



African Journal of Biological Sciences



Evaluation Of Immunomodulatory Activity Of Nagbala (*Grewia Hirsuta Vahl.*) By DTH Method.

Dr. Vaishnavi Wagholikar^{1*}, Dr. Shobha Khilari²

^{1*}Assistant Professor, Department of Dravyaguna Vigyan, Ashtang Ayurved Mahavidyalaya Pune, India.

² Associate professor, Department of Dravyaguna Vigyan, Bharati Vidyapeeth (Deemed to be University) College of Ayurved, Pune, India.

*Corresponding Author : Dr. Vaishnavi Wagholikar

*Assistant Professor, Department of Dravyaguna Vigyan, Ashtang Ayurved Mahavidyalaya Pune, India.

Email address: vaishnavi184@gmail.com

Article History

Volume6, Issue 6, 2024

Received: 16 JAN 2024

Accepted: 24 April 2024

doi.org/10.33472/AFJBS.6.6.2024.5633-5638

ABSTRACT

Background –Nagbala is mentioned as single drug Rasayana in Brihatrayee and used in the management of various diseases.

Grewia hirsuta Vahl., is one of the species considered as Nagbala by different scholars. So it was selected as Nagbala to evaluate its immunomodulatory activity.

Aim – Evaluation of immunomodulatory activity of Nagbala (*Grewia hirsuta Vahl*) root decoction and powder by DTH method in swiss albino mice.

Methodology – Collection, identification and authentication of drug was done.

Immunomodulatory activity was performed on Swiss Albino Mice by DTH model, using Churna and Kwath of Nagbala (*Grewia hirsuta Vahl*) root orally.

Result – Results of screening of immunomodulatory activity showed significant improvement in paw volume, in treatment group. The oral administration of the drug was also found significant increase in WBC count.

Key Words – Nagbala, *Grewia hirsuta Vahl.*, immunomodulatory activity, Rasayan.

Introduction:

Grewia hirsuta vahl. is a small tree or shrub grown in mixed deciduous open and dry evergreen forests, belongs to Malvaceae family¹. Nagbala is mentioned in all major Ayurvedic classical samhitas for Rasayan chikitsa and treatment of various diseases. Nagbala is mentioned in Phala varga in Charak Samhita² and in Vidarigandhadi gana of Sushrut Samhita³. The root of Nagbala has been used in rasayan chikitsa. Rasayana can be correlated with anti-ageing, antioxidant, cognitive enhancers and nootropic, adaptogenic, immunomodulators, haemopoetic, nutritive and anabolic action⁴. *Grewia hirsuta Vahl* is studied for its cardio protective activity on doxorubicin induced cardiotoxicity in rats⁵. Hypoglycemic potential and Antiproliferative effect on HepG2 cell lines⁶.

Number of plants has been screened for Immunomodulatory activity and they are found effective, *Grewia hirsuta Vahl.* is not mentioned in that list. Hence, attempt has been made to scientifically evaluate and validate the immunomodulatory effect of Nagbala (*Grewia hirsuta Vahl.*) Mool Churna and Mool Kwath through animal experimental study.

Three Species are taken as Nagbala by different scholars i.e. *Sida spinosa*, *Sida humilis* Cav, *Grewia hirsuta* Vahl. Balwant Singh Thakur, Acharya Priyavrat Sharma mentioned Nagbala as *Grewia hirsuta* Vahl.^{7,8}

Material and method

Animal: 30 swiss albino mice of either sex weighing between 20–25 gm provided by animal house of SGRS, College Of Pharmacy, Saswad, Pune. Animals were maintained at room temperature, standard laboratory diet were given. Animal experiment was carried out at Seth Govind Raghunath Sable, College Of Pharmacy, Saswad, Pune.

Approval of Institutional Animal Ethical Committee was taken at SGRS, College Of Pharmacy, with approval number –SGRS/IAEC/09/2014–15

Sheep RBCs were used as antigen for the challenge to get an immune response.

Drug preparation – Authentication of plant: Authentication of the plant was done at Agharkar Institute, Pune. Voucher No.– 15–223

Healthy, strong, matured, non–infected plants were collected.

Drying of drugs – Roots were shed dried, after complete drying for 15–20 days weight of dry root was 500gms.

