Toxicological Evaluation of *Rubia cordifolia* Root Extract on Heart and Kidney 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 my/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. I/We have acknowledged all main sources of help.

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Approval

The thesis titled "Toxicological Evaluation of *Rubia cordifolia* Root Extract on Heart and Kidney Function of Rat Model" submitted by Taslia Afrose Eva (20346040) 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

The ethical approval has been given by the Biosafety, Biosecurity & Ethical Committee, Faculty of Biological Sciences, Jahangirnagar University, Savar, Dhaka, Bangladesh (Ref No: BBEC, JU/M 2024/ 11 (154)).

Abstract

Rubia cordifolia (Manjistha), is a plant which has an immense significance in Chinese and Indian medicine. The scientific databases has reported that the plant is widely used in treatment of heart failure, platelet aggregation, myocardial ischemia, kidney dysfunctioning, oxidative damage and can promote blood circulation, cardio protection, protection against nephrotoxicity and other functions. This study attempts to evaluate the toxicological effects on heart and kidney through a biochemical and histopathological study where two (High = 1500 mg/kg and low = 150 mg/kg) doses of plant were used. Therefore, the body weight of rats were increased from day 0-28 observation. Through a biochemical study, the uric acid and creatinine levels were found lower in both high and low dose and the urea level was found higher in high dose of the extract. Through histopathological study in kidney, only mild interstitial infiltrate of lymphocytes were found and not any toxic effects on heart. Therefore, further study can be done to confirm the toxicity profile.

Keywords: *Rubia cordifolia,* therapeutic effects, toxicological effects, clinical applications

Dedication

This work is dedicated to my mother who always support me to overcome challenges and encourage me to achieve my future goals

Acknowledgement

First of all, I would like to express my gratitude to the Almighty Allah to assist me to work hard and complete project work without any disruption. Secondly, I would like to show my gratitude to my supervisor Professor Sharmind Neelotpol, for her kind advice and support throughout the period of my thesis. Without her consistent direction and help, it would have been difficult for me to finish my thesis.

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Table of Contents

List of Figures:

List of Tables:

List of Acronyms

MNJ	Manjista
PAF	Platelet activating factor
3H-PAF	3H-labeled platelet-activating factor
LPO	Lipid peroxidation
CAT	Catalase
SOD	Superoxide dismutase
GST	Glutathione S-transferase
GHS-Px (GPx)	Glutathione peroxidase
GFR	Glomerular filtration rate
ECG	Electrocardiogram
CKD	Chronic kidney disease

Chapter 01

Introduction

Rubia cordifolia, (family: Rubiaceae, Linn. Rubiaceae), a small continuous climbing, plant-based vines, having red rhizomatous roots, bases which have therapeutic plant extracts as well as an essential components in Ayurvedic medicine system. It originated from India, where it is known as Indian madder and locally it is called Manjista (MNJ). Moreover, it is a native plant to southern India, Sri Lanka, northern Barnaby, topical Australia, Pakistan, Japan as well (Wen et al., 2022)

Figure 1: Rubia cordifolia plant (Pulikkottil, 2014)

Figure 2: Rubia cordifolia root (AyurSatwa, 2024)

According to the phytochemistry research, it contains 100 of different chemicals including flavonoids, terpenes, quinoines, polysaccharides, bicyclic peptides and other elements. Among those chemical substances the peptides and quinoines are the highest in quantity as well as have excessive beneficial effects. The current pharmacological research has demonstrated that it claims the anti-oxidative, anti-tumor, anti-platelet aggregation and anti-inflammatory properties (Wen et al., 2022).

R. cordifolia, a medicinal plant which is widely used in Chinese medicine. According to the study, the root extracts of *R. cordifolia* may eradicate pathogenic heat from blood, blood detoxification, renal stone, skin disorders, remove blood stasis and dredge collaterals, and help to manage hemostasis. In traditional medicine, it is primarily employed as a hemostatic medication to treat injuries, damage, strains, hemorrhage, metrorrhagia, and epistaxis This plant extracts are exclusively used in terms of the medical management and treatment in severe conditions like allergic purpura, uterine bleeding, blood disorders, renal hemorrhage, primary menstrual cramping, other reproductive issues etc (Ali et al., 2020; M. Wen, 2022).

In India, during delivery, women received an infusion of the root of *R. cordifolia* to drain the uterine pathways and to treat the blood related illness. Again, this root is used in Korean traditional medicine to treat jaundice, rheumatism and the menstruation problems. After that, to cure the urinary ailments this root is employed widely in the Philippines as well (Priya and Siril 2014).

Effects on Heart: *R. cordifolia* performs significant roles in cardiac health as antiplatelet, calcium channel blocker, effective blood purifier, antioxidant, vasodilator. It also reduce platelet activating factor-induced aggression (Chandrashekar et al., 2018). *R. cordifolia* has proper curing function to blood stasis, promoting good blood circulation and stopping excess bleeding. In addition, it can also work effectively on macrophage inflammation and myocardial ischemia. Besides, it can successfully prevent inflammation and apoptosis and can preserve the functional integrity of cell lines of myocardial and endotheliales (Gao et al., 2023)

Effects on Kidney: *R.cordifolia* has effective protection against nephrotoxicity and showed therapeutic activity towards kidney dysfunctioning. Therefore, *R. cordifolia* was investigated for its capability to give protection against immune response impairment and kidney oxidative damages and alterations (Joy et al., 2008). Moreover, the roots of *R. cordifolia* can assist to decrease the chance of kidney stones and reduce the higher level of calcium and oxalate in the kidneys that limits the formation of urinary stones (Divakar et al., 2010).

