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Acute Toxicity Study of Two Different plants extracts of citrus pulp powder and tecoma stans in zebrafish (Danio Rerio)

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ABSTRACT: The present study was investigated the oral acute toxicity study on two different plant i.e Citrus limon fruit and tecoma stans flower extracts. The tecoma stans(TS) methanolic flower extracted using soxhlet apparatus where citrus pulp podwer(CPP) purchased from suppliers. Oral toxicity test was evaluated with zebrafish as per OECD guidelines 203(june 18 2019). Both the extract shown high toxicity value at 400mg/L concentration and mortality rate is ≤70% in a period of 96 hours. The lethal dose that kills 50% test fish on exposure of both CPP and TS was calculated respectivelly at **312.5mg/L** and **257.5mg/L**. The result indicated that no mortality rate at ≥100mg/L safe to consume.

KEYWORD: zebrafish, tecoma stans, citrus pulp powder.

I. INTRODUCTION

The development of herbal industry nowadays peoples are started to use herbal product and extracts of plants having a no side effects and safe to consume. The many study that not conducted an toxicity of medicinal drugs. the present study was demonstrated the toxicity level study two different plants of citrus pulp and tecoma stans in zebrafish. The toxicity test not only for the allopathic medicine it also used for alternative systems of medicine in the process of drug discovery and clinical or pharmacological activities to reduce adverse effects[1].

The zebrafish are used to evaluate toxicity study in presence of bioactive compound in extracts. the studies are shown that the zebrafish having 85% similarities in the genomic sequences as well as brain patterning with humans. Thus toxicity study makes advantageous assay for zebrafish[2]. Medicinal plants play an important role on human health and potentially give safe drugs as herbal medicine[3].

Tecoma stans is tree Belongs to the family of Bignoniaceae. The Vernacular name of Tecoma stans L. is yellow bells, yellow elder, trumpet flower in English and Piliya in Hindi. it is an important medicinal plant[4]. It was used traditionally for the control diabetics, hepatic, dysenteric, anorexia problems. Tecoma stans are having the sevaral bioactive constituents like alkaloids, phenols, flavonoids, monoterpenes etc. which was shows the great medicinal value of this plant. The plants gives various pharmacological actions due to presence of phytochemicals of various part of the plants are reported to be having like anti-inflammatory, analgesic, anticancer cardio-protective effect, genotoxic, cytotoxicity, wound healing, anti-hyperglycemic, protect CNS, gastric ulcer healing, antiproliferative, antioxidant, anti-microbial, hemolytic activity, anti lipoxygenaseand acetyl- cholinesterase inhibitory activities[5].

Citrus limon (L.) is a tree comes under Rutaceae family with evergreen leaves and yellow edible fruits. The C. limon of the fruit pulp having an particularly the essential oil and vitamin C. C. limon fruit juice has traditionally been used for the solutions of scurvy before the discovery of vitamin C. the lemon juice is traditionally used to cure scurvy, sore throats, fevers, rheumatism, high blood pressure, and chest pain Currently, thus scientific publications focus on the ever extensive pharmacological actions of C. limon fruit extract, juice and essential oil. They include studies of the antibacterial, antifungal, anti-inflammatory, anticancer, hepatoregenerating and cardioprotective activities. The potency of pharmacological action due to presence rich chemical composition in C. limon is determined[6].

The study was conducted to evaluate the acute oral toxicity on dose dependent manner changes of two plant extract in zebrafish. Plants are not generally protected to devour as they may contain harmful substance compounds in the various pieces of the plant. Numerous investigations have uncovered that the many plant

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extricates become harmful to the various organs on fixation subordinate way and can prompt lethality. A significant number of the remedy that are utilized as solution for various diseases are not attempted for their toxicity study level[7].

The present investigation study to understand the toxicity level of two plant extracts. It is hypothesized that higher concentrations of the leaf and fruit extracts could be toxic to the zebrafish. Since there are no published information on the acute toxicity study determination of the two different plant extract exposed to fish the current study was investigation has been undertaken.

II. MATERIALS AND METHODS

1.1 Preparation of flower Extract of Tecoma stans (TS)

Tecoma stans fresh flowers was collected from nearby Vijayapura, Karnataka, India. The fresh flowers of the plant was shade dried for 10 days and then made an fine powdered. The powdered material was extracted with methanol using Soxhlet's apparatus. The solvents was completely evaporated at 40º C using rotary evaporator. The yield of the extract obtained was 20.8% and various concentrations of the test solutions were prepared.

