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The Role of *Withania somnifera* (Ashwagandha) and Omega-3 Fatty Acids on TNF-α and Joint Inflammation in an Animal Model of Rheumatoid Arthritis

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ABSTRACT

Introduction: Rheumatoid Arthritis (RA) is an autoimmune disorder characterised by progressive joint destruction leading to severe disability. The existing management of RA includes Nonsteroidal Anti-Inflammatory Drugs (NSAIDs), Disease-Modifying Antirheumatic Drugs (DMARDs) and biological such as Tumour Necrosis Factor (TNF) inhibitors but search for alternative and safer therapy is still going on.

Aim: To evaluate the efficacy of the combination of *Withania somnifera* (WS) and omega-3 fatty acids on TNF alpha level and joint histopathology in the treatment of Complete Freund's Adjuvant (CFA) induced rheumatoid arthritis in a rat model.

Materials and Methods: Healthy adult male Wistar albino rats were divided into six groups containing six rats in each group (n=36). Group I served as arthritis control, Group II and III received WS in dose of 500 mg/kg and 1000 mg/kg respectively. Group IV received Omega-3 polyunsaturated fatty acids in dose of 100 mg/kg. The combination of WS (1000 mg/kg) and Omega-3 polyunsaturated fatty acids (100 mg/kg) was given to rats of group V. Group VI served as standard treatment group and received Indomethacin 3 mg/kg. Arthritis was induced in groups II to VI by CFA. TNF-α was determined on day 0, 10 and 21. On day 21 all rats were sacrificed and inflamed limbs were excised above the ankle joints and examined for a pathological finding of RA. The data was analysed by one-way analysis of variance (ANOVA) followed by Dunnett's test and Tukey test. The Dunnett's test compares each mean to a control mean and simultaneous comparison between all other pairs was done by Tukey test.

Results: WS in both doses showed significant reduction in TNF-α ($p<0.001$). Among all the treated groups, maximum mean percent TNF-α reduction in group VI (-65.65%) and minimum in group II (-37.35%) was found. Group V containing combination of WS and Omega-3 fatty acid showed a higher percent reduction in TNF-α and minimal cell infiltration as compared with groups II, III and IV.

Conclusion: WS and omega-3 fatty acids suppress the changes produced due to adjuvant induced arthritis and combination of WS and omega-3 fatty acids was more effective than individual drugs alone.

Keywords: Autoimmune disorder, Complete freund's adjuvant, Indomethacin

INTRODUCTION

Rheumatoid Arthritis (RA) is a chronic autoimmune and inflammatory disorder which involves the multiple joints causing pain, immobility and stiffness [1]. About 24.5 million people were affected by RA in 2015 [2]. The existing management of RA includes NSAIDs, DMARDs (like methotrexate, cyclophosphamide, sulfasalazine, hydroxychloroquine), biological such as TNF inhibitors and interleukin-1 receptor antagonists [3].

These available drugs relieve the symptoms and also modify the progression of disease but are required for longer duration and may result into serious adverse drug events. Therefore, patients often seek some alternative modality of treatment which is effective as well as safe in comparison to available medicine. Therapeutic potential of Omega-3 (n-3) polyunsaturated fatty acids (n-3 PUFAs) in chronic inflammatory diseases have been well documented [4]. In Ayurveda, *Withania somnifera* (WS) has been used for arthritis, asthma, hypertension, inflammation and tumours for thousand years [5]. WS and omega-3 fatty acids have been shown effective for the treatment of RA but effect of combination of the two on cytokines level and joint inflammation has not been explored previously [4,6].

Therefore, the purpose of this study was to evaluate the efficacy of the combination of WS and omega-3 fatty acids on TNF alpha level and joint histopathology in the treatment of CFA induced RA in a rat model. CFA-induced arthritis in rats mimics human RA and characterised by chronic synovitis with inflammatory cell infiltration, pannus formation, bone erosion, and cartilage destruction [7].

MATERIALS AND METHODS

This experimental (animal) study was conducted in King George's Medical University, Lucknow, Uttar Pradesh, India, in 2014-15 with duration of one year. It was conducted on 36 healthy adult male Wistar albino rats, weighing between 150-250 gm after being approved by the Institutional Animal Ethics Committee (IAEC Ref. No. 67/IAH/Pharma-14). Animals were housed in Institutional Animal House Facility under standard laboratory conditions of temperature (25±2°C), humidity (55±5%) and 12 hours light-dark cycle controlled environment. Rats were provided pellet food and water ad libitum. Rats were divided into six groups containing six rats in each group.

