TERATOGENIC AND HEPATOPROTECTIVE ACTIVITY OF EDIBLE LIME USING THE ZEBRAFISH MODEL

Submitted in partial fulfillment of the requirements
For the degree of

Bachelor of Pharmacy

BALABHAI IQRA ZAHID NASREEN
16PH06
GUPTA SHUBHAM SURESHCHANDRA
16PH17
PATHAN MUSKAAN MOHIN SHAYADA
16PH35
SHAIKH IKRAR ABRARAHMAD YASMIN
17DPH65

UNDER THE GUIDANCE OF

Asst. Prof. Shaikh Abusufiyan Sirajuddin

CERTIFICATE

This is to certify that the project entitled **"Teratogenic and Hepatoprotective activity of Edible Lime using the Zebrafish model"** is a bonafide work of **BALABHAI IQRA ZAHID NASREEN (16PH06); GUPTA SHUBHAM SURESHCHANDRA PUSHPA (16PH17); PATHAN MUSKAAN MOHIN SHAYADA (16PH35); SHAIKH IKRAR ABRARAHMAD YASMIN (17DPH65)** submitted to the university of Mumbai in partial fulfillment of the requirement for the award of the degree of "Bachelor Of Pharmacy".

Asst. prof. Shaikh Abusufiyan Sirajuddin
Guide
(Assistant Professor, AIKTC, SOP)

Dr.Shariq Syed
Dean, SOP

Dr.Abdul Razzak Honnutagi
Director

PROJECT REPORT APPROVAL FOR B. PHARMACY

This project report entitled **"Teratogenic and Hepatoprotective activity of Edible Lime using the Zebrafish model"** is a bonafide work of Balabhai Iqra Zahid (16PH06); Gupta Shubham Sureshchandra (16PH17); Pathan Muskaan Mohin (16PH35), Shaikh Ikrar Abrarahmad (17DPH65) approved for the degree of "Bachelor of Pharmacy"

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BALABHAI IQRA ZAHID NASREEN 16PH06

GUPTA SHUBHAM SURESHCHANDRA PUSHPA 16PH17

PATHAN MUSKAAN MOHIN SHAYADA 16PH35

SHAIKH IKRAR ABRARAHMAD YASMIN 17DPH65

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ABSTRACT

Many local Vaidya have been giving Edible lime for the treatment of jaundice and other liver diseases. Also, it is infamously consumed along with tobacco for ages. The purpose of this investigation was to determine lethal concentration 10 (LC_{10}) and study the hepatoprotective activity of Edible lime on the zebrafish model. LC_{10} value of Edible lime was calculated by using probit analysis, and it was found to be 95.49 mg/L. Liver toxicity was induced in zebrafish larvae by using paracetamol. Liver atrophy, yolk sac retention, increase in optical density of liver, increased in the level of AST and ALT revealed paracetamol-induced liver toxicity in zebrafish larvae. The Edible lime showed a significant increase in liver size, and decrease in yolk sac retention, reduced liver degeneration, reduced AST and ALT level. The present study revealed the hepatoprotective activity of Edible lime.

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Chapter 1

Introduction

In this new era of drug development, the most of the drugs which are emerging in the market are synthetic one, having a variety of adverse effects on different body organs mainly on heart, liver and, kidney. Since these drugs are synthetically prepared they have a high market price, to overcome such difficulties there is the need for a substance that is efficacious and abundantly available. Calcium hydroxide i.e $Ca(OH)_2$ is an inorganic compound. In crystal form it is colorless whereas in powder form it is white in colour and is form after mixing quicklime with water [1]. It is known as caustic lime, hydrated lime, slaked lime, pickling lime. In addition, the limewater is also the most common name for a saturated solution of $Ca(OH)_2$. The $Ca(OH)_2$is used in food preparation, where it has been given a code (E526).

Edible lime is odourless powder with a Molar mass 74.093 g/mol, melting point of 580 °C, solubility in water is 1.89 g/L at 0 °C, 1.73 g/L at 20 °C, 0.66 g/L at 100 °C and density of 2.211 g/cm3. It is soluble in glycerol, acids and insoluble in alcohol.

