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DETERMINATION OF LETHAL CONCENTRATION FIFTY (LC_{50}) OF WHOLE PLANT ETHANOLIC EXTRACT OF *AMARANTHUS VIRIDIS, CYNODON DACTYLON* & *AERVA SANGUINOLENTA* ON ZEBRAFISH (DANIO RERIO) EMBRYOS

Shabnam Zahir [*1], Tamal Kanti Pal [2], Abhijit Sengupta [3], Shibendu Biswas [4], Shyamal Bar [5] and Samrat Bose [3]

Department of Pedodontics and Preventive Dentistry [1], Department of Oral and Dental Sciences [2], Department of Pharmacy [3], Department of Microbiology [4], Gurunanak Institute of Dental Science & Research, Kolkata - 700114, West Bengal, India.
Guru Nanak Institute of Pharmaceutical Sciences & Research [1], Kolkata - 700110, West Bengal, India.
Department of Orthodontics [5], Burdhaman Dental College and Hospital, Bardhaman - 713101, West Bengal, India.

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Toxicological study, Plant extracts, Zebra fish embryo

Correspondence to Author:
Dr. Prof. Shabnam Zahir

BDS (CAL), MDS (CAL),
Professor,
Department of Pedodontics and Preventive Dentistry Gurunanak Institute of Dental Science & Research, Kolkata - 700114, West Bengal, India.

E-mail:dr.shabnam.zahir@gmail.com

ABSTRACT: Introduction: Most potent toxic elements are derived from nature only. So safety and toxicological evaluation of plant extracts which are otherwise thought to be very safe, are mandatory. The zebrafish embryo has become an important model organism for toxicological research due to its small size, genetic similarity to humans, *etc*. **The Objective:** The objective of the present study is to determine acute toxicity that is lethal concentration fifty (LC_{50}) of whole plant ethanolic extract of *Amaranthus viridis* (A1), *Aerva sanguinolenta* (A2) & *Cynodon dactylon* (A3) on Zebrafish (*Danio rerio*) embryos at a different time interval. **Material and Method:** Acute toxicity testing of the three whole plant ethanolic extracts A1, A2, A3 with six different concentrations each were performed according to OECD guideline 236 on Zebrafish embryos. Embryo development was monitored after 24 h interval for 120 h. After incubation, the living and dead embryos were evaluated using Light Microscope and images obtained with an optical camera. **Statistical Analysis:** LC_{50} values were calculated using the Probit method in the SPSS package. Graphs were plotted using Sigma Plot. **Result***: Amaranthus viridis* (A1), *Aerva sanguinolenta* (A2), *Cynodon dactylon* (A3) whole plant ethanolic extract have mean LC_{50} of 316.22080 µg/ml, 575.42800 µg/ml, 35486.000 µg/ml respectively at a time interval of 24 hours on Zebrafish embryos. **Conclusion:** LC_{50} values of three whole plant ethanolic extracts A1, A2, A3 with six different concentrations each on Zebrafish embryos will aid in determining the therapeutic dose of the plant extracts.

INTRODUCTION: *Amaranthus viridis, Aerva sanguinolenta* & *Cynodon dactylon* are the three medicinal plants with many therapeutic virtues **Table 1**. However, like medicinal plants, *Amaranthus viridis, Aerva sanguinolenta* & *Cynodon dactylon* are not exempt from the risks of intoxication.

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The work of Ugwah-Oguejifor CJ *et al*., revealed that some plant species have hepatotoxic effects [1]. Thus studies on the safety and effectiveness of medicinal plant extract and extract-derived phytoconstituents have become one of the main concerns to guarantee their use for mankind [2].

Rats and mice are traditionally used for the estimation of acute toxicity of substances for mammals. But they are expensive and difficult to manipulate and assess during the embryonic stage. Within the past decade (since 1950), the zebrafish has become an important model organism for toxicological research [3].

Zebrafish (Danio rerio), are vertebrate organisms that are an excellent alternative model due to their small size, rapid external development, optical transparency during early development, permeability to small molecules, genetic similarity to humans, and great fecundity. Zebrafish embryos develop most of the major organ systems present in mammals, including the cardiovascular, nervous, and digestive systems in less than a week [4, 5]. The median lethal concentration, LC_{50} is the amount of a material given all at once, which causes the death of 50% of a group of test animals. The LC_{50} is a way that measures the short-term poisoning potential (acute toxicity) of a material [6].

