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EFFICACY OF KINVA UPANAHA (POULTICE) ON INFLAMMATORY BIOMARKERS IN KNEE OSTEOARTHRITIS – A PILOT STUDY

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ABSTRACT

Introduction: Osteoarthritis is a low grade inflammatory and degenerative joint disease involving varies of molecular interactions between the tissues that form the joint. Janu Sandhigata Vata can be understood in parlance with knee osteoarthritis (OA) which has a great impact on the quality of life. This study is aimed to evaluate the effect of kinva upanaha on inflammatory biomarkers in janu sandhigata vata. **Objectives:** To evaluate the efficacy of Kinva Upanaha on inflammatory biomarkers (TNF- α IL- 6) in Knee osteoarthritis w.s.r to Janu Sandhigata Vata. **Methods:** Basic Phytochemical, Microbial testAmrutarishta Kinva were done and kinva upanaha was done for 20 diagnosed patients of janu sandhigata vata for 7 days for 5 hours duration. Janu Sandhi shola by Visual analogue scale, Knee Range of Movements were assessed on 7th day and 14th day from the day of enrollment, whereas inflammatory biomarkers (TNF- α IL- 6) were assessed on the 1st and 7th day from the day of enrollment. The paired "t" test and the Wilcoxon match-paired test were applied to evaluate the results. **Results:** Kinva Upanaha therapy showed reduction in serum levels of inflammatory biomarkers (TNF-α and IL- 6) and improvement in Knee pain and Knee Range of movements too. **Conclusion**: Kinva upanaha yielded positive subjective

and inflammatory biomarker outcomes in knee osteoarthritis. Kinva is residueand is discarded by most of the pharmacies but this can become an economical and potent upanaha drug in knee osteoarthritis.

Keywords: Inflammatory biomarkers, Knee osteoarthritis, Kinva Upanaha Sweda

INTRODUCTION:

Recent researches concluded OA is low grade inflammatory joint disease in particular of synovitis **[1]**. It is inferred that levels of inflammatory biomarkers (TNF-α, IL-6, IL-1β and hsCRP) are associated with osteoarthritis **[2]**. Upanaha is commonly practiced topical treatment which can be easily adapted with changing time, without compromising its efficacy. Kinva is one of upanaha dravya **[3]** and it is observed that most of the Ayurveda pharmacies discard though it is vatahara, shothahara, ushnaveerya and helps sandhigata vata **[4]**. To tackle high coast variability in OA pain management **[5]** this study was aimed to evaluate the effect of kinva upanaha on TNF-α, IL-6.

Objectives: To evaluate the efficacy of Kinva Upanaha in Knee osteoarthritis w.s.r to Janu Sandhigata Vata.

MATERIAL AND METHODS:

Kinva of Amrutarishta is collected from GMP Certified, KLE Ayurveda Pharmacy, Khasbag Belagavi, and then it is shade dried and powdered in pulverizer then packed into air tight packets after sieving by sieve no 8. Analysis is done at KAHER's Shri B.M.Kankanwadi Ayurveda Mahavidhyalaya. Central Research Facility (AYUSH Approved ASU Drug Testing Laboratory LIC.No.TL-8/2011).

Patients who met all inclusion and exclusion criteria were recruited. Informed written consent was obtained prior to recruitment and patients who received medical care at our hospital's OPD and IPD were included in the planned study. The Institutional Ethics Committee (Protocol ID: BMK/22/KBS/01, KAHER BMK Ayurveda Mahavidyalaya, Belagavi) has provided acceptance for conducting the research. The period of data collecting was from June 2022-December 2022. Throughout the study, the patients were monitored for any adverse events and their records were maintained in a systematic manner.

Preparation of Kinva Upanaha as per standard operating procedures **[10]**. Where kinva 100grms was mixed with 40ml of kanji and heated over a pan and applied to knee joints as shown in **Figure 1**.

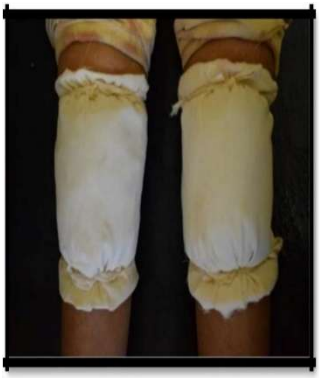

Figure 1: Kinva Upanah

Sample size: A total of 20 patients diagnosed with Knee osteoarthritis and having the classical features of janu shoola, shotha and stambha were recruited. As it was a pilot study, 20 patients were found adequate to draw the conclusions; no specific calculation methods were used to calculate the sample size.

