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INVESTIGATIONS OF *IN-VITRO* THROMBOLYTIC AND ANTI-INFLAMMATORY POTENTIALS OF ETHANOLIC LEAVES EXTRACTS OF *HEVEA BRASILIENSIS*

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ABSTRACT

The study was carried out to investigate the thrombolytic and anti-inflammatory potentials of crude 50% ethanolic extracts of leaves of *Hevea brasiliensis*, using an *in-vitro* clot lysis and protein denaturation method. The thrombolytic potentials of the crude 50% ethanolic extract was performed in different concentrations (100, 200, 300, 400, and 500 µl) using five (05) different nature of human blood samples. Blood samples were gathered from Tripura's youthful volunteers, North East India, and streptokinase was taken as the standard drug and distilled water as the negative control for the validation of the method. The albumin denaturation assay was performed to assess anti-inflammatory activity *in-vitro*. A greater clot lysis result was obtained, i.e., 37.8±0.8 %, 43.3±0.48 %, 51.4±0.33%, 65.4±0.79%, and 75.3±0.98 % at 100, 200, 300, 400, and 500 µl concentrations, correspondingly while the standard drug (streptokinase) showed 49.06±0.93%, 58.5±0.48%, 64.7±0.7%, 73.1±0.56%, 82.8±0.71% clot lysis. Inhibition of protein denaturation was seen in the *in-vitro* anti-inflammatory effect of a 50% ethanol extract of *Hevea brasiliensis*, i.e., 46.1±0.11, 56.03± 0.76,65.7±0.52, 70.7±0.5, 74.7±0.27 at 100, 200, 300, 400, and 500 µl respectively whereas, standard (aspirin) was recorded at 56.4±0.31, 65.6±0.71, 70.5±0.43, 75.1±0.21, 80.1±0.18 at similar various concentrations. Specific polar phytoconstituents may be the cause of the considerable *in-vitro* thrombolytic and anti-inflammatory outcomes that were observed. The preliminary estimations of 50% ethanolic extract

of *Hevea brasiliensis* have been proven the presence of polyphenols and ethanol-soluble flavonoids. However, *in-vivo* thrombolytic, anti-inflammatory potentiality, and active component(s) of the extract are yet to be discovered.

Keywords: ***Hevea brasiliensis, in-vitro thrombolytic, anti-inflammatory*** **activity**

INTRODUCTION

For their basic medical needs, almost 80% of the global population uses herbal or traditional medicine. Herbal therapies are used in Asian nations contributes to a significant category of human interactions with the environment throughout history **[1]**.

Since the beginning of civilization, medicinal plants have been utilised to treat a vast range of human illnesses. Over the years, people's obsession with modern medicine has led them to seek an alternate strategy to improving and maintaining good health **[2]**. Plants are the source of many modern-day vital pharmaceuticals and processed treatments. Medicinal plants contain a variety of medicinal compounds that may have thrombolytic, anti-inflammatory, and other properties **[3]**.

Plants used in traditional medicine have a variety of compounds that can be used to treat both acute and chronic illnesses. The chemical components found in plants that have an influence on how the human body functions make them important in medicine. The most common bioactive chemicals found in plants include alkaloids, flavonoids, tannins, and phenolic compounds. Using medicinal plants as traditional therapies is common in rural areas of developing nations **[4]**.

Traditional healers claim that their treatments are both more inexpensive and more effective than modern medicine **[5]**. Low-income populations in developing nations, such as farmers, people living in rural areas, and members of indigenous tribes, all use traditional medicine to treat common illnesses. Phytochemical research based on ethnopharmacological data is a well-known strategy for discovering novel anti-infective chemicals in higher plants **[6]**.

Hevea brasiliensis contains phytoconstituents such tannins and flavonoids that have thrombolytic and anti-inflammatory activities. A review of the literature revealed that the plant leaf extracts employed in this study had not been tested for anti-inflammatory and thrombolytic characteristics. As a result, the goal of this study was to investigate the thrombolytic and anti-inflammatory activities of *Hevea brasiliensis* extracts.

Thrombosis

The clotting of blood vessels is referred to as thrombosis. The blood vessel will get blocked as a result of the thrombus (clot)

that forms as a result of haemostasis failure in the blood vessels, leading in severe atherothrombotic diseases. Thrombolytic medications are used to prevent this problem **[7]**.

