*ejpmr, 2016,3(12), 396-400*

**SJIF Impact Factor 3.628**

**EUROPEAN JOURNAL OF PHARMACEUTICAL AND MEDICAL RESEARCH**

www.ejpmr.com

Research Article

ISSN 2394-3211

EJPMR

# *IN VIVO* ASSESSMENT OF IMMUNOMODULATORY ACTIVITY OF *WITHANIA COAGULANS* DUNAL FRUITS

**Archana K. Shendkar[1]*, Sugandha G. Chaudhari[1], Usama Khan[1] and Dr. Yogesh K. Shendkar[2]**

[1]Department of Pharmacology, Dr. L.H. Hiranandani College of Pharmacy, Ulhasnagar, District-Thane, India.
[2]Department of Medicine, Virar Homeopathic Medical College, Virar, District-Thane, India.

***Corresponding Author: Archana K. Shendkar**

Department of Pharmacology, Dr. L.H. Hiranandani College of Pharmacy, Ulhasnagar, District-Thane, India.

**Article Received on 11/10/2016** **Article Revised on 02/11/2016** **Article Accepted on 23/11/2016**

**ABSTRACT**

The aim of the study was to investigate the immunomodulatory activity using methanolic and hydro-alcoholic extracts of *Withania Coagulans* dunal fruits i.e. *Withania Coagulans* Methanolic Extract (WCME) and *Withania Coagulans* Hydroalcoholic Extract (WCHAE). Phytochemical analysis has indicated presence of alkaloids, flavonoids, glycosides, steroids, saponins, fixed oil, carbohydrates. Both the extracts have showed immunomodulatory activity by Serum hemagglutinin titer assay. The agglutination reaction between test serum antibodies and antigen (mesh formation) present in Sheep Red Blood Cell's effectively inhibited by 75%, 77.77% in standard (Methotrexate), 40%, 44.44% in WCME-100 mg/kg, 55.55%, 60% in WCME-200 mg/kg, 50%, 66.66% in WCHAE-100 mg/kg, 70%, 75% in WCHAE-200 mg/kg groups in comparison with agglutination of control group wells in micro-titer plates. Both the extracts showed the good immunomodulatory activity but WCHAE showed the most significant immunomodulatory activity than WCME. The findings of the present study suggested that *Withania Coagulans* dunal fruits could be a potential natural source of immunomodulatory activity which is very important against treatment of immune system disease.

**KEYWORDS**: immunomodulatory, *Withania Coagulans*, *serum hemaglutinin* titer, agglutination.

## INTRODUCTION

Maintenance of immune system could be contributed by searching of substances with immunostimulative or immunorestorative effect.[1] Many plants have been evaluated for immunostimulant or immunosuppressive properties.[2] A number of medicinal plants as rasayanas have beenclaimed to possess immunomodulatory activity. Some of the 'Rasayana' drugs known as immunomodulatory agents are *Withania Somnifera*, *Tinospora Cordifolia* and *Mangifera Indica*.[3,4,5] Medicinal plants used for immunomodulation canprovide potential alternatives to conventional chemotherapies for a variety of diseases, especially when the host defense mechanism has to be activated under the conditions of impaired immune response. a variety of plant derivatives such as polysaccharides, lectins, peptides, flavonoids and tannins have been reported to modulate the immune system in various *in vivo* model.[6]

*Withania Coagulans* Dunal, Family-Solanacea is distributed in the east of the Mediterranean region and extends to South Asia, common in drier parts of Punjab, Gujarat, Simla and Kumaon in India, Baluchistan in Iran, Pakistan and Afghanistan and East India. The main component of berries are esterase, fatty oil, essential oils, lignan amino acids such as proline, hydroxyproline, valine, tyrosine, aspartic acid, glycines, aspargines, cysteine and glutamic acid and alkaloids are the phytoconstituents. The most of the activities of the plants is due to the presence of an active component as, Withanolide.[7] Fruits are Carminative, depurative, used for dyspepsia, flatulence and strange; Seeds are anti-inflammatory, emetic, diuretic, emmenaogue. Leaves are febrifuge and showed the analgesic activity.