The present study is performed to determine acute toxicity that is lethal concentration fifty (LC_{50}) of whole plant ethanolic extract of *Amaranthus viridis* (A1), *Cynodon dactylon* (A2) & *Aerva sanguinolenta* (A3) at different time intervals on Zebrafish (Danio rerio) embryos.

The study is also targeted to determine the percentage mortality of Zebrafish (Danio rerio) embryos on exposure to different concentrations of whole plant ethanolic extract of *Amaranthus viridis* (A1), *Cynodon dactylon* (A2) & *Aerva sanguinolenta* (A3) at different time intervals.

TABLE 1: CHARACTERIZATION OF PLANT SPECIMEN (STUDY SAMPLE)

Name of plant	Vernacular name	Family	Genus	Species	Binomial name	Plant form	Ethnomedicinal uses
Amaranthus viridis	Chowlai	Amaranthaceae	*Amaranthus*	*A. viridis*	*Amaranthus viridis* Linn	Annual herb	anti-inflammatory, diuretic, analgesic, antiulcer, antiemetic, laxative, antimicrobial,
Aervasan guinolenta	Safedfulia	Amaranthaceae	*Aerva*	*A. sanguinolenta*	*Aervasan guinolenta* (L) Blume	Herb	anti inflammatory agent, diuretic demulcent, galactogue, anthelmintics, antimicrobial
Cynodon dactylon	Durva grass	Poaceae	*Cynodon*	*C.dactylon*	*Cynodon dactylon* (L) Pers	Perennial grass	Styptics, laxative, coolant, expectorant, carminative, antimicrobial

MATERIALS AND METHODS:

Ethical Clearance: Requisite ethical clearance (GNIDSR/IEC/18-18) & permission to undertake the study was obtained from the institutional ethical committee of Guru Nanak Institute of Dental Sciences and Research, Kolkata.

Collection, Authentication & Pre-treatment of Plant Sample: Whole plant of *Amaranthus viridis* (A1), *Aerva sanguinolant*a (A2) & *Cynodon dactylon* (A3) were collected from medicinal garden of Ramkrishna mission ashram, Narendrapur, Kolkata, West Bengal during the month of December 2018. Herbarium were made and the specific plants were authenticated from Botanical survey of India, Howrah, West Bengal - 711103. The voucher specimen (GNIDSR 002, 003 & 004) were deposited in College Herbarium.

The plant samples were washed, shade dried on paper sheets, chopped into pieces, made into a coarse powder in a mixer grinder (Philips HL), and finally the coarse powder was stored in a sealed, labelled polythene packet in the refrigerator at 4 degree centigrade.

Preparation of Plant Extracts: The coarse powder of *Amaranthus viridis* (A1) (60.8 gm), *Aerva sanguinolanta* (A2) (53.3gm) & *Cynodon dactylon* (A3) (50.29 gm) were subjected to continuous hot extraction with 350 ml, 300ml, and 300 ml ethanol (Mercks) respectively by Soxhlet apparatus (Borosil) with a yield of (26.001gm) (A1), (23.5462g) (A2), (20.546gm) extract by weight. Extracts with solvent were concentrated by distillation using a rotary evaporator (RE100PR0 MFGD silicogex/USA Takashi) with a yield of (14.001gm) (A1), (12.1162g) (A2), (11.006gm) (A3) extract by weight which was stored in a sterilized glass beaker in a refrigerator at 4-degree centigrade. Preparation of plant extracts was performed in the Guru Nanak Institute of Pharmaceutical Science and Technology, Kolkata.

Preparation of Different Concentration of Plant Extracts: Plant extracts were measured on an electronic weighing scale (Model CAS –ME–310). The premeasured amount of the three stored plant extracts (100 microgram) were mixed with a fixed volume (1 ml) of DMSO (Mercks) to prepare the

stock solution. Electrical stirrer (Remi Laboratory Instruments) was used for 30 min for proper mixing of plant extract and solvent. The prepared 100 µg/ml concentration mix was kept as a stock solution. For each plant extract six different concentration of solution (100 micron gram/ millilitre, 200 micron gram/millilitre, 300 micron gram/millilitre, 400 micron gram/millilitre, 500 micron gram/millilitre, 600 micron gram/millilitre) with Dimethyl sulfoxide (DMSO, Himedia) were prepared **Fig 1**.

FIG. 1: PREPARATION OF DIFFERENT CONCENTRATIONS OF PLANT EXTRACT

Acute Toxicity Testing of Whole Plant Ethanolic Extract of *Amaranthus viridis, Cynodon dactylon* & *Aerva sanguinolanta*: According to OECD guideline 236 (Fish Embryo Toxicity Test) acute toxicity that is lethal concentration (LC_{50}) of whole plant ethanolic extract *Amaranthus viridis*, *Cynodon dactylon* & *Aerva sanguinolanta* on Zebrafish (*Danio rerio*) embryos was determined [7].

