based on knee swelling:



The morphology of the knee where knee swelling was calculated using Varner calliper scale shows significant decrease in the knee swelling of the treated rats as compared to the osteoarthritic control.

Histopathological findings:

Histological changes were assessed in all rats that had

received intra-articular injection of MIA on 14th and 28th day of treatment whereas the normal rats at only once.

On 14th day the observed chondrocyte layer was damaged more than 60 % with synovial proliferation in the treated rats and OA control rats, and the inflammatory cells were also present in OA control rats.

On 28th day the chondrocytes layer was completely damaged in the osteoarthritis control group with synovial proliferation due to sepsis formation whereas

in the treated group the chondrocytes were present upto the 50% and no synovial proliferation was observed which shows the protective effect of myostaal against chondrocytes damage.

The histopathology slides of OA control group shows the presence of the inflammatory cells which were absent in the slides of drug treated animals. The absence of the inflammatory cells indicates the suppression of inflammatory response which can be contributed to the anti-inflammatory activity of the test drug

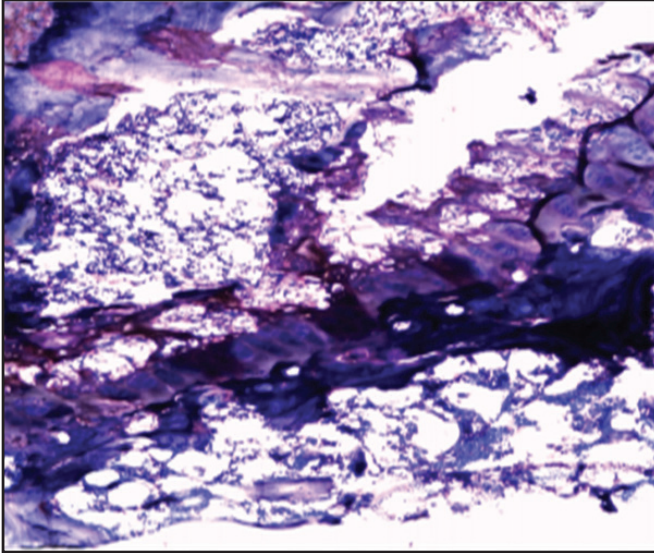
Table: 5. Histopathological findings:

	Score	Normal Control	Osteoarthritic Control		Swarna Mahayoga	
			14 day	28 day	14 day	28 day
Chondrocyte Damage Area	+	0	1/4	0/4	2/4	3/4
	++	0	2/4	0/4	1/4	0/4
	+++	0	1/4	4/4	1/4	0/4
			2	3	1.75	0.75
Chondrocyte Necrosis	+	0	0/4	0/4	2/4	4/4
	++	0	1/4	0/4	2/4	0/4
	+++	0	3/4	4/4	0/4	0/4
			2.75	3	1.5	1
Inflammatory cells in	+	0	0/4	0/4	2/4	2/4
Synovial	++	0	1/4	0/4	1/4	0/4
	+++	0	3/4	4/4	0/4	0/4
			2.75	3	1	0.5
Synovial proliferation	+	0	0/4	0/4	0/4	0/4
	++	0	2/4	0/4	1/4	1/4
	+++	0	2/4	4/4	2/4	0/4
Total score		0.00	2.5 10.00	3 12	2 5.75	0.5 2.75