*Withania Coagulans* dunal fruits family Solanacea have many medicinal and therapeutic benefits although many of them have yet to be proven by science. Hence, current research project was undertaken with an objective to assess the claims of the medicinal use of *Withania Coagulans* dunal fruits as an immunomodulatory activity.

## MATERIALS AND METHODS

### Material

**Plant material:** The fruits of WC belong to the family Solanacea is distributed throughout India. For the present study fruits are collected in the month of August from Mumbai (Maharashtra). The dried fruits was identified, confirmed and authenticated by A. S. Upadhye, Scientist, Plant Drug Authentication Service, Botany group, Plant Sciences Division, Agharkar Research Institute, Pune (V.No. F-180). The dried fruit material was then

pulverized by a mechanical grinder. The resulting coarse powder was then used for extraction.

**Preparation of extracts:** Dried fruits of *Withania Coagulans* Dunal were coarse powdered and packed into Soxhlet column and extracted with methanol for methanolic extract.[8] Whereas the water: methanol (40:60) is used for preparation of hydro alcoholic extract.[9] The extract was concentrated under reduced pressure to get dried powder. The dried extract was stored in airtight container in refrigerator below $10^0$c. Whereas the hydro alcoholic extract is prepared by using water and methanol in ratio of 40:60. To convert into powder, distillation process have been followed to recover solvent and then dried.

**Phytochemical analysis:** Phytochemical studies of methanolic extract and hydro alcoholic extract of fruits part of *Withania Coagulans* Dunal was done. It is subjected to the different chemical tests in ordered to identify the presence of various phytoconstituents. Dragendorff's test, Mayer's test, Wagner's test, Hager's test for alkaloids, Molish's test, Fehling's test for carbohydrates, Legal's Test for glycosides, Liebermann burchard's test for steroidal compounds, Lead acetate Test, Reaction with Sodium Hydroxide for flavonoids, Spot test for fixed oil, foam test for saponin glycosides was carried out for their presence.[10]

**Drugs and chemicals:** Sheep Red Blood Cells (SRBC's) were used for immunization and challenge. Methotrexate injection (Folitrax-15I.P.) marketed preparation of Ipca Laboratories was used as standard drug. Other chemicals used are of analytical grade obtained from local suppliers.

**Experimental animal:** Sprague Dawley rats of either sex weighing 150-200 gm were selected for the anti-inflammatory activity was housed under the uniform laboratory condition fed with commercial diet and provided with water ad-libitum, during the experiment. The animals were procured from from Bharat Serum, Thane, India and Dr. L. H. Hiranandani College of pharmacy and permitted for the study under the Institutional Animal Ethical Committee (IAEC). All protocols of the study were approved by the Institutional Animal Ethical Committee with reference number IAEC/PCOL-08/2013. The IAEC is approved by committee for the purpose of control and supervision of experiments on animals (CPCSEA) with registration number 879/ac/05/CPCSEA.

**Method**

***In Vivo* Assessment of Immunomodulatory Activity**

**Serum Hemaglutinin Titer Assay**

**Procedure**

**Antigen suspension (SRBC suspension):** Blood was collected in alsver's solution and washed twice with buffer saline (PBS). The number of SRBC was then adjusted to concentration $1\times10^8$ cells after the RBC count. RBC count was carried out using Neubers chamber and RBC pipette. This RBC suspension was used for immunization and challenge.

**Serum hemagglutination antibody titer**

Animals were divided into 6 groups of 6 mice each.
Group I: Control receiving normal saline.
Group II: Standard (STD) group administered with METHOTREXATE (0.5 mg/kg) orally.
Group III and IV: Test group were administered orally with WCME of fruits at dose of 100 mg/kg and 200 mg/kg respectively.
Group V and VI: Test group administered orally with WCHAE of fruits at dose of 100 mg/kg and 200 mg/kg respectively.

