Toxicological Evaluation of *Rubia cordifolia* Root Extract on Brain and Spleen of Rat Model

By

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A thesis submitted to the School of Pharmacy in partial fulfillment of the requirements for the degree of Bachelor of Pharmacy

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Declaration

It is hereby declared that

1. The thesis submitted is our own original work while completing degree at Brac University.
2. The thesis does not contain material previously published or written by a third party, except where this is appropriately cited through full and accurate referencing.
3. The thesis does not contain material which has been accepted, or submitted, for any other degree or diploma at a university or other institution.
4. We have acknowledged all main sources of help.

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Approval

The thesis titled "Toxicological Evaluation of *Rubia cordifolia* Root Extract on Brain and Spleen of Rat Model" submitted by Faria Billal Shaolin (20346024), of summer, 2020 has been accepted as satisfactory in partial fulfillment of the requirement for the degree of Bachelor of Pharmacy.

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Ethics Statement

Ethical approval has been granted by the Biosafety, Biosecure and Ethical Committee, Faculty of Biological Sciences, Jahangirnagar University, Savar, Dhaka, Bangladesh [Ref No: BBEC, JU/M 2024/ 11 (154)].

Abstract

Rubia cordifolia, commonly known as Manjistha, is one of the plants which has great significance in traditional medicine systems for its wide use in therapeutic field. However, less studies have been conducted with its toxicity tests. Therefore, we conducted this study with a view to evaluating its toxicity. This evaluation was done by a histopathological study on rat model which involved a control group that was administered with distilled water, a treated group L that was administered with low dose (150 mg/kg) of root extract and a treated group H that was administered with high dose (1500 mg/kg) of the root extract having 6 rats in each group. After 28 days' treatment, no noticeable changes were found in the specimens of either of the organs. However, in the specimen of spleen treated with high dose of Manjistha root extract, a mildly increased megakaryocytes and increased deposition of hemosiderin pigments had been observed. More research in this field needs to be conducted to confirm its toxicity.

Key words: '*Rubia cordifolia L.*', '*Rubia cordifolia* and toxicity', 'characterization of *R. cordifolia*', '*R. cordifolia* and toxicological evaluation'.

Dedication

Dedicated to my family who have been the greatest strength and constant support system of mine in all ups and downs.

Acknowledgement

First, I would like to express my gratitude to the Almighty Allah, for all the opportunities and privileges that I have had in my life.

I would also like to show my earnest gratitude to my supervisor Professor Dr. Sharmind Neelotpol for being extremely kind and supportive throughout this work. Without her constant guidance and motivation, I would not be able to pass this part of my academic journey so smoothly.

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List of Acronyms

WHO	The World Health Organization
TCM	Traditional Chinese Medicine
OECD	Organisation for Economic Co-operation and Development
CNS	Central Nervous System
iNOS	Inducible Nitric Oxide Synthase
TD	Tardive Dyskinesia
GABA	Gamma- aminobutyric Acid
TCP	*Terminalia chebula* Retz. Polysaccharide
RTCP	*Rubia cordifolia* L. processed *Terminalia chebula* Retz Polysaccharide
CTX	Cyclophosphamide
WBC	White Blood Cells
MNJ	Manjistha

Chapter 1

Introduction

According to the World Health Organization (WHO), Herbal Medicine is a major source of primary healthcare for about 80% of people worldwide (Ekor., 2014; Wen et al., 2022). *Rubiaceae* is one among such plant families which has great significance in terms of usage in several traditional medicine systems. About 450 genera and 6,500 species, including trees, shrubs and herbs, make up this group. Approximately among 60 species of *Rubia*, *Rubia cordifolia* is a perennial herbaceous climbing plant with long, cylindrical, red roots (Adwankar et al., 1980; Dengre et al., 1993).

In Africa, tropical Asia, India, China, Japan, Malaysia, and tropical Australia, *R.cordifolia* is widely dispersed (Adwankar et al., 1980). Traditionally, it is known as Indian madder or Manjith or Manjistha in India and *Qiancaogen* and *Chien-tsao* in China, which is extensively dispersed throughout the majority of China, particularly in the provinces of Henan, Hebei, Shaanxi, Anhui, Shandong, Jiangsu, and Zhejiang (Dengre et al., 1993).

A review article stated that *R.cordifolia* is abundant in about 100 chemical components, mainly including anthraquinone glycosides, naphthoquinone glycosides, bicyclic hexapeptides, triterpenoids, polysaccharides and many more due to the presence of which this plant especially exhibits quite diversified pharmacological effects and possesses the potential to cure multiple severe diseases (Son et al., 2008; Itokawa et al., 1993; Rao et al., 2006; Gao et al., 2016; Natarajan et al., 2019; Wen et al., 2022).

Figure 1: *R. cordifolia* Plant (Chandraker et al., 2022).

Figure 2: *R. cordifolia* Root (Adeel et al., 2023).

Many comprehensive studies introduced the pharmacological effects of *R.cordifolia* which include anti-inflammation, antioxidation, anti-proliferation, immunomodulation, neuroprotection, antibacterial, anti-platelet aggregation, anti-nephrotoxicity, anti-tumor, anti-cancer effect etc. (Humbare et al., 2022).

For instance, in Chinese Medicine, *R. cordifolia* is a powerful herb in Traditional Medicine. It's praised for its ability to cool the blood and improve circulation, which helps to stop bleeding. This makes it a go-to remedy for issues like vomiting blood, nosebleeds, excessive menstrual bleeding, and various types of injuries. (Xu et al., 2002; Li et al., 2016; Wen et al., 2022). The plant's water extract is also taken orally to alleviate diarrhea, demonstrating its wide-ranging therapeutic uses (Shi et al., 2014).

Similar to Traditional Chinese Medicine (TCM), the use of *R. cordifolia* as a phytomedicine has been documented in the traditional Indian medicine systems of Ayurveda and Siddha. In India, this drug is used to address conditions like rheumatism (*Shi Feng Bi)*, menstrual discomfort, urinary issues, fluid retention, paralysis, lack of menstrual periods, and jaundice. (Tripathi et al., 1993; Adams et al., 2009). In Ayurveda, *R. cordifolia* is not just valued for its therapeutic properties but is also used as a natural dye for medicinal oils. Additionally, it's applied to inflamed areas, ulcers, and fractures to promote healing (Deshkar et al., 2008; Patil et al., 2009).