Research gap: Upon reviewing the literatures, the majority of the research is in the evaluation of the pharmacological effects and chemical constituents of *R. cordifolia.* However, sufficient and significant toxicological studies are not conducted yet to observe either this plant has any toxicity profiles or not since the toxicity can reveal the harmful effects which are associated with the use of the plant. Therefore, this toxicological study of *R. cordifolia* root extract is conducted with a view to evaluate the toxic activity of the plant.

Aim of the research: The aim of the study is to evaluate and monitor the pharmacological and toxicological changes in the heart and kidney.

Objective of the research:

The objectives of the study are,

1) To review different articles whether this drug has any effects on heart and kidney or not
2) To evaluate the toxicological effects and histopathological changes on heart and kidney

Chapter 02

Methodology

The evaluation of toxicological effects of *R. cordifolia* are observed in the literature review, biochemical test and histopathology. Literatures and scientific databases describe comprehensive reviews on pharmacological effects, therapeutic uses and medicinal properties of *R. cordifolia.*

2.1 Literature Review

From the current studies on the pharmacological and toxicological effects are thoroughly reviewed and evaluated. We searched and examined the PubMed, ScienceDirect, NCBI (National Library of Medicine), and Research gate. Relevant information was found using the key terms such as 'Effects of *R. cordifolia* ', 'Review of *R. cordifolia* ', 'Toxicity study ', 'Renal tissue', 'Therapeutic effects', 'Traditional use', 'Cardiac effect', 'Cardioprotective activity', 'Characteristic of *R. cordifolia*'.

2.2 *R. cordifolia* Root Extraction

Through a solvent extraction method with ethanol this plant root extraction was performed. The fresh and healthy root portions of *R. cordifolia* were collected from Sylhet. Then they were carefully washed, cut into smaller portions, chopped and dried at a temperature of (35°C - 40°C) for a week. After that, those dried portion were pulverized and crushed into powder form with a electric grinder. Then, about 1g of powdered portion was extracted in 4.5ml of ethanol (96%). With the help of a rotary evaporator (under a reduced pressure) the extracted mixture of active components were dried and the complete ethanol extract was received.

2.3 Preparation of Drug Doses

Two oral doses of *R. cordifolia* root extracts were made, one was in high concentration and another was in lower concentration for the treated group H and treated group L accordingly. Therefore, the amounts of the doses of *R. cordifolia* were 150 mg/kg in the lower dose and 10 times more concentrated in the higher dose, that was 1500 mg/kg (Sen et al., 2012).

2.4 Study Design

The healthy male white albino (Sprague-Dawley) rats, weighted about 70-90 gm were taken for the experiment and these rats were kept in the Pharmacology laboratory of Jahangirnagar University. Throughout the experiment, all the rat models were handled effectively and every precaution was taken when handled humanly and all the protocols of the experiment were performed following the OCED Guideline.

The experimental rat models were divided into three groups named Treated group L (for low dose of *R. cordifolia*), Treated group H (for high dose of *R. cordifolia*) and Control group where each group consisted of 6 rats. All the rats were maintained and housed in a cage measuring (60×38×20) cm3. After that, they were kept and monitored at a temperature of (21±5)°C using a 12 hour light/dark cycle. Besides, they had free accessibility of food and water in the cage. All the rats were fed and given the dose of 1ml/day through oral gavage between 10am to 2pm daytime period. With an interval of four days, the body weight of rats were measured and recorded throughout the experiment.

Table 1: Grouping of Experimental Rats

Group	Administered substance	Dose	Frequency of dosing
Group 01 (Control) n=6	Distilled water	1ml/kg body weight	Once a day for 28 days
Group 02 (Treated-MNJ high dose) n=6	High dose of plant extract	1ml/kg body weight	Once a day for 28 days
Group 02 (Treated-MNJ low dose) n=6	Low dose of plant extract	1ml/kg body weight	Once a day for 28 days

2.5 Body Weight of Rats

Before initiating the dosing and dissection of rats, the body weight of all rats were measured carefully through a sensitive scale.

2.6 Biochemical Study

For the analysis, initially the blood sample was taken from the rat. The serum portion was separated from blood through the centrifugation process at 400 rpm (allowing to clot for 10 minutes at 4°C temperature).

Colorimetric Assay was performed to evaluate the biochemical markers of the kidney. For urea, uric acid and creatinine assessment, the Urea Colorimetric Assay kit, Uric acid (UA) Colorimetric Assay kit and Creatinine (Cr) Colorimetric Assay kit were from Elabsceience. After that, by using the kits the samples were evaluated where different reagents were used to quantify the amount of urea, uric acid and creatinine in the sample.

2.7 Histopathology

To perform the experiment, after 28 days all the animals were euthanized in accordance with study procedure, through utilizing an overdose of ketamine (500 mg/kg, i.p.). The anesthetic effect was verified by the "toe pinching". After that, the hearts and kidneys of the rats were removed and preserved by using a 10% neutral buffered formalin solution, the organs were stored and preserved. Following a few days, by using the Haematoxylin and Eosin (H&E) staining procedure, the heart and kidney of the rat model were consistently prepared for the histopathological examination. In the end, for proper visualization, the organs were studied using a light microscope (Olympus, CX43, Japan) and the organ's photomicrographs were obtained with an Olympus D22 camera.