Edible lime has been a part of traditional healing practices over a centuries. It is used traditionally for the treatment of joint pain, arthritis, knee pain, bone fracture, backache, toothache, sciatica, cervical, male impotence, and lack of memory. Lime powder along with sugarcane, orange or pomegranate juice is well known for the treatment of bone weakness and pain. After the age of 50 females suffers from troublesome effects of menopause and need more intake of calcium carbonate. Lime powder is good to cure all associated morbidity of menopause [2].

Our scientific research-based study have been planned on the zebrafish model, as zebrafish embryos are transparent it allow study of internal

organs [3]. Zebrafishes are popularly used as an *in-vivo* model for the screening of drugs due to its rapid organogenesis.

The zebrafish has high degree of genetic conservation, also their molecular basis and organ development has considerable homology to other vertebrates including humans. Due to this, zebrafish model system have attracted the attention of the investigators for the evaluation of the teratogenic effect of the medicaments [4].

Test drugs can be dissolved in fish water. The zebrafish larvae absorb small test drug molecules diluted in the surrounding water through its highly permeable skin and also through gut, and gills. In comparison with other models, we can use relatively high number of zebrafish for each assay and also amounts of drug needed is very small. In addition, its small size, easy maintenance, high productivity, and breeding give zebrafish model edge over other animal models [5]. As compared to cell based assay, as an alternative animal model for drug testing, the zebrafish can greatly decrease costs, speedup the process of drug discovery, and provide more accurate results.

Nowadays with new drugs emerging in the market, there have been various side effects on the body mainly on the liver because it acts as an important organ for drug elimination. Hepatoprotective activity is the ability of any substance to protect liver disorders, and the substances with such activities are referred as hepatoprotectives. Edible lime has been used in the treatment of jaundice and other hepatic disorders by the local Vaidya of Raigad district of Maharashtra. However, scientific evidences to support its use in the treatment of hepatotoxicity is lacking. Therefore, the present study is planned to investigate the hepatoprotective activity of edible lime in zebrafish larvae.

Chapter 2

Literature review

2.1 Selection of Drug

Chuna is a therapeutic modality that addresses biomechanical function, pathology, diagnosis, and theories related to treatment in order to create a balance in orthopedic structure and functions. Physical manipulation of the body has been used to cure human ailments since ancient times. Although its origins remain unclear, but it has been a source of medicine over centuries [6].

2.2 Advantages Zebrafish model for screening hepatotoxicity:

The zebrafish (*Danio rerio*) is a promising model for evaluation of drug-induced toxicity of a variety of organ systems. Well established zebrafish model have frequently been used for measurement of cardiac, g.i.t, the central nervous system functions and also developmental toxicity. Histopathology and clinical chemistry have been used traditionally to report liver toxicity in established animal models. One of the key physiological functions of the liver is oxidative catalytic transformation or metabolism which play vital role in activation or inactivation of many endogenous, exogenous compounds and drugs [7].

Numerous studies showed that the zebrafish mammalian toxicity profiles have strikingly similar to human being. The larval zebrafish transparency also permits direct evaluation of *in vivo* drug toxicity such as liver toxicity. The phenotypic assay of Larval zebrafish is a highly predictive for rapid *in-vivo* evaluation of hepatotoxicity.

The liver toxicity related to drug is a major cause of trouble during both the early and late stages of the drug discovery and marketing. The hepatotoxic drugs induces degeneration of liver, alter liver size and produces retention of yolk sac [8].

Chapter 3

Objectives and Plan of Work

3.1 Objective of work

The main objective of our scientific research is to:

- To find out the MNLC and LC10 of edible lime for the selection of non-toxic doses for further *in vivo* study in zebrafish larvae.
- To investigation the effect of edible lime on visual parameters of hepatotoxicity in zebrafish larvae (Liver atrophy, yolk sac retention, liver degeneration) and biochemical markers of liver toxicity (AST and ALT level).

