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Original Article

EFFECT OF *BALSAMODENDRON MUKUL* GUM RESIN EXTRACT ON PAIN RESPONSE IN OSTEOARTHRITIC RATS

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ABSTRACT

Objective: Osteoarthritis (OA) is one of the prevalent and degenerative disorders of the joints that cause significant pain and functional disability. It is a disease in which not only the articular cartilage of the synovial joint is affected but also the adjacent bone, ligaments, capsule, synovial membrane and even peri-articular muscles are distressed. Worldwide 630 million people or 15% population of the globe are affected with OA. According to WHO report up to 40% of people over 70 year of age suffer from knee osteoarthritis.

Methods: We have taken albino adult female Wistar rats of 200 to 250 gm and segregated into six groups (Control, SHAM, OA, OA+BDM (10%), OA+BDM (20%), OA+BDM (40%) groups. Osteoarthritis was induced by intra-articular injection of Papain and Cystein. The Pain Behavioral tests were performed to evaluate the knee joint pain in all the groups of rats by measuring the Stay Time on Rotarod (STR), Number of Entries (NOE) on photoactometer and Paw Withdrawal Latency (PWL) on eddy hot plate. The results were recorded before and after the induction of OA and treatment.

Results: In both the cases, STR and NOE increased in the rats of OA+BDM (10%), OA+BDM (20%), OA+BDM (40%) groups. The PWL was decreased in the group OA+BDM (10%), OA+BDM (20%), OA+BDM (40%) groups.

Conclusion: Our results suggest that oral dose of BDM gum resin extract have the potential to relieve the osteoarthritic pain, regenerate the cartilaginous matrix and increase sub chondral bone components.

Keywords: Balsamodendron gum resin extract, Number of entries on Photoactometer, Osteoarthritis, Paw withdrawal latency on eddy hotplate, Stay time on Rotarod.

INTRODUCTION

Osteoarthritis (OA) is a degenerative disorder of joints that causes significant pain and functional disability [1,2]. It is one of the most prevalent, disabling, chronic diseases affecting the elderly. Arthritis pain may reflect active inflammation as well as impairment of joint structures [3,4]. Knee OA represents one of the most widespread forms of osteoarthritis. The population-based studies estimated an incidence of this severe radiographic disease amongst 1% of population between the age group25-34, and 30% in those aged 75 and above [5].

Osteoarthritis may induce different types of pain including nociceptive pain and neuroplastic or inflammatory pain [6]. Chronic osteoarthritis may result in allodynia and hyperalgesia. The current therapeutic approach of OA is to lessen the pain and restore the joint function. It is performed by reducing the amount of inflammatory mediators in joint and decreasing the pain sensitivity. This approach may give a primary symptomatic relief temporarily but it will not stop the further progress of osteoarthritis.

Till date, no disease modifying drugs are available for the treatment of OA, which represents a momentous, unanswered clinical conundrum. There are many accessible clinical medications, such as, acetaminophen, non steroidal anti-inflammatory drugs, and narcotics or the surgical procedure but the available treatments do not show significant results without having any side effects. Moreover, the high rate of recurrence of osteoarthritis entails significant cost to society. Direct costs of osteoarthritis include clinical visits, medications, and surgical intervention. Whereas the indirect costs include such items as the loss of time and the physical fitness of the individual that affect the economy of the nation in its own big and small ways. It is because of these reasons that we have proposed the use of an indigenous herbal substitute for the same which is cost effective, free from side effects and natural i.e. *Commiphora mukul* or *Balsamodendron mukul* (Guggul).

