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CLINICAL EVALUATION OF BENEFICIAL EFFECTS OF WITHANIA SOMNIFERA IN PATIENTS WITH RHEUMATOID ARTHRITIS: AN OPEN LABEL SINGLE ARM PILOT STUDY

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ABSTRACT

Medicinal plants comprise an immense potential for producing new drugs of great benefit to mankind and represent a rich source of therapeutically active constituents. Efficacy of many indigenous plants for several disorders has been described by practitioners of traditional medicine. Indian traditional medicine uses the herb Withania Somnifera (WS) as an ailment for variety of dreadful diseases as it seems rich in wide variety of bioactive secondary metabolites. However, still now there is no proper documentary evidence on effect of WS on rheumatoid arthritis with respect to clinical symptoms including biochemical and other immune related parameters. Present investigation aimed at investigating the biochemical and immune effects of WS powder in patients with rheumatoid arthritis. Total of 19 patients identified with rheumatoid arthritis have been selected for the open clinical trial and were treated with WS powder in honey for the period of 48 days. Followed by this clinical, laboratory and immune investigations were performed before and after drug administration. Results of phytochemical investigation have proven that WS powder had Alkaloids, Saponins, Tannins, Phenols, Steroids, Terpenoids and Carbohydrates. HTLC analysis revealed the presence of 8 prominent peaks. Treatment with WS powder reduced the clinical pain score gradually. The reduction in clinical symptoms were associated with the increased haemoglobin levels, reduced ESR and CRP levels, reduced WBC counts, reduced total bilirubin and Direct bilirubin and SGOT, reduced serum urea and serum creatinine including reduction in IL-25 levels without any alteration in IL-8. Thus, WS

significantly reduced the subjective symptoms of rheumatoid arthritis along with a reduction in inflammatory parameters like CRP, ESR. These beneficial effects in patients were also associated with improvement in blood haemoglobin, kidney and liver functions. Hence from the present clinical study it was concluded that usage of WS may halt the progression of arthritis and shall be considered to be first line treatment for the management of rheumatoid arthritis.

KEYWORDS: Rheumatoid arthritis, Withania Somnifera, Phytochemical, HPTLC, Immune parameters.

1. INTRODUCTION

Rheumatoid arthritis (RA) is a chronic inflammatory disease of synovial joints with unknown etiology. This disease was characterised with swelling in joints, pain, deformity and movement problems. The autoimmune destruction of synovial joints with synovial hyperplasia is its major pathological feature. It commonly affects women of childbearing stage (Lu et al., 2015). Though there are number of allopathic medicines to control the symptoms and pathology of RA, most of the times patients are not satisfied as these medicines cannot alleviate the progression of the disease. In addition, these disease modifying anti-rheumatic drugs have numerous adverse effects upon their long term use due to its nature of toxic (methotrexate) and immunosuppressant effects (glucocorticoids) (Leon et al., 2018). As a result more than 60 to 90% of the patients opt for alternative medicine (Ahmed et al., 2005). Among these alternative medicines, herbal formulas are the main options though these herbs are used by different systems of alternative medicine like Ayurveda, Siddha etc. The following review had explored the types of herbs that have been tested in RA patients/mimicking model systems (Soeken et al., 2003). It has been concluded that number of trials were having either poor study design or interpretations (Cameron et al, 2011).

The herbal remedies have been widely used in India since the time of ancient siddhars such as Agasthiyar and Thirumoolar who are pioneers of siddha system of traditional medicines. However, the systematic studies of most of the herbal medicines are mostly unrevealed. The Withania somnifera (also called as ashwagandha or Indian ginseng) is a popular in traditional Indian medicines it has broad spectrum of medicinal properties (Kushwahaet al., 2013). It has been known to possess anti-inflammatory, anti-diabetic, anti-cancer and antimicrobial activities. Withania Somnifera reduces the physical stress and thus increases the endurance

(Sandhu et al., 2010). Further, it has been shown to have anti-tumor effect in many types of cancers and uterine fibroids (Singh N et al., 2011). As it is having cognition improvement property, it has been shown to be useful in a number of neurodegenerative diseases like alzheimer disease. However, its clinical efficacy was not tested with proper controlled study (Singh et al, 2011).

The alkaloids and steroidal lactones are the major chemical contents in the WS plant. The withanine is the major alkaloid though other alkaloids like tropine, Cuscohygrine are also present. The withanolides are major steroidal lactones and withaferin A and withanolide D are two major withanolides present in WS (Elsakka M et al., 1990). Interestingly, a number of studies have been done to explore the anti-inflammatory effects of Withaferin A. For example, Withaferin A inhibits the levels of *H.Pylori* induced increase of pro-inflammatory cytokine IL-1 beta. Interestingly, this inhibition is through the modulating NF-kB and inflammasome (Kim et al., 2015). In another study, W. somnifera extract has been shown to inhibit the expression of potent pro-inflammatory mediators like IL-8 and COX-2 and this was associated with the reduction in the progression of human prostate cancer I in vitro experiments (Setty Balakrishnan et al., 2017). As most of these are key inflammatory mediators in RA, we hypothesized that WS may have beneficial effects.

Based on the literature it was evident that WS contains alsotrans-caryophyllines as a major active compound that has variety of pharmacological activities. It is a bicyclic sesquiterpene that is approved by Food and Drug Administration as a food additive. The biological effects of caryophylline, have been explored very well (Gertsch et al., 2008). It is active as relatively selective agonist for type 2 cannabinoid receptor and thus it has anti-inflammatory activities similar to cannabis alkaloids. As caryophylline cannot bind and activate CB1 receptors, that present in brain, is not having a psychoactive effects. The trans-caryophylline has been shown to cause bronchodilation through inhibiting voltage dependent calcium channels (Pinho-da-Silva et al., 2012).