SRBC's $1\times10^8$ cells/ $mm^3$, obtained from sheep blood administered i.p. to each mice each group on day 0 immunization. The standard and test drug were administered to all the animals from day 0 to 7 as shown above in the standard and test group respectively. Blood samples were collected from individual animals of the entire group by retro orbital bleeding on $7^{th}$ day as per CPCSEA guidelines and serums were separated by centrifugation at 1500 rpm in centrifugation machine. Antibody levels were determined by the hemagglutination technique, this is performed by using 96 wells (12×8) U bottomed titer plate. The wells were marked from 1 to 12. In the first and last well 25 µl of serum collected from treated animals is added and inactivated at 56 $^0$C for 30 minutes. Afterwards 25 µl of PBS was added to all wells except well number 12 and mixed well. Then 25 µl of sample from first well was taken and added to $2^{nd}$ well, again 25 µl from second well was taken and added to third well continued the same procedure up to well number 10. After this 25 µl of sample from well number 10 were discarded. Finally 25 µl of 1% SRBC was added to all wells and kept at room temperature for two hrs. Each well was examined for hemagglutination.[11,12,13]

**Statistical Analysis**

All values are shown as mean ± S.E.M. Statistical analysis was performed using one-way analysis of variance (ANOVA) followed by Dunnett's test *$p<0.05$, **$p<0.01$ was considered statistically significant.

**RESULT**

***In Vivo* Immunomodulatory Activity using Serum Hemagglutination Antibody Titer:** Present study it was observed that on $7^{th}$ day WCE treatment effectively inhibited the agglutination reaction between test serum antibodies and antigen (mesh formation) present in SRBC's by 75%, 77.77% in STD (METHOTREXATE), 40%, 44.44% in TA1,55.55%, 60% in TA2, 50%, 66.66% in TB1, 70%, 75% in TB2 groups in comparison with agglutination of control group wells in micro-titer plates (Table No 1, Figure 1, Figure 2 Figure 3). The probable mechanism behind this activity of WCE might be due to inhibition of antigen uptake by mature

dendritic cells of bone marrow cells of immune system of experimental animals.

**Table No: 1 % Inhibition of serum hemaglutinin antibody titer**

| Experimental Animal | % Immunosupression | | | | |
|---|---|---|---|---|---|
| | STD | TA1 | TA2 | TB1 | TB2 |
| **1** | 62.5 | 12.5 | 25 | 25 | 50 |
| **2** | 66.66 | 33.33 | 44.44 | 44.44 | 66.66 |
| **3** | 75 | 37.5 | 50 | 50 | 75 |
| **4** | 77.77 | 44.44 | 55.55 | 66.66 | 66.66 |
| **5** | 70 | 40 | 60 | 50 | 70 |
| **6** | 75 | 37.5 | 12.5 | 37.5 | 50 |

Values of % immunosupression of STD: Standard, TA1: WCME-100 mg/kg, TA2: WCME-200 mg/kg, TB1: WCHAE-100 mg/kg, TB2: WCHAE-200 mg/kg groups were expressed.

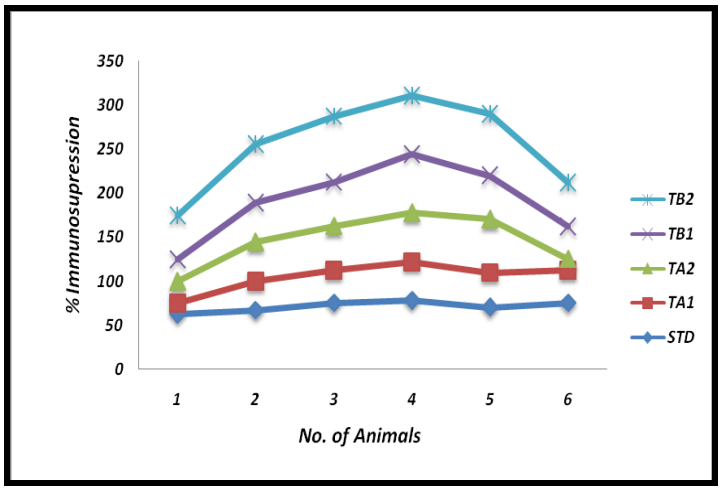



**Figure 1: Effect on % Immunosuppression**

STD: Standard, TA1:WCME-100 mg/kg,TA2: WCME-200 mg/kg, TB1: WCHAE-100 mg/kg, TB2: WCHAE-200 mg/kg groups were expressed.