Commiphora mukul or *Balsamodendron mukul* is a small tree of the Burseraceae family that is indigenous to India. It includes about 185 species of trees and shrubs [7], *Commiphora mukul* grows in their natural habitat in the semi arid Indian states of Madhya Pradesh, Rajasthan, Gujarat, Karnataka and Mysore whereas worldwide *Commiphora mukul* is found in Afghanistan, Arabia, and north-east Africa in rocky dry areas [8-11] Bangladesh, and Pakistan. The herb is mentioned as early as from 3000 to 10,000 years ago in the Vedas, the holy scriptures of India for treating human illnesses [12]. An Indian medical researcher, Dr. G. V. Satyavati was the pioneer to introduce guggul to the scientific world in 1966. The gum resin of the *Commiphora mukul* tree is revered in Ayurveda for its medicinal properties. It is commonly called guggulu or guggul. Gum guggul is the oleoresin of the plant *Commiphora mukul* [7]. The crude gum guggul was found to contain 2% guggulsterones. Guggulsterone (GUG), a resin of the *Commiphora mukul* tree, has been used in ayurvedic medicine for centuries to treat a variety of ailments [13]. Guggul offers a potent herbal remedy for various types of joint problems such as rheumatoid arthritis, gout, pain, swelling and tenderness of the inflamed joints [14]. It has multiple actions on various other systems of the human body with anti hypercholesterolemia, anti hyperlipidemia and anti-obesity properties and show dual profile for the practitioners of modern medicine. Though it is also found to be anti inflammatory, there are very rare reports available regarding the effect of Guggul on Osteoarthritis. Herbal treatment has been identified as safe and effective in treating arthritis and may provide a safe and effective adjunctive therapeutic approach for the treatment of arthritis [15].

The objective of present study is to elucidate the effect of Balsamodendron gum resin extract on mobility and pain status of

knee joint in osteoarthritic rats. There are numerous animal models available for osteoarthritis like mouse, rat, rabbit, cat, goat sheep, primates, guinea pigs and horse. In all these models, rat is an ideal animal model for osteoarthritis studies, as it is tractable, inexpensive, easy to house and manageable. It is not ethical to evaluate the effect of drug/ plant extract on human that is why albino rats are suitable for testing as it emulate a similar physiology as that of human [16].

MATERIALS & METHODS

Animals

All protocols of animal procedure were reviewed and approved by Institutional Animal Ethics Committee [IAEC; approval letter no. 46/1219/ac/Su/IAEC/2012] of Shobhit University, Meerut, India, followed by the Committee for the Purpose of Control and Supervision on Experiments on Animals (CPCSEA), India. To carry out the present investigation, female wistar rats, weighing 200-250 gm were procured from the Central Animal House, Shobhit University, India. The animals were kept in polypropylene cages with five animals per cage and maintained under standard laboratory conditions at 26 ± 2 ºC and 44 – 56% relative humidity. The animals were exposed to light and dark cycles of 10 and 14 hrs respectively, for 5 days for acclimatization before and during the experiment period. They were provided with standard rodent pellet diet (Aashirwad, India) and the food was withdrawn 18 hrs before the experiment though water was allowed, ad libitum. All experiments were performed in the morning time according to current guidelines for the care of laboratory animals and the institutional ethical guidelines for investigations of experimental pain in conscious animal [16].

Study Design

Albino female Wistar rats of 200 to 250 gm were segregated in six groups, namely, Control, SHAM, OA, OA+BDM (10%), OA+BDM (20%), OA+ BDM (40%). Osteoarthritis was induced in all rats of osteoarthrtic (OA) and treatment groups by intra articular injection of papain and cystein on day. The rats in the SHAM group received same volume of isotonic NaCl solution. The Pain Behavioral tests were performed to evaluate the knee joint pain in all the group of rats by Photoactometer, Eddy hot plate and Rotarod on day 5 (before the induction), day 12 (after induction of Osteoarthritis), day 19 (start day of treatment), day 29 (after ten days of treatment), day 39 (after twenty days of treatment) and day 49 (after thirty days of treatment) (Figure 1).

Induction of Osteoarthritis

Osteoarthritis was induced on the 5th, 9th and 12th day of the experiment (with 5th day being taken as the base line), in both the knee joint by intra-articular injections of 0.2 ml of 4% Papain (Shri Ganesh Industrial Enzymes Burhanpur, Madhya Pradesh, India) solution and 0.1 ml of 0.03 M cystein (MERK, USA.) in the patellar ligament of the knee through a 26-gauge, 0.5-inch needle. The SHAM induced rats received the same volume intra-articular injection of saline. The fresh rats of similar age and sex were kept in Control group. Intra articular injection was repeated on the fourth and seventh days of the first injection [17]. The general health of the animals was monitored as per the ethical guidelines of our institution.

Food Pellet

The food pellets were made by kneading 10 gm Balsamodendron gum resin extract (made using Soxhlet apparatus) in wheat flour, making its total weight 100 gm and dividing the kneaded flour in 100 pellets so that each pellet is 1 gm in weight, having 0.1 gm Guggul gum resin. Rats were fed on the food pellet and dose was given according to their groups i.e. OA + BDM (10%), OA + BDM (20%) and OA +BDM (40%) rats received one, two and four pellets respectively, whereas the control, SHAM and OA groups were fed with wheat flour pellets without extract.