Procedure for Acute Toxicity Testing in Zebrafish Embryos: Zebrafish were procured from a local supplier and were maintained in 1 ft aquaria with 12 h light: 12 h dark cycle at ±28ºC and fed twice daily with dry flake food. Egg production, collection of embryos were obtained from spawning zebrafish adults in breeding tanks with a sex ratio 3 male: 5 female. Spawning was induced in the morning, and after 2 h, embryos were collected and washed using embryo medium. Fertilized and dead embryos were separated according to the descriptions of Kimmel [7].

FIG. 2: EXPOSURE OF ZEBRAFISH EMBRYO TO SPECIFIC CONCENTRATION OF PLANT EXTRACTS

Embryos were divided into six different groups with 10 embryos per group. For each of the three plant extracts *Amaranthus viridis*, *Cynodon dactylon* & *Aerva sanguinolenta*, six different concentration of a solution (100-micron gram/ milliliter, 200-micron gram/milliliter, 300-micron gram/milliliter, 400-micron gram/milliliter, 600-micron gram/milliliter, 800-micron gram/milliliter) with Dimethyl sulfoxide (DMSO, Himedia) were prepared, and the embryos were exposed to test compounds **Fig. 2** for a period of 120 h, at a temperature of 28 ºC and twelve dark and twelve light exposure. Plates were sealed with paraffin to minimize evaporation. Embryo development was monitored after 24 h interval for 120 h. After incubation, the living and dead embryos were placed on glass slides, and morphological characteristics were evaluated using Light Microscope and images obtained with an optical camera **Fig. 3, 4, 5, 6.**

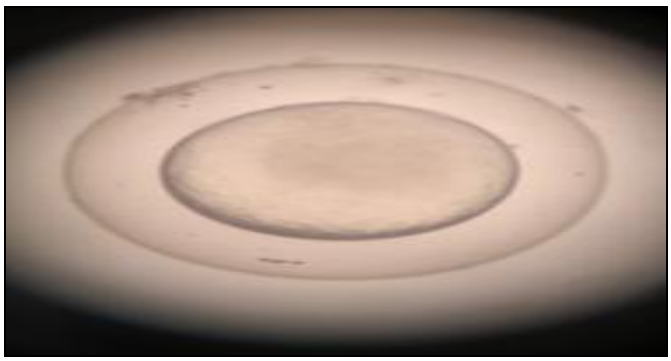


FIG. 3: LIVE ZEBRAFISH EMBRYO IN EARLY DEVELOPMENTAL STAGE

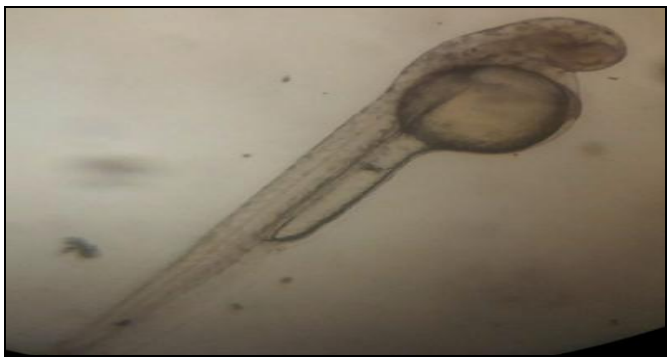


FIG. 4: LIVE ZEBRAFISH EMBRYO IN LATE DEVELOPMENTAL STAGE

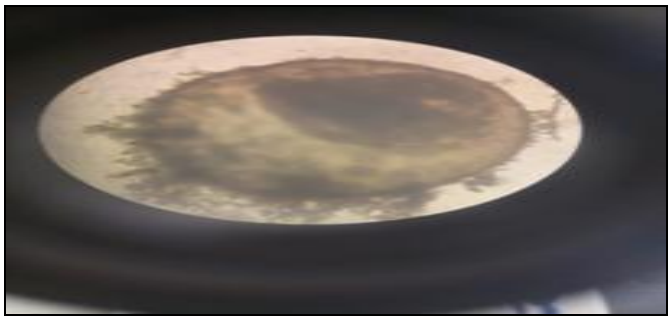


FIG. 5: DEAD ZEBRAFISH EMBRYO IN EARLY DEVELOPMENTAL STAGE

FIG. 6: DEAD ZEBRAFISH EMBRYO IN LATE DEVELOPMENTAL STAGE

Experiments were conducted in triplicates. The maximum concentration at which 50% of the Zebrafish embryos were killed was recorded. The percentage of Zebrafish embryos' mortality for every six concentrations of the three test compounds each was recorded after 24, 48, 72, 96, and 120 h. Embryos were scored as dead if the tissues had changed from a transparent to an opaque appearance and if they were no longer moving **Fig. 5, 6.**

