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# POTENT EFFECT OF LEECH THERAPY AND *ASWAGANDHA GHRITA* ON OXIDATIVE STRESS AND IL-1 LEVEL IN THE PATIENTS OF OSTEOARTHRITIS *(SANDHIGATA VATA)*

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## ABSTRACT

**Introduction:** Osteoarthritis is an inflammatory and most common adult joint disorder that not only leads to articular cartilage loss and joint space narrowing, but also causes pain, loss of function and physical disability thus greatly impairing quality of life. **Aim:** The present study has been carried out for assessing the effect of *Ashwagandha Ghrita* and *Jalaukavacharana* (Leech therapy) on the level of oxidative stress and IL-1 in patients of Osteoarthritis (*Sandhigata Vata*). **Materials and Methods:** In this comparative study total 45 diagnosed cases of osteoarthritis were randomly divided into three groups. In Group-I & II patients were treated with Leech and *Ashwagandha Ghrita* respectively and patients of Group-III were treated with Leech therapy and *Ashwagandha Ghrita* both. Serum samples were analyzed for oxidative stress status and IL-1 level before and after the treatment in all groups. **Result**: Serum oxidants and Serum IL-1 levels were significantly decreased after the treatment in all the three groups likewise serum antioxidants levels were significantly increased in all three groups after the treatment. But Group III (combined therapy) showed better result than other groups. **Conclusion:** The findings indicates that combined effect of Leech therapy and *Ashwagandha Ghrita* prevents progression of the disease by reduction in oxidative stress and IL-1 level in patients of osteoarthritis *(Sandhigata Vata)*. This may show probable disease modifying effect of Leech therapy and *Ashwagandha Ghrita* in osteoarthritis.

**Key words**: *Ashwagandha Ghrita*, Leech therapy, Oxidative stress, IL-1, Osteoarthritis,

## INTRODUCTION

Osteoarthritis (OA), the most common musculoskeletal condition, is a long-term chronic disease involving the thinning of cartilage in joints which results in bones rubbing together, creating stiffness, pain, and impaired movement. Disease progression is associated with cartilage degradation, joint space narrowing (JSN), and bony changes including osteophytes, subchondral sclerosis, and bone marrow lesions. Worldwide estimates are that 9.6% of men and 18.0% of women over the age of 60 years have symptomatic osteoarthritis. Approximately 80% of those with OA will have limitations in movement, and 25% cannot perform their major activities of daily life.[1]

The prevalence of OA is increasing and will continue to do so as the population increases, ages, and is subject to risk factors such as the obesity epidemic. It is a leading cause of disability affecting 60-70% of the population older than 60 years.

The uncontrolled production of free radicals is considered as an important factor in the tissue damage induced by several pathophysiologies[2,3].When the production of damaging ROS exceeds the capacity of the body's antioxidant defenses to detoxify them, a condition known as oxidative stress occurs[4.5]. Antioxidants are compounds that dispose, scavenge, and suppress the formation of free radicals, or oppose their actions [6]. Interleukin (IL)-1 is a cytokine that plays a major role in inflammatory responses in the context of infections and immune-mediated diseases.

Osteoarthritis can be considered as *Sandhigata vata* in *Ayurveda. Acharya charaka* described as *"sandhi gata anila"* under the chapter *Vatavyadhi (Ch. Chi. 28\37)* and defined it as *"Vatapurna driti sparsha"* (on palpation it feels like air filled bag), *"Sopha"* (swelling), *"Prasaranakunchanyoh pravrittischa savedana"* (painful on flexion and extension movement)[7]. Considering *Sandhigata vata* both *Sandhiashrita* and being a *Vataj* disease various researches have been conducted to cure *Vata* aggravated in *Sandhi* with different indigenous drugs and measures including *Snehana* both systemic and external, *Upnaha* and *Agnikarma*, Virechana, *Basti*, *Shamana Yogas* like *Guggulu*, *Vati* preparations etc. In most of the studies significant results were observed [8].

