In-vitro Study to Elicit the Anti-inflammatory Action of Mother Tincture, 3X, 12X and 6C Potencies of Aconitum Napellus and Rhus Toxicodendron by the Human Red Blood Cell (HRBC) Membrane Stabilization Assay

Dr. Perumalla Pavithran¹, K. Harshitha²

¹Associate Professor, Department of Homoeopathic Pharmacy, MNR Homoeopathic Medical college, MNR University, Sanga Reddy, Telangana - 502294, India.

² IV BHMS Student, MNR Homoeopathic Medical College and Hospital, Sangareddy, Telangana, India

Corresponding Author: Dr. Perumalla Pavithran

DOI: https://doi.org/10.52403/gijhsr.20250208

ABSTRACT

Homeopathy, a complementary medicine system, has faced controversy for over 200 years, particularly regarding the efficacy of its remedies and the principle of similia. Despite evidence from human clinical trials supporting the effectiveness of homeopathic medicines, the scientific community often attributes success to placebo effects. This in investigates vitro study the antiproperties inflammatory Aconitum of napellus and Rhus Toxicodendron using the Human Red Blood Cell (HRBC) membrane stabilization assav. Results showed significant anti-inflammatory effects, with potencies 3X, 12X, and 6C demonstrating enhanced HRBC membrane stabilization and reduced hemolysis, particularly Aconitum napellus 12X and Rhus Toxicodendron 3X.

Keywords: in-vitro, Anti-inflammatory effects, HRBC Membrane stabilisation, Homoeopathy.

INTRODUCTION

Homeopathy persistence has led to ongoing clashes with the scientific community and

conventional medical practice, fueling debates and criticisms about its legitimacy effectiveness. According and to the standards of contemporary, evidence-based medicine, homoeopathy is completely ineffective and ought not to be used. In order to deflect criticism from the scientific community, proponents of Homeopathy have appealed to the theory of science for support.^[1]

Historically, homeopathic research focused on clinical effectiveness without exploring scientific validity. However, with advances in scientific knowledge, researchers are now using molecular biology methods (like in experiments) investigate vitro to homeopathic medicines' therapeutic efficacy. In India, homeopathic medications face regulatory issues under the 1940 Drugs and Cosmetics Act, making it essential to conduct in vitro and in vivo trials to provide a scientific foundation for their effects and therapeutic benefits.^[2]

Homeopathy provides medications that have been clinically utilized and proved to totally eliminate or diminish the severity of a wide range of disorders, based on the subjective and objective symptoms of the individual patient.^[3] The modern scientific community

often ignores evidence of the effectiveness of homeopathic medicines, even in cases where in vivo trials on humans confirm their efficacy and attribute the cure to the placebo effect. In the field of therapeutics, conflicting evidence is not uncommon. There is need to provide a scientific foundation for homeopathic medications' effects, particularly in the context of modern medicine. One solution to resolve such contradictions is to establish concrete evidence with reliable potential research techniques such as vitro experimental studies.

The current study results would bridge the gap between Homeopathy and modern medicine by providing scientific evidence therapeutic effectiveness for the of homeopathic remedies Aconitum napellus and Rhus Toxicodendron particularly in reducing inflammation using molecular such as biology methods in vitro experiments. This study addresses regulatory issues in India, explore the potential applications of homeopathic medicines, contribute and to the understanding of their mechanisms of action. In the end, it provides a scientific basis for the therapeutic effectiveness of homeopathic treatments, facilitating for more research and potentially establishing homeopathy as a credible therapy.

In vitro research offers cost-effectiveness, ethical considerations, precision, control, and versatility, making it a crucial alternative to in vivo experimentation. As biological systems become more understood, ethical concerns rise, making in vitro research essential for scientific discovery, innovation, and sustainability.^[4] In the last few decades, Homeopathy has led to a significant number of experimental research at the molecular, cellular, and clinical levels as well as the methodologies used in modern medicine. Inflammation is an area of research and advancement, presumably because it is intimately associated with the conventional "vital force" of the body's capacity for self-healing.^[5]

Inflammation is a biological response of the immune system to harmful stimuli, such as pathogens, damaged cells, toxic compounds, or irradiation.^[6] Inflammation is essential for immune surveillance and host defense, but chronic low-grade inflammation can cause disease or tissue damage in various chronic conditions.^[7,8]Suppressing the inflammatory response is necessary to stop further tissue damage from occurring by halting the transition of acute inflammation.^[9]

One technique to look into the antiinflammatory effects of medications in invitro is the Human Red blood cell (HRBC) membrane stabilization assav. The technique is significant because of the similarities between the lysosomal and HRBC membranes, a medication that stabilizes the former may also stabilize the stabilizing latter. By the lysosomal membrane, the release of pro-inflammatory components is stopped, which in turn reduces the inflammatory response.^[10] Given that this is the most studied model of high-dilution effects, an understanding of the technical intricacies could aid in interpreting the findings.