3.2 Plan of work

In order to study the hepatoprotective activity parameters that were taken into considerations were as follows:

1. MNLC and LC10
2. Liver atrophy (area)
3. Yolk sac retention (area)
4. Liver degeneration (Liver optical density)
5. Levels of enzymes (AST and ALT)

Chapter 4

Materials and Methods

4.1 *Zebrafish Embryos collection and Maintenance*

The zebrafishes were procured from a pet shop from Mumbai. All fishes were breed and eggs were collected as per standard protocol. The research plan was approved by the IAEC (Ref.No: AIKTC/SoP/IAEC/2019/02).

4.2 Determination of LC10, MNLC

To determine LC_{10} and MNLC of the edible lime, 72 hpf to 120 hpf zebrafish larvae were treated with different concentrations of Edible lime. The zebrafish larvae Mortality was recorded. Larvae with no heartbeat under microscope were considered as dead. LC_{10} and MNLC were determine and 3 doses were selected based on MNLC for studying its effect on acetaminophen induced hepatotoxicity.

4.3 Study of hepatoprotective activity of Edible lime

The fertilized eggs were collected and kept in the egg water for 3 days at the temperature of at 28°C. After 3 days post fertilization (dpf), 180 embryos were selected in each group and further exposure was given with test drug till 5 dpf as per following groups:

Group	Treatment
Normal Control	Normal eggs water
Positive Control	Paracetamol (5mMole)
Test Group-I	Eggs water + Paracetamol (5mMole) + Edible lime (D1:1/20th of MNLC, 3.5 ppm)
Test Group-II	Eggs water + Paracetamol (5mMole) + Edible lime (D2:1/10th MNLC, 7 ppm)

Test Group-III	Eggs water + Paracetamol (5mMole) + Edible lime (D3:1/5th MNLC, 14 ppm)
Standard Group	Eggs water + Paracetamol (5mMole) + silymarin (10 µM)

On 6th dpf different biomarkers of paracetamol induced liver toxicity were investigated.

4.4 Percentage Mortality

The % mortality induced due to paracetamol in zebrafish was evaluated. This value was evaluated in triplicate and represented as mean ± SEM.

4.5 Visual assessment of hepatoprotective activity

For visual screening of hepatoprotective activity images of 6 dpf anesthetized larvae were captured by digital microscope. ImageJ software was used for quantitative estimation of these visual changes..

1. **Liver size area**

2. **Yolk sac area**

3. **Liver degeneration**: For measurement of liver degeneration optical density was assessed.

4.6 Biochemical measurement of liver enzymes

Liver enzymes (ALT and AST) were investigated in the larvae homogenate. The 6 dpf zebrafish were collected in triplicate and homogenates of whole larvae homogenate was made in cold saline and centrifuged at 4°C for 7 min, and the supernatant was collected for the study. The concentrations of total protein, ALT, and AST levels were evaluated as per manufacturer instructions of diagnostic kits. The level of ALT and AST in the upper layer was represented in term of U /g of protein.

Chapter 5

Results And Discussions

5.1 Estimation of LC_{10}, and MNLC

LC_{10} and MNLC of Edible lime were calculated based on zebrafish larvae lethality (Figure 1). MNLC and LC10 of Edible lime were found to be 70.79 and 95.49 ppm. Therefore 1/5th of MNLC (14 ppm), 1/10th MNLC (7 ppm), and 1/20th MNLC (3.5 ppm) concentrations of edible lime were used for study of its effect in paracetamol liver toxicity.

