ISSN: 2231-6078
INTERNATIONAL JOURNAL OF DRUG DISCOVERY AND HERBAL RESEARCH (IJDDHR)
2(3): July–September: (2012), 446-455

Research Article

Correlation Studies on the Amount of Sulphur in Arthritic Joint Effusion and its Association with Modern and Traditional Medicinal System

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Abstract

Sulphur contributes substantially in joint tissue and fluid metabolism. Therefore, the present investigation has been carried out to establish the correlation between the levels of sulphur in the joint fluids of arthritis patients consuming Antiarthritic Ayurvedic Drugs (AAD) or allopathic medicines.The subject population has been divided on the basis of allopathic and Antiarthritic Ayurvedic Drug (ADD) treatment. Arthrocentesis has been adopted to tap the knee joint effusion from arthritis patients. Inductively Coupled Plasma Atomic Emission Spectroscopy (ICP-AES) has been used for estimating the levels of sulphur in the biological fluid samples.

It has been established that the arthritic joint fluids contained lower levels of sulphur than controls. Further, it has been evaluated that the patients under the therapy of AAD formulation had significantly altering level of mean sulphur concentration than those under allopathic drug therapy.The screening of sulphur in joint effusion can offer a sensitive and early diagnostic aid in arthritis patients. Also, the study clearly implicated the superior restoration competence of ayurvedic medicine in regaining the appropriate levels of sulphur as compared to allopathic drugs.

Key Words: Bursal fluid, Sulphur, Allopathic medicine, Antiarthritic ayurvedic drug, ICP-AES.

Introduction

Amongst the most broadly identified joint diseases are Osteoarthritis (OA), Rheumatoid arthritis (RA) and Bursitis(B). Sulphur has known to play a major role in joint tissue and fluid metabolism. Also, it assists in the synthesis of Glucosamine and Chondroitin sulphate, a major component in the formation, resiliency and repair of cartilage tissues[1]. Therefore, the estimation of sulphur becomes essential in order to recognize its potential perspective in aetiology of arthritis.

There has been a subsequent line of research reporting variation in the levels of sulphur in synovial fluid from patients with joint trauma, inflammatory arthritis and non-inflammatory arthritis[1-4]. Further, it has been observed by Samantha et.al that the sum of chondroitin sulphate disaccharide concentrations in OA and RA joint fluids was significantly lower than those in normal fluids[5].

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The concentration of sulphur in arthritic cartilage has been shown to be about one-third the level of normal cartilage[6] . O.Donald.et al has illustrated elevated sulphate in joint effusion of degenerative arthritis and decreased concentration in RA compared to serum sulphate[7]. Literature reports marked deficiency of sulphur existing in chronic arthritis, demonstrated by lowered content of cystine in fingernails[8].

It has been reported that the sulphur containing compounds present in herbal plants act as scavengers of reactive oxygen species, and they function as an anti-inflammatory agent[9,10]**.** Hence, the present work utilizes indigenous medication in order to evaluate its therapeutic effect by estimating the alteration in concentration of sulphur in joint fluid of arthritis patients. In addition to that an analogue study was conducted to inspect the efficacy of allopathic drugs in comparison to ayurvedic medication.

Besides, in the consideration of identifying the suitable therapeutic strategies by investigating the amount of element, it is important to adopt a good analytical technique which is devoid of too much chemical interference. Since, there are very few tools for studying the role of sulphur in biological systems; among them Inductively Coupled Plasma Atomic Emission Spectroscopy (ICP-AES) offers a unique non-destructible facility for determining the concentration of element in biological fluids.

Thus, the current study confers the role of sulphur in arthritic joint fluid and examines the difference in the concentration of sulphur in OA, RA and Bursitis patients. Also, the search for better therapy was essential, which might influence the levels of sulphur in different disease processes as led us to distinguish ayurvedic and allopathic medicinal system. The study can be a first step in establishing a relationship between elemental content and restorative potential of effective medicinal system.

Material and Method

All the chemicals of A.R. grade were obtained from Lennetech Laboratory (Mumbai). Distilled, deionized water was used for analytical purposes. Nitric acid (HNO_3) and Perchloric acid ($HClO_4$) used for sample digestion were of Suprapur grade (Merck, Germany).

Study population

The subjects assessed for research were patients with OA, RA and Bursitis with knee joint associated with clinically detected joint effusion. The patients with arthritic disorder were diagnosed on the basis of the criteria laid down by

American College of Rheumatology (ACRC) [11]. The patients under study attended the outpatients department of various hospitals (B.Y.L Nair hospital, Jeevan Rekha Hospital and Navneet hospital, Maharashtra, India.). Declaration of Helsinki (1964) as a statement of ethical principles was followed by the physicians during the research procedure[12]**.** Ethics appraisal was obtained from respective hospital in regards to collection of joint fluid samples in current research work. A written informed consent was acquired from every participant. The patients having serious complications (liver diseases, kidney diseases, hematological diseases, AIDS, Cancer etc.) and pregnant females those incapable of taking oral administration and currently taking other herbal medications were excluded from the study.