2.8 Ethical Approval

The ethical approval has been given by the Biosafety, Biosecurity & Ethical Committee, Faculty of Biological Sciences, Jahangirnagar University, Savar, Dhaka, Bangladesh (Ref No: BBEC, JU/M 2024/ 11 (154)). Throughout the experiment, all the rats were handled humanly and every precaution was taken when handling them in accordance with the internationally recognized guidance.

2.9 Statistical Analysis

Statistical analysis were accomplished through using a portable IBM SPSS Statistics No. 19. All the values were showed as Mean±SEM. The assessment of the variables were observed in 2*4 factorial analysis of ANOVA to terminate the significance difference between the consecutive groups where the significant difference was considered as $p<0.05$. For the metric variables mean, standard deviation and standard errors were expressed as descriptive measures.

Chapter 03

Result and Discussion

3.1 Literature Review on the Effect of *R. cordifolia* in Heart

The toxicological effects of *R. cordifolia* studied in literature review and the current researches explain the enormous amount of information about the pharmacological effects as well. They have developed a way of collecting, evaluating and illustrating the conclusions especially in the field of where researches may show inconsistent results.

3.1.1 *R. cordifolia* as Cardioprotective Agents

A number of researche reveal about the therapeutic effects of *R. cordifolia* which works as a good cardioprotective agents. A report explained that anti-platelet aggregation actions showed significant results. This research reported that the extract of *R. cordifolia* was beneficial in suppressing anti-platelet aggregation, generated through the platelet activating factor (PAF) and impedes the binding of 3H-labeled platelet-activating factor (3H-PAF). Although more pre-clinical studies are conducted in mice models to establish the actual mechanism of actions in giving effective therapeutic actions (Wen et al., 2022).

R. cordifolia extracts reduces the myocardial ischemia-reperfusion injury through protecting the endothelial cell lines from apoptosis, inflammation and work as an effective of cardioprotective agent. This study reported that the animal models were treated with the plant extracts intravenously and they found out that these extracts were effective in reducing the problem as

well as they were nontoxic. However, the doses of *R. cordifolia* were used much less in this study (Gao et al., 2023).

3.1.2 Curing Heart Failure Through *R. cordifolia*

Alloxan, a glucose type analog which is a harmful chemical that can cause the elevation of blood sugar level. However, with the use of *R. cordifolia* root extracts, the problem of hyperglycemia (high blood sugar level) significantly gets reduced. Since, these high blood sugar levels are responsible for blood clotting in arteries, thrombosis which may cause severe heart failure. Moreover, studies reported that the anti-thrombotic properties of extracts can cure the disease related to Blood Stasis Syndrome (BSS) and Heart Failure (Eskandarzadeh et al., 2022).

3.1.3 Protection Against Cardiotoxicity

A research on the evaluation of cardioprotective effects of *R. cordifolia* root extract on rat models to investigate the therapeutic activity against cardiotoxicity. In the study, they reported that upon administering the root extracts *R. cordifolia* to the animal models, they were inspected for Lipid peroxidation (LPO) levels, Catalase (CAT), Superoxide dismutase (SOD) and Glutathione S-transferase (GST) to evaluate the stage of myocardial injury. Here, the root extract showed cardioprotective activity through free radical scavenging where LPO levels were reduced.

Moreover, through preventing the disastrous pathological process this treatment with root extract inhibited the severe cardiac damages like myocardium damage. This study revealed that the plant *R. cordifolia* root contains active compounds such as phosphonate derivatives, uridine diphosphate-N-acetylglucosamine and carbonyl derivatives that play a very significant role in possessing the cardioprotective effects through suppressing the cardiotoxicity activity.

Consequently, those compounds of root extract might contribute to the protective activity against the cardiotoxicity (BS et al., 2018).

3.1.4 Cure Platelet Aggregation Disorder

Through literature studies, the *R. cordifolia* plant is clinically used in terms of problems related to platelet activating factors (platelet aggregation disorders). Therefore, a study was conducted on rabbit platelets to evaluate the pharmacological activity on the PAF activity related problem. The result showed that the extracts of *R. cordifolia* successfully inhibited the platelet aggregation caused by platelet activating factor (PAF) along with 3H labeled-PAF on the platelets and those effects were checked through a dose dependent manner (Meena et al., 2010). Therefore, the effects of *R. cordifolia* were found beneficial in the case of reducing platelet aggregation. In that way, the cardioprotective effects were explained in this research through conducting the clinical use of this herbal plant (Nyeem and Mannan 2018).

3.1.5 *R. cordifolia* Therapeutic Activity as Calcium Channel Blockers

In the isolated tissues, basically in guinea pigs atria and rabbits aorta, the extracts of *R. cordifolia* was tested to evaluate the presence of calcium channel blocker effects. This research explained that the plant extracts reduced the contractions of the aorta that might occur due to norepinephrine and potassium chloride (KCl). Besides, it has some spasmolytic properties which are similar to the verapamil (work to relax the blood vessels) that indicated the existence of the substance like calcium channel blockers. Additionally, the high concentration of potassium ion (K+) which could induce the contraction of blood vessels through the depolarization of smooth muscles and increased the calcium influx through using the voltage operated channels (L type). Consequently, the study found that the extracts of *R. cordifolia* have calcium channel blocking

activity and these activities are benefitted in terms of major heart failures through high blood pressures, to increase oxygen supply and reduce workload load of the heart (Gilani et al., 1994).

