Developing and testing a natural gel that can be applied to the skin to help animals with arthritis.

Abstract

In this study, we aimed to create and test a special herbal gel that contains extracts from Cardiospermum halicacabum and Vitex negundo leaves to see if it can help with arthritis in rats. We made twelve different versions of the gel using different ingredients, and we checked how they looked, their thickness, how easy they could be squeezed out, their acidity, how well they spread, and how the active ingredients were released. We also made sure the gel stayed stable over time and didn't cause skin problems.

Then, we tested the gel on rats with arthritis. We looked at things like their weight, the swelling in their paws, their blood and other body fluids, and we even checked their tissues under a microscope. We found that one of the gel versions, called F4, worked really well in reducing arthritis symptoms in the rats. This means it might have potential as a treatment for arthritis in the future.

Keywords

1. Arthritis treatment with herbal gel

2. Herbal gel with Cardiospermum halicacabum

3. Herbal gel with Vitex negundo

4. Medicinal plants for arthritis treatment.

* + simple words, these are the main topics we are studying:

1. Using herbal gel to treat arthritis

2. Making a gel with Cardiospermum halicacabum

3. Making a gel with Vitex negundo

4. Using plants as medicine for arthritis.

Introduction

Arthritis is a condition where the body's immune system goes haywire and affects a small percentage of people globally. The medicines usually given for Rheumatoid Arthritis have side effects like stomach problems, weakened immune system, and disturbances in the body's defense mechanisms. Traditional Indian systems of medicine, like Siddha and Ayurveda, are becoming more popular as alternatives for treating arthritis. Two plants commonly used in these traditional practices for arthritis are Cardiospermum halicacabum and Vitex negundo. Cardiospermum halicacabum (CH) has been used in Chinese medicine for a long time to treat inflammation, rheumatism, and other diseases. Research has shown that it has anti-inflammatory properties and can reduce pain and fever. It also contains various helpful compounds. Vitex negundo Linn. (VN) is known as Nirgundi in Hindi and is found in wastelands. It contains several natural substances and has been used for its anti-arthritic, anti-inflammatory, and other beneficial effects We decided to make a special gel using extracts from these two plants and test it to see if it could help with arthritis. This gel is easy to apply, works directly where it's applied, doesn't cause much discomfort, and doesn't get broken down by the digestive system. Although these plants have been used for medicine in various forms, we focused on using their leaves for our gel because they are commonly used for arthritis treatment in traditional medicine.

* MATEIALS AND METHODS
* Materials

We collected fresh leaves of Vitex negundo and Cardiospermum halicacabum from Palakkad, Kerala, and made sure they were the right plants with the help of an expert. We also got some chemicals like Freund's complete adjuvant, diclofenac sodium, triethanolamine, propylene glycol, and disodium edetate from Sigma-Aldrich USA. For making our gel, we used substances called Carbopol 934 and Carbopol 940, which we got from Loba Chemie Pvt. Ltd. in Mumbai.

* Preparing the Plant Extracts

First, we cleaned and dried the leaves properly. Then, we used a special method to extract useful stuff from the leaves. For Cardiospermum halicacabum, we used a machine called a Soxhlet extractor with methanol, and for Vitex negundo, we soaked the leaves in methanol for a week. After that, we filtered the extracts and concentrated them under low pressure using a machine called a rotary evaporator. We stored these extracts at a cool temperature for later use.

* Animals

We used Wistar rats, female albino mice, and albino rabbits in our experiments. These animals were kept in a controlled environment with the right temperature, humidity, and lighting conditions. They had their own cages with clean bedding, and they could eat and drink whenever they wanted. We made sure to follow ethical guidelines for using animals in experiments. A committee that oversees animal experiments approved our research plan, and our college's animal ethics committee also gave their approval.

* Making the Gel Base

To create the gel, we first mixed Carbopol 934 with water slowly to avoid lumps. Then, we separately mixed disodium edetate and triethanolamine with water and stirred them. We also mixed propylene glycol with water. Next, we added the disodium edetate and triethanolamine mixture to the Carbopol mixture and adjusted the pH level. Finally, we added the propylene glycol mixture and stirred until we had a clear and consistent gel base.