Streptokinase, urokinase, and tissue plasminogen activator are the most often utilised thrombolytic drugs, even yet these molecules still possess a few adverse effects, such as severe bleeding, bronchospasm, and dyspnea. These effects can be prevented by using herbal products. Because plants contain a wide variety of bioactive substances, herbal remedies have been utilised to treat a wide range of illnesses since ancient times **[8]**.

Inflammation

Inflammation is the body's extreme response to any sort of damage. It's a complex, dynamic process that affects a variety of bodily systems. Pain, redness, heat or warmth, and swelling are all signs of inflammation. Rheumatic illness is becoming a widespread inflammatory disease all over the world. Examples of anti-inflammatory medications include aspirin, celecoxib, diclofenac, ibuprofen, indomethacin, and other NSAIDs **[9]**.

The main drawbacks of today's synthetic anti-inflammatory medications, including steroidal and non-steroidal medications, were their toxicity and the recurrence of problems after medication was stopped. Traditional medicine plays a vital role in healthcare, several plant extracts and individual plant parts work well as anti-inflammatory agents **[10]**.

MATERIAL AND METHODS

Collection of plants

In August 2021, fresh leaves of the selected *Hevea brasiliensis* plant were harvested in the South Tripura area of India. Badal Kumar Datta, a Botanist Taxonomist and Professor in the Department of Botany at Tripura University, assisted in identifying the leaves. The accession number of the specimens is as follows: *Hevea brasiliensis* (Willd. Ex A. Juss.) Mull.Arg. accession no.4424.

Processing of the plants materials

The collected leaves were extensively cleansed with distilled water, cleaned, and chopped into pieces before being thoroughly air dried. The pieces were then ground in a grinder and passed through a sieve (mesh no. 40) to get powder material of the same size. These powders were used in extraction.

Preparation of Plant Extract

In a Soxhlet apparatus, dried leaves powder (30 g) was packed individually. The extracts were made using a sequential extraction procedure that used a succession of natural solvents in a polarity order based on their dipole moments. To extract the polar mixture and -non-polar, a sample plant powder (30 g / 350 mL) was extracted with petroleum ether, n-hexane,

chloroform, methanol, and 50% ethanol using a soxhlet apparatus at 40° C for 72 hours. After extracting residues from petroleum ether, they were extracted in order with n-hexane, chloroform, methanol, and 50 percent ethanol **[11]**. The following solvent was used to extract residues discovered in the previous extraction stage. The extract was filtered using Whatman No. 1filter paper, which was then submerged in the rotary vacuum evaporator at 40° C under decreased pressure (IKA HB 10). For testing, the extract was dried in a water bath at 40° C, measured, and kept at 4° C in storage containers **[12]**.

The percentages of extract yield were pet ether 6.8%, n hexane 1.6%, chloroform 15.6%, methanol 16%, and 50% ethanol 15.66%.

***In vitro* Thrombolysis Activity: Phosphate buffered saline**

5 ml of the commercially available lyophilized streptokinase (15,000,000 I.U.) was added before combining (PBS). The suspension was used as a stock from which appropriate dilutions were made in order to evaluate thrombolytic activity. As mentioned above, experiments on clot lysis were carried out. In summary, 2 ml of venous blood collected from healthy participants was divided into three pre-weighed sterile microcentrifuge tubes (0.5 ml/tube) and incubated at 37°C for 45 minutes **[13]**. Following clot formation, serum was entirely withdrawn (aspirated out without disrupting the formed clot), and each tube containing clot was weighed again to estimate clot weight (clot weight = weight of clot containing tube – weight of tube alone) **[14, 15]**. 100, 200, 300, 400, and 500 µl of ethanolic extract (1 mg/ml) were added to each microcentrifuge tube containing a pre-weighed clot, respectively. Streptokinase was utilised in concentrations of 100, 200, 300, 400, and 500 µl as positive controls and 100 µl of distilled water as a non-thrombolytic, negative control. After that, the tubes were incubated for 90 minutes at 37°C while clot lysis was observed **[16]**. To assess the weight difference following clot breakup, the fluid discharged during incubation was removed, and the tubes were weighed once more. The weight difference recorded before and after clot lysis was reported as a percentage of clot lysis **[17]**.