The WS is one of main components in formula called Amukkarachoornam (AC)(Patra et al., 2010) that has been used in numerous diseases like splenomegaly, anaemia etc. It has been demonstrated that AC had the gastroprotective effects in rat model of gastric ulcer (Patraet al., 2014). It has been shown that AC is having potent anti-inflammatory and anti-oxidant properties (Rajalakshmi et al., 2017; Patraet al., 2010). The AC has also shown to be effective in Chikungunya especially in post pyrexic state to reduce the joint pain and inflammation (A

Technical report, Central council for research in Siddha, Govt. of India). Though it has been recommended in Siddha text for Rheumatoid arthritis there is no scientific evidence for it. In this open label single arm pilot study, we found a gradual reduction in clinical score of pain along with the improvement in various relevant clinical and biochemical parameters with 48 days treatment with WS powder in rheumatoid arthritis patients.

2. MATERIALS AND METHODS

2.1. The plant collection and preparation of powder

The Withania Somnifera plants were collected from Chennai District, Tamil Nadu, India and it was recognized and authenticated by botanist. The collected herb were washed with sterile water and roots of the plants were boiled with milk and then the roots were dried and powdered.

2.2. Phytochemical analysis

The WS powder were subjected to phytochemical analysis for identification of active components as described earlier (Brain and Turner, 1975).

a) Mayer's Test for alkaloids: The mayer's reagent (2 ml) was added to 1 ml test sample and the appearance of white precipitate revealed the presence of alkaloids.
b) Test for coumarins: 1 ml of 10% sodium hydroxide was added with the test sample and appearance of yellow color indicated the presence of coumarins.
c) Test for saponins: The five ml of water was mixed with one ml of WS extract and the mixture was vortexed heavily and the formation of hug lather indicated the presence of Saponins.
d) Test for tannins: The few drops of ten percent of ferric chloride were mixed with 1-2 ml of test sample and appearance of dark blue or greenish black color indicates the presence of tannins.
e) Test for glycosides- Borntrager's Test: To determine the presence of glycosides, first, a hydrolysis of test sample was formed by mixing the test sample with 12 N in water bath for two hours followed by filtration and filtered sample was them mixed with chloroform. After through shaking, chloroform layer was separated and in this layer 10% ammonia solution was added to see the appearance of pink color. The appearance of pink color revealed the presence of glycosides.
f) Test for flavonoids: To determine the presence of flavonoids in the test sample, approximately five ml of ammonia solution was mixed with the test sample. Then few

drops of 24 N H2SO4 were added to this mixture to see the appearance of yellow color and the presence of yellow colour indicates the presence of flavonoids.

g) Lead acetate test for phenols: To determine the existence of phenol in the test sample, 10% lead acetate solution was added to the test sample to see the appearance of heavy white precipitate which revealed the presence of phenols.

h) Test for steroids: To check the presence of steroid in the test sample, chloroform was mixed with the plant extract along with few drops of 24 N H2SO4. This entire mixture was mixed thoroughly. The appearance of red top layer with yellowish green fluorescent bottom layer indicates the presence of steroids in the test sample.

i) Test for Anthocyanin: To determine the presence of anthocyanin, sodium hydroxide (2N) was mixed with the test sample followed by heating at 100°C for at least for 5 minute to see the appearance of bluish green colour.

j) Benedict's test for carbohydrates: To check the presence of carbohydrates, Benedic's reagent was added to the test sample and this mixture was heated for 2 minutes using in a water bath and the carbohydrate existence was confirmed by the appearance of red precipitate.

k) Salkowski test for terpenoids: To determine the presence of terpenoids, test sample was mixed with chloroform and followed by 24 N H2SO4 and the appearance of brownish red at the interface indicated the presence of terpenoids.

2.3. High Performance Thin Layer Chromatography (HPTLC) analysis. HPTLC is a modern sophisticated and automated separation technique derived from TLC. Pre-coated HPTLC graded plates and auto sampler was used to achieve precision, sensitive, significant separation both qualitatively and quantitatively. The HPTLC is a valuable quality assessment tool for the evaluation of botanical materials efficiently and cost effectively. HPTLC method offers high degree of selectivity, sensitivity and rapidity combined with single-step sample preparation. In addition, it is a reliable method for the quantitation of nanogram level of samples. Thus this method can be conveniently adopted for routine quality control analysis. It provides chromatographic fingerprint of phytochemicals which is suitable for confirming the identity and purity of medicinal plant raw materials. Chromatogram development was carried out in CAMAG Twin Trough chambers. Sample elution was carried out according to the adsorption capability of the component to be analysed. After elution, plates were taken out of the chamber and dried. The data obtained from scanning were brought into integration through CAMAG

software. Chromatographic finger print was developed for the detection of phytoconstituents present in each extract and Rf values were tabulated.

Table 1: The HPTLC Chromatographic condition.

Parameters	Details
Extraction Solvent	Chlorofom
Stationary phase	Silica gel GF_{254}
Mobile phase	Toulene: Ethyl Acetate: Acetic Acid (5:1.5:0.25)
Scanning wavelength	254 nm
Sample concentration	10mg/ml
Applied volume	5 µl
Application mode	CAMAG HPTLC

2.4. Human study details. It was an open clinical trial conducted at Ayothidoss Pandithar Hospital, National Institute of Siddha (NIS), Tambaram Sanatorium, Chennai-47, India. The total duration of study was 48days and 19 patients were selected. This powder was given to patients who were asked to take WS powder in honey. Patients reporting to NIS were subjected to screening by screening proforma. After screening they were enrolled for the study fulfilling the inclusion, exclusion and withdrawal criteria mentioned in Table2.

Table 2: The inclusion, exclusion and withdrawal criteria.

Inclusion Criteria	Exclusion Criteria	Withdrawal Criteria
1. Age: 20- 60 years 2. Sex: Both male and female 3. Symmetrical joint involvement 4. Arthritis of three or more joints 5. Rheumatoid factor positive or negative 6. Morning stiffness. 7. Deformities like Swan neck deformity and Buttonhole deformity 8. Swelling especially in the inter-phalangeal joint. 9. Patients willing for admission and stay in IPD or willing to attend OPD 10. Patient willing to undergo Radiological investigation and for laboratory investigation. 11. Patient willing to sign the informed consent stating that he/she will consciously stick to the treatment during 20 days but can opt out of the trial of his/her own conscious discretion.	1. Pregnancy and lactation 2. Tubercular arthritis 3. Any other serious systemicillness 4. Osteoarthritis 5. Psoriatic arthritis 6. Gouty arthritis 7. Diabetic Mellitus 8. Hypertension 9. Hyperlipidemia **10.** Cardiac Disease	1. Intolerance to the drug and development of adverse reactions during drug trial. 2. Poor patient compliance and defaulters. 3. Patient turning unwilling to continue in the course of clinical trial.