Pain Behavioral Tests

Behavioral tests, performed to evaluate the knee joint pain in arthritis models, include Rotarod test, Photoactometer, and Eddy hot plate.

Rotarod Testing

Motor co-ordination (grip muscle strength) was evaluated by a Rota-rod device (Inco, India) as described by Jones and Roberts [18]. Briefly, the motor performance was measured as the latency to fall from an accelerating Rotarod located over plates connected to an automatic counter. Rats were trained to remain on a rotating rod for 2 min as the rod rotated toward them. After the 2-min training period, they were placed for one minute on the rotating rod (25 rpm). When an animal drops onto the individual sensing platforms below, Stay time on Rotarod (STR) were recorded digitally and displayed on the front panel.

Photoactometer Test

Photoactometer (Inco, India) test was employed to assess the effect of drug treatment on spontaneous motor (exploratory) activity of the rats. Each animal was observed for a period of 5 min in a square closed field arena (30 × 30 × 30 cm) equipped with 6 photocells in the outer wall. Interruptions of photocell beams (locomotors/exploratory action) were recorded by means of a six digits counter and number of interruptions was called as number of entries [19] (NOE).

Hot-Plate Test

The hot-plate (Inco, India) test was performed using an electronically controlled hotplate heated to 53 °C (± 0.1 °C). Each mouse was placed unrestrained on hot plate for the baseline measurement just prior to saline or drug administration. Latency to lick a hind paw or jump out of the apparatus was recorded for control and drug treated groups that are called as paw withdrawal latency (PWL). The cut-off time was 30sec [20].

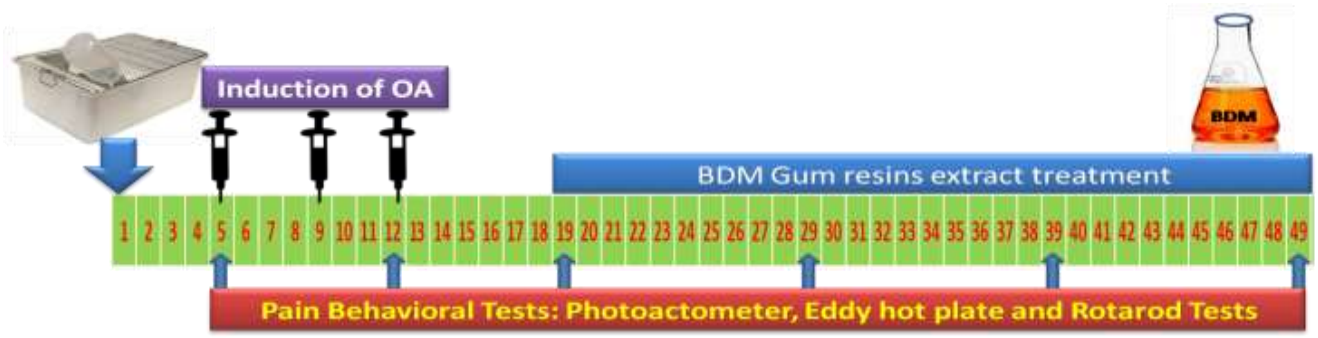

Fig.1: Showing Procurement of Animals, Induction of OA, Treatment Interventions of BDM and Pain Behavioral Tests (on Rotarod, Photoactometer and Eddy Hot Plate) throughout the Experimental Timeline.

Statistical Analysis

The resulting values were analyzed statistically by Mean ± SD or median with range and analysis of Variance (ANOVA) method.

RESULTS

Rotarod Testing

Intra group pain assessment on rotarod showed comparative analysis at different point of observation time with baseline (day5). No significant change was observed in control and SHAM group of rats at any point of observation time (day12, day19, day 29, day 39 and day 49) when compared to their contra lateral baseline data on day 5 except on day 12 of SHAM group of rats. The OA group of rats showed statistically significant change in STR at every point of observation time (day12, 19, 29, 39 and 49). The treatment groups [OA + BDM (10%), OA + BDM (20%) and OA + BDM (40%)] showed improvement in stay time on rotarod (Figure 2). The members of OA+BDM (40%) group of rats, at 49th day of observation (after 30 days of treatment), showed insignificant difference when compared to baseline (day 5). The STR of control and SHAM groups of rats showed very less percentage change at every point of observation time when compared to baseline data of respective groups. However, in case of the remaining OA group of rats, amount of the percentage change at every point of observation was found to be very high (Table 1). In all the treatment groups of rats only OA + BDM (40%) showed very less amount of percentage change in STR at day 39 and 49 (after 20 and 30 days of treatment respectively).