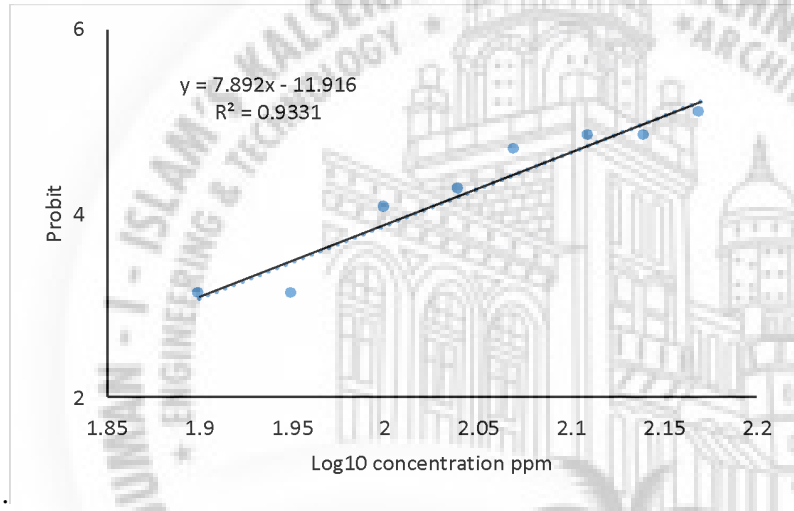

Figure 1: The curve of Log10 Concentration vs probit of Edible lime. LC10 = 95.49 ppm, MNLC=70.79 ppm

5.2 Visual assessment of hepatoprotective activity of Edible lime

The control larvae treated with vehicle exhibit transparent liver with normal tissue architect. Exposure to hepatotoxic concentration of Paracetamol (5mM), the liver becomes dark brown in color and showed reduced in its transparency. The similar changes were observed in the earlier investigation [7]. Edible lime protect zebrafish larvae against paracetamol related changes in the liver area, retention in yolk sac, and liver. The few representative images of this investigations are given below in Figure 2.

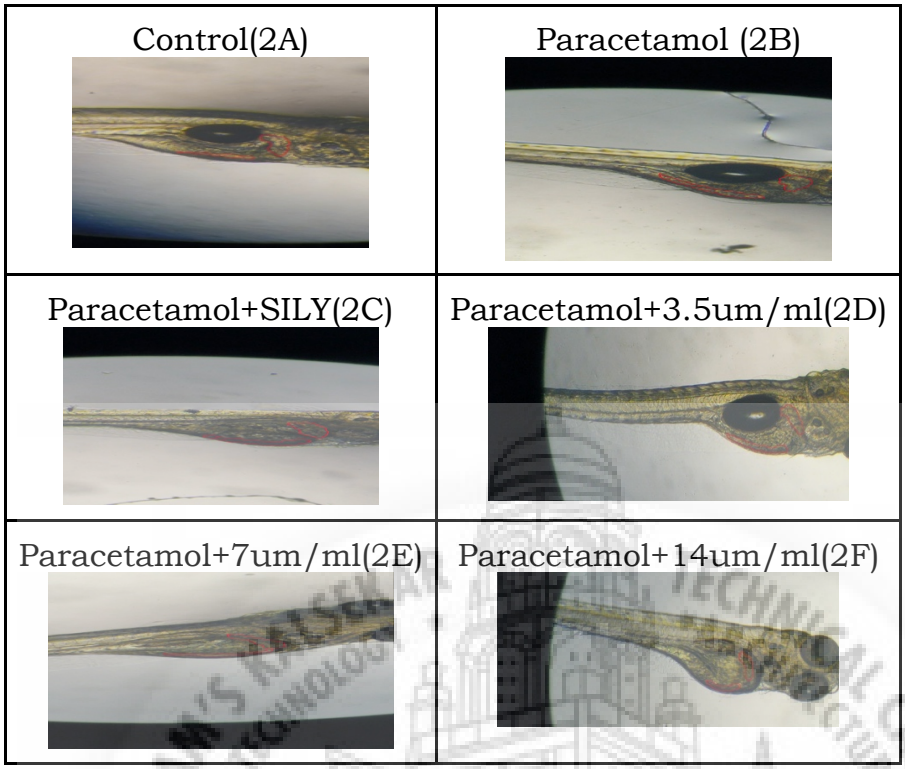

Figure 2 : Representative image of effect of edible lime on liver size area, yolk size area and liver degeneration. Paracetamol treated zebrafish larvae showed significant decrease in liver size area, made liver more darker with increase in yolk sac area which is reversed by both edible lime and silymarin.