Storage of drug – The roots were packed in air tight plastic bags after complete drying to avoid contamination. Plastic bags were kept at cool and dry place to preserve its shelf life. Two dosage forms of *Grewia hirsuta* Vahl. (Churna and Kwath) were prepared by the following methods given in Sharangdhar Samhita⁹ Dried roots were powdered on the grinder. The mesh size of the powder was 80. Fresh Kwath was prepared every day during the experiment.

Dose selection and schedule: Cell mediated immune response was studied using Delayed Type hypersensitivity (DTH) model¹⁰. Two dose forms that is churna and kwath were used in the experiment. Group A was kept as Disease control. The study was carried out at two dose levels for churna high dose (group B) dose is, 1300mg/kg; and for low dose (group C) 650mg/kg. While for kwath high dose (Group D) 10.4ml/kg and for low dose (group E) dose is 5.2ml/kg.

Preparation of animal model –

Duration of experiment 11 days.

Mice were primed with 0.1 ml of SRBC suspension containing 1×10^8 cells intraperitoneally on day 0 Nagbala Mool Churna and Mool Kwath was administered orally on day 0 and continued till day 7 of challenge to the test drug treated group.

On day 7 thickness of right foot pad was measured using Plathysmo meter.

The animals were then challenged on day 7 with 0.1 ml of SRBC suspension containing 1×10^8 cells into right hind paw.

Foot pad thickness was measured again 24, 48, 72 and 96th hour. after the challenge.

The difference between pre and post challenge foot pad thickness expressed in mm was taken as measure of DTH.

Then mean value obtained for treatment groups were compared with that of control group.

The data obtained was subjected to statistical analysis.

Table 1 – Experimental Design – .

Group	Day	Procedure
Group A (DC)	D-0 D1-D6 D7 D8-D9	Immunization of mice by using SRBC On normal diet Challenge with SRBC in hind paw Measurement of paw thickness on 24 th and 48 th hour
Group B Treatment group with Choorna	D-0 D1-D6 D7 D8-D9	Immunization of mice by using SRBC Treated with high dose of Churna Challenge with SRBC in hind paw Measurement of paw thickness on 24 th and 48 th hour
Group C Treatment group with Choorna	D-0 D1-D6 D7 D8-D9	Immunization of mice by using SRBC Treated with low dose of Churna Challenge with SRBC in hind paw Measurement of paw thickness on 24 th and 48 th hour
Group D Treatment group with Kwath	D-0 D1-D6 D7 D8-D9	Immunization of mice by using SRBC Treated with high dose of Kwath Challenge with SRBC in hind paw Measurement of paw thickness on 24 th and 48 th hour
Group E Treatment group with Kwath	D-0 D1-D6 D7 D8-D9	Immunization of mice by using SRBC Treated with low dose of Kwath Challenge with SRBC in hind paw Measurement of paw thickness on 24 th and 48 th hour

Group A: Disease control group; Group B: Treatment group with churna High dose; Group C: Treatment group with churna low dose; Group D: Treatment group with Kwath High dose; Group E: Treatment Group with Kwath low dose.

Statistical analysis:

Result was presented as Mean \pm SEM. Data was quantitative ANOVA test was selected for statistical analysis.

Result:

Observation during the experimental study shows that no specific behavioural change. No specific change in the intake and output (urine and stool) of animals. The food intake of animal was stable throughout the experiment. No specific changes were observed.

In haematological parameter WBC count is supportive parameter for immunity response.

WBC count shows significant difference between disease control group and groups treated with Nagbala. These results have revealed that treated groups show more immune response. Haemoglobin percentage of groups shows that it is slightly more in treated groups but difference in Hb% is not statistically significant. Highest Hb% is found in Kwath high dose group. RBC count of groups shows that there is no significant difference. But treated groups shows slightly higher values than untreated groups. Highest RBC count found in Kwath high dose group. Platelet count in treated group was slightly low than diseased control group but it is not statistically significant.

TABLE 2: Results of haematological parameters are taken by considering mean value of observations:

Group	WBC	RBC	HGB	PLT
A	9.5	5.58	10.72	737.6
B	10.08	6.55	11.35	691.16
C	10.08	5.83	10.08	550.83
D	10.48	7.3	11.81	639.16
E	9.55	6.58	11.36	557

Group A: Disease control group; Group B: Treatment group with churna High dose; Group C: Treatment group with churna low dose; Group D: Treatment group with Kwath High dose; Group E: Treatment Group with Kwath low dose.