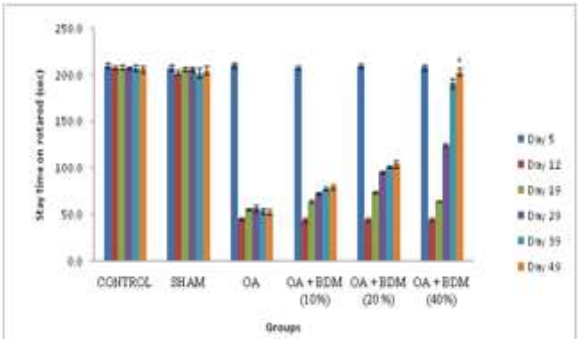

Fig.2: Variation in Stay time on Rotarod in different groups of rats at different time intervals (on Day 5, 12, 19, 29, 39, 49) during the experiment and treatment. *shows insignificant difference between day5 and day49

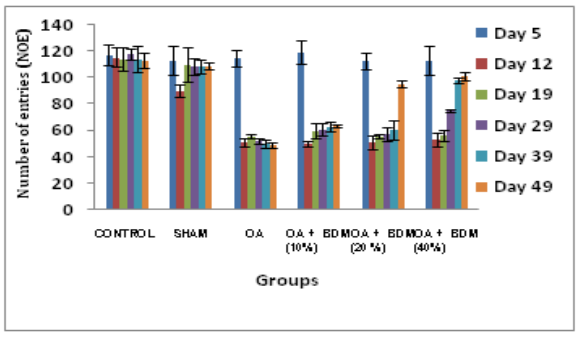

Fig. 3: Variation in exploratory frequency on Photoactometer of different groups of rats at different time intervals (on Day 5, 12, 19, 29, 39, 49) during the experiment and treatment.

Photoactometer Test

The change in spontaneous movement frequency by interruption of infrared beams on Photoactometer showed intra group comparative analysis at different point of observation time with baseline (day 5). No significant change was observed in control and SHAM group of rats at any point of observation time (day12, 19, 29, 39 and 49) when compared to their contra lateral baseline data on day 5 except on day 12 for SHAM group of rats. The OA group rats showed a significant deteriorating change in NOE at every point of observation time (day12, 19, 29, 39 and 49) when compare with NOE of day 5 (Figure 3). The treatment group of rats showed statistically insignificant improvement in NOE on Photoactometer (Table 2).

Hot Plate Test

The comparative analysis of pain threshold at different point of observation time with baseline (day5) showed no significant change in control and SHAM group of rats at any point of observation time (day12, 19, 29, 39 and 49) as shown in Table 3. The PWL of osteoarthric rat increased significantly at every point of observation times when compared to the baseline pain (day 5). In treatment group of rats, the pain threshold was observed to be reduced. The PWL of OA+BDM40% group of rats was almost equivalent to the baseline having statistically insignificant difference (Figure 4).

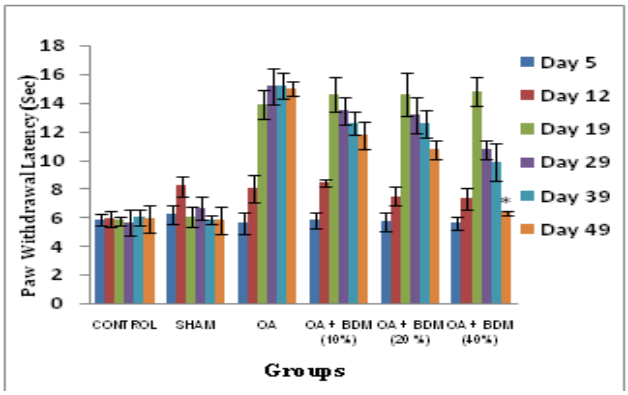

Fig.4: Variation in Paw Withdrawal Latency on Eddy Hot Plate indifferent groups of rats at different time intervals (on Day 5, 12, 19, 29, 39, 49) during the experiment and treatment. *shows insignificant difference between day5 and day49

Statistical Analysis

All the values were compared utilizing one way analysis of variance and followed $p < 0.05$ to be statistically significant. The intra group comparison was performed to evaluate the effectiveness of treatment. The percentage change in pain behavior tests was evaluated by calculating the percentage difference in particular test of day 12 (after induction), day 19 (treatment initiation day), day 29 (after 10 days of treatment, day 39 (after 20 days of treatment) and day 49 (after 30 days of treatment) with respect to day 5 (before induction).