5.3.1 Effect of Edible lime on liver size area

The liver area of larvae treated with paracetamol was significantly (p < 0.001) decreased in comparison with vehicle treated group. The reduced in the liver size is called as liver atrophy. Atrophied liver after paracetamol exposure in the present study was in accordance with the study conducted by Jian-Hui He et al., 2013 [6]. The reduce in the liver size in the paracetamol exposed group might be due to paracetamol-induced necrosis and degeneration of liver. A significant increase in liver size was seen in the Edible lime (14 ppm) exposed zebrafish larvae (P<0.01) and silymarin (10 µM) in comparison with paracetamol treatment group (**Figure 3**).

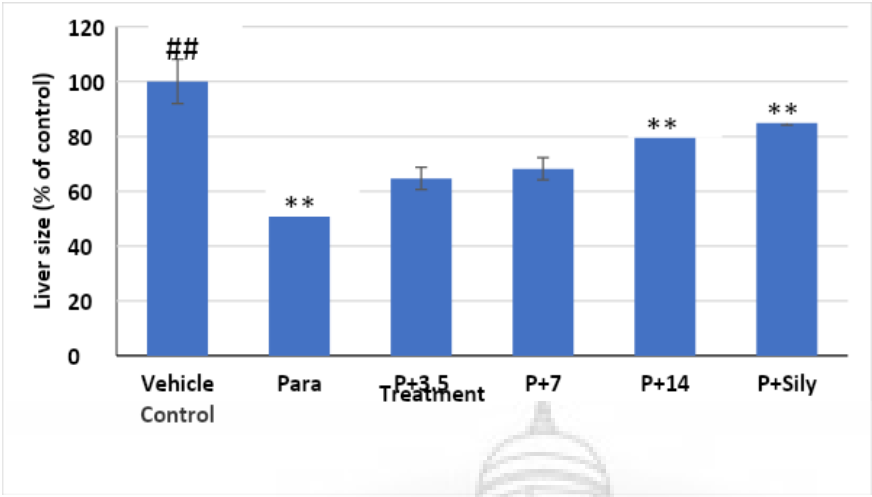

Figure 3: Effect of Edible lime on liver area in paracetamol exposed liver toxicity. Para: Paracetamol exposed; P+3.5: Paracetamol+3.5 ppm Edible lime; P+ 7: Paracetamol+7 ppm Edible lime; P+14:Paracetamol+14ppm Edible lime. The values were presented as Mean ± SEM, (n=6): #P<0.01 in comparison with normal control group; *P<0.05, **P<0.001 in comparison with the paracetamol control group.

5.3.2 Effect of Edible lime on Yolk sac area

The larvae yolk consists of 70 % of neutral lipid [9]. Thus the area of yolk sac has been suggested as one of the endpoints of liver function and yolk absorption could be delayed because of impairment of liver [10]. The area of yolk sac of larvae exposed to paracetamol was significantly increased ($p < 0.001$) in comparison with the normal control which is in accordance with the previous study [8]. A Significant decrease in the area of yolk sac was observed in the Edible lime (14 ppm) exposed larvae (P<0.01) and silymarin (10 µM) in comparison to paracetamol exposed group (Figure 4). The decrease in the area of yolk sac in the Edible lime and silymarin exposed group indicates the absorption of yolk could be due to improved in the function of liver.