To determine the effects of WS, one set of recruited patients were given 2 grams of WS powder and another set with 4 grams of powder. However, when we analyse the data, we could not find any significant different between these two groups. So we considered all patients as a single group.

2.5. Clinical Assessment. Arthritis involving three or more joints, symmetrical joint involvement, morning stiffness, snorexia, spindle shaped appearance of fingers, rheumatoid nodules, depression, swelling of small joints of hands and foot, swan neck deformity, button hole deformity were the assessed clinical parameters.

2.6. Lab Investigations. Blood **(**Hb, Total WBC Count, DLC, Total RBC count, ESR, Blood sugar (Fasting, PP), Serum cholesterol), urine **(**Albumin, Sugar, Deposits), renal function tests **(**Blood Urea, Uric acid**,** Serum Creatinine), liver function tests **(**Serum Total bilirubin, Direct bilirubin, Indirect bilirubin, Serum Alkaline phosphatases, SGOT, SGPT and other specific investigations like CRP, RA factor, ASO Titre and X-Ray of affected joints (AP and Lat view) was performed. It is to be noted that after the investigation of RA factor, the RA diagnosed patients were excluded from the study. So all the participants were RA factor positive.

2.7. ELISA: *The levels of IL-25 and* IL-8(E-biosciences, CA, USA) were performed according to manufacturer's instructions from sera.

2.8. Details of Medicine. Patients reporting at the OPD of NIS with the clinical symptoms of Uthira Vatha Suronitham were examined clinically. Based on the inclusion and exclusion criteria, they were be enrolled for the study. The patients who were enrolled were informed about the study, trial drug, possible objectivesand outcomes of the study in their vernacular language. After ascertaining the patient's willingness, informed consent was obtained in written form. Complete clinical history, complaints and duration, examination findings and laboratory findings were recorded in the prescribed Proformas. Patients were advised to take the trial drug and appropriate dietary advice. Then purgation with Meganaatha Kuligai - 2 early morning with hot water was given for balancing the deranged Mukuttram before starting the treatment(Siddha formulary of India. Part- I). The next day onwards the trial WS powder (Internal) was given for 49 days. OPD patients are asked to visit the hospital once in 7 days. At each clinical visit clinical assessment was done and prognosis was noted. The results were compared at the end of the study.

Laboratory investigations and radiological investigation were done before and after trail. At the end of the treatment, the patients were advised to visit the OPD for follow-up for further 2 months for observing any recurrence. Defaulters were allowed to continue and be withdrawn from the study.

The WS powder was given 2gm for 10 patients and 4 gm for another 10 patients. The dosage was mentioned in page No: 309 in a book (Aathma Ratchamirtham). The medicine was given as packets along with external liquid in Disposable pet bottles. No other external or internal medicines wereused, other than the trial drug during the trial period.

Assessment of pain was performed by Universal pain assessment scale was performed as described earlier (McCaffery, M et al, 1993).

Grade 0 : No Pain
Grade 1-3 : Mild pain
Grade 4-6 : Moderate pain
Grade 7-10 : Severe pain

Other clinical signs and symptoms were assessed by Gradation method.

Grade 1 – Able to perform normal duties
Grade II – Moderate Restriction – Self-care is possible
Grade III – Marked restriction – Limited self-care/some assistance required.
Grade IV – Confined to bed or wheel chair

2.9. Statistics

All the presented data were mean ± SEM; n =19. A p-value less than 0.05 is considered significant. Statistical significance of the differences was tested using paired‘t’ test with PRISM software.

3. RESULTS AND DISCUSSION

3.1. Phytochemical analysis

The phytochemical analysis was performed as described in Materials and Methods.

Table 3: Phytochemical analysis of Withania Somnifera powder.

S.No.	Tests	Observations
1	Alkaloids	+ ve
2	Coumarins	_ ve
3	Saponins	+ ve
4	Tannins	+ ve
5	Glycosides	_ ve
6	Flavonoids	_ ve
7	Phenols	+ ve
8	Steroids	+ ve
9	Terpenoids	+ ve
10	Anthocyanin	_ ve
11	Carbohydrate	+ ve

As shown in Table 1 and Figure 1, it was found that WS powder had the Alkaloids, Saponins, Tannins, Phenols, Steroids, Terpenoids and carbohydrates. The presence of alkaloids is similar to earlier reports where the presence of tropine, cuscohygrine etc. have been reported (Mirjalili et al., 2009). Each alkaloid has been shown to have various medicinal properties. In general, the hairy roots of WS have been shown to have higher alkaloid content compared to callus root (Mirjalili et al., 2009). There are 12 reported alkaloids so far from the WS plant and withanine, somniferine, tropine, choline are few among them. In addition to these 12 types of alkaloids, new alkaloids also have been demonstrated. These alkaloids of WS plant have been demonstrated to have anti-hypertensive and lung stimulatory properties and further investigation have revealed that the observed anti-hypertensive effects could be attributable to inhibiting actions on both autonomic ganglion and higher cerebral centers. The saponins from WS have been shown to have anti-inflammatory properties. Tannins have been shown to have anti-carcinogenic properties (Blyttet al., 1988). The presence of phenol has been shown to be attributable to the presence of phenols in WS. (Ranganathanand Punniamurthy, 2013).