Anti-inflammatory activity

Inhibition of protein denaturation

Protein denaturation inhibition assay was determined by the Baidya *et al*, (2022) method with slight modification. Various concentrations of plant extract (100, 200, 300, 400, and 500 µl) were combined with 500 micro liters of 1% bovine serum albumin **[18]**. The mixture was heated for 20 minutes at 51°C after chilling for 10 minutes at room temperature. A UV spectrometer (Shimadzu Corp. Model No-

01716) fixed at 660 nm was used to measure the resulting solution after it had cooled to room temperature. A positive control was performed using acetyl salicylic acid (100, 200, 300, 400, and 500 µl) **[19, 20]**. The experiment was conducted three times, and determines the % inhibition for protein denaturation.

The thrombolytic and anti-inflammatory properties of *Hevea brasiliensis* leaf extract have been investigated.

RESULTS

The percentage of successful clot lysis for five different concentrations of the plant extract, as well as for the positive thrombolytic control (streptokinase) and the negative thrombolytic control (distilled water), is statistically represented in **Table 1**. **Table 1** shows that when 100, 200, 300, 400, and 500 µl of streptokinase (30,000 I.U.) was used as a positive control, the percentage of clot lysis was 49.06 percent, 58.5 percent, 64.7 percent, 73.1 percent, and 82.8 percent, whereas in the case of distilled water (negative control), the percentage of clot lysis was negligible (13.3 percent). Between positive and negative controls, there was a highly significant difference in mean clot lysis %. When clots were treated with various concentrations (100, 200, 300,400 & 500 µl respectively) of the test sample, moderate clot lysis activity, i.e., 37.8 percent, 43.3 percent, 51.4 percent, 65.4 percent, and 75.3 percent, was observed. When compared with the negative control (water), the mean of percentage (%) of clot lysis was significant for all the concentrations (water). **Figure 1** depicts the percentage of clot lysis following administration of various amounts of the 50% ethanolic extract in addition to suitable controls **[21, 22]**.

The albumin denaturation assay was performed to assess *in-vitro* anti-inflammatory activity, and aspirin was utilised as the reference medication in the study. The *in-vitro* anti-inflammatory activity of a 50% ethanol extract of *Hevea brasiliensis* demonstrated inhibition of protein denaturation (mean %), i.e., 46.1±0.11, 56.03± 0.76, 65.7±0.52, 70.7±0.5, 74.7±0.27 at 100, 200, 300, 400, and 500 µg/ml respectively whereas, standard drug (aspirin) was recorded at similar various concentrations 56.4±0.31, 65.6±0.71, 70.5±0.43, 75.1±0.21, 80.1±0.18. Percentage of inhibition after treatment with different concentrations of the 50% ethanolic extract and standard drug is shown in **Figure 1**.

Table 1: *In vitro* thrombolytic activity of *Hevea brasiliensis* leaves extract

Concentration (µl/ml)	Negative Control (Distilled water)	(*Hevea brasiliensis* 50% ethanolic Extract) (Mean±SEM)	Positive Control (Streptokinase) (Mean±SEM)
100	13.3±0.9	37.8±0.8	49.06±0.93
200	13.3±0.9	43.3±0.48	58.5±0.48
300	13.3±0.9	51.4±0.33	64.7±0.7
400	13.3±0.9	65.4±0.79	73.1±0.56
500	13.3±0.9	75.3±0.98	82.8±0.71