*Ashwagandha* (Withenia somnifera) is one of the most extensively studied Indian Medicinal Plants with substantial confirmed tranquilizer, cardio tonic, antibacterial, antifungal, anticancerous, antiarthritic and immunopotentiation actions.

*Ashwagandha* (Withenia somnifera) is mainly used in vatkaphaj vikara and also has rasayana property along with analgesic and anti inflammatory activity which can be helpful for slow down the processes of osteoarthritis.

**Materials and Methods**

The study was carried out at Department of *Kayachikitsa* and Department of Biochemistry, Institute of Medical Sciences, Banaras Hindu University, Varanasi. Prior to the study, the approval of the institutional ethical committee was obtained. A total 45 patients of osteoarthritis *(sandhigata vata)* were selected from Indoor/Outdoor patients of *Kayachikitsa*, S.S Hospital Varanasi, Uttar Pradesh. The study was undertaken in duration of August 2011 to November 2013. Patients were randomly divided into three groups, 15 patients in each group with care of inclusion and exclusion criteria has been taken, out of 45 patient of them 8 patient were dropped in middle of the trial period and 37 patients completed 3 months of total duration of treatment. Before registration, informed consent has been taken and proper confidentiality has also being maintained regarding sensitive issue, privacy and safety of the subjects throughout the trial.

The case selection was regardless of sex, occupation and socio-economic status. Patients between 35-65 years of age group with diagnosis of osteoarthritis and having persistent osteoarthritic symptoms for at least 6 months, cases of primary osteoarthritis with involvement of knee joint were included for this study.

The patients with diabetes, gouty arthritis, secondary knee osteoarthritis due to inflammatory joint diseases, a history of intra articular corticosteroid injections and a history of oral glucosamine, chronic alcohol consumption, chronic liver and kidney diseases, smokers, oral history of antioxidants vitamin and/or mineral use during the last six months were excluded.

Five ml of venous blood were withdrawn under aseptic precaution, using a sterile disposable syringe from the four patients groups. The blood was allowed to clot in a plain tube at room tem-

perature and then the serum was separated by centrifugation at 3000 rpm for 10 minutes and then kept frozen at -20°C to be analyzed thereafter for the estimation of oxidative stress status and IL-1 levels.

Serum MDA (µmol//l) level was estimated by TBA test [11]. Serum PC (nmol/mg protein) level was estimated by extraction of PC group with dinitrophenylhydrazine[12]. Serum Ascorbic acid (mg/dl) level was estimated by oxidizing it and then derivatizing the product with dinitrophenyhydrazine [DNPH][13]. Superoxide dismutase was estimated by the method described by Marklund & Marklund in 1974 using pyragallol[14].

The biochemical analysis of the collected samples for inflammatory marker (IL-1) was carried out in Department of Biochemistry. Kit (REF IL01b02 LOT IL01b131212) was purchased from Ani Biotec Oy Orgenium laboratories.

**Treatment schedule for group I –** *Ashwagandha ghrita* 5 gram BD with lukewarm water for 90 days.

**Treatment schedule for group II –** Leech therapy, 1 sitting per 15 days interval for 90 days (6 sitting)

**Treatment schedule for group III –** *Ashwagandha ghrita* 5 gram BD with lukewarm water and Leech therapy, 1 sitting per 15 days interval for 90 days (6 sitting)

**Statistical Analysis -** The data obtained was processed on a computer with the help of "SPSS: 17" software package of statistical analysis. Standard statistical methods were used to determine the mean, standard deviation (SD) and the range. Paired t-test was used to compare the results of various biochemical parameters among the patients in the four groups. All value quoted as the mean ± SD and a p-value of $< 0.05$ was considered to be statistically significant and p-value of $<0.01$ or $p < 0.001$ was considered to be statistically highly significant.

**Results**

Table / Fig 1 shows mean and standard deviation of malondialdehyde (MDA) & Protein carbonyl (PC) before and after treatment which is statistically highly significant ($p<0.01$) for all the three groups. The inter group comparison (one way ANOVA) resulted not statistically significant ($p>0.05$) at before and after treatment for all the three groups.

