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Clinical Evaluation of Antioxidant Activity of *Shatavari* (Asparagus racemosus Linn.) in *Janusandhigata Vata* (Degenerative Arthritis of Knee)

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ABSTRACT

Osteoarthritis, also known as degenerative joint disease, is a process of progressive deterioration of joint cartilage and formation of new bone (osteophyte) at the joint surface. Lipid peroxidation mediated byfree radicals is considered to be a major mechanism of cell membrane destruction and cell damage along with *DhatukshayajanyaVataprakopa*.The present study was undertaken to evaluate Antioxidant activity of *Shatavari* (*Asparagus racemosus Linn*) in terms of biological markers namely, SOD (Superoxide dismutase),GSH (Glutathione) &Malionialdehyde in *JanusandhigataVata* (Degenerative Arthritis of Knee).In this trial 30 patients with primary knee osteoarthritis were administered *Shatavari*churna 3 grams once a day empty stomach in morning for the period of 60 days. Preliminary CBC, X-Ray knee was done to assess the severity. Patients were evaluated for 4 sypmtoms namely, *Sandhishool, Aatopa, Sandhishotha&Aakunchanprasaranvedana*in gradations 0 to 4. Oxidative stress markers MDA, SOD & GSH were evaluated before and after the trial.Total percentage improvement in Subjective& Objective parameters was 64.17%& 52.82% respectively.MDA was reduced which in turn was due to increase in antioxidant reserve i.e. SOD & GSH. All the data obtained was statistically evaluated using Students Paired 't' test. It was found that the data was clinically important with 90% showing marked improvement(≤50-75%) & 10 % showing moderate improvement(≤25-50%) as well as statistically significant with P<0.0001.Thus, *Shatavari* was responsible for reducing oxidative stress in patients of *Janusandhigatavata* (Degenerative arthritis of knee).

KEYWORDS

Janusandhigatavata, Degenarative arthritis of knee, Shatavari, Antioxidant activity

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INTRODUCTION

Osteoarthritis is the second commonest musculoskeletal problem and Osteoarthritis of Knee Joint (*JanuSandhigatavata*) is most prevalent joint disease in human beings.

हन्तिसन्धिगत: सन्धिन्शूलाटोपौकरोतिI"

(मा. निवातव्याधि-२२)

Among the elderly, Osteoarthritis of Knee Joint is the leading cause of chronic disability in developed countries.Because of Osteoarthritis, nearly 20%-30% of people in India are affected by some complaints of knee joint during their lives. Females are found more affected by this disorder. Osteoarthritis is considered as a global disease affecting all joint tissues,but cartilage degradation is its end point. The degradation of cartilage results from the combination of mechanical stress and biochemical factors, mainly metalloproteinases and ROS.

In Ayurveda, *Rasayanadravyas*, having *vayasthapana karma*, are considered as nutritional entities responsible for rejuvenation of wear out cells and removal of toxins and free radicals. *'Asthi'&'Sandhi'* both are known as the *moolasthana* or origin of *MajjavahaSrotas.Vatadosha* is mainly situated in *Asth&Sandhi*,hence due to vitiation of *Vatadosha* there are changes or deformity in these *Asthi&Sandhi*. Chronic *vataprakopa* causes *asthikshaya*which is the key *samprapti* of *SandhigataVata*.

मज्जाशुक्रसमुत्थानंऔषधंस्वादुतिक्तम् I

अन्न्व्यायायव्यायामौशुध्दीकालेचमात्राया:II(

च.सू.२८/२६)

Therefore, for management of Denegerative arthritis of knee, a *rasayana* drug having ***madhurtikta rasa*** which is **adaptogenic, chondroprotective, analgesic, free radical scavenging, immunomodulator**& responsible *for* ***saptadhatu&kaphavardhan*** **along with** ***vata shaman***is required.