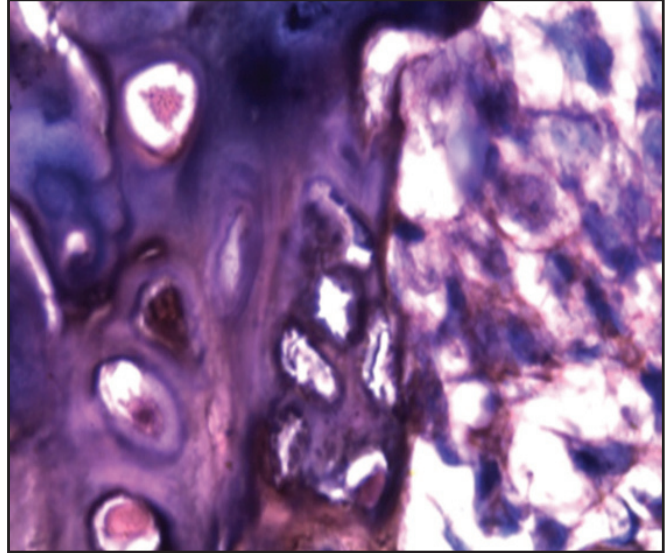
Score: + Slight = 1, ++Moderate = 2, +++Severe = 3

Fig: 3(a) Histopathological findings

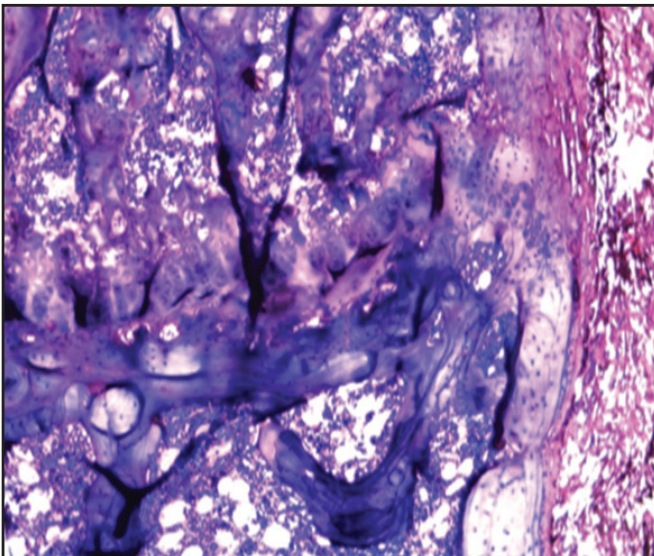
HISTOPATHOLOGICAL OBSERVATIONS (DAY 14)