1.2 Preparation of citrus pulp powder (CPP)

The Citrus pulp powder was purchased from Mevive International Food Ingredients. M3, Mayflower Metropolis, Udayampalayam Road, Sowripalayam, Coimbatore 641028, Tamilnadu, India.

1.3 ANIMAL HUSBANDRY[8]

Zebrafish (Danio rerio) 3 – 4 months old was be obtained from an authorized commercial supplier (Shruti Aqua Culture, Mumbai, Maharashtra, India). Zebrafish was randomly divided into groups and fed twice daily with commercial flake food. After randomization into various groups, all fish used in this experiment was observed in quarantine at least one week before use in experimental studied.

Table 1 Physical parameters and normal values for laboratory Zebrafish[9]

Sl. No.	Parameters	Normal Values
1.	Room Temperature	21-25 ^{0}C
2.	pH	6.0 - 8.5
3.	Photoperiod (hours light)	14:10
4.	Recommended length range (cm)	1 – 2
5.	Light intensity (Lux)	300
6.	Oxygen concentration	at least 80% of air SV
7.	Relative humidity (%)	70
8.	conductivity	8.2 ± 0.2

1.4 DESIGN OF THE ACUTE TOXICITY TEST

The acute toxicity study of zebrafish was investigated by exposed **CPP** and **TS** was performed according to the guideline 203 of OECD ((modified, adopted June 18, 2019)[7]. The fish were exposed to the test extract preferably for a period of 96 hours. The Mortality was recorded at 24, 48 and 96 hours and the concentrations which kill 50 percent of the fish were recorded.

The zebrafish of various genders were randomly assigned to different extract sample by selected (12.5, 25, 50, 100, 200, and 400mg) was prepared by dissolved in 1 liter of chlorinated water. The Control groups (7 fishes) were also included for each treatment. The fish of control and treatment groups were inspected after 24, 48, 72 and 96 hours. The symptoms, visible abnormalities behavior and mortalities were recorded at 24, 48, 72 and 96 hours and concentrations to kill 50% of the fish (LD_{50}) values were calculated[9]. Zebrafish were stopped feeding before 24hour to start toxicity test and fed once a daily with commercial dry diet. If there is no visible movement and touching to fish shows no reactions considered as dead Fish. Dead fishes were removed from tank when mortalities are recorded. Calculated the LD_{50} based on the concentration of the test sample in water which killed $_{50}$ percent of fish within 96 hours of exposure was observed.

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III. RESULTS

Table 2. LD_{50} calculation CPP

SL. No.	Concentration (mg)	% of Mortality	LD 50%
01.	12.5	0	
02.	25	0	
03.	50	0	
04.	100	14.28	**312.5 mg/L**
05.	200	28.57	
06.	400	71.42	

Based on the (y=mx+C) equation y = 0.167x and coefficient is r^2 =0.964 from the graph the LD_{50} was found at **312.5 mg/L.**