Aconitum napellus targets cerebro-spinal nerves, mucous membranes, lungs, tendons, fibrous tissues, and serous membranes for strong inflammation and early fevers. Rhus Toxicodendron acts on stomach, mucous membranes, eyes, mouth, serous fibrous tissue, and lymphatics, treating gastritis, conjunctivitis, and acute mouth inflammation, plus eye, skin, and lung inflammations. Both are chosen based on symptoms in holistic homeopathy. [11,12,13,14,15,16]

Aims and objectives:

 To evaluate and compare the antiinflammatory action of *Aconitum napellus* and *Rhus Toxicodendron* – Q, **3X, 12X and 6C** by HRBC Membrane stabilization method.

2. To investigate the efficacy of proposed in preventing HRBC interventions membrane lysis and to quantify the hemolysis, percentage of thereby determining their anti-inflammatory effects.

MATERIALS & METHODS

Source of data:

MNR Homeopathic medical college Research lab **Study Setting:** Laboratory setting **Type of Study:** Experimental study **Study design:** True Experimental study design **Inclusion/Exclusion criteria** Not Applicable **Proposed interventions:**

- napellus Aconitum and Rhus Toxicodendron – Q, 3X, 12X and 6C were procured from authentic GMP outlet.
- Required chemicals including Alsevers solution, Phosphate Buffer solution, and isosaline solution, were procured from certified and reliable suppliers (Sri Durga Laboratory Equipment supplies, Mangalore) and stored according to manufacturer's instructions.
- Hyposaline solution (0.45% NaCl) was prepared by diluting Isosaline solution (0.9% NaCl) with purified water in a 1:1 ratio.

Study Procedure:

- Equal volumes of Blood from a healthy volunteer and Alsevers solution were mixed.
- The mixture was centrifuged at 3000 rpm for 30 minutes, resulting in the separation of packed cells.
- The packed cells were washed with isotonic saline solution, and a 10% (v/v) HRBC suspension was prepared for hemolysis assay.

- 10 HRBC suspensions were prepared using the aforementioned protocol from blood samples obtained from 10 healthy volunteers.
- Each of the 10 HRBC suspension samples was divided into Nine groups and allocated to control, Aconitum napellus (Q, 3X, 12X, 6C), and Rhus toxicodendron (Q, 3X, 12X, 6C) groups for Hemolysis assay.
- 1ml of Phosphate buffer solution, 2ml of • Hyposaline solution, 20 µl of dispensing alcohol, 20 µl of Aconitum napellus (Q, 3X, 12X, 6C), and 20 µl of Rhus Toxicodendron (Q, 3X, 12X, 6C) were added into their respective experimental groups using a pipette.
- All Assay mixtures were subjected to incubation at 37°C for 30 minutes, then centrifuged at 3000 rpm for 10 minutes.
- Following centrifugation, the decanted supernatant liquid from all experimental groups was applied to a Nanodrop spectrophotometer at 560nm for Hemoglobin content estimation via absorbance measurement.
- The percentage of hemolysis was • estimated by assuming the hemolysis produced in the control group as 100%
- The percentage of Hemolysis of HRBC membrane was calculated as follows: % Hemolysis = (Optical density of test sample) X 100
- **Optical Density of Control**
- The percentage of HRBC membrane • stabilization can be calculated as follows:
- % Protection = $100 \frac{(Optical density of test sample)X 100}{}$ Optical Density of Control
- Optical density = Absorption coefficient • $(\alpha) \ge l$
- Absorption Coefficient (α) = $\frac{2.303 \text{ x Absorbance (A)}}{2.303 \text{ x Absorbance (A)}}$
- The obtained results were tabulated and statistically analyzed using ANOVA (Analysis of Variance) test (p < 0.05) with SPSS software and conclusions were drawn.

STATISTICAL ANALYSIS

SAMPLE	VARIANCE IN ABSORBANCE	VARIANCE IN OPTICAL DENSITY	VARIANCE IN % HEMOLYSIS	VARIANCE IN % MEMBRANE STABILIZATION	F Value	F Crit	Obtained p-value
SAMPLE -1	0.000023	0.000123	907.11	909.2975	22.26	2.901	0.00000564
SAMPLE -2	0.0000242	0.0001288	672.01861	672.01861	26.59	2.901	0.00000803
SAMPLE -3	0.000048	0.000259	848.28	848.28	17.86	2.901	0.00000542
SAMPLE -4	0.000028	0.000153	719.4444	719.4444	21.70	2.901	0.00000748
SAMPLE -5	0.000062	0.00033	854.98	854.98	17.96	2.901	0.00000512
SAMPLE -6	0.000035	0.000186	609.4719	609.4719	25.81	2.901	0.00000112
SAMPLE -7	0.000031	0.000165	959.92	959.92	16.99	2.901	0.00000887
SAMPLE -8	0.000026	0.000138	899.6603	899.6603	18.83	2.901	0.00000327
SAMPLE -9	0.000023	0.000125	724.9236	724.9236	26.35	2.901	0.00000889
SAMPLE -10	0.000056	0.0003	980.6953	980.6953	15.66	2.901	0.00000191