The patients were then divided into two treatment groups:

A) *Subjects under the treatment of Allopathic drugs (n=135)*

Arthrocentesis was employed for aspiration of joint effusions by insertion of needle in joint space. The samples were acquired from 44 AL-OA, 50 AL-RA and 41 AL-B patients. This category of patients was under the oral administration of Non steroidal Anti-inflammatory Drug (NSAID).

B) *Subjects under the treatment of Ayurvedic medicine (n=142)*

The potential subjects screened for current study under this category were treated by Ayurvedic medicine. They were as follows; 42 AY-OA consuming Relistif, 58 AY-RA intaking Rumaquit and 42 AY-B administered Triphala powder.

C) *Controls - healthy adult donors (n=46)*

The samples of controls were used for comparison between diseased and healthy donors.

The three categories of patients were further classified on the basis of gender and age .i.e.

i) Group I: Males with age less than 40 years.
ii) Group II: Males with age more than 40 years.
iii) Group III: Females with age less than 40 years.
iv) Group IV: Females with age more than 40 years.

Every patients joint fluid sample were collected twice ie. before the treatment and after the treatment of 12 weeks. All the subjects were enquired for medical history, physical examination and life style pattern.

Sample pretreatment

After the collection of joint fluid samples it was stored in sterile containers and the volume was recorded. For sample analysis portions of the aliquots were centrifuged and cell debris was separated from each sample until assay. Hemolyzed samples were necessarily rejected because of the higher sulphate values after red-cell disintegration. All the forms of sulphur when digested get oxidized to inorganic sulphate using a wet digestion procedure[13-14]**.** Therefore, the samples for analysis were digested by conc HNO_3 and conc $HClO_4$ (1:1)

Instrumentation

ICP-AES has a multielement detection capability with high sensitivity and wide linear dynamic range for most metallic and metalloid elements. Hence, sulphur could be determined in the major to ultratrace concentration ranges. The quantification of sulphur (wavelength= 185.7nm) in SF/BF samples was performed using ARCOS ICP-AES device (M/s. Spectro, Germany) in Indian Institute of Technology, Sophisticated Analytical Instrument Facility (SAIF), and Mumbai. Standard and Sample solutions were analyzed in triplicate series. The sample solution was analyzed against calibration curve. The sample was then introduced into an atmosphere of argon gas having free electrons induced by high voltage. The high temperature in the plasma raises valence electrons of the elements above their normal stable states and when they return to their original state, they emit photons, which are unique and used to identify and quantify the elements. The Instrumental characteristics and operating parameters are given in Table 1.

Statistical analysis

Data are expressed as mean ± SD. The data from sample were analyzed using oneway ANOVA with treatment specified as the main effect and the Student t test (Wilcoxon's matched pair test) for the remainder of the statistical testing; $P < 0.05$ was considered significant. Spearman correlation test was used to establish the association between sulphur levels in joint fluid of patients with age. These values were determined using Graph pad prism (version:5).

Results and Discussion

The mean concentration of sulphur in all three categories of patients is represented in **Table 2.** All the categories of patients before the treatment showed alterations in the mean amount of sulphur when compared to controls. The variation of sulphur was clearly evident in different diseased states. It was illustrative that the sulphur concentration was lower in OA, RA and Bursitis patients than the controls. The pre and post intervention of patients under the treatment of allopathic drugs demonstrated significant differences ($P<0.05$) in total OA **(Figure 1)**, RA **(Figure 2)** and Bursitis **(Figure 3)** subjects. The concentration of sulphur in three musculoskeleton disorders followed the order RA>OA>Bursitis.

In case of bursitis, it is the first ever attempt made in the path of detection of trace elements in bursal effusion which requires further investigation to exhibit strong scientific evidences. Whereas the reports on sulphur concentration in SF of RA and OA patients is in good agreement with data suggesting decreased concentration of sulphur biomolecules in arthritis patients[15]**.**

The patients under the treatment of ayurvedic drugs from last 12 weeks were administered Relistif tablet to AY-OA patients, Rumaquit tablet to AY-RA patients and Triphala

powder to AY-B patients. A remarkable change was observed in the amount of sulphur in joint fluids of all the patients consuming ayurvedic drugs **(Table 3**).