In Korea, *R. cordifolia* is a popular traditional remedy for menstrual pain, arthritis, rheumatism, bleeding issues, and urinary problems (Do et al., 2013). In Unani medicine, its dark red root is valued for soothing liver conditions, easing menstrual cramps, paralysis, jaundice, amenorrhea, various skin disorders, kidney stones, and purifying the blood. Traditional healers in Uganda use it to treat tuberculosis, while in the Philippines, a root decoction is commonly used to address urinary tract issues (Patil et al., 2009).

Even if there are many established and well proven pharmacological effects of *R.cordifolia* plant especially the root extracts for different organs including heart, liver, uterus, kidney, colon etc. (Eskandarzadeh et al., 2023) which are backed by multiple studies, there have not been sufficient histopathological studies regarding any strong pharmacological effect of this plant root extract on two very crucial organs of human body- brain and spleen.

In addition, along with the pharmacological effects, finding out whether this same root extract can be causing any toxicity to those specific organs or not that needs to be explored too with proper conduction of adequate scientific analysis.

Aim of the study:

The aim of this histopathological study is to evaluate the toxicological effects of *Rubia cordifolia* root extract in brain and spleen.

Objectives of the study:

The objectives of the study are to-

i. review different articles evaluating whether *R. cordifolia* root extract has any effect on brain and spleen.
ii. figure out whether *R. cordifolia* root extract produces any toxicity in brain and spleen.

Chapter 2

Methodology

Pharmacological effects have been reviewed and toxicological effects of *R. cordifolia* root extract on brain and spleen have been examined by histopathological study. In order to achieve the first objective of this research, the existing studies which have been conducted till date for analyzing the biological effects of *R. cordifolia* root on brain and spleen have been thoroughly reviewed. In the meantime, for achieving the second objective, a histopathological study was designed and performed accordingly on male white albino (Sprague-Dawley) model using different doses of *R. cordifolia* root extract to observe even if not in low dose, whether this plant root extract may exhibit any toxic effect in high dose or not.

2.1 Literature Review

ScienceDirect, PubMed database, Google Scholar, Web of Science, ResearchGate have been used for searching and reviewing research articles and journals. In order to search information that are relevant to this study certain key words such as '*Rubiaceae',* '*Rubieae', 'Rubia cordifolia L.*', '*Rubia cordifolia* and toxicity', 'characterization of *R. cordifolia*', '*R. cordifolia* and pharmacological evaluation', 'efficacy on neuro and spleen disease', 'protective effects on spleen', 'immunosuppression', 'alternative medicine', 'munjistin', 'mollugin' etc. were used.

2.2 *R. cordifolia* Root Extraction

R. cordifolia plant material had been collected from Sylhet and then the fresh, healthy root parts were thoroughly washed in water, cut into smaller parts, chopped and shed dried at 35º – 40º C for a week. Then, they were pulverized in an electric grinder to get extractable powder. 1g of the powder was then extracted in 4.5ml of ethanol (96%). Then, using a Rotary evaporator

under reduced pressure the collected mixture of active constituent with ethanol was dried and the final ethanol extract was obtained.

2.3 Preparation of Drug Doses

After obtaining the crude *R. cordifolia* root extract, two separate doses were made with that. Doses were of 150 mg/kg (lower dose) and 1500 mg/kg (higher dose) respectively, which were administered via oral gavage.

2.4 Experimental Animals

Male white albino rats, Sprague Dawley, with a weight range of 70-90 gm were kept in the pharmacology laboratory of Jahangirnagar University. Throughout the experimental period, all rats were caged in a (60 ×38 ×20 cm3) cage and housed with ad libitum access to food and water under a controlled temperature of 21±5°C with a 12-h light/dark cycle. Throughout the experimental period, all the animals were ensured to be treated humanely with maximum care and all of the experimental protocols were performed following the Organisation for Economic Co-operation and Development (OECD) guideline number 407 entitled as "Repeated Dose 28-day oral Toxicity Study in Rodents".

2.5 Study Design

The rats were divided into 3 groups consisting of 6 rats per group. These 3 groups included treated group L which had been treated with a low dose of 150 mg/kg of Manjistha (Siddique et al., 2011), treated group H which had been treated with a high dose of 1500 mg/kg of Manjistha and lastly, a control group which had been given distilled water instead of any dose of Manjistha. All the rats had been fed for 28 consecutive days and had been given dose of 1ml/day by oral gavage during daytime between 10am-2pm. Initial body weights were recorded

and weight measurement kept continued throughout the study period with an interval of four days.

Table 1: Grouping of Experimental Rats:

Group	Administered substance	Dose	Frequency
Group I (Control) n=6	Distilled water	1ml/kg body weight	Once daily for 28 days
Group II (MNJ Low Dose) n=6	Prepared low dose of plant extract	1ml/kg body weight	Once daily for 28 days
Group III (MNJ High Dose) n=6	Prepared high dose of plant extract	1ml/kg body weight	Once daily for 28 days

The body weights of all rats were measured by sensitive scale while acclimatization, before the starting of dose and once on the dissection day.

2.6 Histopathology

After repetitive oral dosing of plant extract for 28 days, on the 29th day rats were euthanized using an overdose of ketamine which was 500 mg/kg, i.p. and the death was confirmed by "toe pinch". The brain and spleen were dissected with care and preserved in 10% neutral buffered formalin.

After two weeks, these brains and spleens of each rat were routinely processed for histological studies for which Haematoxylin and Eosin (H&E) staining method had been used. Lastly, those respective tissues had been examined under a light microscope (Olympus, CX43, Japan) for visualization; followed by taking photomicrographs of them with the help of Olympus, D22 camera.

2.7 Ethical Approval

Ethical approval has been granted by the Biosafety, Biosecurity and Ethical Committee, Faculty of Biological Sciences, Jahangirnagar University, Savar, Dhaka, Bangladesh [Ref No: BBEC, JU/M 2024/ 11 (154)]. Throughout the experimental period animals had been treated and handled following internationally accepted guideline.