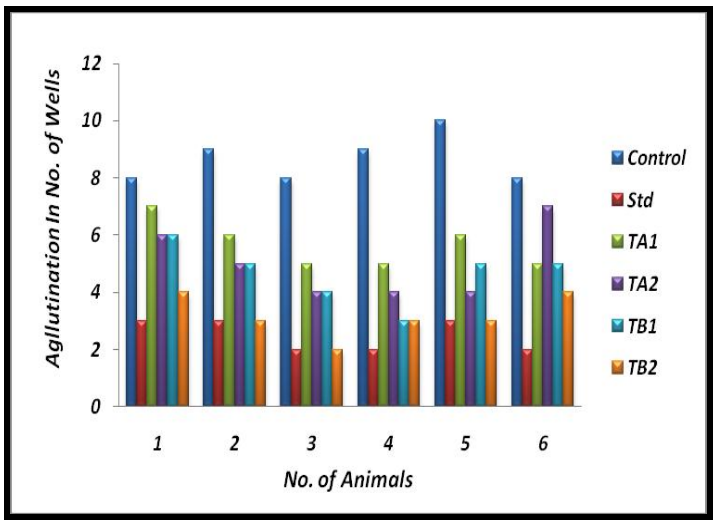
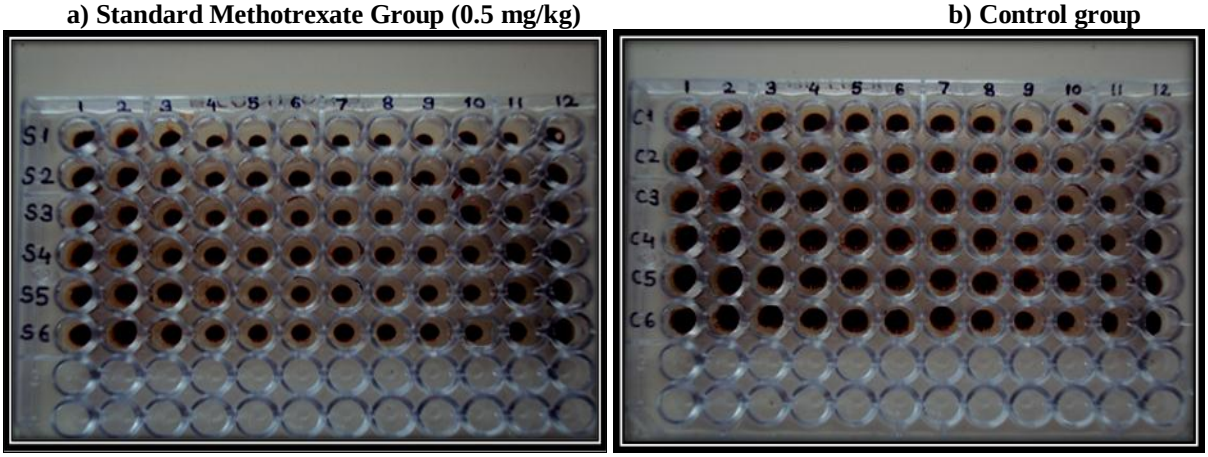