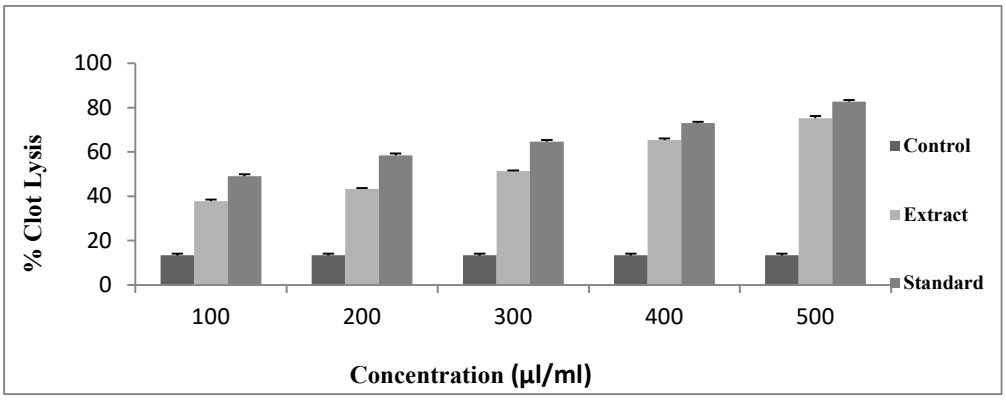

Figure 1: *In vitro* thrombolytic activity of *Hevea brasiliensis* leaves extract

Table 2: *In vitro* anti-inflammatory activity of *Hevea brasiliensis* leaves extract

Concentration (µl/ml)	% Inhibition (*Hevea brasiliensis* 50% ethanolic Extract) (Mean ± SEM)	%Inhibition (Standard) (Mean ± SEM)
100	46.1±0.11	56.4±0.31
200	56.03± 0.76	65.6±0.71
300	65.7±0.52	70.5±0.43
400	70.7±0.5	75.1±0.21
500	74.7±0.27	80.1±0.18

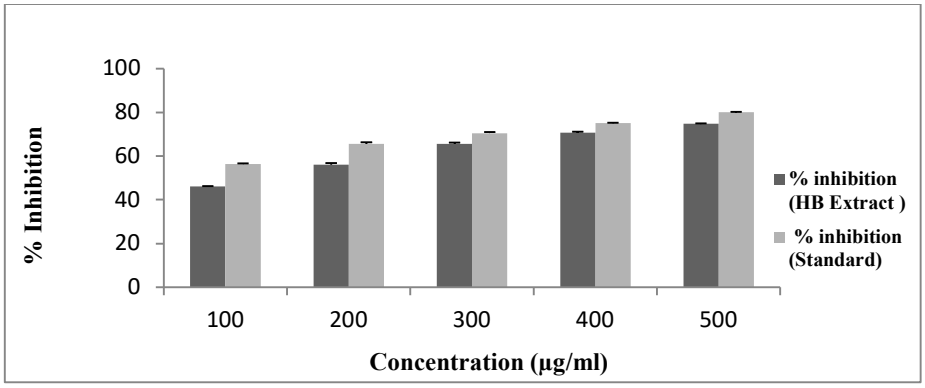

Figure 2: *In vitro* anti-inflammatory activity of *Hevea brasiliensis* leaves extract

DISCUSSION

Platelets serve a crucial role in atherothrombosis by sticking to the disturbed endothelium surface, hence initiating plaque formation and development **[23]**. Plasmin, a natural fibrinolytic substance, aids in the breakdown of fibrinogen and fibrin and begins clot lysis **[24]**. To alter plasmin, SK typically forms a stoichiometric compound with plasminogen. The extractives of *Hevea brasilliensis* were examined as part

of the inquiry into the thrombolytic actions of natural sources; the findings are shown in **Figure 1**. *Hevea brasiliensis* contains alkaloids, tannins, flavonoids, and phenol, which may contribute to blood clot lysis **[25, 26]**.

The fundamental cause of inflammation is protein denaturation. The capacity of the extract to reduce protein denaturation was tested as part of the research into the mechanism of anti-inflammatory effect **[27, 28]**. Selected extracts were helpful in preventing albumin denaturation. The extractives of *Hevea brasilliensis* were examined as part of the study into the anti-inflammatory properties of natural sources; the findings are shown in **Figure 2**.

The study's findings demonstrated that the extract has the capacity to suppress protein denaturation and hence may provide considerable alleviation in inflammation **[29, 30]**. The study's findings revealed that the plant extract may inhibit the synthesis of inflammatory mediators including prostaglandins and leukotrienes. The presence of alkaloids and polyphenols in plant extract may indicate its anti-inflammatory action.

CONCLUSION

The 50% ethanolic extracts have significant thrombolytic and anti-inflammatory effects. As a result, it is possible to conclude that the presence of phytoconstituents in the leaf extracts is responsible for the thrombolytic and anti-inflammatory actions of the 50% ethanolic extracts.