Hence, *Shatavari* was selected for this purpose.*Shatavari* (Asparagus racemosus Linn) has sufficient proof as an antioxidant & is considered a *Rasayanadravya.*

AIMS & OBJECTIVES

- To Study the concept of *Rasayana* with respect to Antioxidation.
- To evaluate of Antioxidant activity of *Shatavari*(Asparagus racemosus Linn) in *Janusandhigatavata* (Degenerative Arthritis of Knee).
- To provide effective medicine for the patients suffering from Degenerative Osteoarthritis of Knee to help them lead a comfortable life.

MATERIALS & METHODS

Ethical Committee Approval No.AMS/1340/16-17 dated 29/4/16

1.Source of Data: Literary search was carried out on *Shatavari,janusandhigatavata* and antioxidant activity through various S*amhitas,* research articles and websites like Pubmetetc.The test drug was authentifiedat Blatter Herbarium of St Xavier's College, Mumbai and standardized at Alarsin pharmaceuticals, Andheri, Mumbai.

2. Patient Selection: The randomized clinical study was undertaken atOPD of Seth RV Rugnalaya, Sion, Mumbai after approval of Institutional ethical committee (IEC approval no- AMS/1950/2018/1).A single group of 30 patients, diagnosed with *Janusandigata Vata* were selected randomly on the basis of predefined inclusion & exclusion criteria.

CRITERIA OF EVALUATION-

Inclusive Criteria:

Patients with *pratyamtmalakshanas* of *nirupathambita janusandhigata vata.*

- ❖ Patients aged between 40 to 70 years.
- ❖ Patients who have signed written & informed consent.

Exclusive Criteria:

- ❖ Patients with secondary osteoarthritis due to obesity (BMI >=30), trauma, postural defects, inflammatory conditions of knee joint,bone deformity & with metallic implants.
- ❖ Patients suffering from Rheumtoid arthritis, Gout, Rheumatic heart disease.
- ❖ Patients with systemic illness like uncontrolled diabetes, uncontrolled hypertension, cardiopulmonary disorders, hypothyroidism, CKD, CLD etc.
- ❖ Pregnant or lactating women or those who are not following contraceptive measures.
- ❖ Patients with immunocompromised states like AIDS, Tuberculosis, Cancer, Hepatitis B & C etc.
- ❖ Patients on antipyretics, analgesics, hypnotics, alcohol or any drug interfering with pain perception.

DRUG ADMINISTRATION:

- ❖ Drug Source: Fine powder of root of Shatavari (Asparagus racemosa Linn)
- ❖ Formulation: *Churna*
- ❖ Mode of administration: Oral
- ❖ Dose: 3 grams Once a day[1]
- ❖ *Anupana*: *Koshnajala*
- ❖ *Kal*: *Abhakata or Rasayanakala* (Empty stomach in the morning)
- ❖ Duration : 60 days

ASSESSMENT

CRITERIA:*SUBJECTIVE –*

A]*Sandhishula* (Knee Joint Pain)

- ❑ No pain- 0

- ❑ Mild pain- 1
- ❑ Moderate pain but no difficulty in walking- 2
- ❑ Slight difficulty in walking due to pain - 3
- ❑ Severe difficulty in walking -4

B]*AkunchanaPrasaranayohVedana*(Restricted Motility)

- ❑ No pain -0
- ❑ Pain without winching of face -1
- ❑ Pain with winching of face-2
- ❑ Prevent complete flexion-3
- ❑ Does not allow passive movement-4

C] *Sandhisphutana* (Atopa, Crepitus)

- ❑ No Crepitus -0
- ❑ Palpable Crepitus-1
- ❑ Audible Crepitus -2

D] *Sandhishotha*(Edema of Knee)

- ❑ No Swelling-0
- ❑ Slight Swelling- 1
- ❑ Moderate Swelling -2
- ❑ Severe Swelling-3

OBJECTIVE: Oxidative Stress markers –

i) Glutathione (GSH)

ii) Superoxide dismutase (SOD)

iii) Malondialdehyde (MDA)

These parameters were evaluated in Department of Biochemistry, Lokmanya Tilak Medical College & Hospital, Sion,Mumbai-22.

ESTIMATION OF MDA, GSH & SOD.