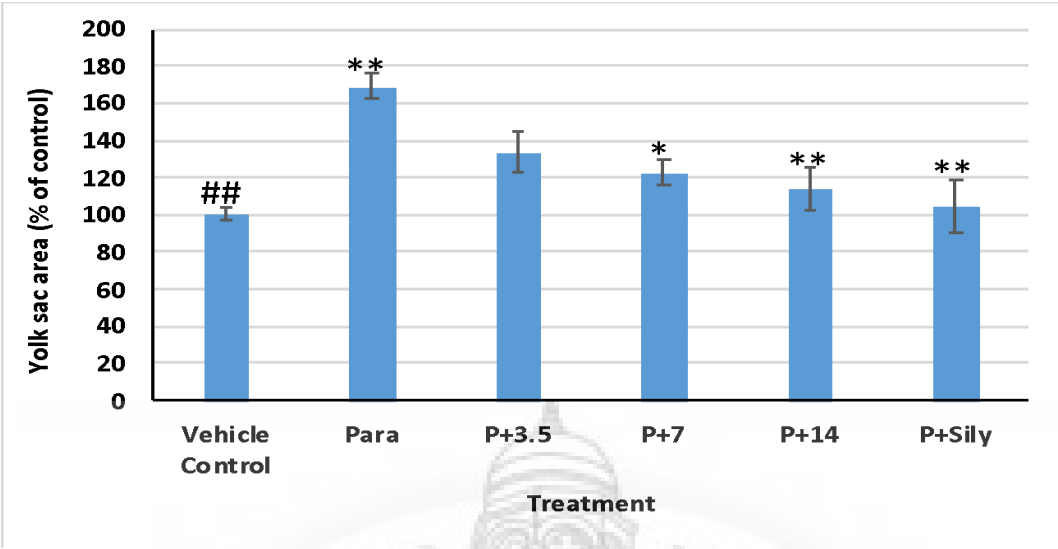

Figure 4: Effect of Edible lime on yolk sac area in paracetamol exposed liver toxicity. Para: Paracetamol control; P+3.5: Paracetamol+3.5 ppm Edible lime; P+ 7: Paracetamol+7 ppm Edible lime; P+14:Paracetamol+14ppm Edible lime. The values were presented as mean ± SEM, (n=6): #P<0.01 in comparison with normal control group; *P<0.05, **P<0.001 in comparison with the paracetamol control group.

5.4 Biochemical investigation of liver enzymes

The AST and ALT are two important biochemical markers of liver impairment. The AST and ALT level in the zebrafish larvae treated with paracetamol was significantly increased ($p < 0.001$) as compared to normal control groups (**Figure 5a and 5b**). Similar increase in the AST and ALT level was observed in isoniazid induced liver toxicity in zebrafish larvae [11]. Significant decrease in the level of AST and ALT was observed in the Edible lime treated zebrafish larvae at the concentration of 14 ppm (P<0.01) and silymarin at the concentration of 10 µM (P<0.01) as compared to paracetamol treatment group which indicate hepatoprotective activity of Edible lime and silymarin.

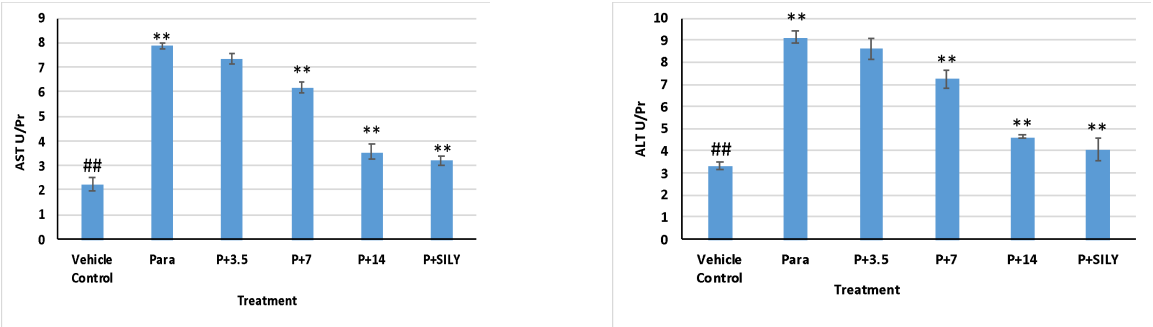

Figure 5: a. Effect of Edible lime on AST level in paracetamol induced liver toxicity in zebrafish larvae. **b.** Effect of Edible lime on ALT level in paracetamol induced liver toxicity in zebrafish larvae. AST: Aspartate Aminotransferase; ALT: Alanine Aminotransferase; U/gp: Unit per gram of protein; Para: Paracetamol control; P+3.5: Paracetamol+3.5 ppm Edible lime; P+ 7: Paracetamol+7 ppm Edible lime; P+14:Paracetamol+14ppm Edible lime. The values were presented as mean ± SEM, (n=6): #P<0.01 in comparison with normal control group; *P<0.05, **P<0.001 as compared to the paracetamol control group.