Group I served as arthritis control, Group II and III received WS in dose of 500 mg/kg and 1000 mg/kg respectively [8]. WS were procured from Himalaya Healthcare Co, India. Group IV received Omega-3 polyunsaturated fatty acids in dose of 100 mg/kg [9]. Capsules of Omega-3 polyunsaturated fatty acid were purchased from Merck Germany. The combination of WS (1000 mg/kg) and Omega-3 polyunsaturated fatty acids (100 mg/kg) was given to rats of group V. Group VI served as standard treatment group and received Indomethacin 3 mg/kg. Indomethacin obtained from Jagsonpal Pharmaceuticals India.

Induction of rheumatoid arthritis: Baseline values were measured on day 0 and arthritis was induced in groups II to VI. CFA was injected (0.2 mL) intradermally in the footpad of their left hind paw. CFA was procured from Sigma Aldrich Chemical Co, USA. Development of arthritis is suggested by swelling appeared in animals' limbs by day 10 [10]. Drug administration in rats was carried out from days 10 to 21. TNF-α was measured on day 0, 10 and 21. On day 21 all rats were sacrificed by using a high dose of Pentobarbitone (150 mg/kg i.p) [11]. The inflammed limbs were excised above the ankle joints and examined for a pathological finding of RA.

Measurement of TNF alpha: Blood samples (1 mL) were withdrawn on day 12 and 21 from the retro-orbital plexus and after serum being prepared by centrifugation for 15 minutes at 3000 rpm. Level of TNF-α was determined by Enzyme-Linked Immunosorbent Assay (ELISA) kits. An ELISA kit for TNF-α was purchased from Diaclone, France. Rat TNF-α ELISA kit was used as per manufacturer's instructions.

Histopathological examination: The left hind paw joint from each rat was cut about 0.5 cm above and below the joint and decalcified by immersion in 3% nitric acid. Then joints were fixed in 10% neutral buffered formalin for 2 days. The decalcified specimens were dehydrated in alcohol series, cleared in xylene and embedded in paraffin. Sections were serially cut at 5-µm thickness and stained with Haematoxylin and Eosin (H&E) and evaluated microscopically.

Destruction of bone and cartilage was evaluated by using the following scoring system [12].

- Synovial proliferation:
  Grade 0- no proliferation
  Grade 1- mild proliferation (2-4 layers of reactive synoviocytes)
  Grade 2- moderate proliferation (4 plus layers with increased mitotic activity)
  Grade 3- severe proliferation (with invasion of joint space)
- Cellular infiltration:
  Grade 0- no changes
  Grade 1- few focal infiltrates
  Grade 2- extensive focal infiltrates
  Grade 3- extensive infiltrates invading capsule with aggregate formation
- Cartilage erosion:
  Grade 0- no changes
  Grade 1- superficial and localised cartilage degradation in more than one region
  Grade 2- deep and localised cartilage degradation
  Grade 3- extensive deep cartilage degradation at several locations
- Pannus formation:
  Grade 0- no changes
  Grade 1- pannus formation (up to 2 sites)
  Grade 2- pannus formation (up to 4 sites with infiltration)
  Grade 3- pannus formation (> 4 sites)

STATISTICAL ANALYSIS

The data was analysed by one-way analysis of variance (ANOVA) followed by Dunnett's test and Tukey test. The Dunnett's test was used to compare each mean with control mean. Statistical significance was based on p-value less than 0.05. Data analysis was performed using SPSS version 16.

The percentage change from baseline to follow-ups was also calculated for each group. All values were expressed as mean±SEM.

RESULTS

Effect of CFA induced arthritis on serum inflammatory factor TNF-α: Induction of arthritis significantly increased the level of TNF-α in all the groups as compared with baseline (day 0) value (p<.001) [Table/Fig-1]. In the absence of any treatment, values of TNF-α kept on rising in control group till the end i.e., 21 day. The mean % change in TNF-α levels from day 10 to day 21 was 30.01 which was significant (p<0.001).