The whole blood from EDTA bulbs were used to estimate reduced GSH levels.Serum and Plasma samples separated from plain bulbs, using a centrifuge machine, was used to estimate MDA, and SOD levels respectively.

- ***Determination of Maliondialdyde (MDA)***

MDA reacts with the thiobarbituric acid in acidic mediumto give a characteristic MDA-TBA coloured complex. The intensity of the pink colour is directly proportional to the amount of MDA which in turn measures the generation of free radicals. Lipids are separated from serum proteins using 40 % Trichloracetic acid to determine their amount using TBA. The absorbance is read colorimetrically at 530 nm.

- ***Determination of Superoxide Dismutase (SOD)***

Superoxide anion is involved in auto-oxidation of Pyrogallol solution at alkaline pH of 8.5. The SOD inhibits auto oxidation of Pyrogallol which can be determined as an increase in absorbance at 420nm.

- ***Determinaton of Glutathione (GSH)***

This method is based on the development of a relatively stable yellow color when 5.5'-dithio-bis- (2- nitro benzoic acid) or DTNB is added to sulphydryl compounds, read at 412nm filter.

FOLLOW UP: Clinical follow up was advised 15,30,45 & 60 days after first visit.

STATISTICAL ANALYSIS: The Objective parameters were statistically evaluated using Student's Paired 't' test.

WITHDRAWAL CRITERIA: 1) Left against medical advice (LAMA).

2) Development of complications due to presenting illness or otherwise.

3) Aggravation of symptoms.

4) Pronounced toxic side effects

TOTAL EFFECT OF THERAPY: It was assessed asfollows-

- Complete Remission ≥ 75%
- Marked Improvement ≥ 50%
- Moderate Improvement ≥ 25%
- No Improvement = 0%

Table 1 Percentage relief of Subjective Parameters

SYMPTOMS	PERCENTAGE	SUBJECTS SHOWING IMPROVEMENT
Sandhishool	73.85 %	29 OUT OF 30
Aatopa	67.65 %	17 OUT OF 30
Sandhishotha	85 %	19 OUT OF 20
Aachunchana Prasaran Vedana	76.19 %	28 OUT OF 30

RESULTS

Out of 30 patients (Table 1), 29 patients showed symptomatic improvement in *sandhishool*. Average percentage improvement was 73.85%. Seventeen patients showed symptomatic improvement in *aatopa*, while 13 patients showed no improvement. Average percentage improvement was 67.65%. Twenty patients presented with the complains of *sandhishootha*, out of these nineteen patients showed symptomatic improvement. Average percentage improvement was 85%. Twenty eight patients showed symptomatic improvement in *akuncha prasarana vedana*. Average percentage improvement was **76.19%.**

Out of 30 patients (Fig 1), Moderate increase in SOD was found in 3 patients i.e 10% & only in 27 patients i.e 90% significant increase in SOD was observed.7 patients i.e 23.33% low grade increase was observed, mild grade increase i.e 50% in 15 patients , moderate increase in 07 patients i.e 23.33% & only in 1 patients i.e 3%, significant increase in GSH was observed.

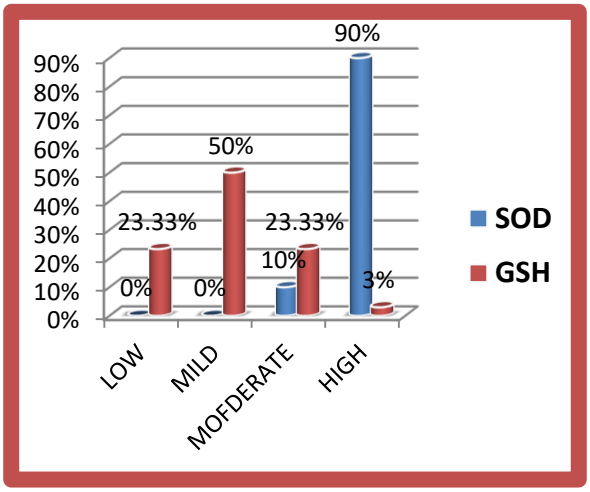


Fig 1. Graphical representation of Percentage increase in SOD & GSH

In (Fig 2) one patient low grade (0-25%) decrease(16.67%) in MDA was observed,

mild grade 25-50%) decrease i.e 44 % in 13 patients , moderate (50-75%) decrease in 14 patients i.e 47% & only in 2 patients i.e 6%, significant (75-100%) decrease in MDA was observed.