The average amount of sulphur after 12 weeks treatment with ayurvedic drug demonstrated remarkable variation in post treatment groups than pre treatment groups. Significant changes were observed in pre and post treatment groups of AY-OA ($P<0.05$), AY-RA ($P<0.05$) and AY-B ($P<0.05$) **(Figure 4, 5 & 6).** The post treatment groups when compared to controls illustrated slight variation may be due to higher activity of disease which probably requires more time span to display an accurate recuperation impact.

The total of all four groups of OA, RA and Bursitis subjects as shown in **Table 2 and 3** gives a glimpse of pre and post treatment effect in **(Figure 7, 8 and 9)** patients intaking allopathic and ayurvedic drugs.

In order to estimate the relative efficiency of allopathic and ayurvedic medication, percent differences were assessed for pre and post intervention. It was observed that **(Figure 7)** AL-OA groups had 5.3% increase in levels of sulphur whereas AY-OA had 27.2% increment in concentration of sulphur after the treatment by ayurvedic medication. Similarly in RA patients the amount of sulphur post-treatment by allopathic medicine **(Figure 8)**, augmented by 5.3% and 17.6% in patients intaking ayurvedic medicine. In case of bursitis the amount of sulphur was raised by 7.4% in AL-B subjects **(Figure 9)** whereas in AY-B it improved by 32.2%. This clearly indicates the exceptional therapeutic potential of ayurvedic medicine in regaining the appropriate levels of sulphur as compared to allopathic drugs. As ayurveda is an holistic science of medicine, its efficacy is very much evident in the present work; due to its ability of regaining the suitable levels of vital mineral in diverse musculoskeleton disorders.

A negative correlation in OA ($r = -0.6894$, $P<0.05$) **(Figure 10)**, RA patients ($r= -0.5272$, $P<0.05$) **(Figure 11)**, and in Bursitis ($r = -0.2352$, $P < 0.05$) **(Figure 12)** indicates increase in sulphur inadequacy with age**.** Thus, the depletion in the levels of sulphur was very much evident in older group of patients.

In the current study reduced levels of sulphur was observed in joint fluid of aged group of patients. The possible explanation lies in diminished amount of chondroitin sulphate, which is a sulphated glycosaminoglycan (GAG), present in synovial fluid of arthritis patients. It was reported by Bayliss etal. that sulphur decreases with age due to the differences in the sulphation pattern, caused by lower ratio of Δdi-6S: Δdi-4S (Chondroitin 4- and 6-sulphated disaccharides) at this site[15].

Sulphur is an integral component in connective tissue. The major route of entry of sulphur is through sulphur-containing amino-acids, methionine and cystine or in smaller amount as inorganic sulphate. The oxidation of the sulphur-containing amino-acids in the body results in the production of inorganic sulphate. The cytosolic enzyme which converts S-carboxy methyl cysteine to its sulphoxide metabolites is believed to be identical with cysteine oxygenase, which converts cysteine to cysteine sulphinic acid. This is the first step in the pathway which forms inorganic sulphate levels, as the supply of this anion in vivo is believed to be largely controlled by cysteine oxidation via the cysteine oxygenase route. Thus, decreased availability of inorganic sulphate would be expected to affect production of biochemical components which have a large number of sulphated residues[16].

Sulphur is present in synovial fluid in the form of glucosoamine and chondroitin sulphate(CS) which are responsible for resiliency of cartilage and maintainance of sturdy joint health. Furthermore, the metabolic turnover during the inflammatory and degenerative process in the joint space is reflected in synovial-fluid changes[17]

The data in the present work illustrates significantly lower levels of sulphur in OA and RA when compared to controls. It has been proclaimed that in osteoarthritis, the joint capsule becomes thickened and the synovial fluid contains diminished concentration of glucosamine restraining the formation of hyaluronic acid (HA) and CS[18].

Glucosamine is a amino monosaccharide used to make glycoseaminoglycans (GAGs) and proteoglycans (PGs), an essential ground substance of the connective tissue. Therefore any disturbance in concentration of sulphur may in turn deteriote the load of sulphur containing compounds in the joint fluid leading to degenerative joint pathology.

Also in case of patients with RA there is low capacity to retains sulphur in the tissues. The diminution in sulphur content of RA patients are so far controversial, but may depend on the decreased amount of sulphur-rich protein(s) [19]. Also studies by Ropes and co-worker has reported similar levels of changes in sulphur status in RA and OA patients[20].

Furthermore, the result describes lower concentration of sulphur in BF than control. No evidences have been recorded in the literature regarding the possible role of sulphur in BF. Thus, in OA, RA and Bursitis condition depleted levels of sulphur containing compounds results in worsening of joint pathology and in turn augments the illness. However, post-treatment by NSAID showed significant variation in levels of sulphur though not comparable to controls.