Effect of *Withania somnifera* (Ashwagandha) on serum inflammatory factor TNF-α: After treatment with WS in doses of 500 mg/kg and 1000 mg/kg, TNF-α levels decreased significantly (p<0.001) [Table/Fig-1]. The mean % change were -37.35 and -45.63 respectively. WS at both doses decreased the level of TNF-α as compared with arthritic control group (p<0.001) but better effect was seen with higher doses [Table/Fig-2].

Effect of Omega-3 fatty acids on serum inflammatory factor TNF-α: TNF-α levels decreased significantly (p<0.001) after treatment with Omega 3 fatty acids at the dose 100 mg/kg. The mean % change was -45.02 [Table/Fig-1]. Omega 3 fatty acids decreased the level of TNF-α as compared with arthritic control group (p<0.001) [Table/Fig-2].

Effect of combination of *Withania somnifera* (Ashwagandha) and Omega-3 fatty acids on serum inflammatory factor TNF-α: TNF-α levels decreased significantly (p<0.001) after treatment with combination of Withania somnifera (1000 mg/kg) and Omega-3 fatty acids (100 mg/kg). The mean % change was -56.57 [Table/Fig-1]. Combination of WS and Omega-3 fatty acids decreased the level of TNF-α as compared with arthritic control group (p<0.001) [Table/Fig-2].

Effect of indomethacin on serum inflammatory factor TNF-α: TNF-α levels decreased significantly (p<0.001) after treatment with indomethacin (3 mg/kg). The mean % change was -65.65 [Table/Fig-1].

Histopathological Analysis

Histology of joints of arthritic control rats showed greater score for synovial proliferation, cellular infiltration in the sub synovial region, cartilage erosion and pannus formation compared to treatment groups. Treatment with the combination group showed minimal synovial cell infiltration and less erosion of cartilage and bone. WS (1000 mg/kg) and omega-3 fatty acids treated rats showed moderate synovial cell infiltration and significantly reduced bone and cartilage destruction. WS (500 mg/kg) reduced the histological scores to a lesser extent when compared to higher dose of WS [Table/Fig-3].

	Groups	Days of study			Day 0-10		Day 10-21	
		Day 0	Day 10	Day 21	Mean % change	p-value	Mean % change	p-value
TNF-α	I	10.46±0.01	61.68±0.10	80.12±0.02	489.32	<0.001	30.01	<0.001
	II	11.23±0.01	60.72±0.03	38.03±0.01	440.50	<0.001	-37.35	<0.001
	III	11.19±0.05	59.51±0.07	32.36±0.02	431.79	<0.001	-45.63	<0.001
	IV	10.29±0.02	60.43±0.01	33.23±0.01	487.20	<0.001	-45.02	<0.001
	V	10.10±0.02	60.46±0.25	26.25±0.02	498.67	<0.001	-56.57	<0.001
	VI	10.86±0.01	59.25±0.02	20.35±0.03	445.24	<0.001	-65.65	<0.001

[Table/Fig-1]: TNF-α (pg/mL) values as mean ± SEM on day 0, 10 and 21.

	Groups		Day 21	
			Mean difference	p-value
TNF-α	I Vs	II	42.16	<0.001
		III	47.84	<0.001
		IV	46.97	<0.001
		V	53.94	<0.001
		VI	59.85	<0.001

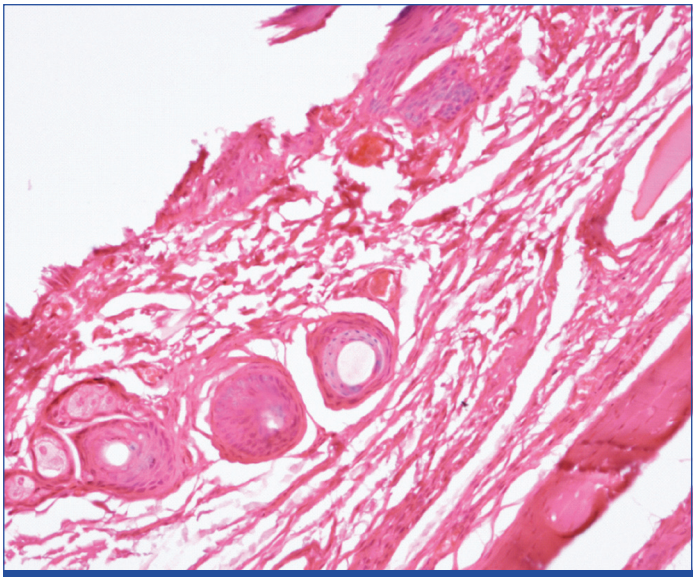
[Table/Fig-2]: Comparison of TNF-α (pg/mL) values of arthritic control group with treatment groups on day 21.