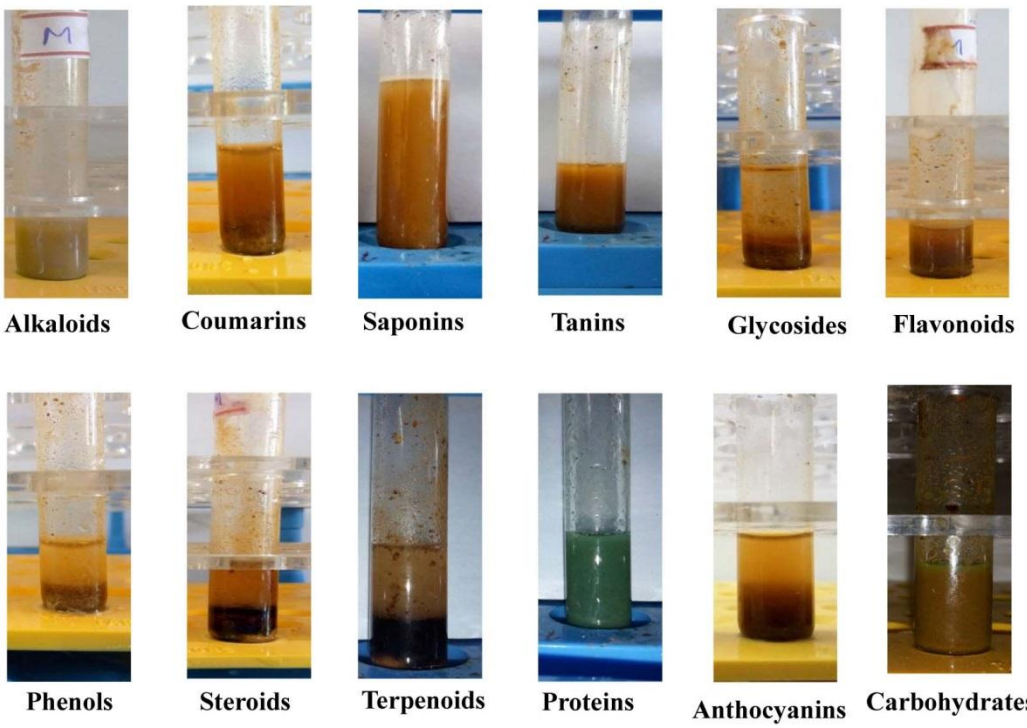
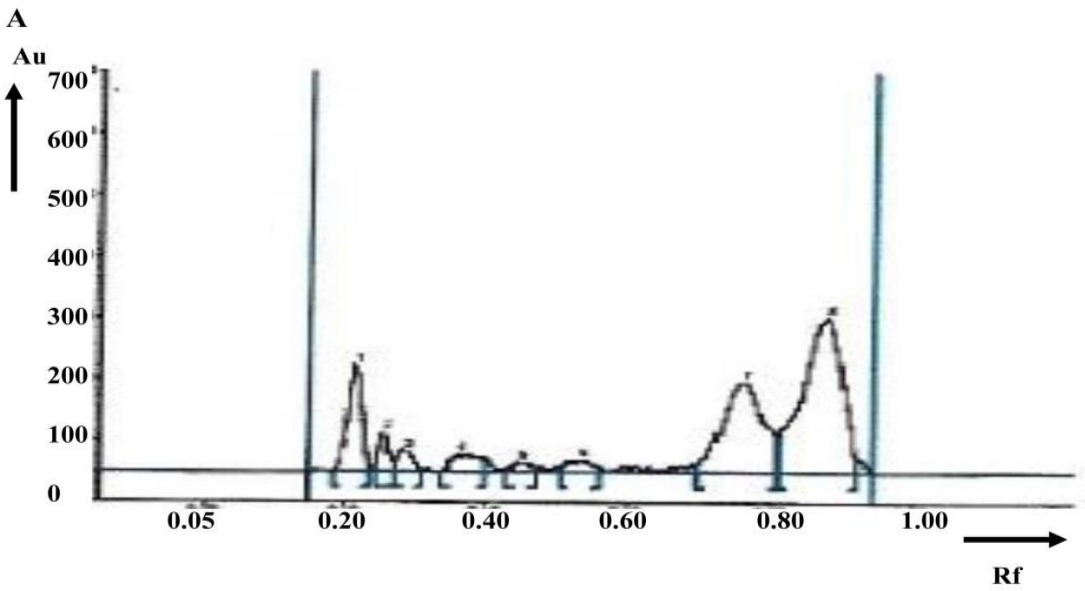


Figure 1: Phytochemical analysis of Withania Somnifera powder. The WS powder was analysed to see the major phytochemical components with different chemical tests.

The HPTLC experiment was performed as described in Methods. The obtained chromatogram (Figure 2A, 2B), was derived using Toulene: Ethyl Acetate: Acetic Acid (5:1.5:0.25) solvent combination. To get the chromatographic fingerprinting, we have used the aqueous extract of WS. The HPTLC fingerprinting of WS extract had shown 8 major peaks (Figure 2A, 2B) with Rf values ranged from 0.18 to 0.81 (Table 4). The highest area of phytoconstituent was 47.69% with the respective Rf value of 0.81. When we exposed the HPTLC plate under 254 nm, there were few dark bands in green background image (Figure 2C).

Table 4: Phytochemical constituents of Withania Somnifera powder derived by HPTLC chromatogram.

Peak	Start Rf	Start Height	Max Rf	Max Height	Max %	End Rf	End Height	Area	Area %
1	0.18	0.5	0.22	175.0	23.68	0.24	2.4	2623.5	11.61
2	0.24	25.8	0.26	63.7	8.62	0.27	17.5	718.0	3.18
3	0.27	17.9	0.28	35.8	4.84	0.31	0.5	580.5	2.57
4	0.34	0.3	0.36	28.5	3.86	0.40	19.6	849.9	3.76
5	0.43	4.0	0.45	17.0	2.30	0.47	3.5	322.6	1.43
6	0.51	7.5	0.54	19.1	2.59	0.56	6.2	534.4	2.37
7	0.70	14.3	0.76	147.9	20.01	0.81	60.1	6191.3	27.4
8	0.81	60.2	0.88	251.9	34.10	0.92	21.1	10776.3	47.69