As there is continued wear and tear of normal cartilage and age related decline in sulphur containing compounds. It may be helpful to compensate the normal metabolic turnover of the extracellular matrix and to prevent the degradation of cartilage by stimulating the healing process by green medication. Ayurvedic medication serves as a source of sulphur which synergistically enhances the health of connective tissue. Our study on human subject supplemented with AAD showed augmentation of sulphur reserves. The polyherbs present

in Relistif are Cyperus Rotundus, Curcuma Longa and Apium graveolens which may act as a source glycoproteins for OA patients. As many glycoproteins are sulphur containing compounds, this eventually proves, that the elevated levels of sulphur in synovial fluid[21,22] can be ascribed to the post-treatment effect of AAD. Studies suggest that these herbs may block cyclo-oxygenase and lipoxygenase activity, thereby inhibiting inflammatory prostaglandin and leukotriene release. However, more information is required on the biochemical aspect of present study in order to draw firm evidences.

Likewise, the therapeutic effect of Rumaquit may be due to anti-inflammatory effects of the polyherbs; Curcuma longa[23], Tinospora cordifolia[24], Withania Somnifera[25], Tribulus terrestris[26] and Ginger officinale as they are known to probably exert an inhibitory effect on some of the mediators of inflammation. Also, these herbs in sync may attribute to play a key role in catering a defense against oxygen radical pathology[27, 28]. It might be suggested that the antioxidant effect of all the polyherbs may be due to peroxiredoxins, a sulphur containing biochemical, which is present in the chloroplasts of plant. Peroxiredoxins are antioxidant enzymes that control cytokine induced peroxide which is very much prevalent in RA pathological conditions. Thus the possible therapeutic effect which showed elevated sulphur levels in joint fluid of RA patients may possibly due to intake AAD.

Also, the augmented level of sulphur was remarkable in Bursitis patients after the treatment with Triphala. Little information exists on the molecular and biochemical pathophysiology of knee bursitis. Clinical data suggests that elevated levels of expression of cytokines (interleukin [IL]-1β, IL-1, IL-6, tumor necrosis factor [TNF] α, small inducible cytokines), metalloproteases, and cyclo-oxygenases contribute significantly to bursitis[29]. Triphala may inhibit enzymes that destroy connective tissue. In addition to that researches have reported subsequent lines of number of clinical and biochemical data demonstrating the antioxidant activity of Triphala by inhibiting the lipid peroxidation[30]. Perhaps due to lack of adequate data in the literature concerning the effects of triphala in bursitis patients, further studies are needed to establish more substantial evidences.

Conclusion

The present research explored the potential role of allopathic and ayurvedic medicinal system in elevating the levels of sulphur in joint effusion of arthritic patients. The possible curative effect seen in patients under the treatment of Anti-Arthritis ayurvedic drugs might be due to bioavailable sulphur present in medicine exhibiting anti-inflammatory properties. To ascertain a direct role of sulphur in producing a probable therapeutic effect, it had to be estimated by suitable analytical method such as ICP-AES. It was clearly evident that the ayurvedic system served as a appropriate choice in bringing restoration in the health of Osteoarthritis and Bursitis patients by augmenting the levels of sulphur. Whereas in case of Rheumatoid arthritis a profound effect indicating the effectiveness of the drug action was unclear. Further clinical trials can provide better scientific evidences in evaluating the most promising therapeutic treatment for joint diseases. It is of interest that, the discrete status of sulphur may assist in characterizing diverse musculoskeleton joint disorder. Thus investigation of sulphur in joint effusion is an important finding in understanding the pathogenesis of arthritis.

Acknowledgements

The present authors express sincere thanks to Vikram Londhe(B.Y.L nair Hospital), Shriram Savrikar (R.A.Podar Ayurvedic college), Mukund Sabnis(Jeevan Rekha Chikitshalay, Aurangabad, Dr.Vividh Makwana and Dr.Vijay Patil Navneet hospital, for providing joint fluid samples and medicinal samples.

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Table 1: ICP-AES instrument characteristics and operating parameters.

PARAMETERS		SETTING
RF Generator	:	1000 watts
Power required	:	220±10 V
Flame Temperature	:	11000 K
Plasma	:	Argon
Spectra Range	:	189-800 nm
Coolant Flow	:	12 L/min
Auxillary Flow	:	1 L/min
Nebulizer	:	0.8L/min
Sensitivity	:	ppb level of detection

Table 2: The levels of Sulphur (Mean±SD) in Joint fluid of Controls and patients under the Treatment of Allopathic drug with its Pre and Post intervention.