For estimation of results of immune response observations of paw thickness on 24th and 48th hour was considered as per standard protocol given in DTH model. And 72 hours and 96 hours reading were taken to observe appearance of immune response in disease control group and also duration of response.

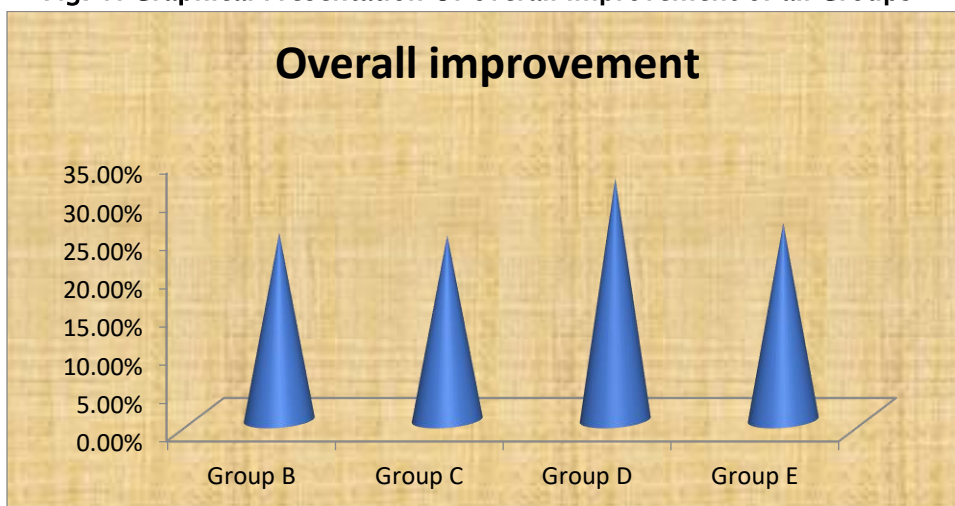
After going through statistical analysis results were found as follows

TABLE 3: Following table shows Mean ± SEM for paw values of animals.

Group	Initial	24th hour	48th hour	72th hour	96th hour
Group A	0.9916±0.1096	1.084±0.1213	1.164±0.1154	1.132±0.1138	1.12±0.1168
Group B	0.87±0.0824	1.34±0.3733	1.2566±0.3011	1.211±0.3410	1.1516±0.3088
Group C	0.9266±0.0496	0.9683±0.3152	1.025±0.2765	1.2583±0.3511	1.2216±0.2792
Group D	0.8416±0.1307	1.3316±0.4132	1.14±0.32912	1.185±0.3035	1.1333±0.31366
Group E	0.93166±0.01834	1.5±0.4601	1.3283±0.2999	1.333±0.2814	1.3616±.02314

Group A: Disease control group; Group B: Treatment group with churna High dose; Group C: Treatment group with churna low dose; Group D: Treatment group with Kwath High dose; Group E: Treatment Group with Kwath low dose.

Fig. 1: Graphical Presentation Of overall improvement of all Groups -



From Above diagram we found that Group D that is high dose of Nagbala (*Grewia hirsuta* Vahl.) Mool Kwath is more effective than other groups.

DISCUSSION:

Rasayana drugs described in Ayurveda may possess immunomodulatory, antioxidant and rejuvenating effects. Also they may prevent diseases and promotes healthy life. Different Rasayana yogas described in Samhitas, are plant originated and mostly consists of single drug. Nagbala is one of the single drug Rasayana mentioned in Brihattrayee. Hence this topic is selected for evaluation of its immunomodulatory activity.

DTH (Delayed Type Hypersensitivity) method is used for screening of immunomodulatory activity of Nagbala root which is associated with T-cell mediate immunity. This mechanism is observed in Tuberculosis and leprosy.

In Ayurveda Nagbala is prescribed diseases of respiratory system like in Kasa, Kshaya and Kshata Chikitsa which validates to use DTH model for evaluation of immunomodulatory activity of the drug. As Churna and Kwath of root is mentioned for Rasayana Chikitsa in Samhitas. So, these Kalpana are used by oral rout in treatment groups. Two dose levels are selected in experiment from which high dose of Churna and Kwath are taken as per in Samhita, while low doses are being advised in day to day practice. Results of screening of immunomodulatory activity shows that a group treated with high dose of Kwath (Human dose 80 ml), immunity response is higher and slightly lower in high dose of Churna (human dose 10gm).