3.2 Literature Review on the Effect of *R. cordifolia* in Kidney

3.2.1 Evaluation of *R. cordifolia* to Assess Renal Tissue Damage Through Oxidative Stress

A study has been conducted to evaluate the effect of ethanolic extract of *R. cordifolia* to investigate the renal tissue of mice models treated with lead nitrate. The study observed that there was a drastic increase in LPO levels and decrease in Glutathione peroxidase (GSH-Px), CAT and SOD levels in renal tissue of mice which could create free radical induced toxicity and these modifications happened due to the alterations in the peroxidative pathway with the lead administration (Wen et al., 2022). However, administering the root extracts along with the lead nitrate significantly reduced the presence of lead in the antioxidative system. It basically lowered the LPO level while increasing the GHS-Px, CAT and SOD levels in renal tissue of mice models that were beneficial for cellular antioxidant defense systems towards the overproduction of free radicals. Therefore, these results explained that the plant has a good anti-peroxidative property and can provide in vivo protection against the lead induced oxidative stress (Lodi et al., 2011).

3.2.2 Cisplatin Induced Nephrotoxicity Evaluation of *R. cordifolia*

A study conducted on cisplatin induced nephrotoxicity evaluation in Swiss Albino mice by *R. cordifolia* extracts to determine the neuroprotection activities. In that study, the cisplatin induced mice models were observed having nephrotoxicity and the free radicals influenced the renal damage caused by cisplatin. Additionally, this study reported that, due to the declining

antioxidant defense system the nephrotoxicity happened and cisplatin might be responsible for the case of losing proper amounts of copper and zinc from the kidney which provoked the decrease of the renal tissues SOD and G-Px activity (Joy et al., 2008, Kumari et al., 2021).

Therefore, upon administering the extracts of *R. cordifolia,* it showed inhibition of cisplatin induced peroxidation in renal tissues and protection against the toxicity effects of cisplatin through restoring the SOD and GPx activities. Since, it has been observed that extracts have significant free radical scavenging properties and antioxidant effects (Joy et al., 2008; Kumari et al., 2021)

3.2.3 *R. cordifolia* Root Effectivity Against Urolithiasis

A study was performed on rats to observe the protective effects of the hydro alcoholic liquid extracts of *R. cordifolia* root over the ethylene glycol induced urolithiasis usually referred to as kidney stone. Ethylene glycol causes hypocalciuria, hyperoxaluria and excessive excretion of phosphate from the kidney. However, administration of hydro alcoholic liquid root extracts of *R. cordifolia* substantially lowered the urine oxalate, calcium and phosphates in a dose dependent manner as well as efficiently reduced the excess calciums and oxalates in renal tissues. Moreover, through giving protection over ethylene-glycol induced urolithiasis, the hydro alcoholic liquid root extracts of *R. cordifolia* could prevent the recurrence of that disease. Additionally, it effectively functions by lowering and preventing formation of kidney stones since it has been demonstrated having effects on early stages of renal stone growth (Divakar et al., 2010).

3.2.4 Evaluation of Biochemical Markers and Activities

The diuretic activity of root extract of *R. cordifolia* to evaluate the biochemical markers creatinine clearance and activity of Glomerular filtration rate (GFR). The diuretic effects of the root extracts *R. cordifolia* explained the activity on urine volume, creatinine, Na+, K+ and Cl- through promoting effective excretion of those electrolytes and increase urine volumes. Therefore, it was probable that the therapeutic components of roots operate similarly to furosemide. After treatment of plants root extracts on Wister Albino rat models, there was an increased level of sodium and potassium ions (Pawar et al., 2009).

Additionally, the root extract of *R. cordifolia* described as having diuretic effect by increasing GFR and inhibiting salt reabsorption. Both *R. cordifolia* root extract and furosemide significantly increased GFR. This increased GFR due to the extract could be related to (1) a detergent-like reaction with structural properties of glomerular membranes (2) a decrease in renal perfusion pressure, resulting from the reduction on the resistance of the afferent arteriole, and increases the resistance of the efferent arteriole and (3) the impact of the arteriole wall on the blood flow of glomerular (Divakar et al., 2010).

3.3 Biochemical Markers and kidney Function Evaluation

3.3.1 Body Weight

Table 2: Body weight of rats treated with *R. cordifolia*:

Record of 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

In this observation, the control group of rats showed that their body weight increased from day 0 to day 28. Similarly, in the treated group L (low dose of *R. cordifolia*) showed an increased level of body weight of the rats. Lastly, the treated group H (high dose of *R. cordifolia*) also reported increased body weight. However, in the comparison of the body weight of all rat groups, the treated group L showed a more drastic increase in weight than the control group and treated group H.

3.3.2 Kidney Function Parameter Evaluation

Table 3: Effects of *Rubia cordifolia* on kidney function specific parameters:

Parameters	Control	MNJ Low	MNJ High
	Mean (±SEM)		
Urea	21.57 (0.95)	16.80 (0.97)	17.80 (0.58)
Uric Acid	1.93 (0.13)	1.67 (0.07)	.01 (0.11)**
Creatinine	0.46 (0.04)	0.54 (0.04	0.44 (0.02

Data were analyzed by one way ANOVA. Values are expressed as Mean±SEM, n=6. $p<0.001$=***: MNJ treated group (High dose) the uric acid level is significantly different from control group and treated group (low dose) of rats.

The effects of *R. cordifolia* reported alterations on kidney function specific parameters which were urea, uric acid and creatinine levels. In the study, the urea level in the kidney showed slightly higher in treated group H than the treated group L. Consequently, if urea cannot filter out from the kidney properly, it may cause higher level of urea which is an indicator of Chronic Kidney Disease (CKD) (Vanholder et al., 2017).