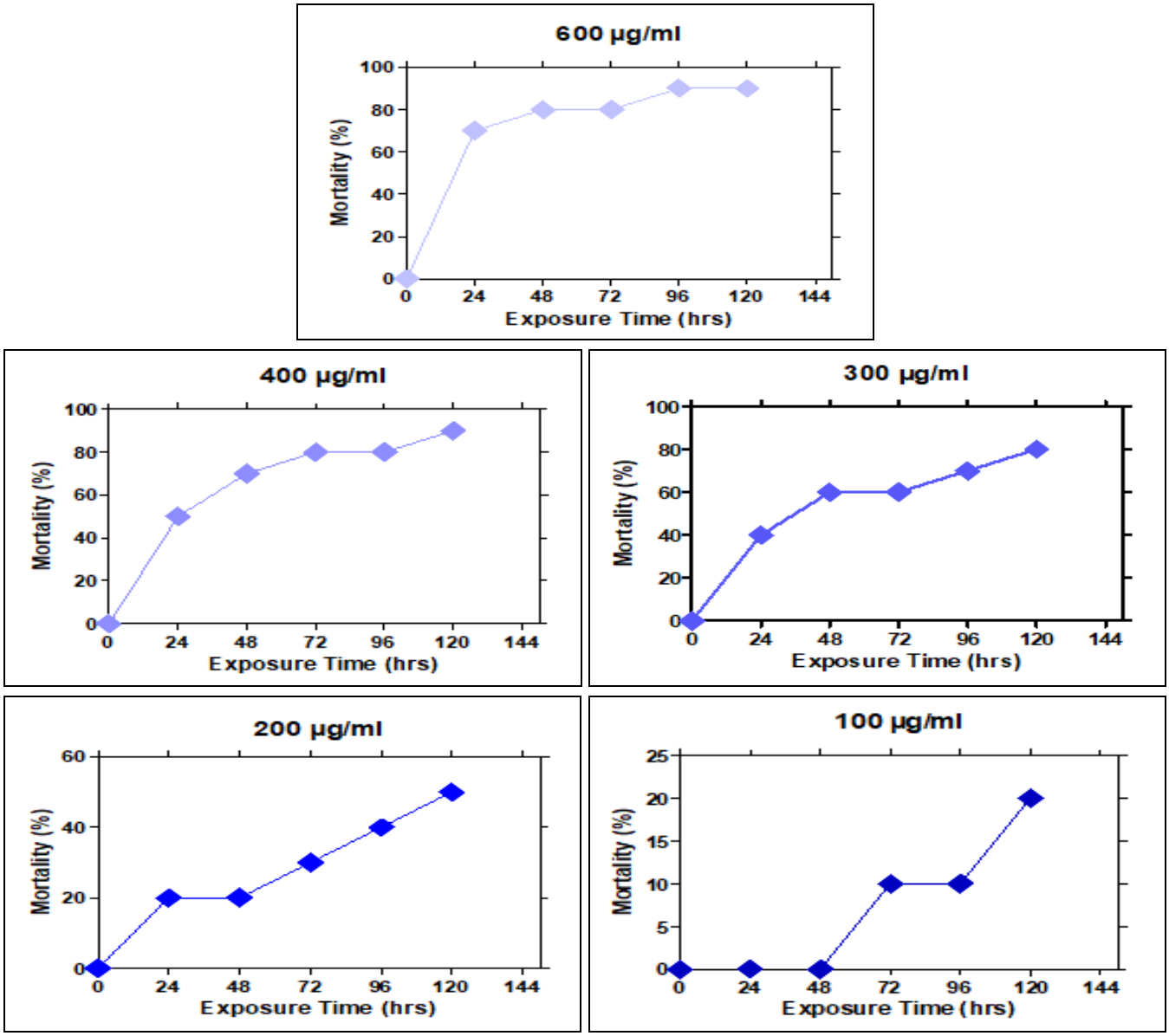
Calculation of LC_{50}: LC_{50}, median lethal concentration (LC_{50}) were statistically analyzed by Graph Pad PrismVersion 6 software. LC_{50} value is determined based on probit analysis which is a graphical method for calculating the LC50. In this Log dose or Log concentration on the X-axis *vs*. Probits of mortality on the Y-axis were plotted for a varied range of doses.