Total percentage improvement in Subjective & Objective parameters is 64.17% & 52.827, respectively.

Therefore (Table 2), the total effect of therapy depicts that 27 patients (90%) showed marked improvement (≤ 50-75 %), while 3 patients (10%) showed moderate improvement (≤ 25-50%). None of the patients showed complete or no improvement.

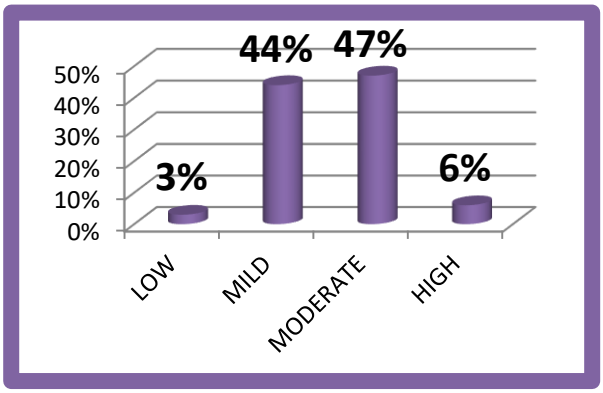


Fig 2. Graphical representation of Percentage decrease in MDA

Table 2: Total Effect of Therapy

CATEGORY	CRITERIA	NO OF PATIENTS	PERCENTAGE
Complete Remission	≥ 75%	0	0%
Marked Improvement	≥ 50%	27	90%
Moderate Improvement	≥ 25%	03	10%
No Improvement	= 0%	0	0%

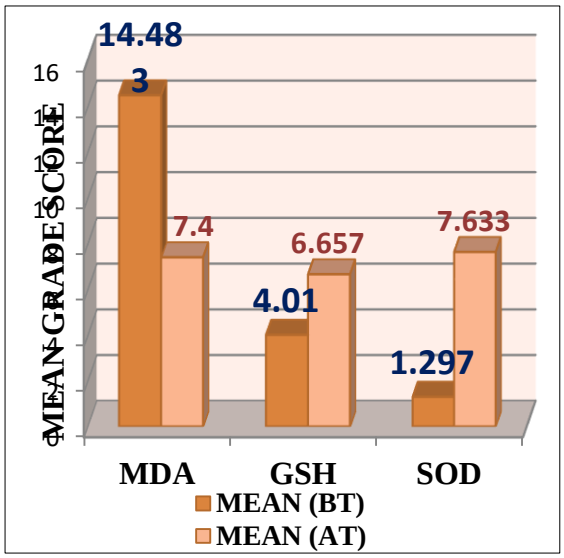


Fig 3 Statistical Difference in Objective Parameters

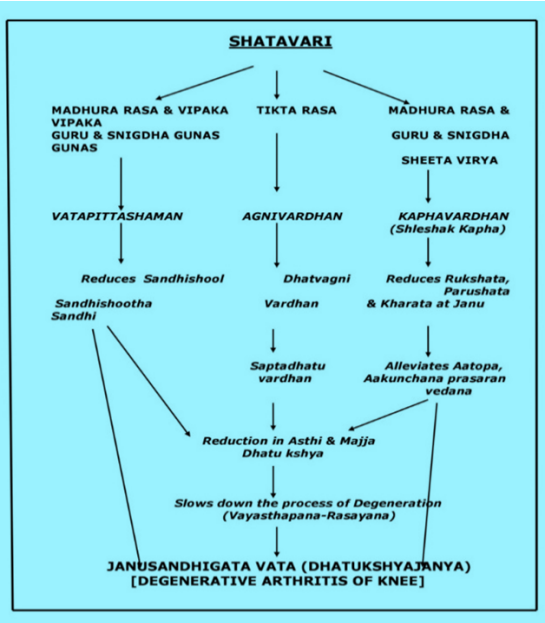
Objective parameters (Fig 3) were statistically evaluated by using paired 't' test. It was found that t value, with 29 degrees of freedom(*n*-1), for MDA,SOD & GSH were 13.990, 23.878 & 11.123 respectively, whose p value is < 0.0001is considered extremely significant (at 95% confidence level).