Groups	Sulphur in Joint fluid of patients under Allopathic Treatment*						
	AL-OA		AL-RA		AL-B		Controls
	Pre	Post	Pre	Post	Pre	Post	
I	413.2±4.0	481.9±1.5	535.0±2.2	596.2±3.5	314.9±3.2	385.1±3.4	833.7±3.1
II	402.0±1.8	426.6±2.4	516.0±3.3	559.0±5.5	304.3±3.1	373.7±3.3	857.8±5.6
III	405.2±1.6	466.5±5.9	521.6±5.5	585.0±3.7	320.3±5.1	392.5±5.1	812.4±7.3
IV	391.0±1.3	416.2±2.7	506.6±5.9	524.0±4.18	318.5±7.9	352.5±3.0	808.4±4.9
Total	**402.9±13.2**	**446.9±27.7**	**518.4±12.8**	**562.4±27.8**	**314.9±7.8**	**376.9±15.8**	**829.0±19.8**

Mean±SD (mg/L)

Table 3: The levels of sulphur (Mean±SD) in Joint fluid of Controls and patients under the Treatment of Ayurvedic drug with its Pre and Post intervention.

Groups	Sulphur in Joint fluid of patients under Ayurvedic Treatment*						
	AY-OA		AY-RA		AY-B		Controls
	Pre	Post	Pre	Post	Pre	Post	
I	423.7±4.3	685.4±7.1	534.3±3.7	722.7±3.9	327.7±5.6	656.3±6.3	833.7±3.1
II	412.5±2.3	604.5±3.1	514.4±3.1	604.0±3.4	318.7±2.0	522.5±4.0	857.8±5.6
III	405.0±1.5	654.1±3.6	508.3±3.1	703.1±3.1	330.6±3.4	629.6±1.6	812.4±7.3
IV	387.2±3.3	594.2±4.1	492.9±4.5	616.6±2.1	306.8±3.0	545.4±3.2	808.4±4.9
Total	**406.9±13.2**	**632.4±36.6**	**511.0±14.9**	**657.8±52.2**	**321.4±9.6**	**588.0±57.8**	**829.0±19.8**

Mean±SD (mg/L)

Figure 1: Variation in the levels of sulphur in Joint fluid of Controls and AL-OA patients under Allopathic Treatment.

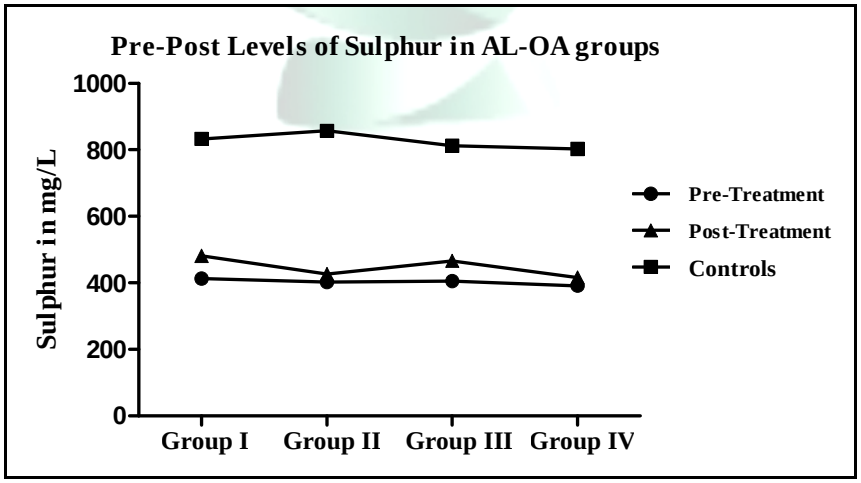

Figure 2: Variation in the levels of sulphur in Joint fluid of Controls and AL-RA patients under Allopathic Treatment.

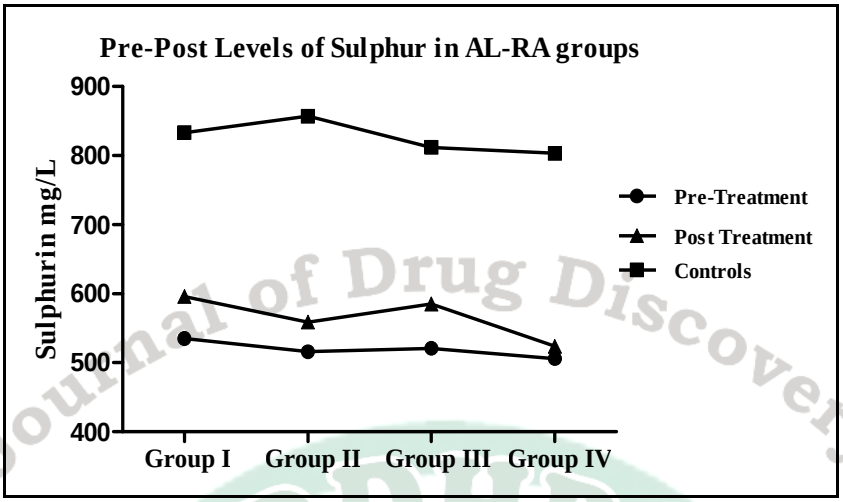

Figure 3: Variation in the levels of sulphur in Joint fluid of Controls and AL-B patients under Allopathic Treatment.