After that, the uric acid level and creatinine level were found lower in treated group H compared to treated group L. As a result, the extracts of that plant may cure kidney dysfunctioning. Since higher levels of uric acid can worsen kidney functions by producing inflammation, endothelial lining dysfunction, renal tissue damage and alterations. A meta-analysis discovered that uric

acid-lowering medication could improve renal outcomes of the patients who are the sufferers of Chronic Kidney Disease (CKD) (Goncalves et al., 2022).

The plant extracts help to reduce creatinine levels as higher levels of this biomarker cause compromised kidney function, which can progress to CKD. Creatinine is a waste product generated by muscles that healthy kidneys filter out of the blood (Salazar, 2014).

3.4 Histopathological Evaluation

3.4.1 Histopathology of Heart and Kidney

Through the microscope observation of the control group, treated group L and treated group H, the findings were crucially evaluated. Here, the control group was basically treated with distilled water and showed no remarkable effects to the heart of rats. After that, in the treated group L were given a low dose of *R. cordifolia* root extract and the result showed unremarkable effects on heart. Therefore, there were no abnormalities or significant changes and toxicity effects of *R. cordifolia* in that group. Similarly, the treated group H showed no changes or effects where high dose *R. cordifolia* of was given. Accordingly, following the lower dose, the higher dose of *R. cordifolia* reported no unusual alterations in the heart of rat groups.

In the case of kidney, the microscope observation of the control group, treated group L and treated group H, the findings were crucially assessed. The control group was treated with distilled water, reported no remarkable results on the kidneys of rats. In the treated group L and treated group H were given a low dose and high dose of *R. cordifolia* root extract accordingly, showed mild interstitial infiltrate of lymphocytes in kidney.

3.5 Microscopic Observation

3.5.1 Microscopic observation of Kidney

Control group:

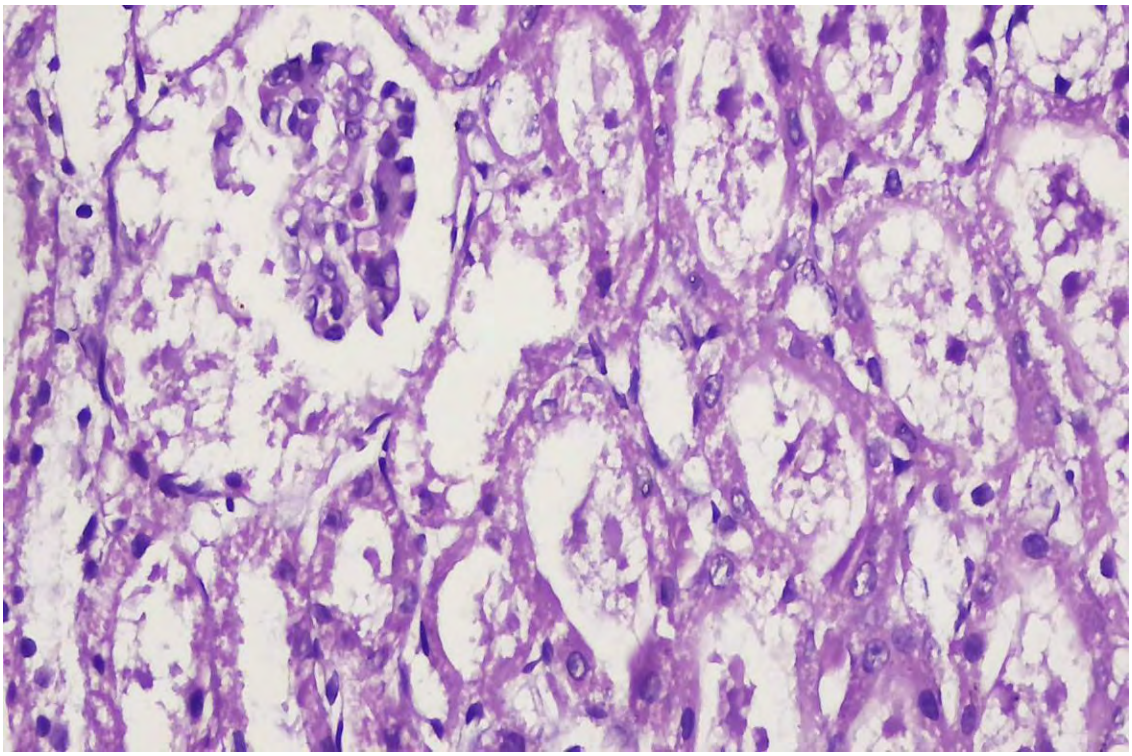

Figure 3: Microscopic picture of kidney (Control group-40x)

In the microscopic observation of the kidney the control group of rats (treated with distilled water) showed no remarkable results.

Treated group L:

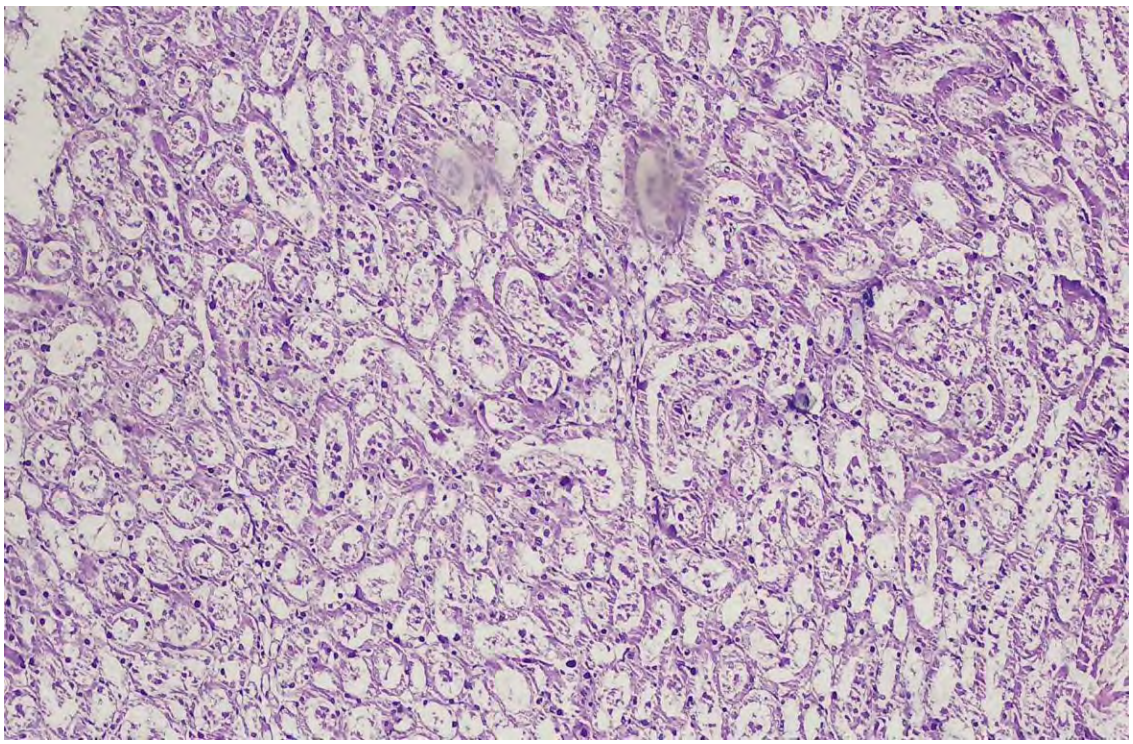

Figure 4: Microscopic picture of kidney (Treated group L-10x)

The treated group L (low dose of *R. cordifolia*), showed mild interstitial infiltrate of lymphocytes in the kidney (inflammation in renal interstitium or lymphoma infiltration).

Treated group H:

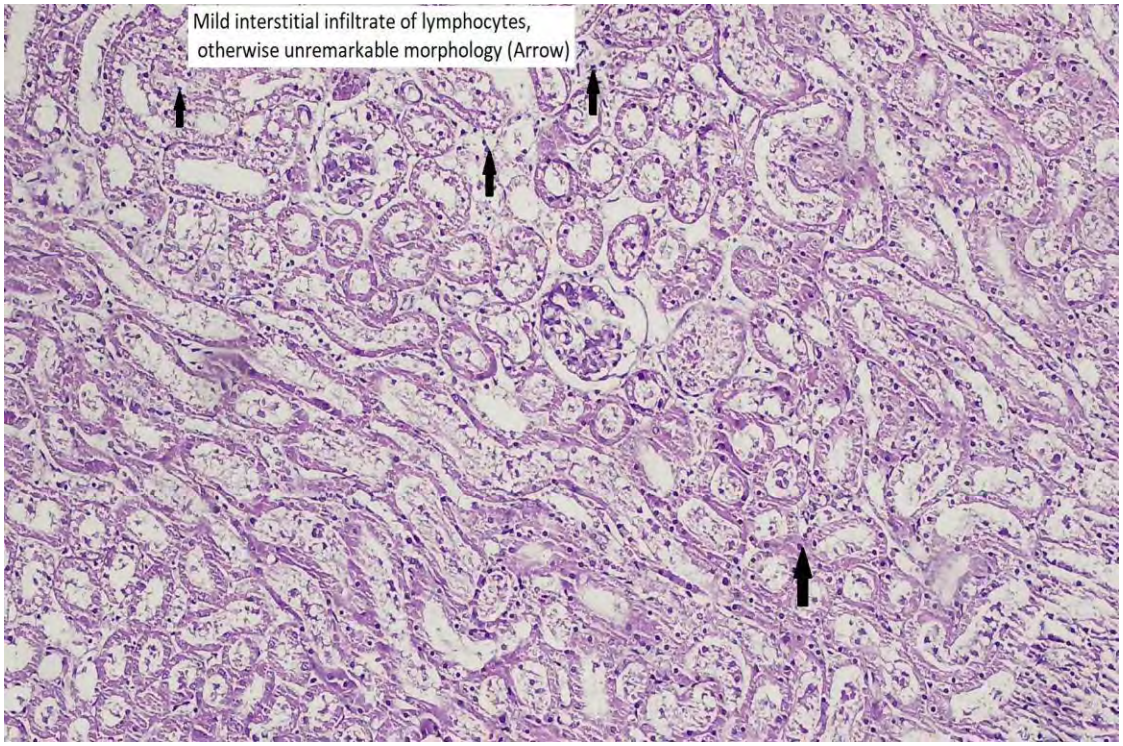

Figure 5: Microscopic picture of kidney (Treated group H-10x)

The microscopic observation of the treated group H (High dose of *R. cordifolia*), found mild interstitial infiltrate of lymphocytes in kidney (inflammation in renal interstitium or lymphoma infiltration).

This lymphoma infiltration (both in high and low dose) of the kidney may result in tubular atrophy and necrosis and compression or enlargement of the tubular lumen which finally leads to renal tubular dysfunction (Jhamb et al., 2007). However, this research did not find any other nephrotoxicity or renal dysfunctioning with the root extracts of *R. cordifolia*.