The current study's findings confirm the ancient use of *Hevea brasiliensis* ethanolic extracts in the treatment of thrombolytic and inflammatory diseases.

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DECLARATION

Conflict of interest

The authors state that there is no conflict of interest with this study.

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REFERENCES

[1] Handin RI, 2005. "Chapter 53-bleeding and thrombosis", In Kasper DL, Braunwald E, Fauci AS, *et al*, Harrison's Principles of Internal Medicine.

[2] Fahy JV, 2006. Anti-IgE- Lessons learned from effects on airway inflammation and asthma exacerbation, The Journal of Allergy and Clinical Immunology.pp. 117.

[3] CK Kokate, 2008. Pharmacognosy, Nirali Prakashan, 55th ed, Warangal, India, pp-601-607.

[4] Mohammad AS, Md Rafiqul I, Tahmida KC, *et al*, 2017. Investigation of in vivo Analgesic, Anti-Inflammatory, in vitro Membrane Stabilizing and Thrombolytic Activities

of Atylosia scarabaeoides and Crotalaria spectabilis Leaves, Journal of Pharmacology and Toxicology, 12,120-128.

[5] Baidya M, Anbu J, Akhtar MS, *et al*, 2020. Antimicrobial Evaluation of Ethanolic Extract of Selected Seed Shells, International Journal of Current Pharmaceutical Research, 12, pp.74-6.

[6] Sarvan KG, Narender M, Umasankar K, *et al*, 2021. Phytochemical investigation and In vitro Thrombolytic activity of Terminalia pallida Brandis leaves, Research Journal of Pharmacy and Technology. 14, pp.879-882.

[7] Das M, Ram A, Ghosh B, 2003. Luteolin alleviates bronchoconstriction and airway hyperreactivity in ovalbumin sensitized mice, Inflammation Research, 52, pp.101-106.

[8] Baidya M, Jayaraman A, Maji HS, Ramya Krishna PS, Das D, 2022. Evaluation of acute and sub-acute toxicity of sivanar amirtham in albino mice and wistar albino rats, Journal of Medical Pharmaceutical and Allied sciences, 11, pp. 4196 - 4204. doi: 10.22270/jmpas. V11I1.2217.

[9] Ramya Krishna PS, Jayaraman A, Baidya M, Nayak DA, 2022. Toxicological evaluation of fucoidan, a polysaccharide isolated from *Turbinaria conoides* (J.agardh) Kutzing procured from mandapam coastal area, tamilnadu, Journal of Medical Pharmaceutical and Allied Sciences, 11, pp.4629–4635.doi: 10.55522/jmpas.V11I2.2511.

[10] Islam MA, Mahmud ZA, Rahman SMA, *et al*, 2013. Evaluation of Thrombolytic activity and Brine Shrimp Lethality Bioassay of Methanol extract of stems of *Tinospora crispa*, International Journal of Pharmaceutical Science and Research, 4, pp. 1148-1153.

[11] Baidya M, Maji HS, Bhatt S, Das D, 2022. In-vitro Evaluation of the Thrombolytic and Anti-inflammatory Activity of *Capparis sepiaria* Root Extracts, Journal of Medical Pharmaceutical and Allied Sciences, 11(1), pp. 4166-4171. doi: 10.22270/jmpas.V11I1.1879.

[12] Mandal SK, dawn S, Mondal S, Sarkar S *et al*.,2021. The therapeutic versatility of quinolines, International Journal of Biology, Pharmacy and Allied Sciences, 10(7), pp.2161-2181.doi.org/10.31032/IJBPAS/2021/10.7.5528.

[13] Elumalai A, Chinna EM, Vidhyulatha CCH, *et al*, 2012. Screening of Thrombolytic Activity of *Bougainvillea glabra* Leaves Extract, Asian Journal of Research in Pharmaceutical Sciences, 2, 134-6.

[14] Mandal SK, Biswas D, Sony A, *et al*, 2021. Plants and phytochemicals in the treatment of colorectal Cancer: recent updates, International Journal

of Biology, Pharmacy and Allied Sciences, 10(10), pp.3502-3521.doi.org/10.31032/IJBPAS/2021/10.10.5657.