Chapter 3

Results and Discussion

3.1 Literature Review

While going through the existing research articles related to Manjistha, some credible information regarding the pharmacological effects of the plant root on brain and spleen have been found.

Verma et al. (2019) stated in one of his studies that rubiadin suspension which is isolated from *R. cordifolia* root, at a higher concentration (i.e. 250 mg/kg, p.o.) had been observed to significantly delay the onset of action of convulsions. This in turn presented the anti-convulsant activity of the root extract.

In a review about the *Rubia* genus, Eskandarzadeh et al. (2023) thoroughly presented the variety of therapeutic effects that *R. cordifolia* root may have on brain. It stated that Alzheimer's disease is linked to the buildup of amyloid-beta and tau proteins in the Central Nervous System (CNS), leading to cognitive decline. Injecting β-amyloid into the brain's ventricles can cause such dysfunctions, but this harmful effect is counteracted by the administration of an ethanolic extract from the root of *Rubia cordifolia* Linn. This extract has shown promise in preventing the cognitive issues associated with Alzheimer's (Chitra et al., 2009).

As well as, oxidative stress causes several pathophysiological condition in the body which start with free radicals and lead to apoptosis, necrosis, and cell death that potentially cause a CNS neurodegenerative disease. By restoring glutathione peroxidase activity, reducing the expression of Inducible Nitric Oxide Synthase (iNOS), and raising the antioxidant gene Cu-Zn superoxide dismutase, extracts of Rubia species roots showed promise as antioxidants (Rawal et al., 2004).

Potential neuroprotective and antioxidant activities of *R. cordifolia* root extract with methanol were proved on orofacial dyskinesia induced by reserpine administration. Tardive dyskinesia (TD) is caused by reserpine administration with the symptom of orofacial dyskinesia characterized by vacuous tongue protrusion, chewing movements and orofacial bursts. Extraction of the roots of *R. cordifolia* along with methanol remarkably inhibited orofacial dyskinesia and catalepsy induced by reserpine (Patil et al., 2012).

The review further explained that neuropathic pain can result from nerve damage affecting nociceptive receptors and descending pathways of CNS. Paclitaxel, a chemotherapy drug, is known to cause neurotoxicity, leading to such pain. However, the alcoholic extract from the roots and rhizomes of *Rubia cordifolia* Linn. showed both preventive and protective effects against paclitaxel-induced neuropathic pain in a dose-dependent manner. This extract worked significantly by inhibiting neuropathic pain through the Gamma-aminobutyric Acid (GABA) neurotransmitter and its antioxidant properties (Diwane et al., 2015).

A study by Chitra et al. (2009) stated that *R. cordifolia* extract administration noticeably reduced the β-amyloid induced cognitive and memory dysfunction. It also mentioned that long with possessing many therapeutic activities on brain, the extract decreases the neurodegeneration and helps in memory retention activity as well.

Since, this study involves evaluating pharmacological effects of Manjistha or *R. cordifolia* root extract on both brain and spleen, research works on spleen had also been reviewed. For instance, a study by He et al. (2023) revealed the efficacy of *R. cordifolia* root extract being used as *Rubia cordifolia* L. processed *Terminalia chebula* Retz Polysaccharide (RTCP) compared to *Terminalia chebula* Retz Polysaccharide (TCP) in protecting spleen from Cyclophosphamide (CTX) induced injury by promoting the recovery of spleen organ index and repairing the spleen tissue structure.

Another study was conducted by Kannan et al. (2009) for immunological investigation of *R. cordifolia* root. This study on one side, proved the increased hematopoiesis which also indicated certain obvious improvements that took place due to *R. cordifolia* in spleen functions as spleen is a major organ playing crucial role in hematopoiesis (Coppin et al., 2018) ; on the other side, the study showed remarkable preventive effect of the plant root extract on immunosuppressant by which we could again speculate the efficacy of this root extract on

spleen since spleen is directly associated with filtering blood and improving immunosuppressive diseases (He et al., 2023).

3.2 Body Weight of Rats

Table 2: Body Weight of Rats Treated with MNJ and Observed for 28 Days:

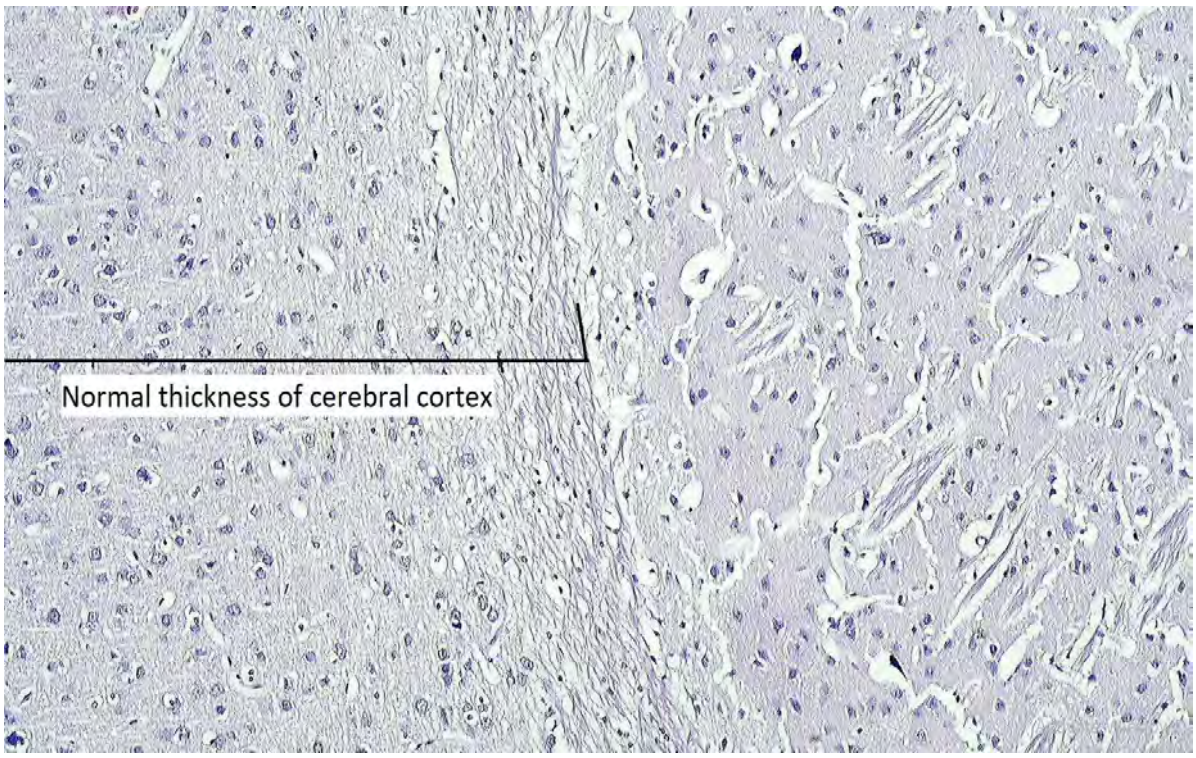
Days	Control (gm)	MNJ Low (gm)	MNJ High (gm)
	(Mean ± SEM)		