3.5.2 Microscopic observation on Heart

Control Group:

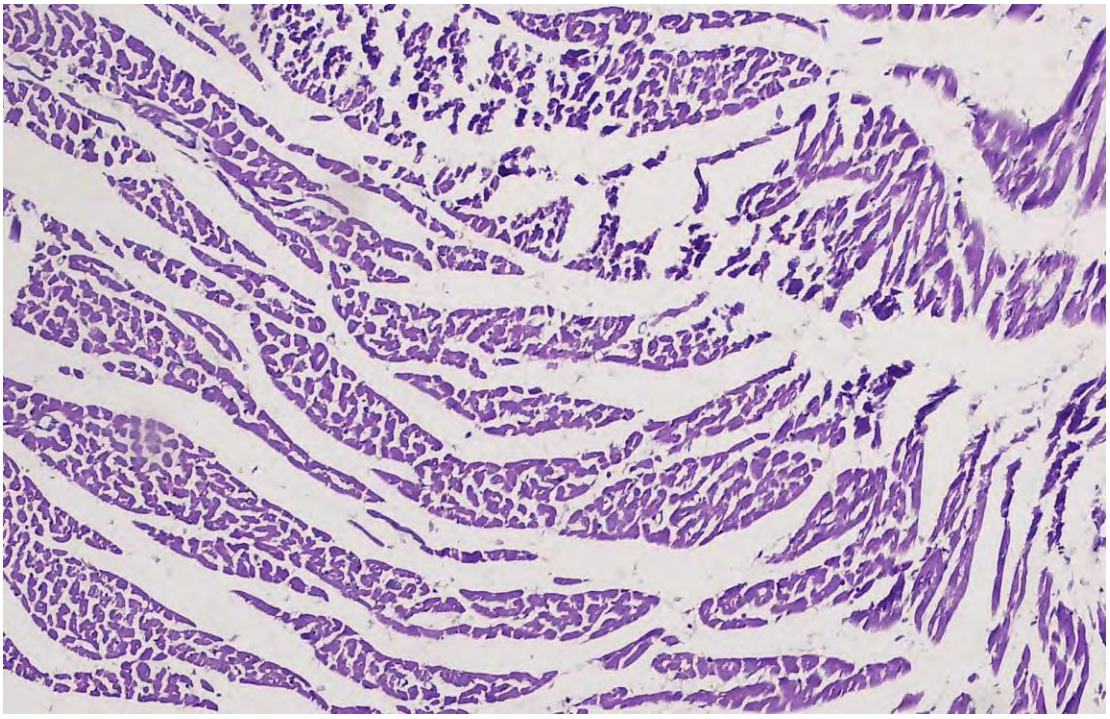

Figure 6: Microscopic picture of Heart(Control group-40x)

In the microscopic observation of the heart, the control group of rats (treated with distilled water) showed no remarkable results.

Treated group L:

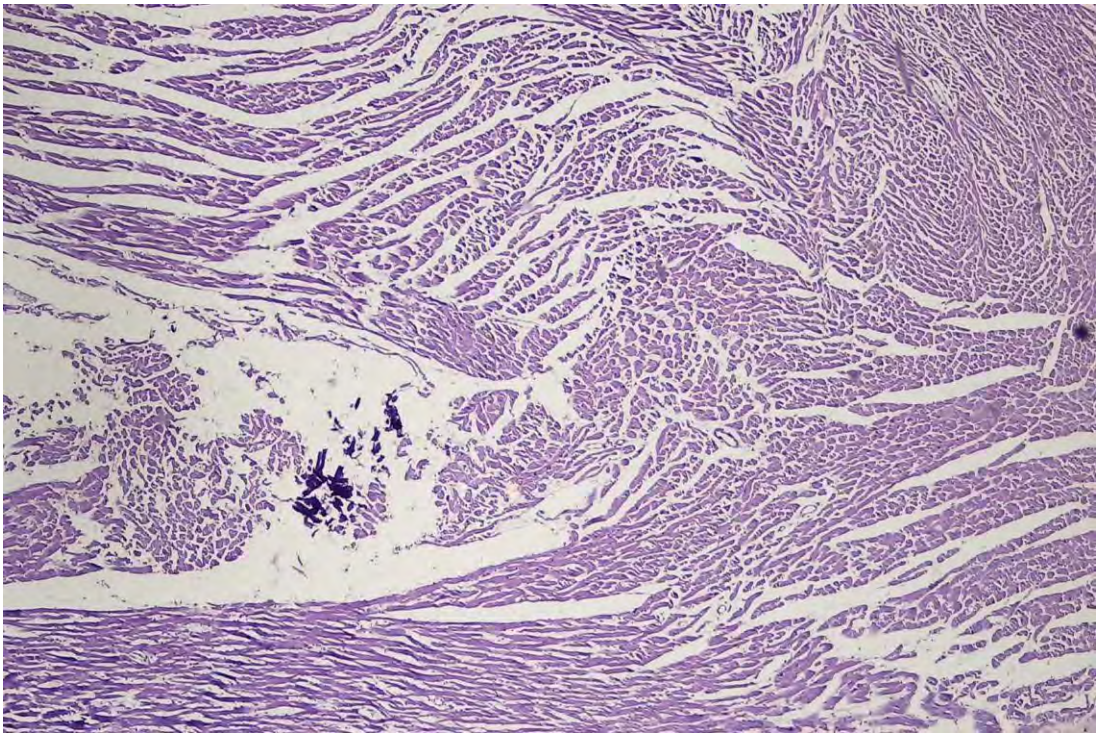

Figure 7: Microscopic picture of Heart (Treated group L-40x)

The microscopic observation of the treated group L (low dose of *R. cordifolia*), found unremarkable results. Therefore, there were no signs of tissue or cell injury and other cardiotoxicity effects observed.