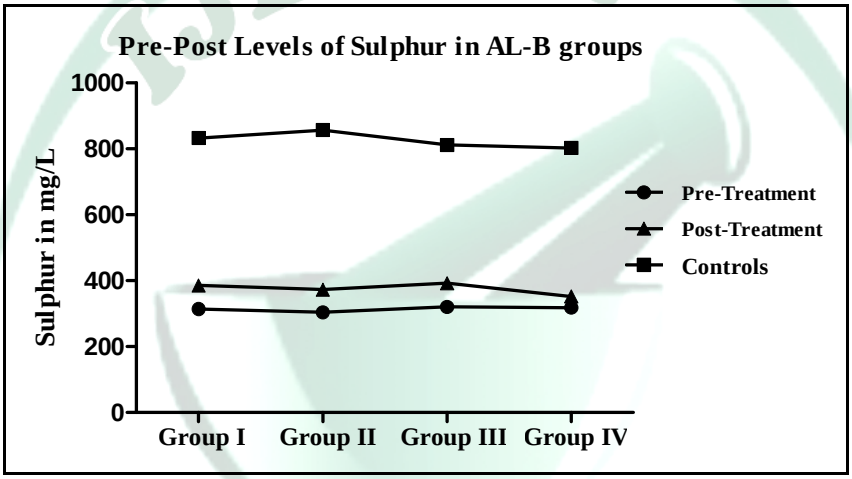

Figure 4: Variation in the levels of sulphur in Joint fluid of Controls and AY-OA patients under Ayurvedic drug Treatment.

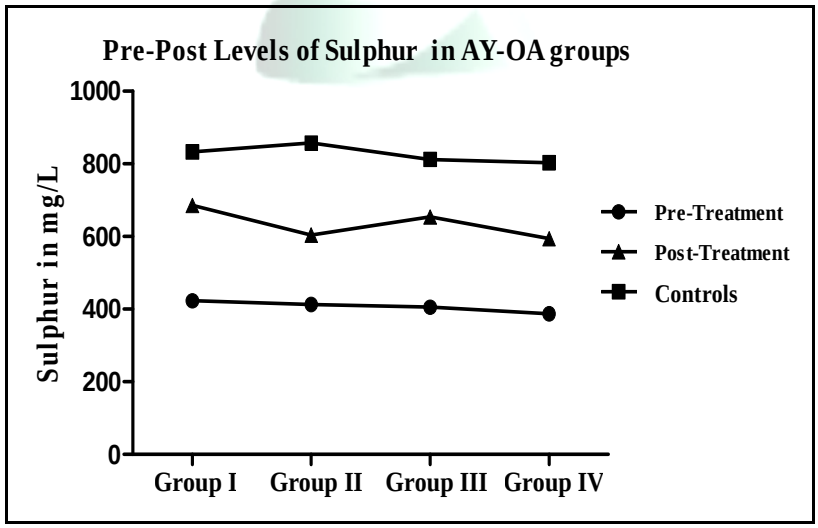

Figure 5: Variation in the levels of sulphur in Joint fluid of Controls and AY-RA patients under Ayurvedic drug Treatment.

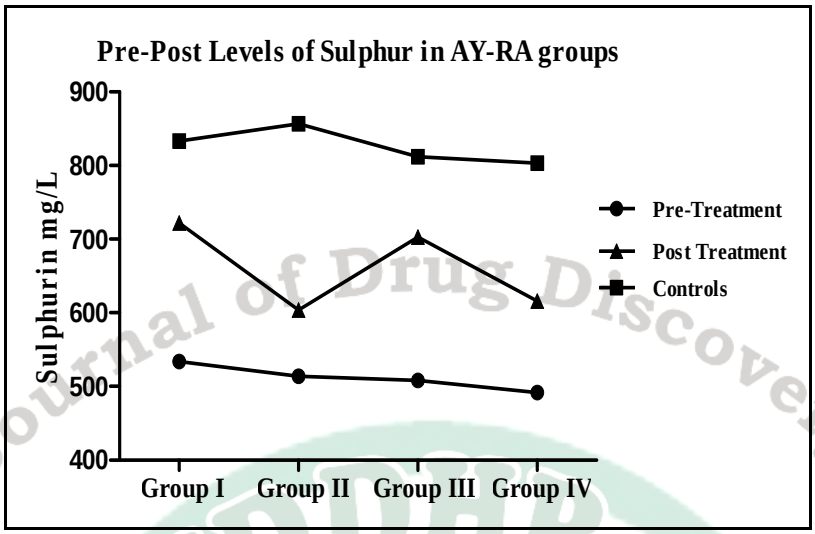

Figure 6: Variation in the levels of sulphur in Joint fluid of Controls and AY- B patients under Ayurvedic drug Treatment.