Day 0	75±2.50	74±2.45	79±6.59
Day 1	79.5±4.60	80.6±4.70	82.8±6.42
Day 4	82.42±4.80	87±5.38	85±7.13
Day 8	82.85±5.85	93±4.83	86.4±7.54
Day 12	88.14±6.24	105.4±5.37	94.8±8.30
Day 16	93.71±6.36	113.8±5.22	103.8±7.17
Day 20	108.14±6.82	131.6±4.88	116±5.35
Day 24	117.14±7.16	137.6±4.61	121±5.68
Day 28	120.85±7.33	143.6±5.19	124.2±4.39

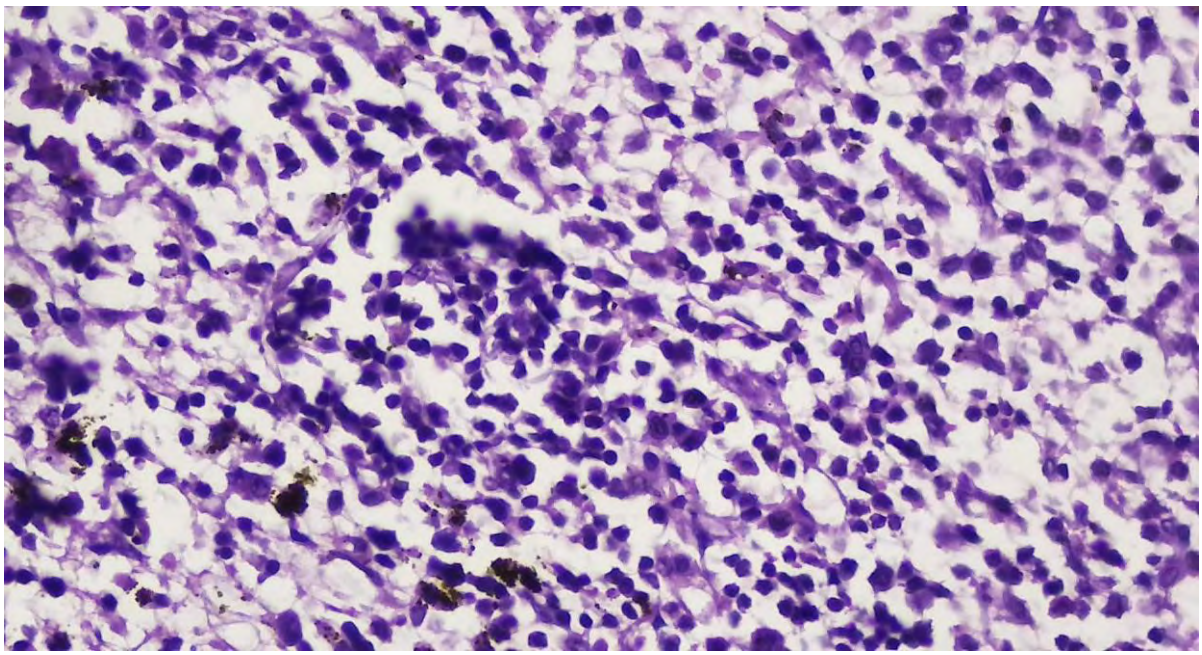
3.3 Histopathology

The microscopic observation of the control group specimens was unremarkable for both brain and spleen. Additionally, the observation of the treated group L (treated with low dose of Manjistha root extract) exhibited unremarkable change both in spleen and brain; whereas even

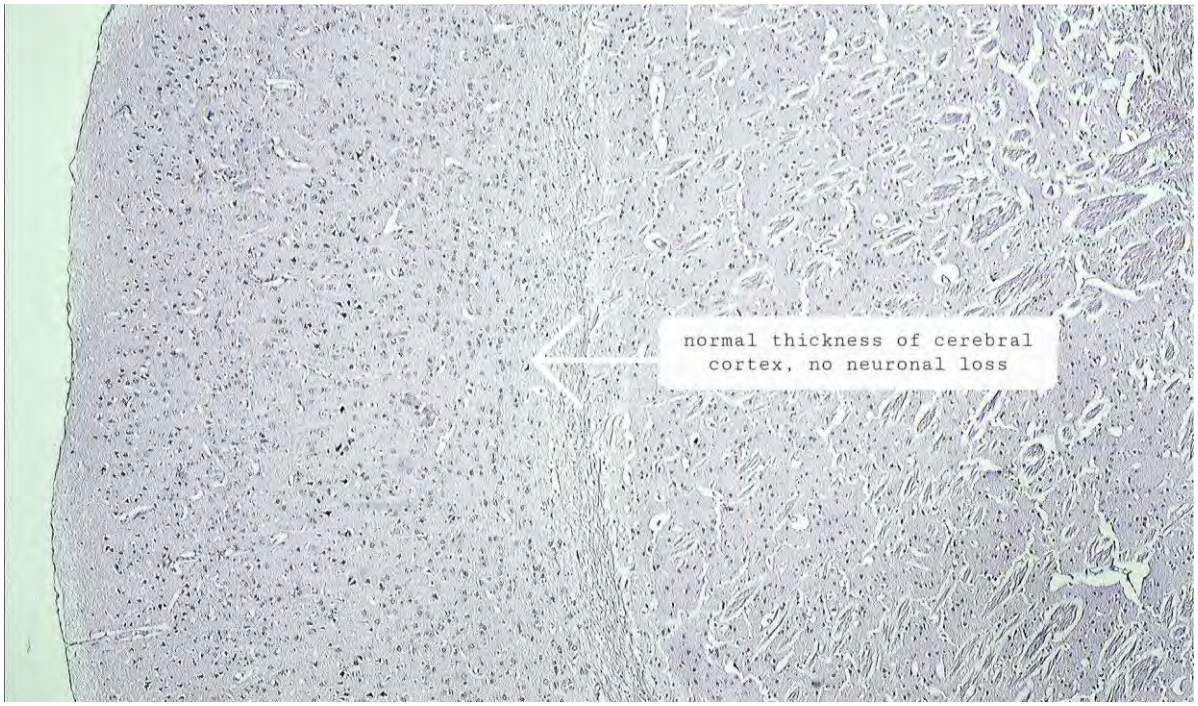
though mildly increased megakaryocytes and mildly increased deposition of hemosiderin pigments in spleen had been observed in treated group H, no noticeable change could be observed in brain in treated group H (treated with high dose of Manjistha root extract).