MDA which is one of the products of lipid peroxidation[15] causes free radicals generation in the body, combined therapy (Group III) shows better result in reduction in MDA level.

Protein carbonyls most commonly used marker of protein oxidation, this PC level, when compared between the three groups, found more decrease in patients of combined therapy (Group III) after treatment.

**[Table/Fig-1]: Serum MDA and PC in patients of OA *(Sandhigatavata)***

| | | | Group-I (n=11) | Group-II (n=13) | Group-III (n=13) |
|---|---|---|---|---|---|
| **MDA [µmol/l]** | Mean ± S.D | BT | 0.55±0.032 | 0.61±0.036 | 0.59±0.032 |
| | | AT | 0.31±0.031 | 0.37±0.021 | 0.25 ±0.015 |
| | p-Value | | p < 0.01 | p < 0.01 | p < 0.01 |
| **PC [nmol/mg Protein]** | Mean ± S.D | BT | 3.03±0.232 | 3.18±0.177 | 3.02±0.222 |
| | | AT | 1.51±0.12 | 2.23±0.156 | 1.07±0.115 |
| | p-Value | | p < 0.01 | p < 0.01 | p < 0.01 |

[MDA - Malondialdehyde; PC - Protein carbonyls; BT -Before Treatment; AT - After treatment; n-Number of patients; S.D-Standard deviation]

Table/Fig (2) shows mean and standard deviation of SOD (Superoxide dismutase) and Ascorbic acid before and after treatment which is statistically highly significant ($p<0.01$) in all

three Groups. For Ascorbic acid p-Value is highly significant ($p<0.01$) for all the three groups. The inter group comparison (one way ANOVA) resulted not statistically significant ($p>0.05$) at before and after treatment for all the four groups.

SOD is an enzymatic and ascorbic acid is a major non-enzymatic anti oxidants in the body. This antioxidant level, when compare between the three groups, combined therapy (Group III) shows better result.

**[Table/Fig-2]: Serum SOD and Ascorbic acid in patients of OA *(Sandhigatavata)***

| | | | Group-I (n=22) | Group-II (n=21) | Group-III (n=20) |
|---|---|---|---|---|---|
| SOD [U/0.1ml] | Mean ± S.D | BT | 0.46±0.045 | 0.44±0.056 | 0.42±0.047 |
| | | AT | 0.97±0.087 | 0.66±0.089 | 0.91±0.105 |
| | p-Value | | p < 0.01 | p < 0.01 | p < 0.01 |
| A.Acid [mg/dl] | Mean ± S.D | BT | 1.97±0.137 | 1.98±0.131 | 2.12±0.144 |
| | | AT | 2.97±0.215 | 2.30±0.149 | 3.75 ± 0.256 |
| | p-Value | | p < 0.01 | p < 0.01 | p < 0.01 |

[SOD – Superoxide dismutase; A.Acid-Ascorbic Acid; BT - Before Treatment; AT - After treatment; n-Number of patients; S.D-Standard deviation]

Table/Fig (3) shows mean and standard deviation of IL-1 (pg/ml) before and after treatment which is statistically highly significant ($p<0.01$) for all the three groups. The inter group comparison (one way ANOVA) resulted not statistically significant ($p>0.05$) at before and after treatment for all the three groups.

This IL-1 level when compared between the three groups, better result is observed in combined therapy (Group III).

**[Table/Fig-3]: Serum IL-1 Level in patients of OA (*Sandhigatavata)***

| | | | Group-I (n=11) | Group-II (n=13) | Group-III (n=13) |
|---|---|---|---|---|---|
| IL-1 [pg/ml] | Mean ± S.D | BT | 0.61±0.074 | 0.71±0.044 | 0.73±0.037 |
| | | AT | 0.43±0.069 | 0.36±0.026 | 0.32±0.0211 |
| | p-Value | | p < 0.01 | p < 0.01 | p < 0.01 |

[IL-1-Interleukin- I, OA - Osteoarthritis; BT - Before Treatment; AT - After treatment; n-Number of patients; S.D-Standard deviation]

## DISCUSSION

*Ashwagandha* (*Withania somnifera*, fam. Solanaceae) is commonly known as "Indian Winter cherry" or "Indian Ginseng". It is one of the most important herb of *Ayurveda* (the traditional system of medicine in India) used as a *Rasayana* for its wide ranging health benefits. It is also used as an analgesic, astringent, antispasmodic and immuno-stimulant while being used to treat inflammation, cancer, stress, fatigue, diabetes and cardiovascular complications.