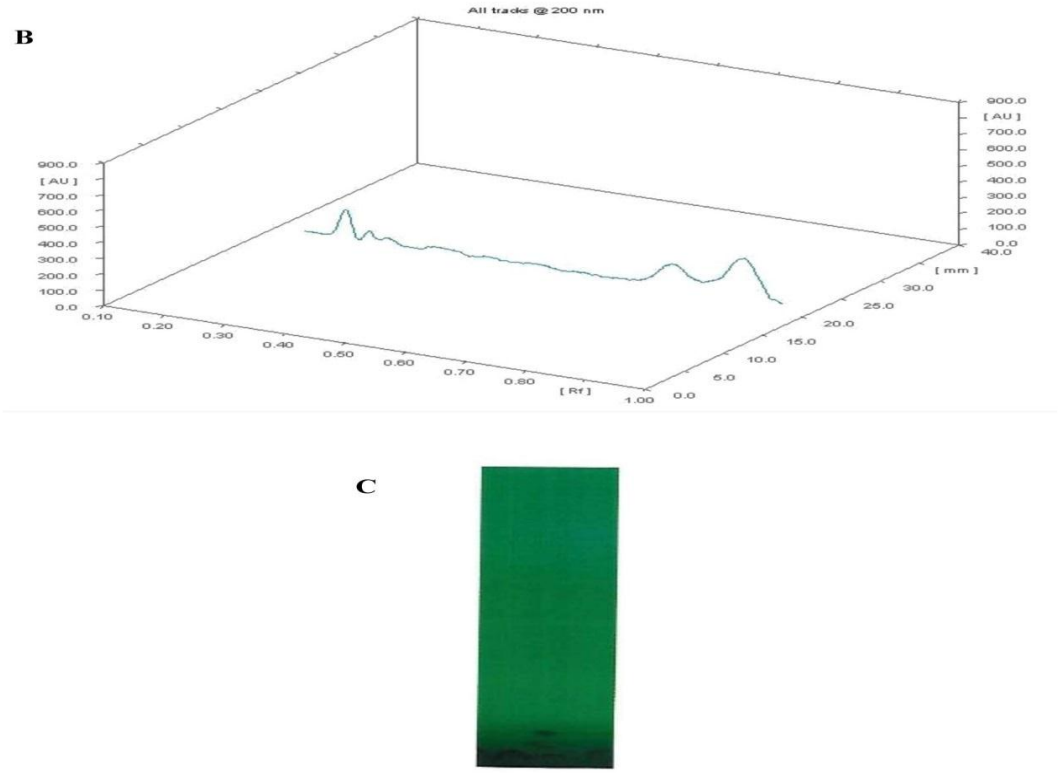


Figure 2: HPTLC and TLC analysis of Withania Somnifera powder. A) HPTLC chromatogram for separating different phytoconstituents in WS aqueous extract. All peaks are recognized. The x axis was Rf (retentions factor) values and y axis was absorption unit (Au). B) 3D HPTLC chromatogram shows different phytoconstituents in WS aqueous extract. C) TLC chromatogram in UV (254 nm) of the phytoconstituents in WS aqueous extract.

3.2. Withania Somnifera powder reduces the pain scale

To understand the efficacy of WS powder on pain, we have recorded the pain scale as described earlier with some modifications. The pain scale was recorded every week since the time of recruitment. At the time of recruitment the scale was severe in most of the patients and it became mild on Day 49 and there was a gradual reduction in pain scale. This indicates that WS powder significantly reduced the pain scale (Figure 3).

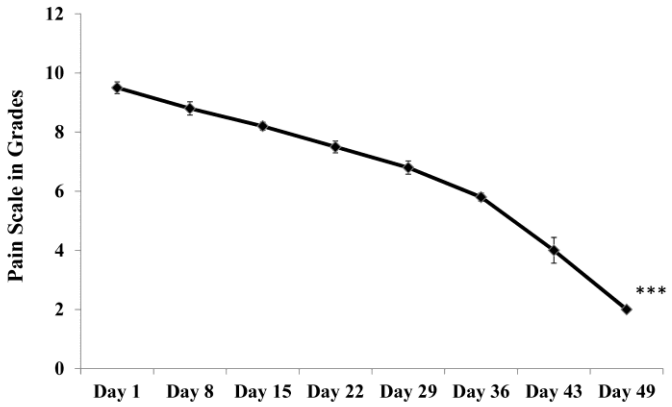


Figure 3: Effects of Withania Somnifera powder on Pain scale in Rheumatoid arthritis patients. ***p < 0.0001 vs. Day 1.

3.3. Withania Somnifera powder reduces subjective joint symptoms

The thorough questionnaire analysis revealed that the joint symptoms like morning stiffness that exist for more than one hour, restricted movements, tenderness of joints, feeling of muscle spasm and low grade fever were present at Day 1 for almost all patients. However, after WS treatment these symptoms have reduced gradually. For example, these symptoms were present from Day 22 to Day 29 after WS treatment. In any event these symptoms were absent from Day 36 till the end of the study.

3.4. Withania Somnifera powder increases hemoglobin levels

Though the levels of haemoglobin has minimum to do with the pathophysiology of RA, reduced Hb levels have been shown to be associated with the severity of RA and (Smyrnova G et al, 2014). It could be possible the resultant pain in RA may be associated with reduced Hb levels and also indicate the need for the improvement in Hb levels in these patients. It is to be noted that Withania Somnifera has been described as adaptogen. However few studies have demonstrated with scientific proof for this. For example, WS root powder has been shown to increase the endurance of hockey players. This increased adaptogenic property of WS root powder was associated with an increased in increased in Hb significant with increased VO2 Max (Malik et al., 2013). Similarly, even 8 week WS supplementation has been shown to improve the endurance of Indian cyclists (Shenoyet al., 2012; Choudhary B et al., 2015). Thus higher ergogenic property of WS root powder could be attributable to increased Hb and its consequent effects on best oxygen utilization. Indeed, this increased Hb content can be also be attributable to increased cardiovascular performance of athletes. It is

well known that polycythemia vera with increased Hb is attributable to increased endurance in sprinters. In this study, we found that WS powder increased the basal Hb levels significantly (Figure 4A). This indicates that the reduction in joint pain scale and joint symptoms may also be attributed to this increased Hb levels.