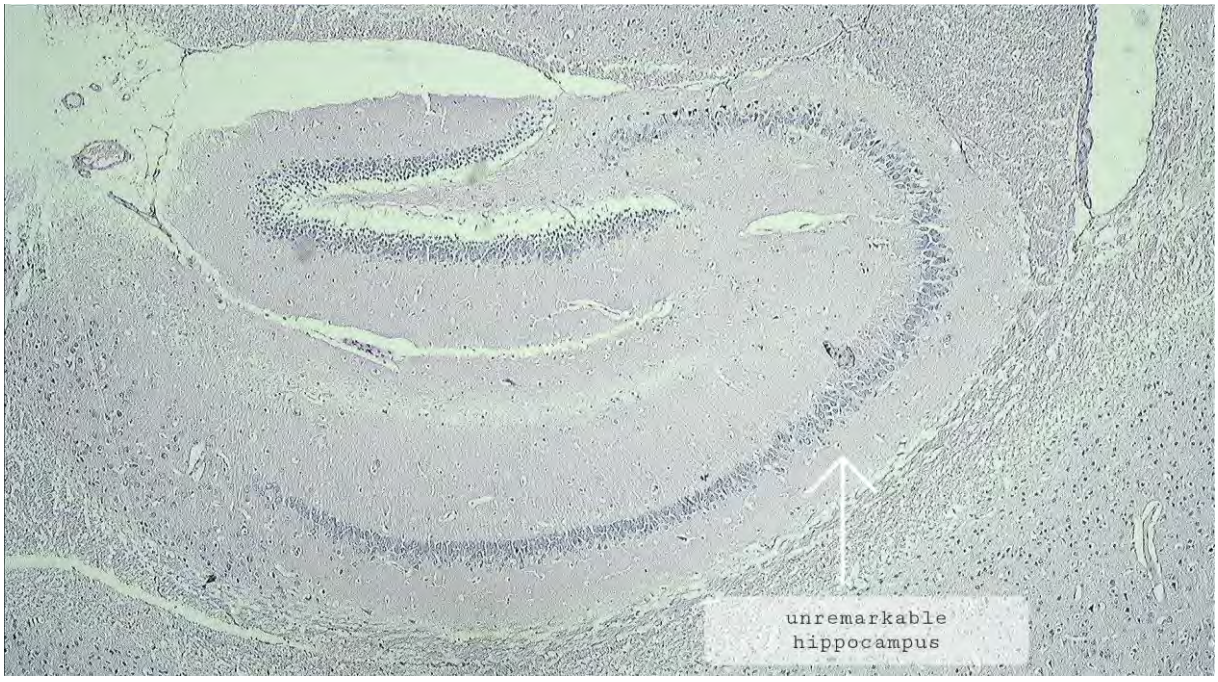
(A) Unremarkable microscopic observation in control group of brain (40X).



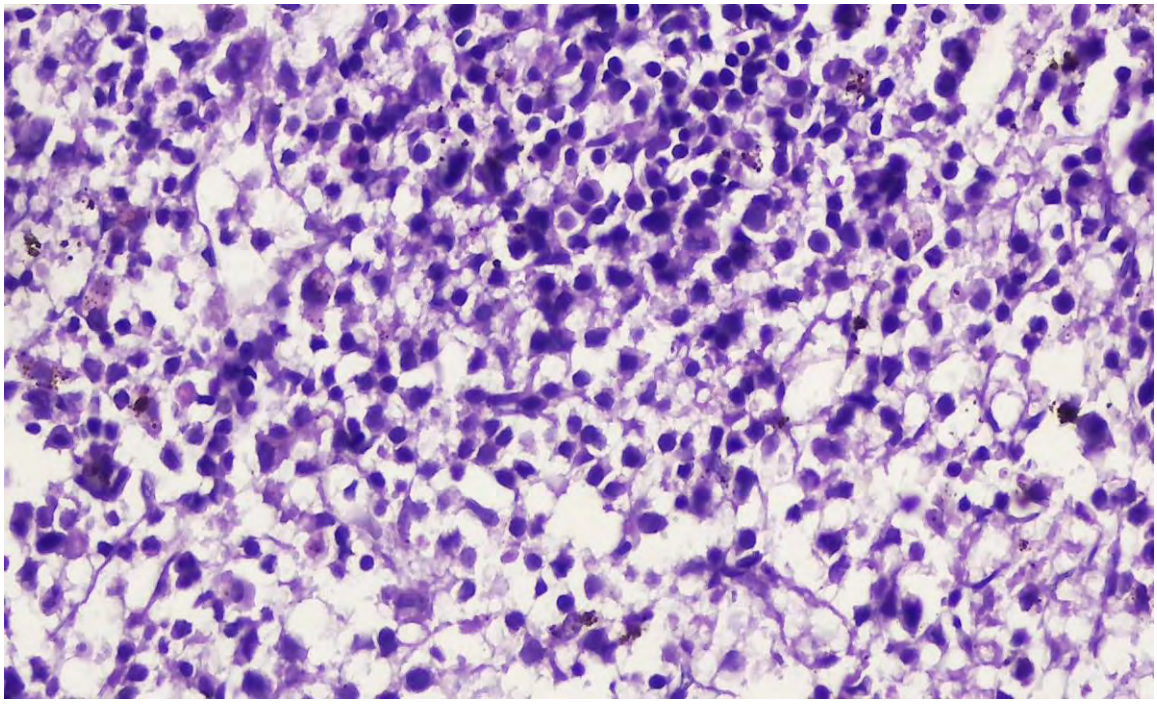
(B) Unremarkable microscopic observation in control group of spleen (40X).