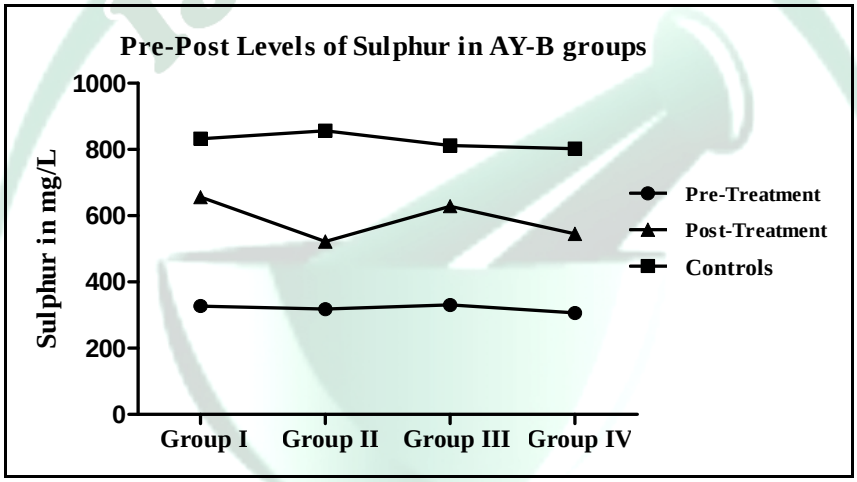

Figure 7: Variation in the levels of sulphur in joint fluid of Controls and OA patients under Allopathic and Ayurvedic drug Treatment.

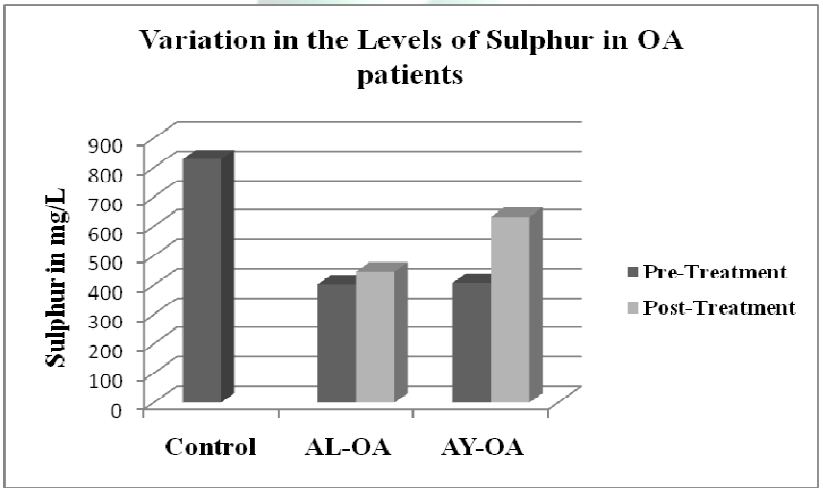

Figure 8: Variation in the levels of sulphur in Joint fluid of Controls and RA patients under Allopathic and Ayurvedic drug Treatment

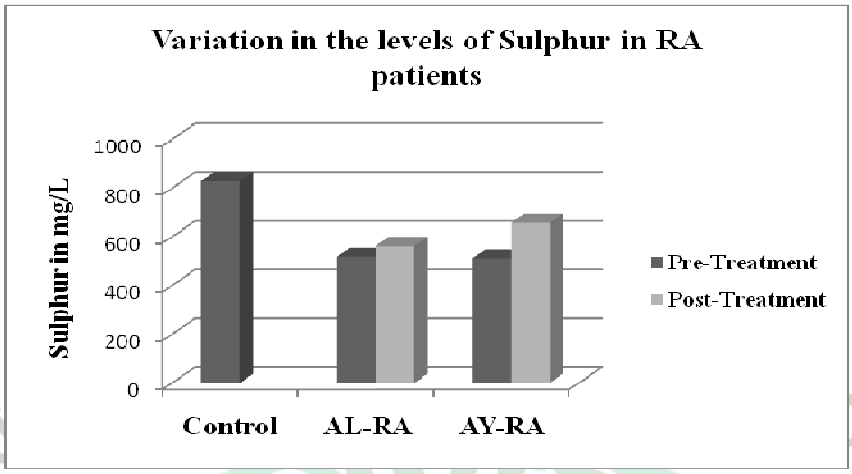

Figure 9: Variation in the levels of sulphur in Joint fluid of Controls and Bursitis patients under Allopathic and Ayurvedic drug Treatment