DISCUSSION

Osteoarthritis is the most common form of arthritis affecting millions of people around the globe. Several plant extract mediated therapies are proposed in the recent past to treat osteoarthritis and related disorders [21,22]. Our observation

of the present study provides the evidence that the administration of BDM gum resin extract, a *Commiphora mukul* extract has an effective analgesic effect on acute nociceptive pain induced by osteoarthritis in adult female rats [23,24] as observed in behavior analysis performed after 10, 20 and 30 days of BDM gum resin extract treatment. We also report the relief in pain by the resin of *Commiphora mukul* in fibroblast-like synoviocytes. It has been suggested that the Guggulsterone can modulate the IL1 beta-mediated inflammatory responses by suppressing NF-kB activation [14, 25].

Pain induction was done by Papain and Cysteine injections. Papain is a proteolytic enzyme that causes OA by releasing chondrotin sulphate from protein polysaccharide of articular cartilage matrix [26,27]. Cathepsin k a member of the Papain super family, which degenerate type II collagen by cleaving intra helical region and induce the inflammatory osteoarthritis [28,29]. In this study papain and cycteine were administered at the suggested doses [18]. Earlier Robert *et al.*(1958) also showed that papain can disintegrate the cartilaginous matrix in vivo [30].

After induction of inflammatory osteoarthritis we have analyzed the pain behavior tests. A numbers of pain behavior tests have been performed earlier by several workers to know the status of joint inflammation. Rats with knee osteoarthritis have an increased PWL which shows a significant reduction in thermal sensitivity to noxious heat [31-33]. Hans *et al*. (2005) measured the locomotion of osteoarthritic rats with boitelmetry system and observed the similar results [34]. Force mobilization was evaluated to characterize the osteoaarhritic model by Appleton *et al*. (2007) [35]. We have used hot plate to investigate thermal sensitivity, photoactometer to evaluate spontaneous mobility and rotarod to see the response of forced mobilization of joint. After induction of OA the PWL was significantly increased whereas NOE and STR were decreased.

Previous workers have found the antiarthritic activity of Guggul resin, showing anti-inflammatory action of *Commiphora mukul*. Singh *et al*. (2001) found the pharmacological effect of Salai guggul extract in inhibition of inflammation [36-38]. Duwiejua *et al*. (1993) also observed the resin of Burseraceae plant extract to have inhibitory response against the inflammation [39]. They have concluded that the resin is a worthy anti inflammatory herbal drug. In the present study the BDM gum resin extract intervention causes significant decline of PWL at hot plate and increment in mobility at rotarod as well as at photoactometer (Table 4). Our result shows the inhibitory response of BDM gum against the OA generated inflammation. The above mentioned parameters were restored, when the OA induced rats were treated with BDM extract which indicate the therapeutic potentials of BDM gum extract in management of osteoarthritis and its related inflammatory-pain.

Table 1: Percentage Variations in Stay Time on Rotarod in Different Groups of Rats at Various Time Intervals

Sr. No.	Stay time on Rotarod (STR)	CONTROL	SHAM	OA	OA + BDM (10%)	OA + BDM (20 %)	OA + BDM (40%)
1.	% Change on day 12	1.07	2.48	78.45	78.71	78.65	78.57
2.	% Change on day 19	0.97	2.30	73.41	69.11	64.59	69.36
3.	% Change on day 29	1.23	1.98	73.09	65.04	54.24	40.10
4.	% Change on day 39	1.36	2.68	74.20	62.47	51.76	8.17
5.	% Change on day 49	1.96	1.42	74.86	61.60	50.14	2.37

Table2: Percentage Variation in Exploratory Frequencies on Photoactometer in Different Groups of Rats at Various Time Intervals