5.5 Effect of edible lime on liver optical density (Liver degeneration)

The percentage of degeneration of liver in larvae treated with paracetamol was significantly ($p < 0.001$) increased in comparison with normal control group which is in accordance with the previous study [6]. The significant reduced in the liver degeneration was seen in the Edible lime (14 ppm) exposed zebrafish larvae and silymarin (10 µM) exposed in comparison with paracetamol exposed group (Figure 6).

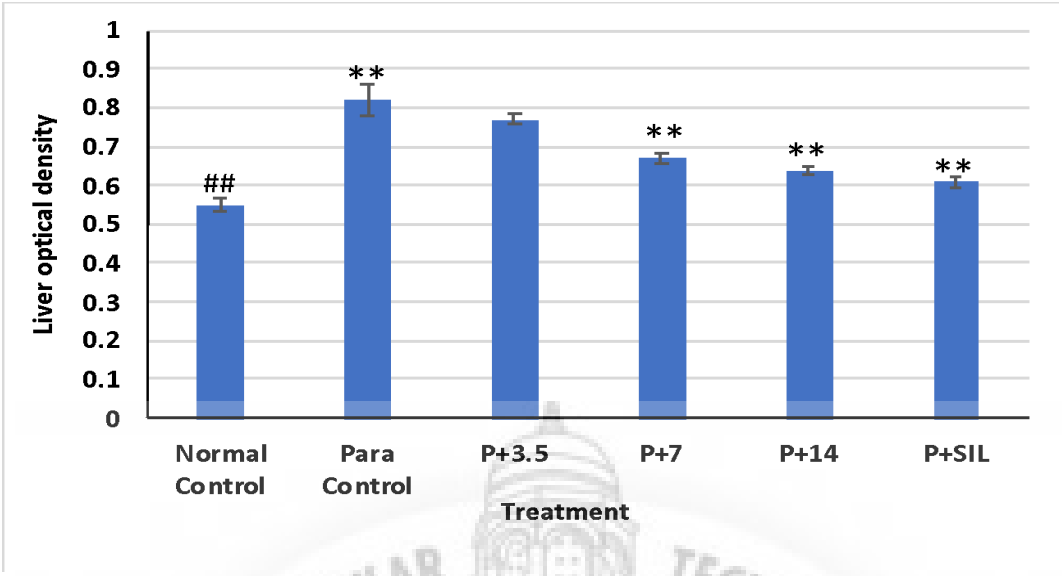

Figure.6: Effect of Edible lime on % degeneration of liver in paracetamol-induced liver toxicity. Para: Paracetamol control; P+3.5: Paracetamol+3.5 ppm Edible lime; P+ 7: Paracetamol+7 ppm Edible lime; P+14:Paracetamol+14ppm Edible lime. The values were presented as mean ± SEM, (n=6): #P<0.01 in comparison with normal control group; *P<0.05, **P<0.001 in comparison with paracetamol control group.

Chapter 6

Conclusion

In current study, we have evaluated the hepatoprotective activity of Edible lime on paracetamol induced hepatotoxicity in zebrafish models. Visual parameters of liver toxicity such as yolk sac retention, liver atrophy, and liver degeneration in zebrafish larvae were reduced with the use of Edible lime. In addition, Edible lime protects larvae zebrafish against mortality induced by paracetamol. Also, levels of ALT, and AST were reduced that indicate the hepato-protective activity of Edible lime. Our study provides scientific evidence to support traditional use of edible lime by local Vaidya for the treatment of liver diseases.

Chapter 7

REFERENCES

1] Herbpathy,(May 2015). Uses and benefits of calcium carbonate. https://herbpathy.com/Uses-and-Benefits-of-Calcium-Carbonate-Cid4198.