Inclusion: Subject who submitted written informed consent and of either sex, 30-70 years, patients with classical lakshanas of Sandhigatavata were selected irrespective of religion, sexand occupation.

Exclusion: Patients with K/C/O rheumatoid arthritis (RA), gouty arthritis, and psoriatic arthritis and with H/O Secondary knee osteoarthritis by underlying tuberculosis, syphilis, AIDS, leprosy and other infections. Patients with acute trauma, open wound and skin lesions over knee joint.

Assessment Parameters:

1. Sandhi shola was assessed by Visual Analogue Scale and ROM was recorded bygoniometer.
2. Biomarker Tumor Necrosis Factor-α (TNF- α) and Interleukin-6 test kits of Krishgen Biosystems Company were used. Plain blood serum in polypropylene vials stored at -20°C and analyzed at KLE's Dr. Prabhakar Kore Basic Science Research Center. A Research Facility at KAHER (Deemed to be University).

Study design:

A single-group, open-label clinical trial with a pre- and post-test design. Written consent was taken from the enrolled patients after orienting the detailed procedure, possible risks and follow ups.

Duration of Kinva Upanaha - 7 daysFollow up on 14th day

Duration of study – 14 days

Sample size: 20 Patients

Primary outcomes: whereas inflammatory

biomarkers (TNF- α IL- 6) were assessed on 1st and 7th day from the day of enrollment.

Secondary outcomes: The severity of knee pain is assessed by VAS. The range of movements were assessed in degrees by Goniometer. both the parameters were assessed at various time points, i.e.7th day and 14th day from the day of enrollment.

Statistical methods: Using the Wilcoxon match-paired test, the changes in VAS, range of motion was compared from baseline to two time points, i.e., the seventh and fourteenth days. Using the paired "t" test, changes in biomarker values were compared from baseline to two time points, particularly the baseline and seventh day. All tests were considered statistically significant at $p<0.05$, with values expressed as mean ± standard deviation.

OBSERVATION AND RESULTS:

The study was completed by 20 patients in total; no patients dropped out. There were no adverse events during the course of the study.

Table 1: Organo - leptic character of Kinva	
Character	**Result**
Consistency	**Hard**
Color	**Blackish Brown**
Odor	**Aromatic**
Physicochemical property of Kinva	
Test Parameter	**Result**
Loss on drying	**10.203%**
Water soluble Extract	**54.400%**
Alcohol soluble Extract	**41.280%**
pH Value (5% solution)	**4.53**

Table 2: Specified Micro – Organisms in Kinva		
Specified Micro – Organism	**Limits (As per IP)**	**Results**
E Coil	**Absent/100ml**	**Absent**
S aureus	**Absent/100ml**	**Absent**
P aeruginosa	**Absent/100ml**	**Absent**
S abony	**Absent/100ml**	**Absent**
Microbial Limit Test for Kinva		
Specified Micro – Organism	**Limits (As per IP)**	**Results**
Total Bacteria Count	**30-300cfu/ml**	**49 cfu/ml**
Total Fungal Count	**10-100cfu/ml**	**06 cfu/ml**
Preliminary Phyto-chemical Screening		
Tests	**Water**	**Alcohol**
Carbohydrates	**Positive**	**Negative**
Reducing sugar	**Positive**	**Positive**
Monosaccharides	**Negative**	**Negative**
Pentose Sugar	**Positive**	**Negative**
Proteins	**Negative**	**Negative**
Amino Acids	**Negative**	**Negative**
Steroids	**Positive**	**Positive**
Flavonoids	**Positive**	**Positive**
Alkaloids	**Positive**	**Negative**
Tannins	**Negative**	**Negative**
Test for Glycosides		
Glycosides	**Water**	**Alcohol**
Cardiac Glycosides	**Positive**	**Positive**
Anthraquinone Glycosides	**Positive**	**Positive**

Kinva of amrutarishta was subjected to preliminary phytochemical evaluation showed presence of steroids and flavonoids in both water and alcohol extract.