DISCUSSION

Probable mode of action of Shatavari (Fig 4)

Sandhigata vata, a disorder of *Asthi–Majjavaha Srotas* is degenerative in nature where in degeneration of *Asthi Dhatu* (osseous tissue) in the joints occur. *Vata.* It will be more severe in *Vata Kala and Vata Prakruti* individuals. *Vata-*

Asthisthanasamraya is the chief event in osteoarthritis. In osteoarthritis *Asthikshya*is the key samprapti due to chronic *vataprakopa*. On a close analysis ***the kharaguna* of *Asthi* is lost, leading to degeneration of the joint**. The *prithav imahabhoota* related *sarabhava* of asthi is gradually undergoing *kshaya* which is seen in radiological pictures as *asthisaraheenatha.*



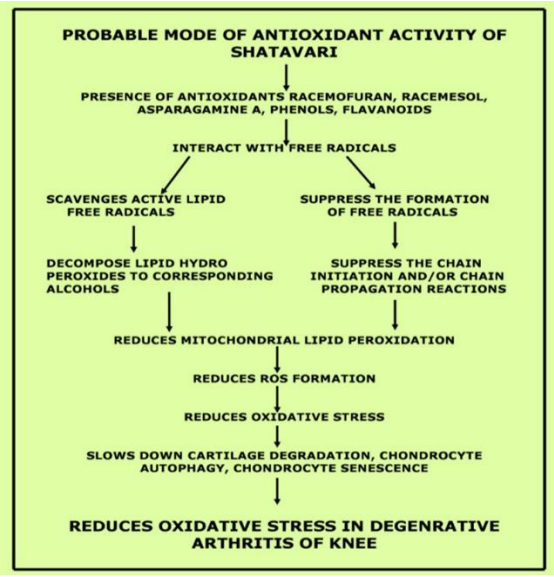


Fig 4 Probable mode of action of Shatavari

Sandhigata Vata is *Madhyama Roga Margagata Vatika* disorders in which vitiated *Vata* gets lodged in *Sandhi*. Hence to treat *Sandhigata Vata* drugs acting on both *Vata*and *Asthisandhi* should be selected. According to *Charaka,* in *Asthi Dhatu Dushti* the treatment should be given is *TiktaDravya.*

अस्थ्याश्रयाणांव्याधिनांपंचकर्माणिभेषजम्।

बस्तयः क्षीरसर्पीषितिक्तकोपहितानिच॥(च.सू. २८/२५)

Excess use of *Tiktaras* causes *Dhatukshay* and *Vatavyadhi* . This states that *Tiktaras* has ability to reach to *Asthidhatu* and deal with *vatadosha*. So, here we can say that *Tiktaras* has definite effect on *Asthi* and *Vata*.

धातुक्षयानिलव्याधीनतियोगात्करोतिसः।

(अ.हृ.सू.१०/१६)

Tikta Rasa increases the *Dhatvagni* (metabolic stage). As *Dhatvagni* increase, nutrition of all the *Dhatus* will be increased. As a result *Asthi Dhatu, Majja Dhatu* may get nourished and *Asthi* and *Majja Dhatu Kshaya* will be decreased. So degeneration in the *Asthi Dhatu* may not occur rapidly. It can be said that it slows down the degeneration processes. *Tikta Rasa* has got *Deepana, Pachana* and *Rochana* properties. So it helps in the improvement

of the general condition of health and thus strengthen the whole body as well as joints. *Tikta ras* of *Shatavari brings kharatva* to *asthi* while *Madhura ras, vipaka & sheetavirya* ensures *snigdhata at* the *janu sandhi.*

Madhura rasa & *vipaka ,snigdha gunas* causes *vata shaman*& these along with *sheeta virya, guru gunas* causes *kapha vruddhi*i.e specially of *shleshak kapha*. This s*hleshak kapha* is responsible for lubrication of joints, which reduces the *rukshata, parsuhta & kharata* at *janusandhi* by increasing *snigdhata.* This alleviates *aatopa* (crepitus i.e friction between the joints), *aakunchan prasaran vedana & sandhihool*.