* Checking the Quality of the Gel

We wanted to make sure that the gel we prepared had the right amount of active ingredients. To do this, we took a little bit of each gel formulation (1 gram) and mixed it with a special liquid called methanol. We shook it well to dissolve the active ingredients. Then, we filtered the solution to get rid of any solid bits, and we took a tiny amount (0.1 mL) of the filtered liquid. We made this tiny amount a bit bigger (10 mL) by adding more methanol to it. Next, we used a special machine that measures how much of the active ingredients are in the liquid by shining a light at it. This light had a specific wavelength of 275 nm, which is the best for measuring these ingredients.

* Extrudability

We wanted to see how easily the gel comes out of the tube. So, we took a tube of the gel, squeezed it, and measured how much came out. Then, we calculated the percentage of gel that came out.

* pH Measurement

We measured the acidity or alkalinity of the gel using a digital pH meter. We dipped a special glass probe into the gel to get this measurement. We did this three times and took the average.

* Appearance and Homogeneity

We looked at the gel to see how it appeared and if it was mixed well.

* Viscosity

We measured how thick the gel was using a machine called a Brookfield viscometer. It tells us how easily the gel flows.

* Spreadability

We tested how well the gel spreads. We put the gel between two glass slides and pressed them together. Then, we measured how long it took for the top slide to slide off. We did this three times and took an average.

* In vitro Diffusion Profile

We studied how the active ingredients in the gel move through rat skin using a special setup. We used rat skin as a barrier and placed the gel on it. We had a liquid on the other side of the skin that was like what's inside our bodies. We took samples of the liquid at different times to see how much of the active ingredients had moved through the skin.

* Release Kinetics

We looked at how the active ingredients were released from the gel over time and tried to understand the pattern.

* Stability Studies

We checked how the gel's quality changed over 6 months when stored at different temperatures and humidities. We looked at things like color, smell, how well it mixed, pH, thickness, the amount of active ingredients, and if any microbes grew in it.

* Anti-arthritic Activity

We tested if the gel worked to reduce arthritis in rats. We divided the rats into groups, applied different things to their joints, and measured their weight and paw size to see if the gel helped with arthritis pain.

* Hematological Parameters

We took blood from the rats and checked things like red and white blood cell counts, hemoglobin, and other markers in their blood to understand how the gel affected their health.

* Biochemical Estimations

We measured different substances in the rats' blood to see how the gel affected their overall health.

* Histopathological Investigations

We looked at the rats' thymus, spleen, and ankle joints under a microscope to see if there were any changes after using the gel.

* Statistical Analysis

We used math to analyze the data and see if the results were statistically significant.

* Skin Irritation Study

We applied the gel to the skin of rabbits and checked if it caused any irritation or reaction on their skin over several days.

* Conclusion

The gel we made for arthritis treatment seems to work because it contains certain natural substances called luteolin and apigenin. Among our different gel formulations, the one which has 2% of both CHME and VNME and 1.5% of carbopol 934, showed the most promise in treating arthritis. But, we need more studies with real patients to be sure it works well for people with joint problems.

* Acknowledgment

We want to thank the Defense Research and Development Organization in New Delhi for providing the funds we needed to do this research. We also appreciate the support from the Chairman and Secretary of the Kovai Medical Centre Research and Educational Trust in Tamil Nadu, who gave us the facilities we needed for our work.