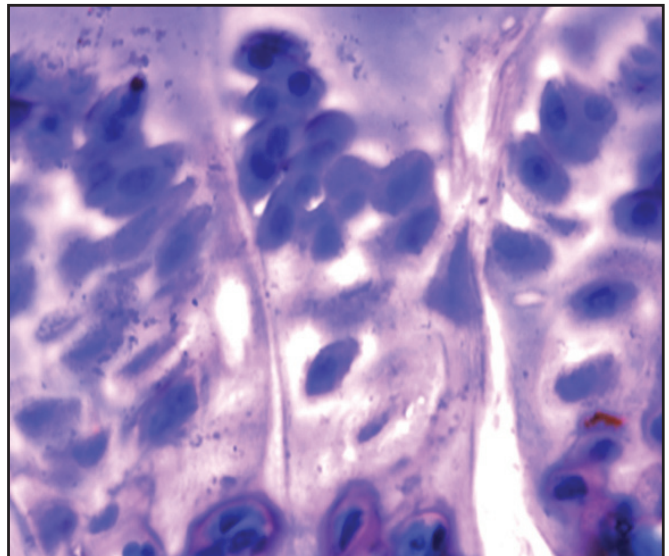
Osteoarthritis Control 40X



Osteoarthritis Control 100X



Swarna Mahayoga 40X

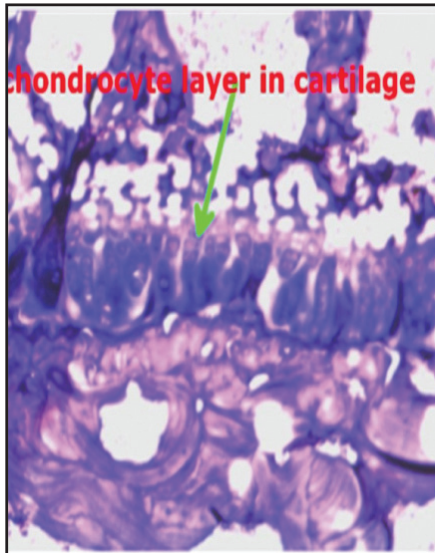


Swarna Mahayoga 100X

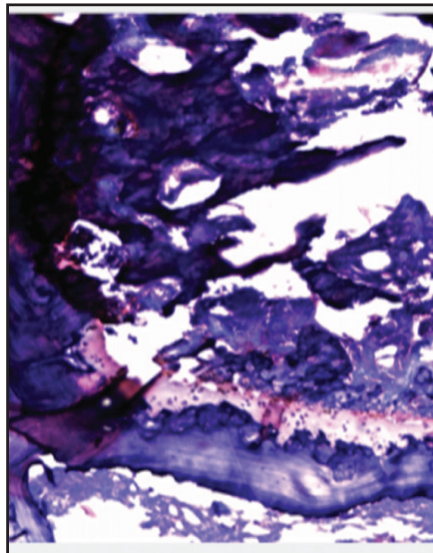
Fig.3a. Histopathological observations in the rat knee showing chondrocyte layer in the knee cartilage of OC, and Swarna Mahayoga treated rats on 14th day of study.

Fig: 3(b) Histopathological findings

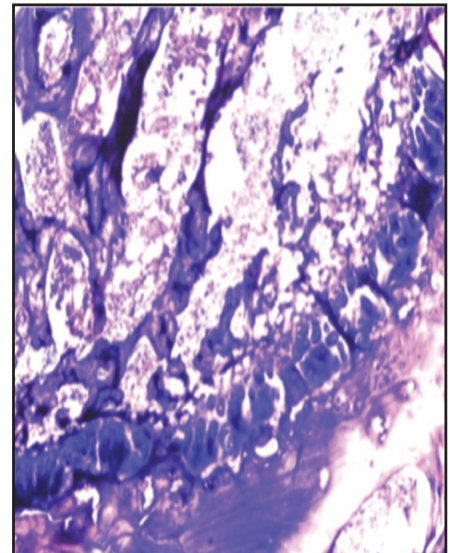
HISTOPATHOLOGICAL OBSERVATIONS (DAY 28)