Histopatho-logical changes	Arthritic control	WS (500 mg/kg)	WS (1000 mg/kg)	Omega 3 Fatty Acids (100 mg/kg)	WS 1000 + Omega 100 mg/kg	Indo-methacin (3 mg/kg)
Synovial Proliferation						
Grade 0	-	-	-	-	1	2
Grade 1	-	-	3	2	3	3
Grade 2	-	4	3	4	2	1
Grade 3	6	2	-	-	-	-
Total	18	14	9	10	7	5
Mean	3	2.33	1.5	1.66	1.16	0.83
Cellular Infiltration						
Grade 0	-	-	-	-	2	4
Grade 1	-	2	5	3	3	2
Grade 2	1	3	1	3	1	-
Grade 3	5	1	-	-	-	-
Total	17	11	7	9	5	2
Mean	2.83	1.83	1.16	1.5	0.83	0.33
Cartilage Erosion						
Grade 0	-	-	-	-	-	2
Grade 1	-	1	2	1	3	4
Grade 2	1	3	3	5	3	-
Grade 3	5	2	1	-	-	-
Total	17	13	11	11	9	4
Mean	2.83	2.16	1.83	1.83	1.5	0.66
Pannus Formation						
Grade 0	-	-	1	1	1	2
Grade 1	-	-	2	1	3	3
Grade 2	-	4	3	4	2	1
Grade 3	6	2	-	-	-	-
Total	18	14	8	9	7	5
Mean	3	2.33	1.33	1.5	1.16	0.83

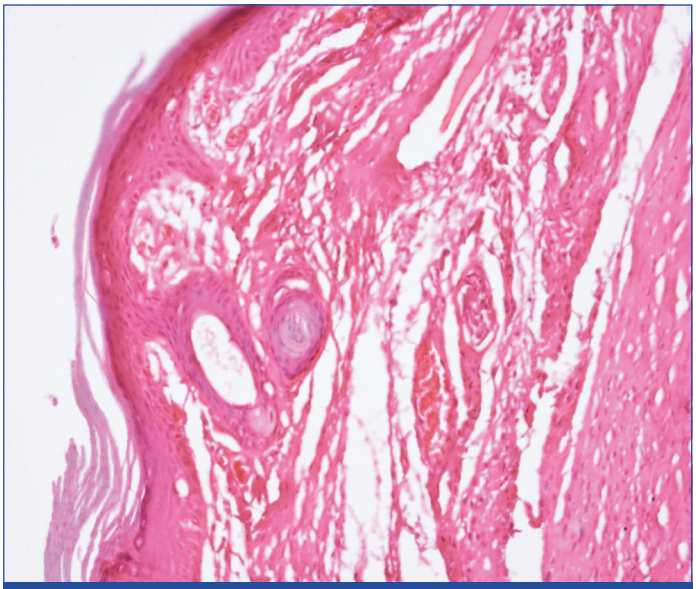
[Table/Fig-3]: Histopathological grading in control and experimental groups.

Synovial proliferation score: Maximum synovial cell proliferation was observed in arthritic control rats [Table/Fig-4]. Arthritic rats treated with WS (500 mg/kg and 1000 mg/kg) showed decline in synovial cell proliferation score (14 and 9 respectively) in comparison to arthritic control group. Omega-3 fatty acids treated group also showed greatly reduced score (10) as compared to arthritic control group. Combination of WS and omega 3 fatty acid showed minimal synovial proliferation score (7) in comparison to other test groups [Table/Fig-5].