Sr. No.	Number of Entries on Photoactometer (NOE)	CONTROL	SHAM	OA	OA + BDM (10%)	OA + BDM (20 %)	OA + BDM (40%)
1.	% Change on day 12	1.44	19.62	55.36	57.71	54.83	52.80
2.	% Change on day 19	2.64	2.87	51.46	50.01	50.90	49.87
3.	% Change on day 29	-0.81	4.11	54.09	48.78	48.99	33.38
4.	% Change on day 39	2.05	3.75	56.10	46.92	46.31	12.80
5.	% Change on day 49	3.07	3.02	57.27	46.29	15.42	10.12

Table3: Percentage Variation in Paw Withdrawal Latency on Eddy Hot Plate in Different Groups of Rats at Various Time Intervals

Sr. No.	Paw Withdrawal Latency on Eddy hot plate (PWL)	CONTROL	SHAM	OA	OA + BDM (10%)	OA + BDM (20 %)	OA + BDM (40%)
1.	% Change on day 12	-1.13	-33.48	-44.85	-46.00	-30.61	-31.66
2.	% Change on day 19	0.99	2.10	-151.52	-147.57	-166.17	-166.67
3.	% Change on day 29	2.82	-5.97	-175.43	-133.85	-130.45	-92.52
4.	% Change on day 39	-2.55	4.36	-174.83	-118.87	-121.35	-76.89
5.	% Change on day 49	-1.94	5.72	-171.54	-103.65	-89.44	-13.08

CONCLUSION

OA is one of the severe diseases affecting the increasing number of people leading to an amplified personal and socio-economic encumbrance. Until now, the only non-operative measures available to treat the OA-related symptoms are pain medication and physical therapy. The present study demonstrated that the artificial induction of OA significantly ($p<0.05$) affected the tested parameters whereas the treatment interventions pertaining *Balsamodendron mukul* gum resin extract helped in reinstating the values of the test parameters to the initial levels as was present before the OA induction. This shows the anti osteoarthritic properties of the *Balsamodendron mukul* gum resin extract which seems to be very promising in the light of the fact that with the rising concentration of

the extract, as mentioned above, the testing parameters were significantly restored with the original values.

CONFLICT OF INTEREST

The authors have declared that there is no conflict of interest.