2] Life in vedas. Health benefits of chuna. https://www.lifeinvedas.com/health-benefits-chuna/.

3] Rajaretinam RK, Appadurai MI, Samuel GPV. Chemical genetic effects of Sargassum wightii during embryonic development in zebrafish. Indian J Pharmacol. 2015; 47(2):195-198.

4] Abusufyan S, Ibrahim M, Mohib K. Comparative *in vitro* antidiabetic and antioxidant activity of various extracts of *Ficus* species. Pharmacog J. 2018; 10(2):349–54.

5] Westerfield M. The zebrafish book, 5th Edition; A guide for the laboratory use of zebrafish (Danio rerio), Eugene, University of Oregon Press. Paperback. 2007.

6] Tae YP, Tae WM, Dong CC, Jung CC, Jung HL, Youn SK et al. An introduction to chuna manual medicine in Korea: History, insurance coverage, education and clinical research in Korean literature. Integr Med Res. 2014; 3(2):49-59.

7] Bastiaan Vilegenthart AD, Carl ST. An introduction to chuna manual medicine in Korea: History, insurance coverage, education and clinical research in Korean literature. British J Clinical Pharmacol, 2014;(78).

8] Jian HH, Sheng YG, Feng ZH Jing Z.A zebrafish phenotypic assay for assessing drug-induced hepatotoxicity. J of pharmacol toxicol met, 2013;(67)25-32.

9] Jones, K. S., Alimov, A. P., Rilo, H. L., Jandacek. A high throughput live transparent animal bioassay to identify non-toxic small molecules or genes that regulate vertebrate fat metabolism for obesity drug development. Nutrition & metabolism J.(London), 2008 ; 5 :23.

10] Hill A. Zebrafish use in drug discovery. 47th Annual Meeting of the Society of Toxicology, M Seattle, Washington, USA. Toxicol Sciences J,2008;102:(S-1):407 .

11] Zhang, Huang, W., Chua, S. S.wei..Modulation of acetaminophen-induced hepatotoxicity by the xenobiotic receptor CAR. Science J, 2002; 298(5592):422–424.

8.FUTURE SCOPE

A study was conducted on the hepatoprotective activity of edible lime using zebrafish larvae. Although this study gives Favorable results on zebrafish models but to further confirm its activity, the study on higher rodent models is recommended. Edible lime's hepatoprotective activity was evaluated using various experimental parameters such as AST-ALT levels, liver atrophy, liver degeneration, and yolk sac edema. The effect of edible lime on antioxidant parameters and other biomarkers of hepatotoxicity can be further investigated.

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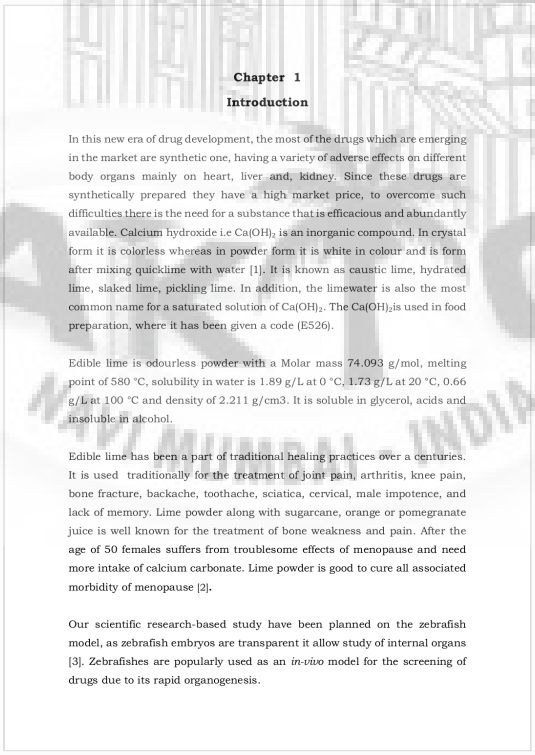

Chapter 1

Introduction

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