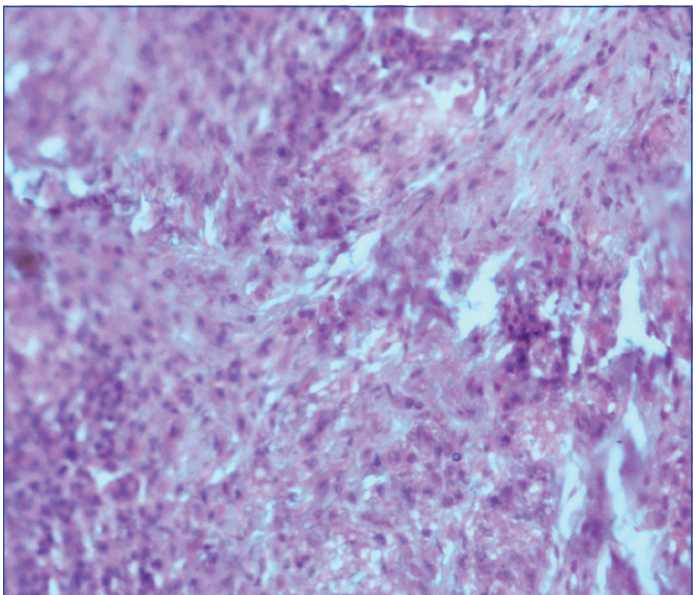
Cellular infiltration score: Cellular infiltration score was maximum (17) in arthritic control rats in comparison to treatment groups. Score of 11 was observed with the lesser dose of WS (500 mg/kg). WS (1000 mg/kg) treated rats showed a score of 7 [Table/Fig-6]. Omega treated rats showed a score of 9. Arthritic control group showed extensive infiltrates invading the capsule with aggregate formation while few focal infiltrates were found in the combination group (score of 5) [Table/Fig-7].



[Table/Fig-4]: Control group showed ulceration and damage of cartilage and pronounced synovitis (X20).

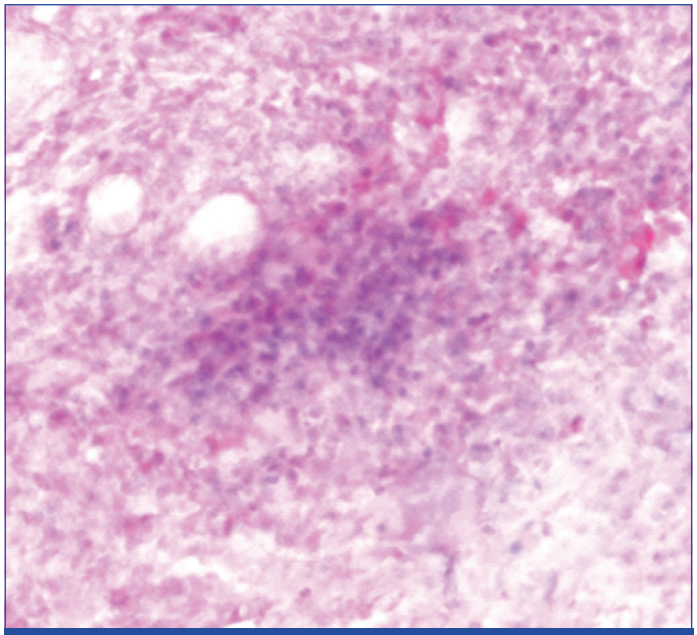


[Table/Fig-5]: *Withania somnifera* and Omega-3 fatty acid treated group showed intact cartilage and synovium (X20).



[Table/Fig-6]: *Withania somnifera* treated group showed moderate infiltration of macrophages and plasma cells (X20).

Cartilage erosion score: The cartilage destruction score was found maximum (17) in arthritic control rats as compared to that of the treatment groups [Table/Fig-4]. The combination group (score of 9)



[Table/Fig-7]: *Withania somnifera* and Omega 3 fatty acid treated group showed mild infiltration of plasma cells and macrophages (X20).

showed superficial localised cartilage degradation while deep cartilage degradation was present in arthritic control group [Table/Fig-4,5].

Pannus formation score: Mean Pannus formation score was maximum (18) in arthritic control group. Extensive pannus formation was seen in more than 4 sites in arthritic control group. Pannus formation up to 2 sites was present in the combination group.

DISCUSSION

RA is a chronic inflammatory and autoimmune disorder characterised by progressive joint destruction leading to severe disability. It mainly affects the distal joints symmetrically which become tender, swollen and stiff. Stiffness usually worsen in the mornings. Extra-articular manifestations also present in RA affecting eyes, skin, lungs, heart and blood vessels [13].

Anti-inflammatory and antioxidant activities of WS have been demonstrated in earlier studies [14,15]. In our study, Serum TNF-α was significantly increased in all groups after 10 days of injecting CFA to induce arthritis and inflammatory changes in rats. TNF-α has a significant role in mediating inflammation and bone degradation and it is found elevated in synovial fluid in RA [16,17].