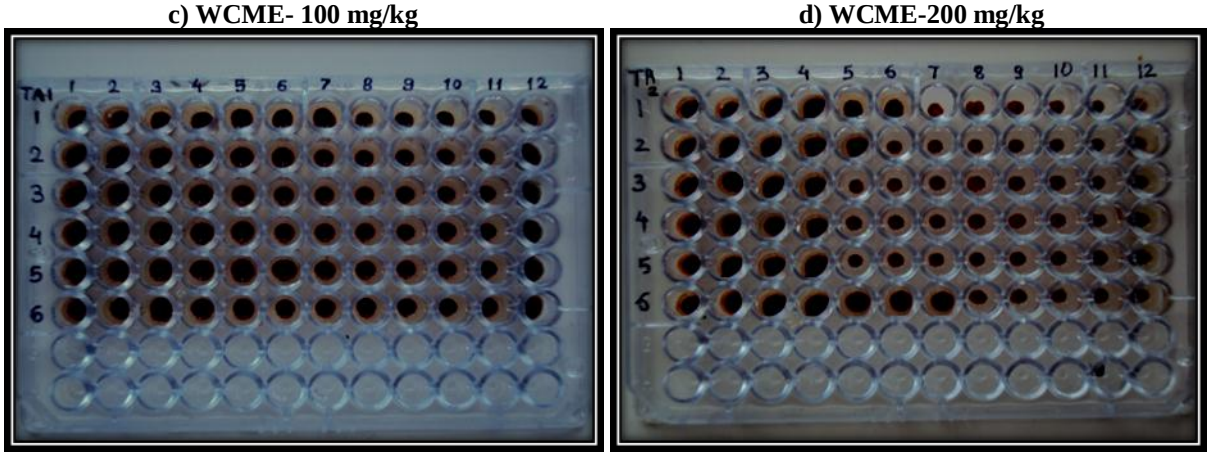

**Figure 2: Effect on Antibody Agglutination Level**

STD: Standard, TA1: WCME-100 mg/kg, TA2: WCME-200 mg/kg, TB1: WCHAE-100 mg/kg, TB2: WCHAE-200 mg/kg groups were expressed.

**a) Standard Methotrexate Group (0.5 mg/kg)** **b) Control group**



**c) WCME- 100 mg/kg** **d) WCME-200 mg/kg**



**e) WCHAE-100 mg/kg** **f) WCHAE-200 mg/kg**

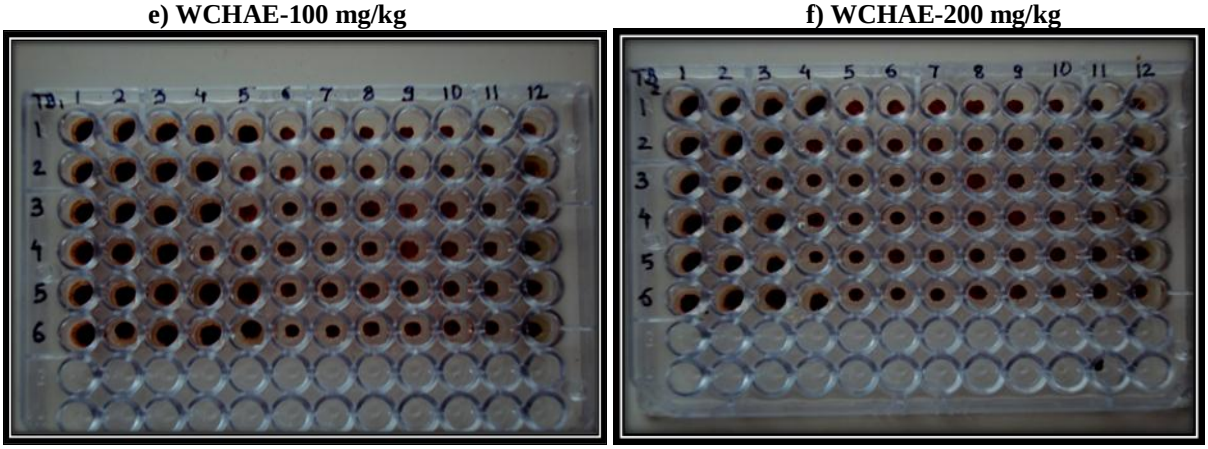


**Figure 3: Agglutination in Micro-titer plates**

## DISCUSSION

Serum hemagglutination antibody titer model is used to determine the humoral type immunity response against SRBC as antigen. The humoral immunity involves interaction of B cells with the antigen and their subsequent proliferation and differentiation into antibody-secreting plasma cells. Antibody functions as the effector of the humoral response by binding to antigen and neutralizing it or facilitating its elimination by cross-linking to form clusters that are more readily ingested by phagocytic cells.[14] At neutral pH, red blood cell possess negative ion cloud that make the cells repel from one another, this repulsive force referred to as zeta potential. Because of its size and pentameric nature, IgM can overcome the electric barrier and get cross link to red blood cells, leading subsequent agglutination. The smaller size and bivalency of IgM, however for, IgM being more effective than IgG in agglutinating red blood cells.[15] Major part of drug therapy of rheumatoid arthritis includes the drug like methotrexate and cyclophosphamides which are having immunosuppressant actions as it is an auto-immune disease, these drugs will be useful for the prevention of

auto-immunity along with inhibition of hazards associated with it.