Nagbala is Madhur Rasa, Madhur Vipak, Sheeta Veerya Dravya and also possesses Rasayana, Vrushya, Balya karma, it helps to produce potent Saptadhatus as well as proper nourishment of Dhatus; which ultimately promotes the vyadhikshamatva.

According to phytochemical analysis, *Grewia hirsuta* Vahl. contains flavonoids, B sitosterols in moderate amount. Flavonoids have been shown to decrease the rate of arterial aging and immune-system aging. Flavonoids seem to help your immune system's memory bank maintain the files it keeps on old foes (such as bacteria and viruses that cause illness) longer¹¹.

The mechanism by which B sitosterol and beta sitosterol glucosides would improve the immune response include increase in the proliferative response of blood lymphocytes and in the lytic/cytotoxic activity of natural killer cells, a modulation of the T-helper 1/T-helper 2 (Th1/Th2) Balance [60,65], as well as effect on macrophages¹².

Present study supports the immunomodulatory activity of Nagbala (*Grewia hirsuta* Vahl) root and useful in immunomodulation for controlling autoimmune diseases.

Conclusion -

Experimental study shows significant immunomodulatory activity of the drug. Though both dosage forms that is Churna and Kwath can be used for immunomodulatory activity but, Kwath Kalpana is more effective in both doses. Hence it is necessary to access both the forms Kwath and Churna for clinical study of Rasayan karma of Nagbala (*Grewia hirsuta* Vahl.)

Fig.2:



Injection in paw



Dosing



Paw thickness measurement

Acknowledgement – I am thankful to, the teaching faculties of Seth Govind Raghunath Sable, College of Pharmacy, Saswad, Pune. For their constant support, valuable suggestions all through the experimental study.

References:

1. Heywood, V.H., Brummitt, R.K.; Chulham, A. and Seberg (2007) Flowering plant families of the world. Firefly books, Richmond Hill, Ontario, Canada. ISBN-I-55407- 206-9
2. Charak Samhita with Ayurved dipika Vyakha (edition 2001) edited by Yadavji Trikanji Acharya, published by Chaukhamba Surabharti (Varanasi).
3. Sushrut Samhita with Nibandha Sangraha Vyakha (2010) by Yadavji trikamji Acharya and Narayanrama Acharya Kavyateertha, published by Chaukhamba Surbharti (Varanasi).
4. Dessertation by Dr. Neeta Nikam, Guide- Dr.Shobha Khilari; Study of Vanshalochana with the help of Pharmacognostic and physicochemical analysis; BVDU, COA, 2007.
5. Author- sheikh jilani, ravena Siddappa, sheikh bhasha, veena v, daya sagar (2015, Nov- Dec) pre- clinical evaluation of cardioprotective activity of Grewia Hirsuta on doxyrubicin induced cardiotoxicity in rats [ISSN NO.-2321-5674;2320 34714]
6. Author- Sharma ema, M.sheikh kumar, S.sindhu, Jayanthi Rebecca, A. anbarasi (2013,June) Evaluation of antiproliferative effect of Grewia Hirsuta on HepG2 cell lines; Journal of Academia and Industrial research; ISSN NO.- 2278-5213, Vol.2, issue-1.
7. Glossary Of Vegetable Drugs In Brahatrayi (Second Edition:1999), by Thakur Balwant Singh and Dr.K.C. Chunekar, Published by Chaukhamba Amarabharti Prakashan,(Varanasi).
8. DravyaGuna-Vijnana (Vol II and V) (2005), by Prof. P. V. Sharma, Published by Chaukhambha Bharati Academey Academey. (Varanasi)
9. Sharangdhar Samhita (Third edition,1983) by Pandit Sharangdharacharya with the commentary Adhamalla's Dipika and Kashirama's Gudartha Dipika, Published by Chaokhabha Oriantialia (Varanasi).
10. Drug discovery and evaluation (second edition, page no 800) by H.G.Vogel, springerverlang New York.
11. A review: immunological potential of bioactive flavonoids and flavonoid containing fractions isolated from medicinal plants; Cibtech Journal of Bio-Protocols ISSN: 2319-3840;2016 Vol. 5 (2) May-August, pp.7-14/Edewor
12. Auther- Elke A. Trautwein, Isabelle Demonty, Phytosterols: natural compounds with established and emerging health benefits 5 sept-oct 2007; OCL VOL.14 No]