Table 3: Observation on temperature retention of Kinva Upanaha:	
Time	**Kinva Upanahatemperature in Celsius**
15th Minute	**42^0 C**
30th Minute	**41^0 C**
1st Hour	**36^0 C**
2nd Hour	**36^0 C**
3rd Hour	**36^0 C**
4TH Hour	**36^0 C**
5th Hour	**36^0 C**

Figure 2: Temperature Recording of Upanaha

Subject characteristics:

Age: There were 20 patients, 08 men and 12 women. 6 patients had an average age of less than 50 and 14 patients were more than 50 years old, respectively.

Occupation: 10 of the patients were home makers and the rest of the patients included people who worked as farmers, teachers and other jobs.

Religion: 16 patients were Hindu and 4 patients were Muslim in the present study.

Table 4: Comparison of Different treatment time points with VAS							
Comparison of different treatment time points with VAS scores by Wilcoxon matched pairs test							
Time Points	**Mean**	**SD**	**Mean Diff**	**SD Diff**	**% of change**	**Z-Value**	**P-Value**
Baseline	**6.2**	**1.14**	**1.8**	**1.54**	**46.46**	**3.4078**	**0.0007***
7th Day	**4.4**	**1.04**					
Baseline	**6.2**	**1.14**	**4.7**	**1.58**	**77.78**	**3.4079**	**0.0007***
14th day	**1.5**	**1.10**					

Pain:

Assessment of pain by VAS from baseline to 7th day and 14th day was done with Wilcoxon matched pairs test t. The mean value of VAS on baseline was 6.2 which decreased to 4.4 on 7th day and 1.5 on 14th day with significant p value of 0.0007. The adopted treatment has given significant results i.e. $p<0.05$ in the pain parameter assessed by VAS.

Table 5: Comparison of Range of movements at various time points.						
Comparison of different treatment time points with Right Knee FLEXION scores by Wilcoxon matched pairs test						
Time Points	**Mean**	**SD**	**Mean Diff**	**% of change**	**Z-Value**	**P-Value**
Baseline	**46.25**	**9.71**	**20.0**	**72.73**	**3.2958**	**0.0010***
7th Day	**66.25**	**10.49**				
Baseline	**46.25**	**9.71**	**38.27**	**86.68**	**3.4091**	**0.0022***
14th day	**84.52**	**7.05**				
Comparison of different treatment time points with Left Knee FLEXION scores by Wilcoxon matched pairs test						
Time Points	**Mean**	**SD**	**Mean Diff**	**% of change**	**Z-Value**	**P-Value**
Baseline	**46.25**	**9.71**	**20.0**	**72.73**	**3.2948**	**0.0010***
7th Day	**66.25**	**10.49**				
Baseline	**46.25**	**9.71**	**48.27**	**84.62**	**3.4081**	**0.0022***
14th day	**94.52**	**9.07**				

Range of motions:

Assessment of range of motions flexion from baseline to 7th and on 14th day was done with by Wilcoxon matched pairs test. The mean value of right knee flexion scores on baseline was 46.25 which increased 66.25 to on 7th day and 84.52 on 14th day with significant *p* value of 0.0010 and 0.0022 respectively. The mean value of left knee flexion scores on baseline was 46.25 which increased to 66.25 on 7th day and 94.52 on 14th day with significant *p* value of 0.0010 and 0.0022 respectively. The adopted treatment has given significant result i.e. $p<0.05$ on 7th and 14th day in the range of motion parameters.

Table 6: Comparison of Different treatment time points with Inflammatory Biomarkers					
Comparison of TNF- α different treatment time points with Paired 't' test.					
Time Points	**Mean**	**SD**	**Mean Diff**	**t Value**	**pValue**
Baseline	**135.24**	**54.65**	**85.74**	**8.6731**	**0.0001***
7th Day	**49.50**	**27.43**			
Comparison of Interleukin-6 different treatment time points with dependent 't' test.					
Baseline	**116.17**	**13.97**	**71.14**	**9.6925**	**0.0001***
7th day	**45.03**	**23.68**			

Inflammatory Biomarkers:

Inflammatory biomarkers were assessed from baseline to 7th day was done with paired t test. The mean value of **TNF- α** on baseline was 135.24 which reduced 49.50 to on 7th day with significant *p* value of 0.000. The mean value of **Interleukin-6** on baseline was 116.17 which reduced 45.03 to on 7th day with significant *p* value of 0.0001. The adopted treatment has given significant result i.e. $p<0.05$ on baseline and 7th day in the inflammatory biomarker levels.