[15] Banerjee S, Chanda A, Adhikari A, *et al*, 2014. Evaluation of Phytochemical Screening and Anti-Inflammatory Activity of Leaves and Stem of *Mikania scandens* (L.) Wild, Annals of Medical and Health Science Research, 4, pp. 532–6.

[16] Uddin MS, Millat MS, Islam MS, *et al*, 2020. Exploration of in vitro thrombolytic, anthelminthic, cytotoxic and in vivo anxiolytic potentials with phytochemical screening of flowers of Brassica *nigra*, Future Journal of Pharmaceutical Sciences, 6, pp.73.

[17] Vaibhav PU, Sushil YR, Sachin MH, *et al*, 2021. Pharmacological assessment of antiulcer activity of Gloriosa Superba linn tubers in experimentally induced gastric ulcers, Journal of Medical Pharmaceutical and Allied Sciences,10,pp.2852-2856.

[18] Mizushima Y, Kobayashi M, 1968. Interaction of anti-inflammatory drugs with serum proteins, especially with some biologically active proteins, Journal of Pharmacy and Pharmacology, 20, pp.169-73.

[19] Vaibhav PU, Sushil YR, Sachin MH, *et al*, 2021. Pharmacological assessment of antiulcer activity of Gloriosa Superba linn tubers in experimentally induced gastric ulcers, Journal of Medical Pharmaceutical and Allied Sciences, 10,pp. 2852-2856.

[20] Uddin MS, Millat MS, Islam MS, *et al*, 2020. Exploration of in vitro thrombolytic, anthelminthic, cytotoxic and in vivo anxiolytic potentials with phytochemical screening of flowers of Brassica *nigra*. Future Journal of Pharmaceutical Sciences, 6,pp.73.

[21] Mizushima Y, Kobayashi M, 1968. Interaction of anti-inflammatory drugs with serum proteins, especially with some biologically active proteins, Journal of Pharmacy and Pharmacology, 20, pp.169-73.

[22] Rajeswari S, Vidhya R, 2017. Evaluation of in vitro thrombolytic and antiproteinase activities of *wedelia trilobata* (linn), Innovare Journal of Life Sciences. 5, pp.6-10.

[23] Edeoga HO, Okwu DE, Mbaebie BO, 2005. Phytochemical constituents of some Nigerian medicinal plants, African Journal of Biotechnology, 4, pp. 685-8.

[24] Yada D, Sivakkumar T, Srinivas N, 2021. Aqueous extract of whole plant of hyptis suaveolens (l) Poit- An antiulcer agent, Journal of Medical Pharmaceutical and Allied Sciences, 10 (4), pp. 4187–3190.

[25] M. H. Sanad, F. A. Marzook*a*, S. K. Mandal, M. Baidya, 2022.

Radiocomplexation and Biological Evaluation of [99mTc] Tricarbonyl Rabeprazole as a Radiotracer for Peptic Ulcer Localization, Radiochemistry, 64(2), 210–21. doi: 10.1134/S1066362222020138.

[26] Sakat S, Juvekar AR, Gambhire MN, 2010. *In vitro* antioxidant and anti-inflammatory activity of methanol extract of *Oxalis corniculata* Linn, International Journal of Pharmacy and Pharmaceutical Sciences,2 (1), pp.146-55.

[27] Shinde UA, Phadke AS, Nari AM, *et al*, 1999. Membrane stabilization activity- a possible mechanism of action for the anti-inflammatory activity of *Cedrus deodara* wood oil, Fitoterapia, 70 (3), pp. 251-7.

[28] Biresh Kumar Sarkar, Ravi Kumar, Reeta, SC Verma, *et al*, 2017. Evaluation of *in vitro* anti-inflammatory activity and HPTLC analysis of plant *Phyllanthus fraternus*, International Journal of Current Pharmaceutical Research, 9 (5), pp.198-200.

[29] Ramadevi M, Sivasubramanian N, Selvan AT, *et al*, 2014. Screening of *in vitro* anti-inflammatory activity of *Ficus virens* bark, Journal of Global Trends in Pharmaceutical Sciences, 5, pp.2034-6.

[30] Giri SN, Biswas AK, Saha BP *et al*, 1998. Studies of the anti-inflammatory action of *Bougainvillea glabra* leaves. Indian Journal of Pharmaceutical Sciences, 50, pp.42-49.