* References
1. ASHA, V.V.; PUSHPANGADAN, P. Anti-fever activity of Cardiospermum halicacabum. Indian J. Exp. Biol., v.37, n.4, p.411-414, 1999.
2. BABU, K.C.V.; KRISHNAKUMARI, S. Cardiospermum halicacabum reduces the production of TNF-α and NO by human immune cells. Afr. J. Biomed. Res., v.9, p.95-99, 2006.
3. BLONCO-FLONTE, H.; ANGUIANO-IGEA S.; OTERO-ESPINAR, F.J.; BLANCOMENDEZ, J. In-vitro stickiness of carbopol gel. Int. J. Pharm., v.142, p.169-174, 1996.
4. CHOI, E.M.; LEE, Y.S. Luteolin suppresses IL-1b-induced cytokines and MMPs production in human synovial sarcoma cells. Food Chem. Toxicol., v.48, n.10, p.2607-2611, 2010.
5. FELDMANN, M.; MAINI, S.R. Role of chemicals in rheumatoid arthritis: an explanation in the science of disease. Immunol. Rev., v.223, p.7-19, 2008.
6. GHOSH, M.N. Basics of experimental pharmacology. Kolkatta: Scientific Book Agency, 1984. p.156-157.
7. GIINTER, S.; IRMARGD, M.; UTE, W.; CHISTOPH, M.S. Anti-cancer effects of the flavonoid luteolin. Molecules., v.13, n.10, p.2628-2651, 2008.
8. GOPALAKRISHNAN, C.; DHANANJAYAN, R.; KAMESWARAN, L. Studies on the effects of Cardiospermum halicacabum. Indian J. Physiol. Pharmacol., v.20, p.203-206, 1976.
9. GUPTA, M.; MAZUMDER, U.K.; BHAWAL, S.R. CNS activity of Vitex negundo Linn in mice. Indian J. Exp. Biol., v.37, n.2, p.143-146, 1999.
10. JAIN, S.; PADSALG, B.D.; PATEL, A.K.; MOALE, V. Formulation development and evaluation of fluconazole gel in various polymer bases. Asian J. Pharm., v.1, p.63-68, 2007.
11. JEYADEVI, R.; SIVASUDHA, T.; RAMESH KUMAR, A.; DINESH KUMAR, L. Anti-arthritic activity of the Indian leafy vegetable Cardiospermum halicacabum in Wistar rats and UPLC-QTOF-MS/MS identification of the putative active phenolic components. Inflamm. Res., v.62, n.1, p.115-26, 2013.
12. KIM, J.Y.; SONG, J.Y.; LEE, E.J.; PARK, S.K. Rheological properties and microstructures of carbopol gel network system. Colloid Polym. Sci., v.281, n.7, p.614-623, 2003
13. KUMAR, E.; MASTAN, S.K.; AMRANDER REDDY, G.; RAGUNANDAN, N.; SREEKANTH, N.; CHAITANYA, G. Anti-arthritic property of the ethanolic leaf extracts of Cardiospermum halicacabum Linn. Biomed. Pharmacol. J., v.1, p.2, 2008.
14. KUMARAN, A.; KARUNAKARAN, R.J. Antioxidant activities of the methanol extract of Cardiospermum halicacabum. Pharm. Biol., v.44, n.2, p.146-151, 2006.
15. LAIRD, J.M.A.; CARTER, A.J.; GRAUERT, M.; CERVERO F. Pain relief activity of a new sodium channel blocker, crobenetine, in rats with immune arthritis. Br. J. Pharmacol., v.134, n.8, p.1742-1748, 2001.
16. LOGANATHAN, V.; MANIMARAN, S.; JASWANTH, A.; SULAIMAN, A.; SHIVAPRASADHA, R.M.V.; SENTHIL KUMAR, B.; RAJASEKARAN, A. The effects of polymers and permeation enhancers on releases of flurbiprofen from gel formulations. Indian J. Pharm. Sci., v.63 n.3, p.200-204, 2001.
17. MARTIN, A. Physical pharmacy, kinetics. First Indian reprint. New Delhi: B.I Waverly, 1994.
18. MIZUSHIMA, Y.; TSUKADA, W.; AKIMOTO, T. A Modification of rat adjuvant arthritis for testing anti-rheumatic drugs. J. Pharm. Pharmacol., v.24, n.10, p.781-785, 1972.
19. MURPHY, C.T.; MCCARROLL S.A.; BARGMANN, C.I.; FRASER, A.; KAMATH, R.S.; Genes that act downstream of DAF-16 to influence the lifespan of Caenorhabditis elegans. Nature, v.424, p.277-283, 2003.
20. NAIR, A.M.; SARAF, M.N. Inhibition of antigen and compound 48/80 induced contraction of guinea pig trachea by ethanolic extract of the leaves of Vitex negundo linn. Indian J. Pharmacol., v.27, n.4, p.230-233, 1995.
21. NAIR, C.K.N.; MOHENAN, N. Medicinal plants in India with special reference to Ayurveda. Delhi, India: NAG Publisher, 1998.
22. NANDGUDE, T.; THUBE, R.; JAISWAL, N.; DESHMUKH, P.; CHATAP, V.; HIRE, N. Formulation and evaluation of pH induced in situ nasal gel of salbutamol sulphate. Int. J. Pharm. Sci. Nanotechnol., v.1, n.2, p.177-83, 2008.
23. NAPPINNAI, M.; PAKALAPATI, S.; ARIMILLI, R. Roficoxib gels-preparation and evaluation. Indian Drugs., v.43, p.513-15, 2006.
24. NAYAK, S.H.; NAKHAT, P.D.; YEOLE, P.G.