REFERENCES

1. Sprangers MA, de Regt EB, Afaq F, Agt AHM, Bijl RV, de Boer JB, *et al*. Which chronic conditions are associated with better or poorer quality of life? J Clin Epidemiol 2000; 53(9): 895-907.
2. Rice JR, Pisetsky DS. Pain in the rheumatic diseases. Practical aspects of diagnosis and treatment. Rheum Dis Clin North Am 1999; 25(1): 15-30.
3. Arden N and Nevitt M. Osteoarthritis: Epidemiology. Best Prac Res Clin Rheumatol 2006; 20(1):3-25.
4. Kidd BL, Photiou A and Inglis JJ. The role of inflammatory mediators on nociception and pain in arthritis. Novartis Found Symp. 2004; 260: 122-133.
5. McAlindon TE, Cooper C, Kirwan JR, Dieppe PA. Determinants of disability in osteoarthritis of the knee. Ann Rheum Dis 1993; 52(4): 258-262.
6. Siddiqui MZ, Mazumder PM. Comparative study of hypolipidemic profile of resinoids of Commiphora mukul/Commiphora wightii from different geographical locations. Indian J Pharm Sci 2012; 74(5): 422–427.
7. Ramesh B, Karuna R, Reddy SS, Haritha K, Sai MD, Bhusana RBS, *et al*. Effect of Commiphora mukul gum resin on hepatic marker enzymes, lipid peroxidation and antioxidants status in pancrease and heart os streptozotocin induced diabetic rats. Asian Pac J Trop Biomed 2012; 2(11): 895-900.
8. Goyal S, Khilnani G, Singhvi I, Singla S, Khilnani SA. Guggulipid of Commiphora mukul, with antiallodynic and antihyperalgesic activities in both sciatic nerve and spinal nerve ligation models of neuropathic pain. Pharm Biol 2013; 51(12):1487-98.
9. Varier VPS. Indian Medicinal Plants: A Compendium of 500 Species, Universities Press 2012; 2: 416.
10. Atal CK, Gupta OP, Afaq SH. Commiphora mukul source of gugal in Indian systems of medicine. Econ. Bot 1975; 29(3): 209-218.
11. Siddique M. Guggul an excellent herbal panacea. Asian J Health and Pharm Sci 2011; 1: 35 -38.
12. Deng R. Therapeutic Effects of guggul and its constituent guggulsterone: cardiovascular benefits. Blackwell Publishing Inc 2007; 25(4): 375-390.
13. Choubey J, Patel A, Verma MK. Phytotherapy in the treatment of arthritis: a review. Int J Pharma Sci Res 2013; 4(8): 2853-2865.
14. Sarfaraz S, Siddiqui IA, Syed DN, Afaq F, Mukhtar H. Guggulsterone modulates MAPK and NF-kB pathways and inhibits skin tumorigenesis in SENCAR mice. Carcinogenesis 2008; 29 (10): 2011–2018.
15. Little CB, Smith MM. Animal Models Osteoarthritis. Current Rheumatol Rev 2008; 4.
16. Zimmermann M. Ethical guidelines for investigations of experimental pain in conscious animals. Pain 1983; 16: 109-110.
17. Nergis M, Bahattin K, Sermin O, Vasfi K, Sedef G. Quantification of papain-induced rat osteoarthritis in relation to time with the Mankin score. Acta Orthop Traumatol Turc 2007; 41(3): 233-237.
18. Jones BJ, Roberts DJ. A rotarod suitable for quantitative measurements of motor incoordination in naive mice. Naunyn Schmiedebergs Arch Exp Pathol Pharma 1968; 259(2): 211.
19. Goddard M, Zheng Y, Darlington CL, Smith PF. Locomotor and exploratory behavior in the rat following bilateral vestibular deafferentation. Behav Neurosci 2008; 122: 448-459.
20. Omar Abdel-Salam ME, Baiuomy AR, Nada SA. Effect of spironolactone on pain responses in mice. EXCLI Journal 2010; 9: 46-57.
21. Verma SK, Kumar A. Therapeutic uses of Withania somnifera (Ashwagandha) with a note on withanolides and its pharmacological actions. Asian J Pharm Clin Res 2011; 4(1): 1-4.
22. Karthik M, Gayathri C. Effect of ethanolic extract of Hibiscus cannabinus leaf on high cholesterol diet induced obesity in female albino rats. Asian J Pharm Clin Res 2013; 6(4): 65-67.
23. Kobayashi K, Imaizumi R, Sumichika H, Tanaka H, Goda M, Fukunari A, *et al*. Sodium idoacetate induced experimental osteoarthritis and associated pain mode in rats. J Vet Med Sci 2003; 65(11): 1195-1199.
24. Huang MH, Ding HJ, Chai CY, Huang YF, Yang RC. Effects of sonication on articular cartilage in experimental osteoarthritis. J Rheumatol 1997; 24: 1978-84.
25. Lee YR, Lee JH, Noh EM, Kim EK, Song MY, Jung WS, *et al*. Guggulsterone blocks IL-1β-mediated inflammatory responses by suppressing NF-κB activation in fibroblast-like synoviocytes. Life Sci 2008; 82: 1203-9.
26. Pap G, Eberhardt R, Sturmer I, Machner A. Development of osteoarthritis in the knee joints of Wistar rats after strenuous running exercise in a running wheel by intracranial self-stimulation Pathol Res Pract 1998; 194: 41-47.
27. Havdrup T, Henricson A, Telhag H. Papain-induced mitosis of chondrocytes in adult joint cartilage: an experimental study in full-grown rabbits. Acta Orthop Scand 1982; 53: 119- 24.
28. Konttinen YT, Mandelin J, Li TF, Salo J, Lassus J, Liljeström M, *et al*. Acidic cysteine endoproteinase cathepsin K in the degeneration of the superficial articular hyaline cartilage in osteoarthritis. Arthritis Rheum 2002; 46: 953-960.
29. Dejica VM, Mort JS, Laverty S, Antoniou J, Zukor DJ, Tanzer M *et al*. Increased type II collagen cleavage by cathepsin K and collagenase activities with aging and osteoarthritis in human articular cartilage. Arth Res Ther 2012; 14: 113.
30. Robert T, Cluskey Mc, Thomas L. The removal of cartilage matrix, in vivo, by papain. Identification of crystalline papain protease as the cause of the phenomenon. J Exp Med 1958; 108(3): 371–384.
31. Hong Y, Ji H, Wei H. Topical ketanserin attenuates hyperalgesia and inflammation in arthritis in rats. Pain 2006; 124: 27-33.
32. Sluka KA, Westlund KN. Behavioral and immune histochemical changes in an experimental arthritis model in rats. Pain 1993; 55: 367-377.
33. Sluka KA, Milton MA, Willis WD, Westlund KN. Differential roles of neurokinin 1 and neurokinin 2 receptors in the development and maintenance of heat hyperalgesia induced by acute inflammation. Br J Pharmacol 1997; 120: 1263-1273.
34. Hans JS, Bird GC, Li W, Neugebauer V. Computerized analysis of audible and ultrasonic vocalizations of rats as a standardized measure of pain-related behavior. Neurosci Meth 2005; 141: 261-269.
35. Appleton CTG, McErlain DD, Pitelka V, Schwartz N, Bernier SM, Henry JL, *et al*. Forced mobilization accelerates pathogenesis: characterization of a preclinical surgical model of osteoarthritis. Arth Res Ther 2007; 9: R13.
36. Singh BB, Mishra L, Aquilina N, Kohlbeck F. Usefulness of guggul (*Commiphora mukul*) for osteoarthritis of the knee: An experimental case study. Altern Ther Health Med 2001; 7(2): 120, 112-4.
37. Singh BB, Mishra LC, Vinjamury SP, Aquilina N, Singh VJ, Shepard N. The effectiveness of Commiphora mukul for osteoarthritis of the knee: an outcomes study. Altern Ther Health Med 2003; 9(3): 74-9.