3.5. Withania Somnifera powder decreases ESR

The ESR is considered as acute phase reaction to pathological or physiological conditions. Though the use of ESR in many inflammatory conditions is waning, the ESR is useful till now for assisting the diagnosis for specific diseases like rheumatoid arthritis and also for the prognosis of various infectious and inflammatory diseases (Brigden et al., 1999). Like ESR, an index of acute inflammation, another acute phase reactant, C-reactive protein (CRP), also used as a marker though non-specific of inflammation. The CRP levels also are being used as index of disease activity and prognosis. Earlier, ESR was considered as a part of calculating disease activity score in case of rheumatoid arthritis. Nowadays, both ESR and CRP can be used interchangeably to calculate DAS28 scale. The other components to calculate the DAS20 are tender joint count, swollen joint count patent's global health(Inoue et al., 2007; Wolfe 1997, Silva et al., 2010). In this study, we found that ESR (measured both at 30 minutes and 60 minutes) is drastically reduced after WS powder treatment (Figure 4C, D). The reduction in ESR was along with the drastic reduction in another acute phase reactant, C-reactive protein. Indeed, CRP was negative in almost all the patients with WS treatment though CRP was positive for all the patients before treatement. However, there was no change in total RBC counts with WS treatment.

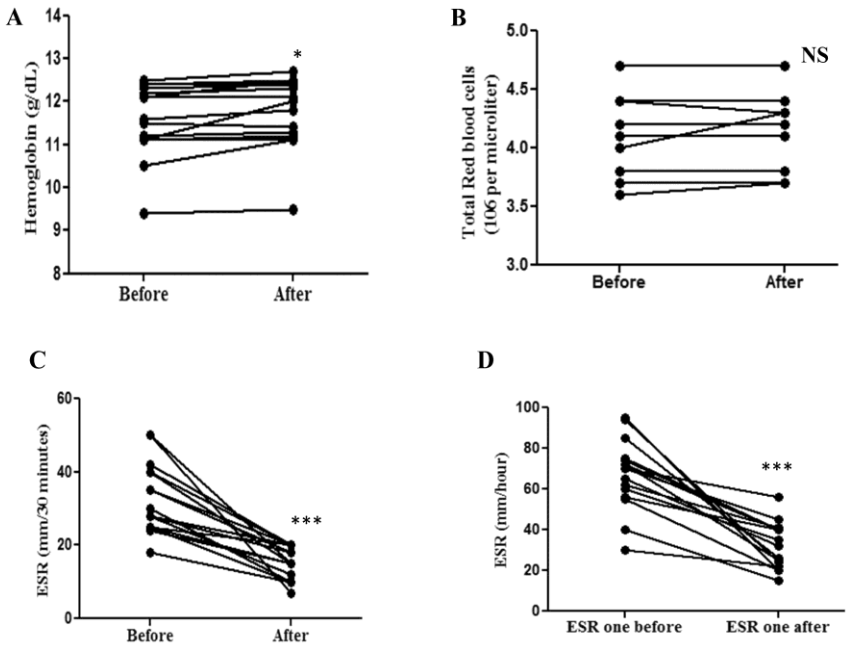


Figure 4: Effects of *Withania Somnifera* powder on Hemoglobin levels, total RBCs and ESR in Rheumatoid arthritis patients. The levels of haemoglobin (A), RBC counts (B), ESR at 30 minutes (C) and ESR at 60 minutes (D) were determined in blood samples of patients who were treated with WS powder. *p=0.0047 vs. "Before", NS, non-significant, ***p=< 0.0001 vs. vs. "Before"

3.6.Withania Somnifera powder decreases total WBC

In most of the studies, WS powder has been shown to have immunomodulatory activities against many diseases. In most of the conditions, the increased immunomodulatory propertieshave been shown to be attributable to increased WBC like lymphocytes. For example, cyclophosphamide caused a drastic reduction in total WBC count and this is well known to have cytotoxic affect and interestingly, WS powder administration to these cyclophosphamide treated animal have been shown to increase total WBC (Ali et al, 2015). Similarly in another study, when azoxymethane treated colon cancer condition was treated with WS extract had also shown increased in total WBC (Muralikrishnan et al., 2010). However, in our study, we found a decreased in total WBC with WS treatment (Figure 5). This indicates that the effects of WS could be depends on the conditions. As RA is characterized by the accumulation of reactive T lymphocytes and in this condition there is a necessity to reduce such auto-reactive leukocytes. However, the reduction in total WBC need to be investigated carefully as it could invite opportunistic infections. So the effect in normal control individuals and immune-compromised also are needed to confirm such effects.

Whether it could lead to general bone marrow suppressive effects is to be investigated. However, DLC revealed that there was no significant change in both neutrophils and lymphocytes and reduction in total WBC could be attributable to reduction in monocytes and eosinophils (data not shown). The reduction in eosinophil at basal levels is interesting as WS extract has been shown to reduce allergic airway inflammatory condition where eosinophil is one of the main culprits in the pathogenesis of asthma (Giri, 2016). In any event, this needs further investigations. However, our study also found there is no such in change In RBC count. This indicates that there may not be any general bone marrow suppressive activities.