Role of TNF-α in pathogenicity of RA is endorsed by the fact that neutralisation of monoclonal anti-TNF antibodies diminish collagen-induced arthritis in mice [18]. TNF-α antagonists, like infliximab, etanercept, adalimumab and Certolizumab Pegol (CZP) have been used for the treatment of RA. In our study, WS in both doses (500 mg/kg and 1000 mg/kg) showed significant reduction in TNF-α ($p<0.001$). A previous study showed that administration of WS extract for 10 days significantly decreases TNF-α concentration [19]. Malekshahi MA et al., showed that short-term omega-3 fatty acid administration decreases serum levels of TNF-α which is in agreement to our study which also showed decrease in TNF-α level after omega-3 fatty acid administration in dose of 100 mg/kg [20].

Combination of WS (1000 mg/kg) and omega-3 fatty acid (100 mg/kg) significantly decreased the TNF-α expression indicating synergistic anti-inflammatory effect on pathology of the disease. Combination of WS and omega-3 fatty acids was more effective than individual drugs alone. Though the mean % change in TNF-α levels was lower than that of indomethacin, both group showed statistically significant decrease in its levels.

Inter group comparison analysis showed a dose dependent response with maximum mean percent reduction in group VI (-65.65%) and minimum in group II (-37.35%). Group V showed a higher percent reduction in TNF-α as compared with groups II, III and IV.

TNF increases the expression of chemokines and adhesion molecules and there by attracts recruitment of neutrophils [21]. Local inflammation is also induced by TNF-α leading to pannus formation and bone destruction [22].

Histopathological evaluation revealed similar findings as observed by Gupta A et al., [6]. Histology of the joints of arthritic control rats showed synovial cell proliferation, pannus formation and infiltration of mononuclear and neutrophil infiltration in sub synovial region. Pannus formation caused destruction of bone and cartilage. Elevation of synovial cell infiltration was observed in arthritic control group. Arthritic rats treated with combination of WS and omega-3 fatty acids showed minimal cell infiltration, pannus formation and no sign of bone and cartilage destruction. Reduction in mean histological score in the combination group may be attributed to its anti-inflammatory and antioxidant properties [23].

LIMITATION

Animal model of disease does not exactly represent the real disease in human beings so further clinical studies should be conducted. Though ideally both the doses of WS should be chosen in combination with omega-3 fatty acid, only higher dose of WS was chosen (to reduce the number of animals). As this study was of short duration and evaluated only treatment of disease, other studies of longer duration need to be planned to explore the preventive role of WS and omega-3 fatty acids in RA.

CONCLUSION

The results of the present study are encouraging and may reveal the importance of the WS and omega-3 fatty acids which suppress the changes produced during adjuvant induced arthritis. Combination of WS and omega 3 fatty acids was more effective than individual drugs alone.