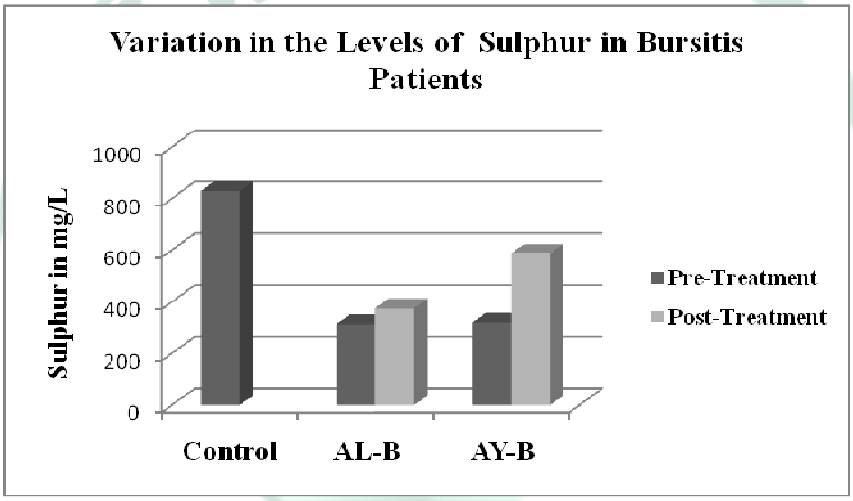

Figure 10: Correlation in the levels of sulphur in OA patients with age

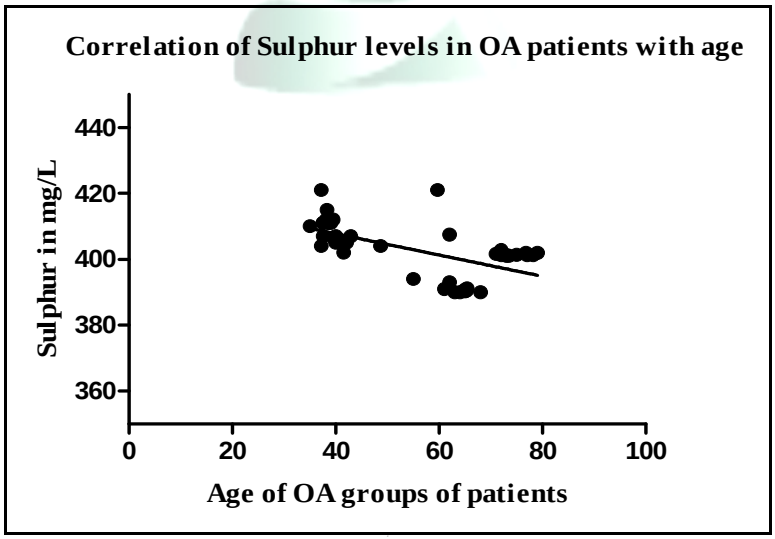

Figure 11: Correlation in the levels of sulphur in RA patients with age

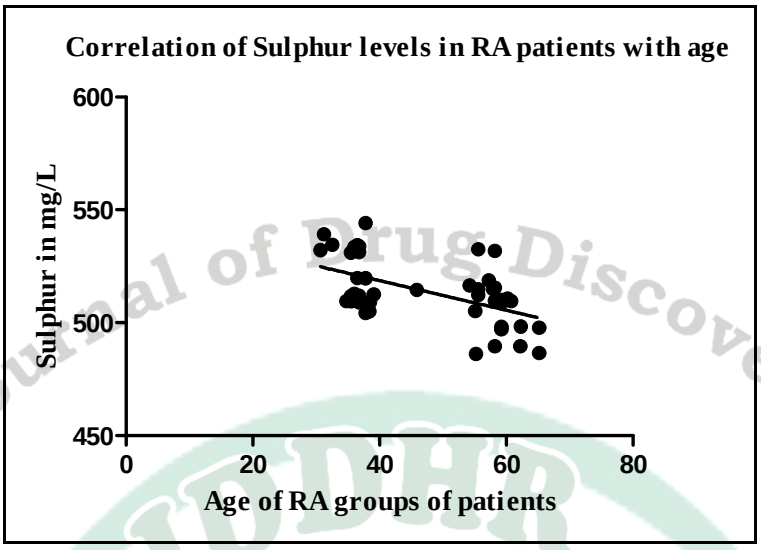

Figure 12: Correlation in the levels of sulphur in Bursitis patients with age