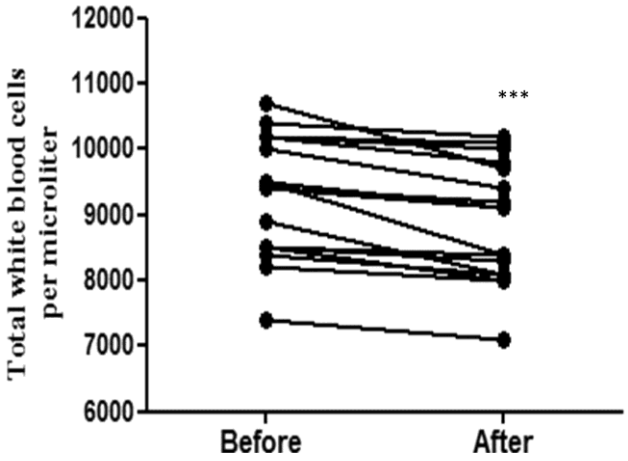


Figure 5: Effects of Withania Somnifera powder on Total WBC count in Rheumatoid arthritis patients.*p=< 0.0001 vs. vs. "Before".**

3.7. Withania Somnifera powder decreases blood glucose

This is interesting that W. Somnifera has been shown to be useful to treat the diabetes and its related metabolic consequences. A number of pre-clinical and clinical trials have been conducted to show the efficacy of WS against diabetes (Kumar et al., 2017). In addition to its hypoglycaemic effects, hypolipidemia effects have also been demonstrated in alloxan stimulated diabetes mellitus rats (Udayakumar et al., 2009). Thus, we wanted to determine the effects of WS treatment on basal levels of blood glucose. Though there was no statistical change in fasting blood glucose, there was a borderline reduction in the levels of postprandial blood glucose after WS treatment (Figure 6A, B).

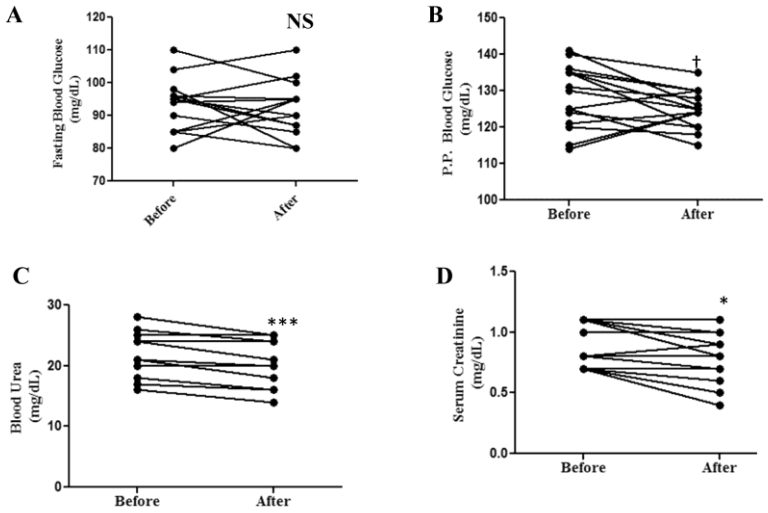


Figure 6: Effects of *Withania Somnifera* powder on key Liver functions in Rheumatoid arthritis patients. The fasting and postprandial blood glucose (A & B) levels, serum urea and creatinine (C & D) were determined in blood samples of patients who were treated with WS powder.*p < 0.05 vs. "Before", NS, non-significant.

3.8. *Withania Somnifera powder decreases the basal levels of* serum urea and creatinine

It has been demonstrated that WS root extract acts as a nephroprotective agent against nephrotoxic inducing stimuli. For example, gentamycin induced nephrotoxicity with kidney swelling, tubular necrosis, increased levels of serum urea and creatinine were significantly reduced by WS extract (Jeyanthi et al., 2009, Choudhury et al., 2018). In this context, we wanted to effect of WS powder on the basal levels of serum urea and creatinine. As shown in Figure 6C & D, there was a significant reduction in the basal levels of serum urea and creatinine.

3.9. Withania Somnifera powder decreases the basal levels of SGOT and direct bilirubin

It has been demonstrated that root powder of WS improves the liver function against the hepatotoxic stimuli. For example, when bromobenzene administered rats which were having hepatotoxic parameterswith elevated liver enzymes, increased bilirubin and increased pro-inflammatorymediators treated with WS root powder, these rats attenuated the parameters of hepatotoxicity (Vediet al., 2014). Similarly, WS treatment was able to reverse the hepatotoxic effects induced by paracetamol overdose treatment by reducing the oxidative stress in rates (Sabina, 2013). Further, it has been shown that WS was able to restore the liver function including increased SGOT in cisplatin treated mice (Sachdeva et al., 2013). In addition, the

elevated levels of SGOT by anti-tubercular treatment regimen, DOTS, have been shown to be reduced when DOTS medicines were given along with WS (Kumar et al., 2017). In our study, we found that WS treatment had reduced the total and direct bilirubin though there was no change in indirect bilirubin levels (Figure 7A-C). Similarly, SGOT levels were also reduced significantly with WS treatment (Figure 7D) though there was no change in SGPT and alkaline phosphatase.