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REFERENCES

[1] Gabriel SE. The epidemiology of rheumatoid arthritis. Rheum Dis Clin North Am. 2001;27:269-81.
[2] GBD 2015 Disease and Injury Incidence and Prevalence Collaborators. Global, regional, and national incidence, prevalence, and years lived with disability for 310 diseases and injuries (1990-2015) A systematic analysis for the global burden of disease study. Lancet. 2015;388(10053):1545-602.
[3] Gibofsky A. Combination therapy for rheumatoid arthritis in the era of biologicals. HSS J. 2006;2:30-41.
[4] Yates CM, Calder PC, Ed Rainger G. Pharmacology and therapeutics of omega-3 polyunsaturated fatty acids in chronic inflammatory disease. Pharmacol Ther. 2014;141:272-82.
[5] Durg S, Dhadde SB, Vandal R, Shivakumar BS, Charan CS. *Withania somnifera* (Ashwagandha) in neurobehavioural disorders induced by brain oxidative stress in rodents: A systematic review and meta-analysis. J Pharm Pharmacol. 2015;67:879-99.
[6] Gupta A, Singh S. Evaluation of therapeutic potential of *Withania somnifera* root powder on oxidative stress and cartilage damage in collagen induced arthritis in rats. World J Pharm Sci. 2015;3:929-40.
[7] Snekhalatha U, Anburajan M, Venkatraman B, Menaka MZ. Evaluation of complete Freund's adjuvant-induced arthritis in a Wistar rat model. Comparison of thermography and histopathology. Rheumatol. 2013;72:375-82.
[8] Prabhu PC, Panchapakesan S, Raj CD. Acute and sub-acute oral toxicity assessment of the hydroalcoholic extract of *Withania somnifera* roots in Wistar rats. Phytother Res. 2013;27:1169-78.
[9] Meganathan M, Madhana GK, Sasikala P, Mohan J, Gowdhaman N, Balamurugan K, et al. Evaluation of hepatoprotective effect of Omega 3-fatty acid against paracetamol induced liver injury in albino rats. Glob J Pharmacol. 2011;5:50-53.
[10] Liu YL, Lin HM, Zou R, Wu JC, Han R, Raymond LN, et al. Suppression of complete Freund's adjuvant-induced adjuvant arthritis by cobratoxin. Acta Pharmacol Sin. 2009;30:219-27.
[11] Recommended methods of euthanasia for common laboratory animals. Available at www.tau.ac.il/~karena/.../mice_Euthanasia 20 of % 20 laboartory% 20 animals.doc. Accessed 8 July 2014.
[12] Shen L, Wang P, Guo J, Du G. Anti-arthritic activity of ethanol extract of Fagopyrum cymosum with adjuvant-induced arthritis in rats. Pharmaceutical Biology. 2013;51:783-89.
[13] Cojocaru M, Cojocaru IM, Silosi I, Vrabie CD, Tanasescu R. Extra-articular manifestations in rheumatoid arthritis. Maedica (Buchar). 2010;5(4):286-91.

[14] Alhindawi MK, Alkhafaji SH, Abdulnabi MH. Antigranuloma activity of Iraqi *Withania somnifera*. J Ethanopharmacol. 1992;37:113-16.
[15] Bhattacharya A, Ghosal S, Bhattacharya SK. Anti-oxidant effect of *Withania somnifera* glycowithanolides in chronic footshock stress induced perturbations of oxidative free radical scavenging enzymes and lipid peroxidation in rat frontal cortex and striatum. J Ethanopharmacol. 2001;74:1-6.
[16] Fütterer A, Mink K, Luz A, Kosco-Vilbois MH, Pfeffer K. The lymphotoxin beta receptor controls organogenesis and affinity maturation in peripheral lymphoid tissues. Immunity. 1998;9:59-70.
[17] Visvanathan S, Rahman MU, Keystone E, Genovese M, Klareskog L, Hsia E, et al. Association of serum markers with improvement in clinical response measures after treatment with golimumab in patients with active rheumatoid arthritis despite receiving methotrexate: results from the GO-FORWARD study. Arthritis Res Ther. 2010;12:R211.
[18] Maini RN, Brennan FM, Williams R, Chu CQ, Cope AP, Gibbons D, et al. TNF-alpha in rheumatoid arthritis and prospects of anti-TNF therapy. Clin Exp Rheumatol. 1993;11 Suppl 8:S173-75.
[19] Davis L, Kuttan G. Effect of *Withania somnifera* on cytokine production in normal and cyclophosphamide treated mice. Immunopharmacology and Immunotoxicology. 1999;21:695-703.
[20] Malekshahi MA, Saedisomeolia A, Djalali M, Djazayery A, Pooya S, Sojoudi F. Efficacy of omega-3 fatty acid supplementation on serum levels of tumour necrosis factor-alpha, C-reactive protein and interleukin-2 in type 2 diabetes mellitus patients. Singapore Med J. 2012;53:615-19.
[21] Mizgerd JP, Spieker MR, Doerschuk CM. Early response cytokines and innate immunity: Essential roles for TNF receptor 1 and type I IL-1 receptor during Escherichia coli pneumonia in mice. Journal of Immunology. 2001;166:4042-48.
[22] Redlich K, Hayer S, Ricci R, David JP, Tohidast-Akrad M, Kollias G, et al. Osteoclasts are essential for TNF-alpha-mediated joint destruction. J Clin Invest. 2002;110:1419-27.
[23] Pal A, Kumar M, Sharan V, Bhushan B. Anti-oxidant and free radical scavenging of ashwagandha (*Withania somnifera L.*) leaves. J Global Biosci. 2015;4:1127-37.

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