Sandhishootha in *dhatukshyajanya janusandhigata vata* is basically of *Vataj* type, where pain is the predominant feature. It gets pacified on its own & gets resurfaced on its own. On pressing & releasing it quickly rises to the surface. It is *vatapurnadrutisparsha*. Such type of *sandhishotha* gets pacified by *Madhura-tikta rasa* & *snigdhaguna* of *shatavari*.

*Shatavari*is a plant belonging to *anupa* or *sadharandesha*. Its *panchbhautik* constitution indicates it being *Prithavi &Aap mahabhutapradhan dravya*. Thus, it brings stability & compactness to the knee joint as it increases the *sarabhava* of *asthi* which is gradually undergoing *kshaya* in osteoarthritis.*Shatavari* acts on *Rasa dhatu,* thereby enchancingit's quality & quantity. Once the *rasa dhatu* is well established, it nourishes the remaining *dhatus* according to *Kedarkulyanyaya*, leading to *saptadhatuvardhan*. It exhibits anabolic properties to give strength to the body. It delays aging, thereby is a potent *Vayasthapana dravya*. *Shatavari churna* was administered once a day in morning empty, which is considered as *Rasayanakala*, as *agni* is strongest during this period. Hence it helps in proper digestion & assimilation of *dravya.*

Also, according to *Acaraya Sushruta,* 40 to 70 years is termed as *Madhyamavastha,* which is *pitta* predominant. Degenerative Arthritis of Knee is a disease of old age (i.e above 70 yrs as per *Ayurveda*). But owing to the current lifestyle & reduced life span, the prevalence of arthritis is more in 40 to 70 years age group. This can be termed as *AkalajJara* (i.e premature aging). *Shatavari* being a *vayasthapanadravya* pacifies *pitta*dosha, hence it was found beneficial in this trial.

Oxidative stress, defined as the imbalance between the production and degradation of ROS, is considered to play an important role in mechanism of cartilage degradation. Free radicals can be regarded under the category of *ama. Ama* constitutes of *Ama rasa*, *Amadosha & Amavisha*. Free radical

is a form of Amavisha[2]. Studies suggest that Phenols present in *Shatavari* are well established to exhibit antioxidant activity, contribute to human health. Flavonoids are good free radical scavengers, donate hydrogen atoms. *(B Priyanka et. Al. 2012).*New antioxidants isolated from its roots namely **Racemofuran, Racemosol & Asparagimne A** are also responsible for the antioxidant activity of Shatavari[4].In this study all the patients with Osteoarthritis showed increased levels of lipid perioxidation product MDA & Decreased levels of natural antioxidants SOD & GSH. After administration of *Shatavari* for 60 days, MDA was reduced which in turn was due to increase in antioxidant reserve i.e SOD & GSH. Thus, it can be inferred that antioxidants present in *Shatavari* increases the action of antioxidant enzymes i.e *Dipana* & also inhibits lipid perioxidation i.e *Pachan*[3].

CONCLUSION

Patients of *Dhatukshyajanya janusandhigata vata* showed marked improvement in symptoms, of which maximum relief was found in *sandhishootha,* followed by *aakunchan prasaran vedana,* then *sandhishool* & lastly *aatopa*. It was also found that the activity of antioxidant enzymes SOD & GSH were significantly lower while that of MDA, a highly reactive byproduct of polyunsaturated fatty acid peroxidation, was raised in patients with knee osteoarthritis. And after the administration of *Shatavari*, levels of SOD & GSH increased significantly and those of MDA were reduced. Thus, Shatavari does exhibits antioxidant activity in *Janusandhigata Vata.*

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