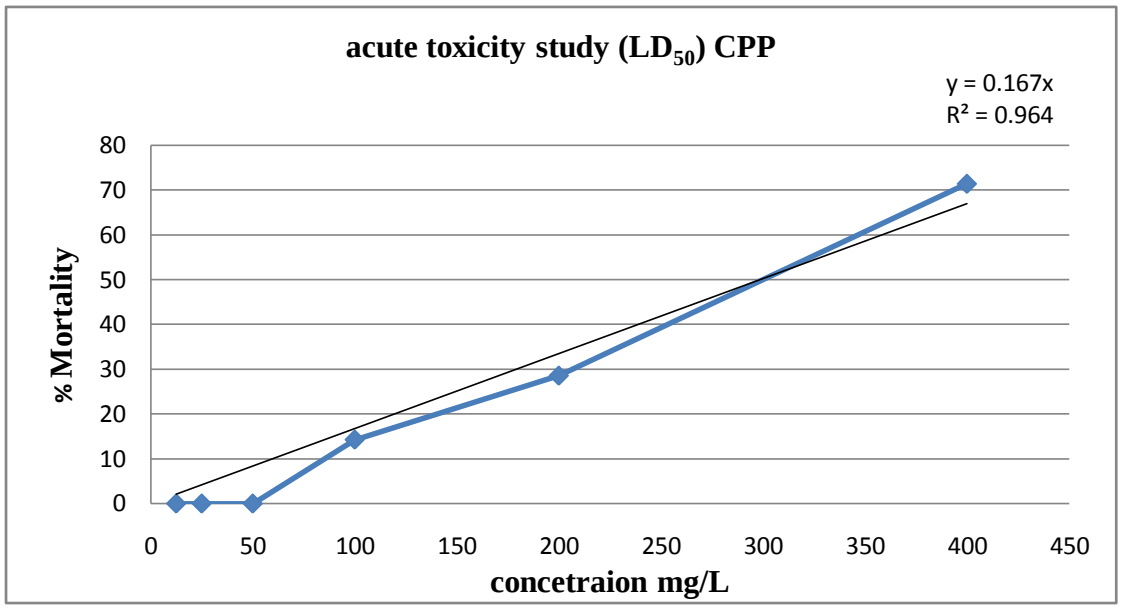

Table 3. LD_{50} calculation TS

SL. No.	Concentration (mg)	% of Mortality	LD 50%
01.	12.5	0	
02.	25	0	
03.	50	0	
04.	100	14.28	**257.5 mg/L**
05.	200	28.57	
06.	400	85.71	

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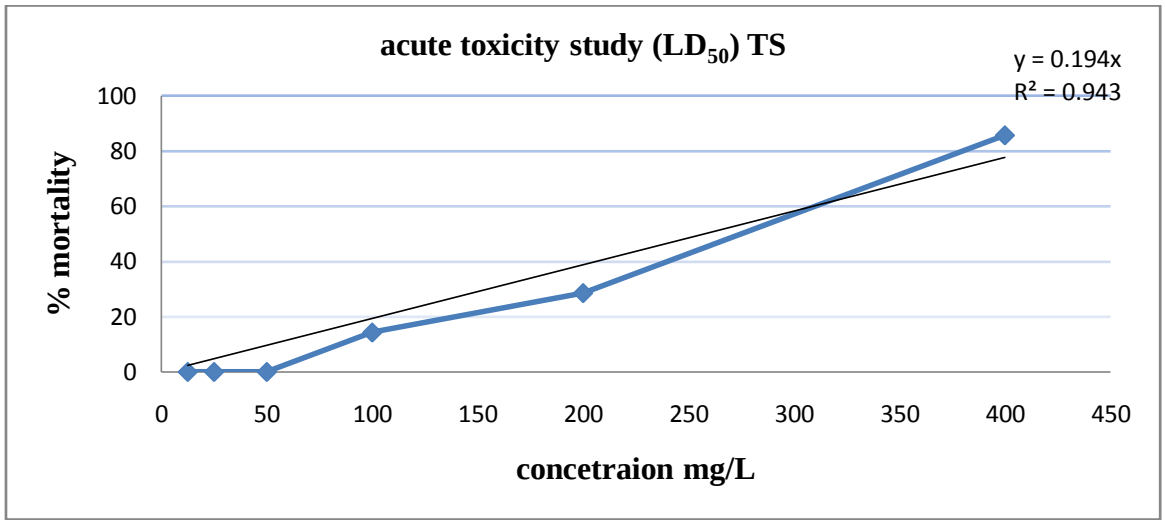

Based on the (y=mx+C) equation y = 0.167x and coefficient is r^2 =0.964 from the graph the LD_{50} was found at **257.5 mg/L.**

No behavioral changes were observed in control fish. The study of acute oral toxicity was conducted on both extracts of CPP and TS was more toxic to zebrafish. The medium lethal concentration (LC_{50}) value of CPP and TS was founded at **312.5mg/L** and **257.5mg/L** respectively for 96 hours exposure. The partial coefficient of CPP is 0.963 and TS is 0.964. The plant extract of both CPP and TS toxicity was observed at concentration of 400mg/L >70% mortality was found. The lower concentrations that are 12.5mg/L, 25mg/L, and 50mg/L are no mortality and consider as safe for zebrafish.

IV. DISCUSSION AND CONCLUSION

The objectives of study to demonstrated the acute toxicity of **CPP** and **TS** and zebrafish shown lethality was found at **312.5 mg/L** and **257.5 mg/L** which is highest LC_{50} value than already reported on plant E. fluctuans leaf extract[10]. From the above data we concluding that the both extracts at different concentrations are toxic to zebrafish at dose depended manner. The low concentration extract was safe to consume and avoid the toxicity. The current study was investigated the safe use of CPP and TS on animal model for the further pharmacological screening activities.

V. ACKNOWLEDGMENT

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