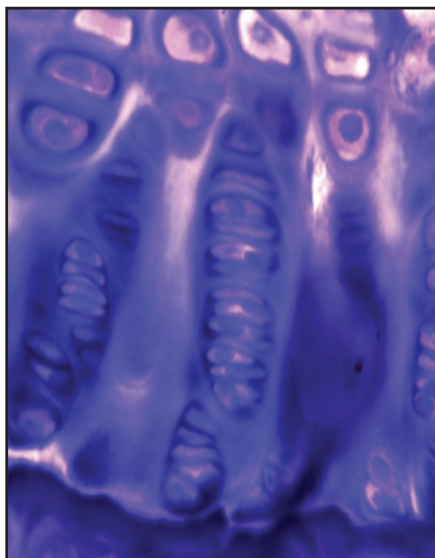
Normal Rat cartilage 40X



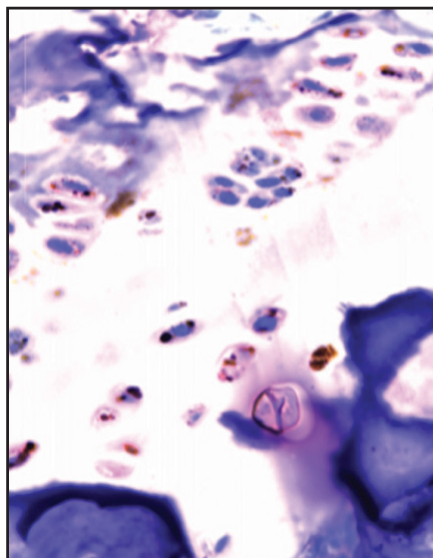
Rat cartilage in Osteoarthritis 40X



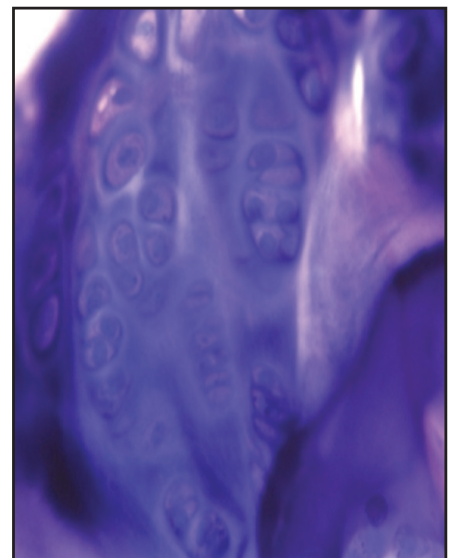
Swarna Mahayoga 40X



Normal Rat cartilage 40X



Rat cartilage in Osteoarthritis 100X



Swarna Mahayoga 100X

Fig.3b. Histopathological observations in the rat knee showing chondrocyte layer in the knee cartilage of NC, OC, and Swarna Mahayoga treated rats on 28th day of study.

Discussion:

Swarna Mahayoga a patent proprietary product of Shree Dhootapapeshwar Limited which contains Commiphora wightii (Guggulu) as main constituent which is well known for its hypolipidemic (Nancy L. et al 2003) and anti inflammatory action (MS Bagul et al 2005).

The guggulu have been also reported for its chondroprotective activity in experimentally induce osteoarthritis (Jahromy et.al.2009)The formulation is enriched with gold which also known for its immunomodulatory and anti inflammatory actions. A clinical study sponsored by a the Empire Rheumatism Council confirmed the effectiveness of gold compounds against rheumatoid arthritis (Simon P Pricke,2005) and since then it is been a used in modern medicine for treatment and management of various painful arthritic conditions.

In the present study Swarna Mahayoga has shown a time dependant action against chondrocytes damage in rat model of MIA induce osteoarthritis.

The animals of the osteoarthritic control group show upto 70% degradation of the chondrocyte layer. The synovial proliferation and presence of inflammatory cells in synovial was observed on 14th day of the osteoarthritis induction which leads to the complete degradation of the chondrocyte layer along with increase in synovial proliferation and presence of inflammatory cells in synovial on 28th day of the osteoarthritis induction.

Whereas in the animals treated with Swarna Mahayoga, the observed 60% chondrocyte necrosis, on 14th day was not progressed further and about 50% of the chondrocytes were preserved on the 28th day of the treatment. The inflammatory cells and synovial proliferation present on the 14th day were also absent on the 28 the day of the treatment.

All the observations of the treated, control and the normal group show the test substance has significant effect in the protection against chondrocyte damage in osteoarthritis.

The absence of inflammatory cells with decreased

synovial proliferation indicates the significant anti-inflammatory action of the Swarna Mahayoga which can be helpful for the improvement of the quality of life in the osteoarthritic patients

Conclusion:

From the above results it can be concluded that the Swarna Mahayoga shows the time dependant protection against damage to the chondrocyte layer and palliate the inflammation and discomfort of the osteoarthritis.

References:

1. Jahromy MH, Kermani RM, Nobakht F. Influence of commiphora mukul resin on the knee articular cartilage of rats in experimental osteoarthritis induced by iodoacetate. Pak J Med Sci 2009;25(2): 269-273.
2. Nancy L. Urizar and David D. Moore., GUGULIPID: A Natural Cholesterol-Lowering Agent. Annual Review of Nutrition Vol. 23: 303-313 (Volume publication date July 2003) DOI: 10.1146/annurev.nutr.23.011 702.073102.
3. MS Bagul, H Srinivasa, NS Kanaki, M Rajani. Antiinflammatory activity of two Ayurvedic formulations containing guggul. Indian Journal Of Pharmacology 2005.Vol :37,6, 399-400
4. Simon P Pricker. Medical Uses of Gold Compounds:Past present and future. Gold bulletin 1996 29(2).