Treated group H:

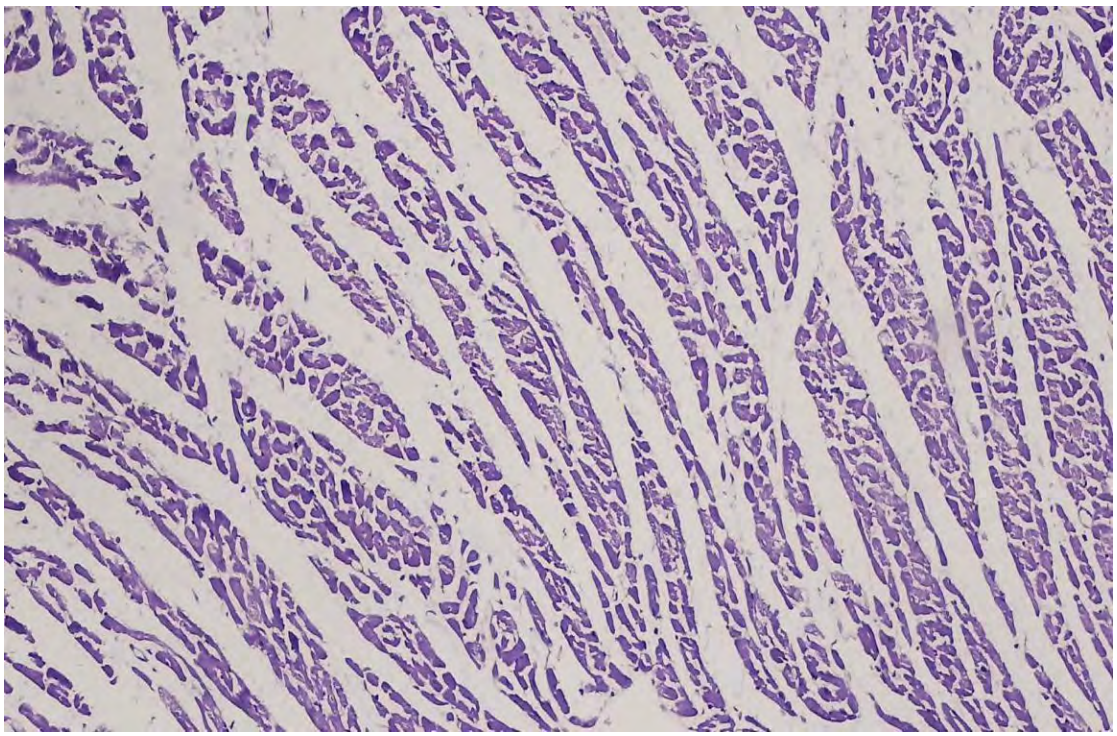

Figure 8: Microscopic picture of Heart (Treated group H-40x)

The microscopic observation of the treated group H (High dose of *R. cordifolia*), found no remarkable changes in heart. Therefore, this group of rats showed no signs of tissue or cell injury and other no cardiotoxicity.

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Chapter 04

Conclusion

4.1 Conclusion

This study was performed to evaluate the toxicological effects of the root extracts of *Rubia cordifoli*a on heart and kidney. Through the biochemical study, the biochemical markers (urea, uric acid and creatinine) of kidney reported decreased levels of uric acid and creatinine levels upon administering higher doses of *R. cordifoli*a compared to other doses and it was explained as a beneficial effect in the kidney. The body weight observation reported increased body weight in three consecutive rat groups.

The histopathological study reported that with the administration of the root extract of *R. cordifoli*a in the kidney showed mild changes in lymphocytes of kidney (inflammation in renal interstitium or lymphoma infiltration) and no other nephrotoxicity activity in both treated group L and treated group H. However, in the heart, there was no significant toxicological activity found through this study. Consequently, this study demonstrated that all the doses for the consecutive groups named treated group L (low dose) and treated group H (high dose) reported no potential or remarkable alterations and any cardiotoxicity on heart. Therefore *R. cordifoli*a root extracts found to be effective for the heart considering that no histopathological and toxicological changes occurred.

4.2 Limitation of this Study

This study could have been done with more dosing ranges and the evaluation of those doses might have come more satisfactory. In this study, only two of doses were selected. In the toxicological studies, dose levels, measurements are required assessments where more precise level of dosing and measurements shows desired accuracy of results (Borgert et al., 2021). However, more than two doses could have been taken, due to financial constraints only two dose measurements were taken to evaluate the toxicity effects.

4.3 Future Prospects of this Study

*Rubia cordifoli*a also known as Manjistha which is described as a detoxifying herb, has many uses as Ayurvedic medicine. Through a clinical trial, a toxicological evaluation can be done to investigate the beneficial outcomes and toxicity effects of *R. cordifoli*a. Therefore, the research can be done through taking blood samples and observing them to check whether *R. cordifoli*a gives any toxicity or not as an Ayurvedic preparation. Accordingly, to evaluate the effects on the heart, the healthy heart parameters need to be assessed such as blood pressure, resting heart rate, cholesterol, physical exercise, Electrocardiogram (ECG). Moreover, the biochemical markers of the kidney for example serum creatinine, urea, uric acids, GFR can be analyzed to estimate their proper values because they are directly related to healthy kidney function.

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