TABLE No .1 SUMMARY OF ONE-WAY ANOVA TEST RESULTS OF 10 SAMPLES

- From the above table it is evident that of One way Analysis variance indicates significant (ANOVA) differences between the Control and homeopathic treatments (Aconitum napellus and Rhus Toxicodendron) in mother tincture and various potencies (3X, 12X, 6C), as evidenced by calculated F-values greater than the critical F-value (2.901)at 5% significance, with degree of freedom v1 = 3 and v2 = 32.
- The obtained p value is less than 0.05, which is strong evidence to reject the null hypothesis and accept the alternate hypothesis.
- This research study is highly significant as it demonstrates the potential antiinflammatory effects of Mother tincture, 3X, 12X, and 6C potencies of Aconitum napellus and Rhus Toxicodendron.

RESULT





STEP -6 FOLLOWING INCUBATION, THE ASSAY MIXTURES WERE CENTRIFUGED AT 3000 RFM FOR 10 MINUTES.

SAMPLES AFTER CENTRIFUGE

Fig. 5





STEP -7

FOLLOWING CENTRIFUGATION, THE DECANTED SUPERNATANT LIQUID FROM ALL EXPERIMENTAL GROUPS WAS APPLIED TO A NANODROP SPECTROPHOTOMETER AT 560nm FOR HEMOGLOBIN CONTENT ESTIMATION VIA ABSORBANCE MEASUREMENT



Fig. 6





DISCUSSION

The scientific community broadly agrees Homeopathy system underlying that assumptions are either disproven or lack plausibility. In evidence-based medicine, it is a fundamental pragmatic approach to the actual effectiveness of determine therapeutic methods separately from their plausibility.^[1] theoretical То address criticism from conventional medicine, with advances in scientific knowledge, researchers employing molecular are biology techniques, such as in vitro experiments, to investigate the therapeutic efficacy of homeopathic medicines. One of the keys focuses of scientific evaluation in homeopathic medicine is assessing antiinflammatory activity in vitro.^[2]

Considering the potential therapeutic antiinflammatory indications of Aconitum napellus and Rhus Toxicodendron, this study was aimed at investigating their antiinflammatory effects in various potencies using the Human Red Blood Cell (HRBC) membrane stabilization assay, thereby contributing to the evidence base literature for homeopathic therapeutics.

I. Aconitum napellus group exhibited significant anti-inflammatory potential, as evidenced by enhanced membrane stabilization and reduced hemolysis in its homeopathic potencies. Specifically, the following combinations demonstrated higher membrane stabilization compared to the mother tincture:

- 12X potency exhibited high membrane stabilization in 5/10 samples (range: 65.8-93.8%)
- 3X potency showed high membrane stabilization in 4/10 samples (range: 75-88.9%)
- 6C potency demonstrated moderate membrane stabilization in 3/10 samples (range: 87.5-94.1%)
- Mother Tincture showed low membrane stabilization in 10/10 samples (range: 12.5-61.1%)

II. RhusToxicodendron group exhibited significant anti-inflammatory potential, as evidenced by enhanced membrane stabilization and reduced hemolysis in its homeopathic potencies. Specifically, the following combinations demonstrated highest membrane stabilization compared to the mother tincture:

- 3X potency showed high membrane stabilization in 6/10 samples (range: 70.6-93.8%)
- 6C potency demonstrated high membrane stabilization in 3/10 samples (range: 74.1-83.3%)
- 12X potency exhibited moderate membrane stabilization in 2/10 samples (70.8-83.3%)
- Mother Tincture showed low membrane stabilization in 10/10 samples (range: 12.5-57.9%)

Consistent High Membrane Stabilization:

- Aconitum napellus 12X potency showed high membrane stabilization (mean: 75.34 ± 15.7)
- Rhus Toxicodendron 3X potency showed high membrane stabilization (mean: 77.17 ± 9.4)

CONCLUSION

Homeopathy, a complementary medicine system, has been involved in controversy for over 200 years. A longstanding debate surrounds the efficacy of homeopathic medicines, particularly the requirement for a precise symptomatic match between patient and drug pathogenesis, adhering to the principle. Despite evidence similia supporting the efficacy of homeopathic medicines in human clinical trials, the scientific community often dismisses these findings, attributing success to the placebo effect.