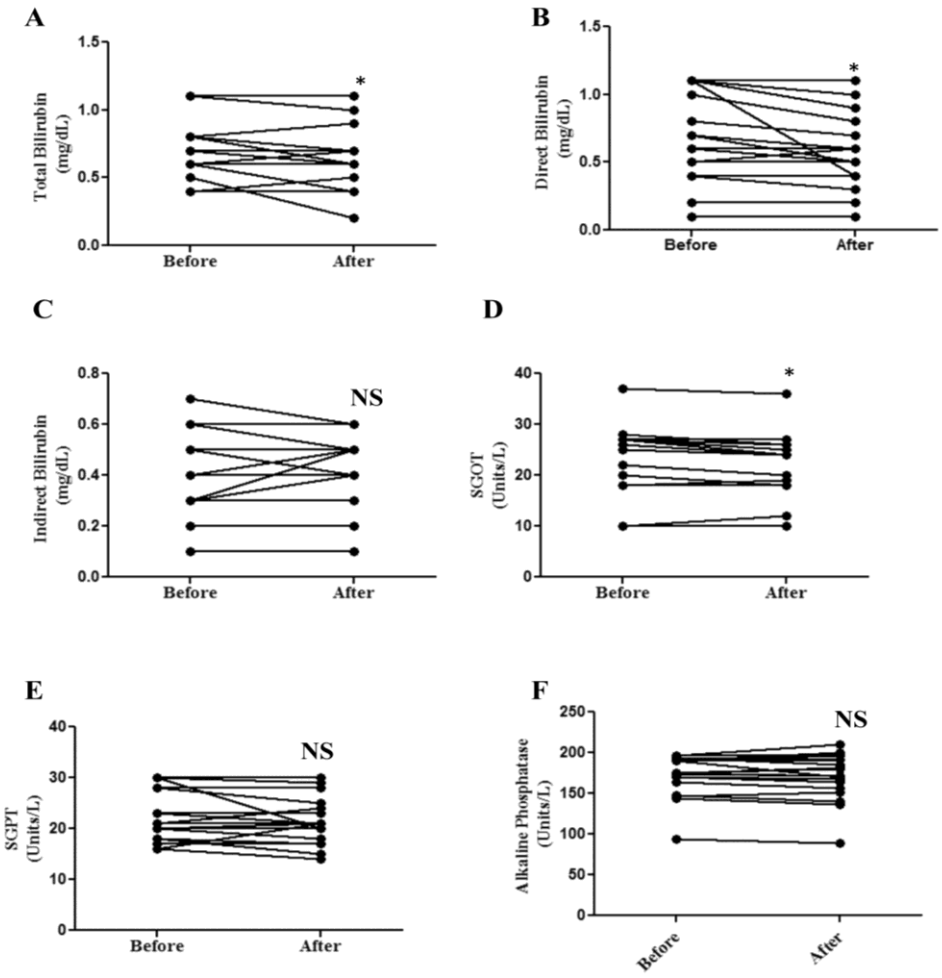


Figure 7: Effects of Withania Somnifera powder on key blood glucose and kidney functions in Rheumatoid arthritis patients. The levels of total, direct and indirect bilirubin (A-C), SGOT (D), SGPT (E) and Alkaline phosphatase (F) were determined in blood samples of patients who were treated with WS powder.*p < 0.05 vs. "Before", † p = 0.06 vs. "Before", ***p=< 0.0001 vs. vs. "Before"

3.10. Withania Somnifera powder decreases the IL-25

The role of IL-25 in Rheumatoid arthritis is controversial. It is known that IL-25 acts as Th17 suppression and thus beneficial in reducing the features in RA (Liu et al., 2016). On the other

hand, increased IL-25 levels have been shown to be positively associated with bone erosion in RA patients (Lu et al., 2017). In any event, increased IL-25 levels have been found to be increased in RA patients though the exact role in RA pathogenesis is not clear. In our study we wanted to determine the levels of IL-25 in RA patients who were treated with WS powder. We found that WS powder reduced the levels of IL-25 in sera. As WS powder leads to reduce the features of RA, we believe that the reduction of IL-25 could be attributable to reduction in symptoms of RA. In any event, the detailed investigations would be required to determine the exact role of IL-25 in RA. But the reduction of IL-2 by WS powder is interesting and this needs further investigations.

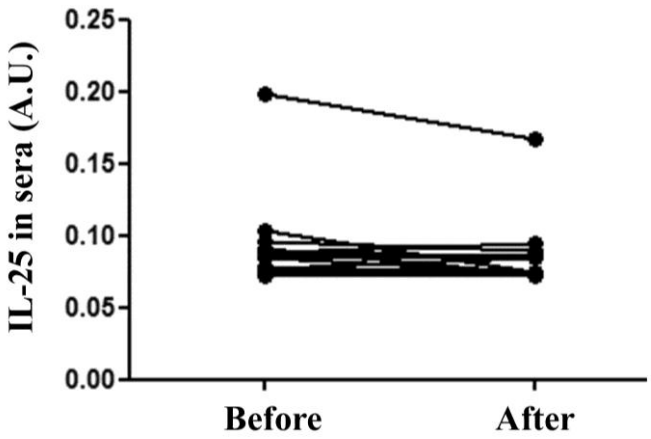


Figure 8: Effects of Withania Somnifera powder on IL-25 levels. The levels were significantly (P < 0.05) reduced after treatment.

3.11. Withania Somnifera powder does not affect the levels of IL-8

It is well known that IL-8 is strongly associated with the pathogenesis of RA. Numbers of studies have demonstrated the increased IL-8 levels in synovial fluids of RA patients (Cascão et al, 2010). However, the increased levels in sera of RA patients were found mostly in the very early stage of the disease. In any event, we wanted determine the effect of WS powder on IL-8 levels. We found that there was no change in the levels of IL-8 with WS powder treatment in RA patients. We need to perform IL-8 levels in synovial fluid in future to determine the exact effects of WS powder. It also is possible that WS powder treatment mediated beneficial effects may be independent of IL-8.

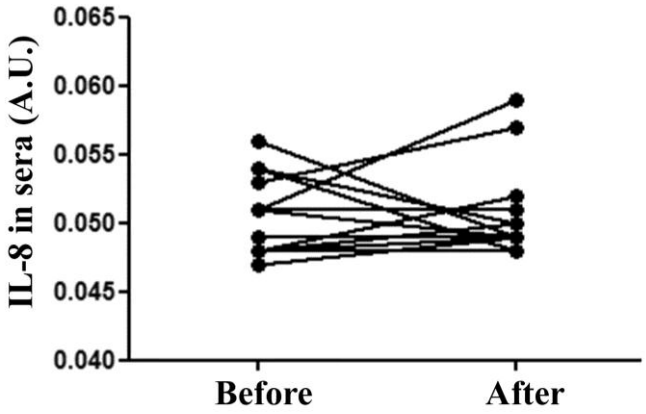


Figure 9: Effects of Withania Somnifera powder on IL-8 levels. The levels were not significantly (P > 0.05) altered after treatment.

4. CONCLUSION

In this study, we demonstrated that 2-4 gram dosing of WS powder in honey had reduced the symptoms of RA in human patients. This reduction in symptoms was associated with the reduction in acute phase reactants like ESR and CRP, WBC count, postprandial blood glucose, blood urea and creatinine, bilirubin and SGOT and the increase in levels of hemoglobin. Thus, our data suggest that WS powder could be potent pharmacological agent to reduce the symptoms and signs of RA patients. Further studies are warranted to decipher the mechanism underlying beneficial effects of WS powder.

Conflict of interest

The authors declare that they have no conflict of interest.

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