Immunosuppression is important parameter in prevention of RA, hence WCME and WCHAE assessed for its immunosuppressant activity by serum hemagglutinin titer assay with methotrexate as a standard drug. It was observed that on 7$^{th}$ day both WCME and WCHAE treatment effectively inhibited the agglutination reaction between test serum antibodies and antigens (mesh formation) present in SRBC's in comparison with agglutination of control group wells in microtiter plates. But WCHAE treatment more effectively inhibited the agglutination reaction than WCME treatment. The probable mechanism might be due to the inhibition of antigen uptake by mature dendritic cells of bone marrow cells of immune system.

**ACKNOWLEDGEMENTS**

The Authors are grateful to extend special thanks to Dr. Parrag Gide, Principal and Dr. L.H. Hiranandani College of Pharmacy for his constant encouragement and support throughout the work.

**REFERENCES**

1. Yeap SK, Omar AR, Ali AM, Ho WY, Beh BK, Alitheen NB, Immunomodulatory Effect of Rhaphidophorakorthalsiion natural killer cell cytotoxicity. eCAM, 2012; 2012: 1-7.
2. Ramesh VK, Padmavathi K. Assessment of immunomodulatory activity of Euphorbia Hirtal. Indian J Pharm Sci., 2010; 72: 621-625.
3. Dahanukar SA, Thatte UM. Current status of Ayurveda in phytomedicine. Phytomed. 1997; 4: 359-368.
4. Davis L, Kuttan G. Immunomodulatory activity of Withania Somnifera. J. Ethnopharmacol. 2000; 71: 193-200.
5. Makare N, Bodhankar S, Rangari V. Immunomodulatory activity of alcoholic extract of Mangifera Indica L. in mice. J. Ethnopharmacol. 2001; 78: 133-137.
6. Shivaprasad HN, Kharya MD, Rana AC, Mohan S, Preliminary immunomodulatory activities of aqueous extract of Terminalia Chebula. Pharmaceutical Biology. 2006; 44: 32–34.
7. Gupta PC. Withania Coagulans Dunal- An Overview. Int J Pharm Sci Rev and Res. 2012; 12(2): 68-71.
8. Prasad SK, Kumar R, Patel DK and Hemalatha S. Wound healing activity of Withania Coagulans in Streptozotocin- Induced Diabetic Rats. Pharm Biol. 2010; 48(12): 1397–1404.
9. Sangwan et al. Process for Isolation for Withaferin-A from Plant Material and Product Therefrom. U.S. Patent US 7,108,870, B2; 2006.
10. Khandelwal KR. Practical Pharmacognosy Techniques and Experiments. 12$^{th}$ ed. India: Nirali Prakashan, 2008.
11. Makare N, SubhashBodhankar, Vinod Rangari. Immunomodulatory Activity of Alcoholic Extract of Mangifera Indica L. in Mice. Journal of Ethnopharmacology. 2001; 78: 133–137.
12. Puri A., Saxenaa R, Saxena RP, Saxena KC, Tandonb JS, Vandita Srivastava. Immunostimulant Activity of Nyctanthes Arbor-tristis L. Journal of Ethnopharmacology. 1994; 42: 31-37.
13. Vaghasiya J, Datani M, Nandkumar K, Malaviya S, Jivani N. Comparative Evaluation of Alcoholic and Aqueous Extracts of Ocimum Sanctum for Immunomodulatory Activity. Int J Pharm Biol Res. 2010; 1(1): 25-29.
14. Dashputre NL, Naikwade NS. Immunomodulatory Activity of Abutilon Indicumlinn on Albino Mice. IJPSR. 2010; 1(3): 178-184.
15. S. Agarwal, Khadase S and Talele G. Bioactive Immunomodulatory Fraction from Tridax Procumbens. Asian J Biol Sci. 2010; 3(3): 120-127.