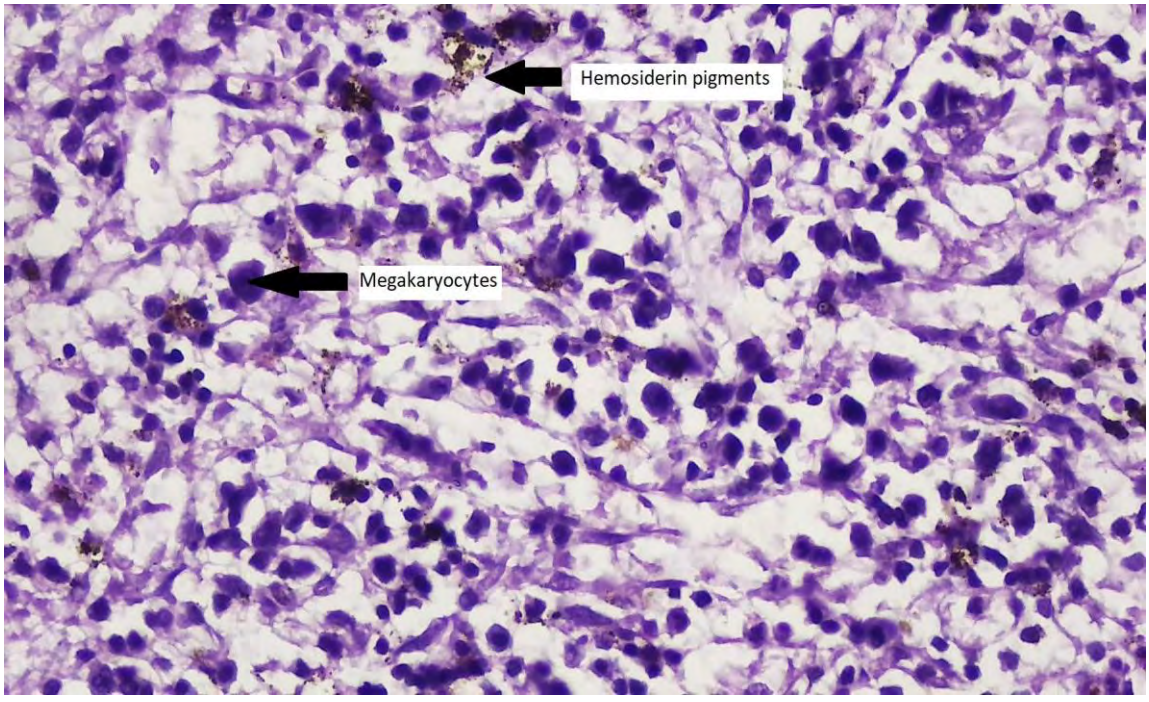
(C) Unremarkable microscopic observation in in treated group L of brain (40X).



(D) Indifferent microscopic observation in treated group H of brain (40X).



(E) Unremarkable microscopic observation in treated group L of spleen (40X).



(F) Mildly increased megakaryocytes and mildly increased deposition of hemosiderin pigments observed in treated group H of spleen (40X).

Figure 3: Histopathology of Rat Brain and Spleen

In one side, reviewing the relevant literatures, has brought up certain important effects of Manjistha or *R. cordifolia* root, specifically on brain and spleen; which means each of the found pharmacological effects (such as neuroprotection, antioxidation, memory retention etc. on brain and spleen organ index promotion, anti-immunosuppression, improved hematopoiesis etc. on spleen), are backed by reliable studies that enhances the credibility of those information.

Having said that, while treating with Manjistha root extract the body weights of rats were measured for 28 days long with a 4- day interval. This showed that the weight varied significantly in the treated groups relative to the control group. For instance, the body weight of the control group, treated group L and treated group H before starting the treatment were 75 ±2.50 gm, 74 ±2.45 gm and 79 ±6.59 gm. On day 1 of the treatment the weights became 79.5±4.60 gm, 80.6 ±4.70 gm and 82.8 ±6.42 gm respectively. On day 28, the weights were 120.85±7.33 gm for control group, 143.6±5.19 gm for treated group L and 124.2±4.39 gm for treated group H. This showed that with time the weights observed in treated groups kept increasing noticeably compared to the control group.

After that, the conducted histopathological studies exhibited unremarkable changes in control group for both respective organs. As well as, in treated group L, followed by the administration of a low dose of the plant root extract no significant change was noticed either in spleen or in brain: whereas, in case of treated group H, which was inserted with high dose of *R. cordifolia* root extract, mildly increased megakaryocytes and mildly increased deposition of hemosiderin pigments had been observed in the specimen (spleen). However, the high dose used in treated group H still did not bring any significant change in brain. Thus, even upon the provision of a high dose of *R. cordifolia* root extract did not render any significant damage to the organs; nor exhibited any potential sign of any toxic effect. Therefore, *R. cordifolia* root can be considered to be free of any toxicity induction potential on brain and spleen; hence a safer therapeutic option for several diseases related to those respective organs.

Chapter 4

Conclusion

4.1 Conclusion

R. cordifolia is a significant plant among the plants that have been used in the history of traditional medicine systems for centuries. In fact, phytochemical analysis revealed identification of eight major phytotherapeutic compounds in methanol root extract of *R. cordifolia* (Chandrashekar et al., 2018). Being inspired by the richness of therapeutic constituents in roots, in this study we tried to explore the strong pharmacological effects of this root extract specifically on brain and spleen by which from literature review we got to know that *R. cordifolia* root extract renders several major effects including neuroprotection, antioxidation, memory retention etc. on brain and spleen organ index promotion, anti-immunosuppression, improved hematopoiesis etc. on spleen.