To address this disparity, comprehensive scientific investigations are necessary. To achieve this objective the current invitro study was aimed to justify the clinical use of homeopathic medicines Aconitum napellus and RhusToxicodendron in various potencies as anti-inflammatory drugs as

measured by the Human red blood cell (HRBC) membrane stabilization assay and provide concrete, reliable scientific evidence.

This study findings validates Homeopathic principles and therapeutic potential by demonstrating significant anti-inflammatory effects of Aconitum napellus and Rhus Toxicodendron in various potencies. The that Homeopathic studv demonstrates potencies (3X, 12X, 6C) exhibit enhanced bioactivity, supporting the concept of potentization. Using an HRBC membrane stabilization assay, the study establishes a standardized evaluation method and contributes to evidence-based Homeopathic practice. Overall, positive findings from this research study strengthens Homeopathy scientific foundation, encouraging further investigation and potential mainstream recognition. This research result contributes to the growing literature on evidence-based homeopathy therapeutic efficacy.

Declaration by Authors Ethical Approval: Approved Acknowledgement: None Source of Funding: None Conflict of Interest: The authors declare no conflict of interest.

REFERENCES

- 1. Grams N. Homeopathy—where is the science? A current inventory on a pre-scientific artifact. EMBO reports. 2019 Mar;20(3): e47761.
- Gupta P, Sundaram E, Sharma M, Prajapati S, Arya B, Khurana A, Manchanda R. Pre-clinical pharmacology: An important aspect in homoeopathic research. Indian Journal of Research in Homoeopathy. 2018;12(3):164-79.
- Garg KL. Use of homoeopathic drugs as antifungal agent for the protection of books and paper materials. In: Aranyanak C, Singhasiri C, editors. Biodeterioration of Cultural Property-3, proceedings of the 3rd International Conference on Biodeterioration of Cultural Property held at. Bangkok, Thailand: Conservation

Science Division, The Fine Arts Department; 1995. p. 103-15.

- 4. Cerulean. Unlocking the potential: Advantages of in vitro research overinvivostudies [Internet]. Linkedin.com.2024[cited2024Jul6].
- Bellavite P, Conforti A, Piasere V, Ortolani R. Immunology and homeopathy.
 Historical background. Evidence-Based Complementary and Alternative Medicine. 2005;2(4):441-52.
- 6. Medzhitov R. Inflammation 2010: new adventures of an old flame. Cell. 2010 Mar 19;140(6):771-6.
- 7. Hotamisligil GS. Inflammation and metabolic disorders. Nature. 2006 Dec;444(7121):860-7.
- Chen L, Deng H, Cui H, Fang J, Zuo Z, Deng J, Li Y, Wang X, Zhao L. Inflammatory responses and inflammation-associated diseases in organs. Oncotarget. 2018 Jan 1;9(6):7204. Medzhitov R. Inflammation 2010: new adventures of an old flame. Cell. 2010 Mar 19;140(6):771-6.
- Headland SE, Norling LV. The resolution of inflammation: Principles and challenges. In Seminars in immunology 2015 May 1 (Vol. 27, No. 3, pp. 149-160). Academic Press. Hotamisligil GS. Inflammation and metabolic disorders. Nature. 2006 Dec;444(7121):860-7.
- 10. Yesmin S, Paul A, Naz T, Rahman AA, Akhter SF, Wahed MI, Emran TB, Siddiqui SA. Membrane stabilization as a mechanism of the anti-inflammatory activity of ethanolic root extract of Choi (Piper chaba). Clinical Phytoscience.2020 Dec; 6:1-0.
- Burt WH. Physiological Materia Medica, Containing All that is Known of the Physiological Action of Our Remedies: Together with Their Characteristic Indications and Pharmacology. Gross & Delbridge; 1881
- 12. Boericke W. New manual of homoeopathic materia medica and repertory. B. Jain Publishers; 2001.
- 13. Allen HC. Keynotes and Characteristics with Comparisons of some of the leading

Remedies of the Materia Medica with Bowel Nosodes. B. Jain Publishers; 2002.

- 14. Nash EB. Leaders in homoeopathic therapeutics with grouping and classification. Jain; 1985.
- 15. Farrington EA. Condensed Materia Medica by Constantine Hering
- 16. Clarke JH. A Dictionary of Practical Materia Medica: In 3 Volumes. Jain; 1992

How to cite this article: Perumalla Pavithran, K. Harshitha. In-vitro study to elicit the antiinflammatory action of mother tincture, 3X, 12X and 6C potencies of aconitum napellus and rhus Toxicodendron by the human red blood cell (HRBC) membrane stabilization assay. *Gal Int J Health Sci Res.* 2025; 10(2): 79-86. *DOI: https://doi.org/10.52403/gijhsr.20250208*