DISCUSSION:

Knee osteoarthritis results in marked work disability and it is a common degenerative joint disorder seen in practice affecting more than 70% adults between 45-78 years of age **[6]**. Now it is understood that the OA is a chronic degenerative low grade inflammatory condition. It has been evidently found that the synovitis is related with structural progression of OA by degeneration of cartilage and formation of osteophytes leading to joint pain and dysfunction **[7]**. Cytokines present in synovial fluid of OA patients **[7]** hastens joint destruction and pain by activating innervating nociceptors **[8]**, research works done on functional MRI of brain have revealed the arthritic pain response in the brain is due to neutralization of TNF-α **[9]**.

Kinva is an important residue with various biological properties obtained after filtration of the final product of fermentation is called „surabeeja" or „kinva" which is dried and stored for further use **[10]**. Kinva of amrutarishta is blackish brown in colour having aromatic odor with hardconsistency. Loss on drying of kinva is 10.203% which is suggestive of no fungal growth. Solubility test indicates about the bioavailability and it was seen that water soluble extract is 54.400% which was more than alcoholic extract 41.280%. Microbial study showed absence of, *E-coli, Salmonella abony, Pseudomonas aueruginosa* and *Pseudomonas aureus*. Preliminary Phyto-chemical Screening found the presences of flavnoids are therapeutically proved as anti- inflammatory, antioxidant.

It is observed that Kinva Upanaha has maintained 41^0 C of temperature after 30 minutes of application and is maintained at 36^0 of heat till five hours [**Table 3**] might be due to self-generated alcohol content and ushna, tikshna guna. It is known that sustenance of increased local temperature for 30 minutes helps in improving vascular supply of affected joint. Heat induced by kinva upanaha swedana indirectly acts on the autonomic nervous system and may reduse knee joint pain, which is evedent in patients by marked improvement seen in knee pain.

Improvementis also seen in knee flextion and extension by strenthgenng the muscules

supporting the joints due to increased local temperature hastens the trans-dermal delivery of various drugs by enhancing skin permeability, body fluid circulation, vascular wall permeability and drug solubility **[11]**. Flavonoids are naturally occurring polyphenols having anti-inflammatory properties **[12]**. Because of Lipophilic property of flavonoids, they are readily absorbed by cutaneous route and this route is the best delivery approach for flavonoids **[13]**. Studies revealed that the flavonoids interfere the production of various pro-inflammatory cytokines by blocking nuclear factor kappa B translation via COX-2 synthesis **[14]**, so flavonoids present in the kinva might have reduced the inflammation which is evident in reduction of inflammatory biomarkers (IL-6 and TNFα) as they are proved having anti-inflammatory, anti-oxidant properties.

CONCLUSION:

In recent years anti-inflammatory therapy has become an effective strategy for the therapeutic management of Osteoarthritis. Kinva is residue and is discarded by most of the pharmacies but this can become an economical and potent upanaha drug, as kinva of amrutarishta has shown the marked effect in reducing the pain, improving the knee joint range of movements and oninflammatory biomarkers as well. Efforts have been made to provide evidence-based cost-effective pain management pre and post evaluation of inflammatory biomarkers concerned to knee pain and joint degeneration. Additionally, there is a room for clinical trials of kinva of various preparations and more researches in this area are still needed.

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Conflicts of Interest: The authors declare that there is no conflict of interest.

Author Contribution: Both the authors provided their contributions in treating the patients.

Dr. Kavita B S involved in the collection of data. Analysis, interpretation of the data was done by both the authors. Manuscript drafting was done by Dr Kavita B S, and review, correction of manuscript was done by Dr Pradeep L Grampurohit and Dr Kavita B S. Approval from both the

authors were provided for the submitted manuscript.

Abbreviations: VAS: Visual Analogue Scale; TNFα: Tumor Necrosing Factor α; IL-6: Interlukine-6.

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