At the same time, we conducted a histopathological study to assess if the same root extract at higher dose may cause any toxicity on the same organs or not. In this study, we noticed that neither of the organs were damaged or undergone severe changes upon administration of a high dose of 1500 mg/kg except that mildly increased megakaryocytes and mildly increased deposition of hemosiderin pigments had been observed in the specimen of spleen.

4.2 Limitations of the Study

The most prominent limitation of this study was that the study only included two doses (150 mg/kg and 1500 mg/kg) of the root extract. If more doses with larger variation could be incorporated, the results might have been varied markedly and the credibility of the results would have been much more as well. In that case, doses much higher than 1500 mg/kg could be used and whether this extract exhibit any toxic changes in those doses or not that could have been observed. The more doses used, the stronger the results become.

However, due to the financial constraints we could not incorporate such a variety of doses to be confirmed about the potentiality of toxicity induction of the constituents of *R. cordifolia* root extract.

4.3 Future Prospect

Nowadays, the phytotherapeutic effects of *R. cordifolia* have been coming to the attention of researchers; therefore, many studies are being conducted focused on this plant. Since major roles of different constituents of this plant root have already been found out for preventing or treating crucial diseases of brain and spleen, Ayurvedic medicine system should give more emphasis on the usage of this plant handling those conditions.

Furthermore, conduction of more toxicological studies of this plant root extract can provide confirmation regarding its capability of causing any toxicity. If no toxicity induction potentiality seemed, then passing through further tests and clinical trials, *R. cordifolia* root extract should be a well-recognized plant extract in the upcoming days to be used simultaneously with Ayurvedic medicine system in the modern medicine system as well.

References:

Adams, M., Berset, C., Kessler, M., & Hamburger, M. (2009). *Medicinal herbs for the treatment of rheumatic disorders—A survey of European herbals from the 16th and 17th century*. Journal of Ethnopharmacology, 121(3), 343–359. https://doi.org/10.1016/j.jep.2008.11.010

Adwankar, M. K., Chitnis, M. P., Khandalekar, D. D., & Bhadsavale, C. G. (1980). *Anti-cancer activity of the extracts of Rubia cordifolia Linn.* (NSC b668893). Indian Journal of Experimental Biology, 18(1), 102. https://pubmed.ncbi.nlm.nih.gov/7399571/

Anantharaman, A., Priya, R. R., Hemachandran, H., Akella, S., Rajasekaran, C., Ganesh, J., Fulzele, D. P., & Siva, R. (2015). *Toxicity study of dibutyl phthalate of Rubia cordifolia fruits:in vivo and in silico analysis.* Environmental Toxicology, 31(9), 1059–1067. https://doi.org/10.1002/tox.22115

Bana, S., Kumar, N., Sartaj, A., Abdulsalam Alhalmi, Ashraf Ahmed Qurtam, Nasr, F. A., Al-Zharani, M., Singh, N., Gaur, P., Mishra, R., Bhardwaj, S., Ali, H., & Goel, R. (2023). *Rubia cordifolia L. Attenuates Diabetic Neuropathy by Inhibiting Apoptosis and Oxidative Stress in Rats.* Pharmaceuticals, 16(11), 1586–1586. https://doi.org/10.3390/ph16111586

Bhat, B. A., 1, Shergojri, F. A., 1, Gaur, M., 2, Shammi, Q. J., 3, *A comprehensive review on Rubia cardifolia (Manjistha)*. https://www.ijarse.com/images/fullpdf/1522672998_GGSP5059ijarse.pdf

Chandraker, S. K., Lal, M., Khanam, F., Dhruve,P., Singh, R. P., & Shukla, R. (2022). *Therapeutic potential of biogenic and optimized silver nanoparticles using Rubia cordifolia L. leaf extract.* Scientific Reports, 12(1). https://doi.org/10.1038/s41598-022-12878-y

Chandrashekar, B., Prabhakara, S., Mohan, T., Shabeer, D., Bhandare, B., Nalini, M., Sharmila, P., Meghana, D., Reddy, B. K., Hanumantha Rao, H., Sahajananda, H., & Anbazhagan, K. (2018). *Characterization of Rubia cordifolia L. root extract and its evaluation of cardioprotective effect in Wistar rat model*. Indian Journal of Pharmacology, 50(1), 12–21. https://doi.org/10.4103/ijp.IJP_418_17

Chitra V., Pavan Kumar K., (2009). *Neuroprotective studies of rubia cordifolia Linn. On β-amyloid induced cognitive dysfunction in mice.* Int. J Pharm Tech Res. 2009;1(4):1000-9. https://citeseerx.ist.psu.edu/document?repid=rep1&type=pdf&doi=7d6867377d684e96128935ebda718b5f87fe4267

Coppin, E., Florentin, J., Vasamsetti, S. B., Arunkumar, A., Sembrat, J., Rojas, M., & Dutta, P. (2018). *Splenic hematopoietic stem cells display a pre-activated phenotype*. Immunology and Cell Biology. https://doi.org/10.1111/imcb.12035

Dengre, R. G., Patel, K. N., & Chauhan, M. B. (1993). *Comparative studies of Rubia cordifolia linn. And Rubia tinctorum linn (rubiaceae).* Ancient Science of Life, 13(1-2), 165–179.

https://pubmed.ncbi.nlm.nih.gov/22556644/

Diwane, C., Patil, R., Vyavahare, P., & Bhambar, R. (2015). *Protective effect of Rubia cordifolia in paclitaxel-induced neuropathic pain in experimental animals*. Indian Journal of Pain, 29(3), 150. https://doi.org/10.4103/0970-5333.165833

Do, M. T., Hwang, Y. P., Kim, H. G., Na, M., & Jeong, H. G. (2013). *Mollugin inhibits proliferation and induces apoptosis by suppressing fatty acid synthase in HER2-overexpressing cancer cells*. Journal of Cellular Physiology, 228(5), 1087–1097. https://doi.org/10.1002/jcp.24258

Ekor, M. (2013). *The growing use of herbal medicines: issues relating to adverse reactions and challenges in monitoring safety.* Frontiers in Pharmacology, 4(177). https://doi.org/10.3389/fphar.2013.00177