38. Singh GB, Atal CK. Pharmacology of an extract of salai guggal ex-Boswellia serrata, a new non-steroidal anti-inflammatory agent. Agents Actions 1986; 18(3-4):407-12.

39. Duwiejua M, Zeitlin IJ, Waterman PG, Chapman J, Mhango GJ, Provan GJ. Anti-inflammatory activity of resins from some species of the plant family Burseraceae. Planta Med 1993; 59(1):12-6.

Table 4: Intra group comparison of pain behavior parameters of STR, NOE and PWL using Rotarod, Photoactometer and Eddy hot plate respectively by One Way ANOVA, where, p < 0.05, showing significant values

Parameters	*Groups*	Day 5 Vs day 12	Day 5 Vs day 19	Day 5 Vs day 29	Day 5 Vs day 39	Day 5 Vs day 49th
ROTAROD	Control	0.11	0.18	0.07	0.14	0.07
	SHAM	0.02	0.38	0.40	0.07	0.25
	OA	1.93×10^{-14}	4.34×10^{-14}	5.29×10^{-13}	2.81×10^{-13}	4.6×10^{-13}
	OA +BDM 10%	1.68×10^{-14}	1.49×10^{-14}	1.17×10^{-14}	1.08×10^{-13}	$`1.26 \times 10^{-13}$
	OA +BDM 20%	9.51×10^{-15}	2.32×10^{-14}	1.08×10^{-13}	1.78×10^{-13}	2.21×10^{-11}
	OA +BDM 40%	3.57×10^{-14}	4.61×10^{-14}	6.66×10^{-12}	0.0009	0.07
PHOTOACTOMETER	Control	0.723452	0.59	0.88	0.62	0.36
	SHAM	0.00317	0.69	0.35	0.41	0.42
	OA	2.52×10^{-8}	3.33×10^{-8}	2.11×10^{-8}	2.62×10^{-8}	1.68×10^{-8}
	OA +BDM 10%	1.76×10^{-7}	1.77×10^{-6}	1.42×10^{-6}	1.06×10^{-6}	8.04×10^{-7}
	OA +BDM 20%	1.12×10^{-7}	4.12×10^{-8}	4.17×10^{-7}	2.19×10^{-6}	0.0005
	OA +BDM 40%	4.01×10^{-6}	5.03×10^{-6}	5.49×10^{-5}	0.02	0.04
EDDY HOT PLATE	Control	0.83	0.77	0.66	0.69	0.89
	SHAM	0.002	0.77	0.49	0.35	0.48
	OA	0.002	4.23×10^{-7}	2.06×10^{-7}	8.48×10^{-7}	1.49×10^{-8}
	OA +BDM 10%	1.43×10^{-5}	5.96×10^{-7}	3.08×10^{-7}	2.78×10^{-7}	1.79×10^{-6}
	OA +BDM 20%	0.002815	1.44×10^{-6}	1.37×10^{-6}	1.23×10^{-6}	2.24×10^{-6}
	OA +BDM 40%	0.003	7.18×10^{-8}	5.96×10^{-7}	0.0001	0.11