*Ashwagandha* improves the body's defense against disease by improving the cell-mediated immunity. It also possesses potent antioxidant properties that help protect against cellular damage caused by free radicals.

*Ashwagandha* has a *vatakapha shamaka* action and in pharmaceutical form of *Ghrita*, it becomes *tridoshshamak* as *Ghrita* is very good *pittashamak. Charaka* has mentioned that *Ghrita* is *Vatashamaka* due to its *Snigdhata*, *Pittashamaka* due to its *Shaityata* and *Kaphashamaka* due to its *Sanskara*..

The biologically active chemical constituents of *Withania somnifera* (WS) include alkaloids (isopelletierine, anaferine, cuseohygrine, anahygrine, etc.), steroidal lactones (withanolides, withaferins) and saponins (Mishra, 2000 et al., 2000).

Withaferin A and 3-b-hydroxy-2, 3-dihydrowithanolide F isolated from *Withania somnifera* show promising antibacterial, anti-tumoral, immunomodulating and anti-inflammatory properties (Budhiraja and Sudhir, 1987).

*Ashwagandha* is an analgesic that soothes nervous system from pain response (Twajj et al., 1989). The powerful anti-arthritic properties (Singh et al. 1984, 1986) of *Ashwagandha* are now widely accepted and documented; it is furthermore found to be effective as antipyretic as well as analgesic also.

*Sushruta*, Father of Indian Surgery, in his treatise, *'Sushrut Samhita'* has given all the information regarding bloodletting in detail. *Raktamokshan*, i.e., bloodletting is one of the ancient and important parasurgical procedures described in Ayurveda for treatment of various diseases. Of them, *Jalaukavacharana* or leech therapy has gained greater attention globally, because of its medicinal values. The saliva of leech contains numerous biologically active substances like bdellins, eglins, hirudin, calin, destabilase, kallikrein, histamine like Substances, acetylcholine, anesthetics substance, which has anti-inflammatory as well as anesthetic properties. Probably these substances help to decrease the symptoms such as pain, tenderness, inflammation and restriction of the joints. Vasodilator and antithrombotic action of leech saliva increases microcirculation by decreasing the blood viscosity at the near vicinity of bite. By this way the substances which are present in leech saliva increase the microcirculation, decrease the inflammation as well as stiffness and restriction of movement of the joints which are the main complaints in OA patients.

## CONCLUSION

Osteoarthritis is a chronic progressive disease that is one of the leading causes of disability among elderly populations throughout the world. It causes pain, disability and impaired movement, which places a large burden (both in terms of health and economics) on individuals, communities, and health systems. While there are several therapies available for symptomatic treatment that mitigate pain, there are no medicines that can reverse or halt the progression of the disease. Allopathic treatment has its own limitation in managing this disease. It can provide either conservative or surgical treatment and is highly symptomatic and with troublesome side effects.

The finding of this study suggested that combined therapy of *Ashwagandha Ghrita* and Leech therapy for OA patients causes reduction in oxidants levels and increase levels of antioxidants of these patients. Combined therapy also helps in reduction of IL-1 level which plays a central role in the processes underlying the pathogenesis of osteoarthritis and has a stronger influence in the context of inflammation.

On the basis of the above study we can conclude that this *Ayurvedic* therapy is beneficial in reduction of pain, tenderness, stiffness, crepitus, and swelling in the patients of OA and we can avoid the hazards of prolong use of analgesic, anti-inflammatory drugs and improve the quality of life of the arthritis patients.

So, further researches in the field of antioxidants, anti-inflammatory and analgesic principles of *Ashwagandha Ghrita* and Leech saliva may lead to the development of new effec-

tive substances for complete management of Osteoarthritis.

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