Eskandarzadeh,M., Esmaeili, A., Nikbakht, M. R., Yukio Hitotsuyanagi, & Fard. (2023, January 1). *Genus Rubia: Therapeutic Effects and Toxicity: A Review.* https://www.researchgate.net/publication/364153433_Genus_Rubia_Therapeutic_Effects_and_Toxicity_A_Review

Gao, M., Yang, J., Wang, Z., Yang, B.-Y., Kuang, H.-X., Liu, L., Wang, L., & Yang, C. (2016). *Simultaneous Determination of Purpurin, Munjistin and Mollugin in Rat Plasma by Ultra High Performance Liquid Chromatography-Tandem Mass Spectrometry: Application to a Pharmacokinetic Study after Oral Administration of Rubia cordifolia L. Extract.* 21(6), 717–717. https://doi.org/10.3390/molecules21060717

Gupta, S. (2023, November 21). *IAFA Ayurveda*. IAFA for Allergy. https://www.iafaforallergy.com/dravya-herbs-part-a/manjistha-rubia-cordifolia/

He, M., Wu, Q., Wei, J., Zhang, Y., Guo, H., & Guo, X. (2022). *Research Note: Effect of Rubia cordifolia L. processed Terminalia chebula Retz polysaccharide on the histological structure and apoptosis in the spleen in immunosuppressed Chinese yellow quail.* Poultry Science, 102(2), 102416–102416. https://doi.org/10.1016/j.psj.2022.102416

Humbare, R. B., Sarkar, J., Kulkarni, A. A., Juwale, M. G., Deshmukh, S. H., Dinesh Amalnerkar, Chaskar, M. G., Maria Cristina Albertini, Rocchi, M., Kamble, S. C., & Ramakrishna, S. (2022). *Phytochemical Characterization, Antioxidant and Anti-Proliferative Properties of Rubia cordifolia L. Extracts Prepared with Improved Extraction Conditions.* 11(5), 1006–1006. https://doi.org/10.3390/antiox11051006

Itokawa, H., Ibraheim, Z. Z., Qiao, Y. F., and Takeya, K. (1993). *Anthraquinones, Naphthohydroquinones and Naphthohydroquinone Dimers from Rubia cordifolia and Their Cytotoxic Activity*. Chemical and Pharmaceutical Bulletin, 41(10), 1869–1872. https://doi.org/10.1248/cpb.41.1869

Natarajan, S., Mishra, P., Malayaman Vadivel, Basha, M., Kumar, A., & Sundaresan Velusamy. (2019). *ISSR Characterization and Quantification of Purpurin and Alizarin in Rubia cordifolia L.* Populations from India. 57(1), 56–72. https://doi.org/10.1007/s10528-018-9875-4

Patil, R. A., & Kasture, S. B. (2011). Protective effect of Rubia cordifoliaon reserpine-induced orofacial dyskinesia. Natural Product Research, 1–3. https://doi.org/10.1080/14786419.2011.635341

Patil, R., Mohan, M., Kasture, V., & Kasture, S. (2009). *Rubia cordifolia: a review*. Oriental Pharmacy and Experimental Medicine, 9(1), 1–13. https://doi.org/10.3742/opem.2009.9.1.001

Rao, G. M. M., Rao, C. V., Pushpangadan, P., & Shirwaikar, A. (2006). *Hepatoprotective effects of rubiadin, a major constituent of Rubia cordifolia Linn.* Journal of Ethnopharmacology, 103(3), 484–490. https://doi.org/10.1016/j.jep.2005.08.073

Rawal, A. K., Muddeshwar, M. G., & Biswas, S. K. (2004). *Rubia cordifolia, Fagonia cretica linn and Tinospora cordifolia exert neuroprotection by modulating the antioxidant system in rat hippocampal slices subjected to oxygen glucose deprivation.* BMC Complementary and Alternative Medicine, 4(1). https://doi.org/10.1186/1472-6882-4-11

Sandip Kumar Chandraker, Lal, M., Khanam, F., Preeti Dhruve, Singh, R. P., & Shukla, R. (2022). *Therapeutic potential of biogenic and optimized silver nanoparticles using Rubia cordifolia L. leaf extract*. Scientific Reports, 12(1). https://doi.org/10.1038/s41598-022-12878-y

Shi, Y., & Ji, H. (2014). *Kernel canonical correlation analysis for specific radar emitter identification*. Electronics Letters, 50(18), 1318–1320. https://doi.org/10.1049/el.2014.1458

Siddiqui, A., Tajuddin, Amin, K. M. Y., Zuberi, R. H., & Jamal, A. (2011). Standardization of Majith (Rubia cordifolia Linn.). *Indian Journal of Traditional Knowledge*, *10*(2), 330–333. https://www.researchgate.net/publication/288062897_Standardization_of_Majith_Rubia_cordifolia_Linn

Son, J. K., Jung, S. J., Jung, J. H., Fang, Z., Lee, C. S., Seo, C. S., Moon, D. C., Min, B. S., Kim, M. R., & Woo, M. H. (2008). *Anticancer Constituents from the Roots of Rubia cordifolia L.* Chemical and Pharmaceutical Bulletin, 56(2), 213–216. https://doi.org/10.1248/cpb.56.213

Tripathi, Y. B., Pandey, S., & Shukla, S. D. (1993). *Anti-platelet activating factor property of Rubia cordifolia Linn.* Indian Journal of Experimental Biology, 31(6), 533–535. https://pubmed.ncbi.nlm.nih.gov/8406601/

Verma, A., Mahalwal, V. S., & Kumar, B.(2019, May 31). *Antiepileptic activity of Rubidian isolated from the roots of Rubia cordifolia in mice.* International Journal of Pharmaceutical sciences and research. https://ijpsr.com/bft-article/antiepileptic-activity-of-rubiadin-isolated-from-the-roots-of-rubia-cordifolia-in-mice/

Wen, M., Chen, Q., Chen, W., Yang, J., Zhou, X., Zhang, C., Wu, A., Lai, J., Chen, J., Mei, Q., Yang, S., Lan, C., Wu, J. & Wang, L. (2022). *A comprehensive review of Rubia cordifolia L.: Traditional uses, phytochemistry, pharmacological activities, and clinical applications.* Frontiers in Pharmacology. https://doi.org/10.3389/fphar.2022.965390