Statistical Analysis: Statistical analysis was performed using the SPSS program with one-way ANOVA and Tukey test. A significant difference was considered as $p<0.05$ - Significant, $p<0.001$ - Highly significant

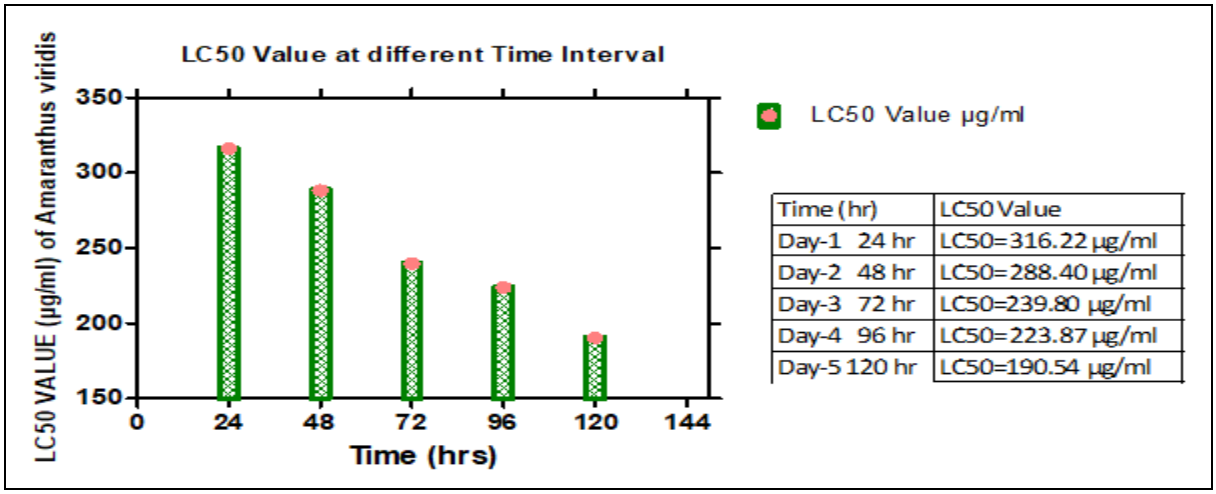
RESULT: Graph 1,3,5 reveal the percentage of mortality of Zebrafish embryos treated with 6 different concentrations (100-micron gram/ml, 200-micron gram/ml, 300-micron gram/ml , 400-micron gram/ml, 600-micron gram /ml, 800-micron gram/ml) of *Amaranthus viridis* (A1), *Aerva sanguinolanta* (A2) & *Cynodon dactylon* (A3) plant extracts respectively each at a time interval of 24 hours, 48 h, 72 h, 96 h, and 120 h. For all the three plant extracts the lowest (100µg/ml) concentration exposure to Zebrafish embryos produced Percentage of mortality of zebrafish embryo 0% after a time interval of 24 h, but after 120 h plant extract A1, A2, A3 produced Percentage of mortality of 20%, 10%, and 0% respectively. For the three plant extracts, mortality rate of embryos increased as their concentrations increased.

For all the three plant extracts, the highest (800µg/ml) concentration exposure to Zebrafish embryos produced Percentage of mortality of zebrafish embryo 100% after a time interval of 120 h, but after 24 h plant extract A1, A2, A3 produced Percentage of mortality of 100%, 80%, and 10% respectively. On comparing the three plant extracts *Cynodon dactylon* (A3) produced the lowest, *Aerva sanguinolanta* (A2) produced moderate and *Amaranthus viridis* (A1), which produced highest mortality rate of embryos at both highest and lowest concentrations and at shortest and longest time durations. Graphs 2, 4, 6 shows median lethal concentration (LC_{50}) of ethanolic whole plant extracts of *Amaranthus viridis* (A1), *Aerva sanguinolanta* (A2) & *Cynodon dactylon* (A3) after 24 h, 48 h, 72 h, 96 h and 120 h time interval. **Table 1, 2, 3, 4, 5** shows a comparison of LC50 values at different time intervals in terms of {Mean (SD)} among all the groups using ANOVA test. At a time interval of 24 h, 48 h, 72 h, 96 h and 120 h there was a statistically significant difference observed between the three groups (p value<0.001). Further using Tukey's post hoc analysis, significant diffe-rence was observed between all the three groups.

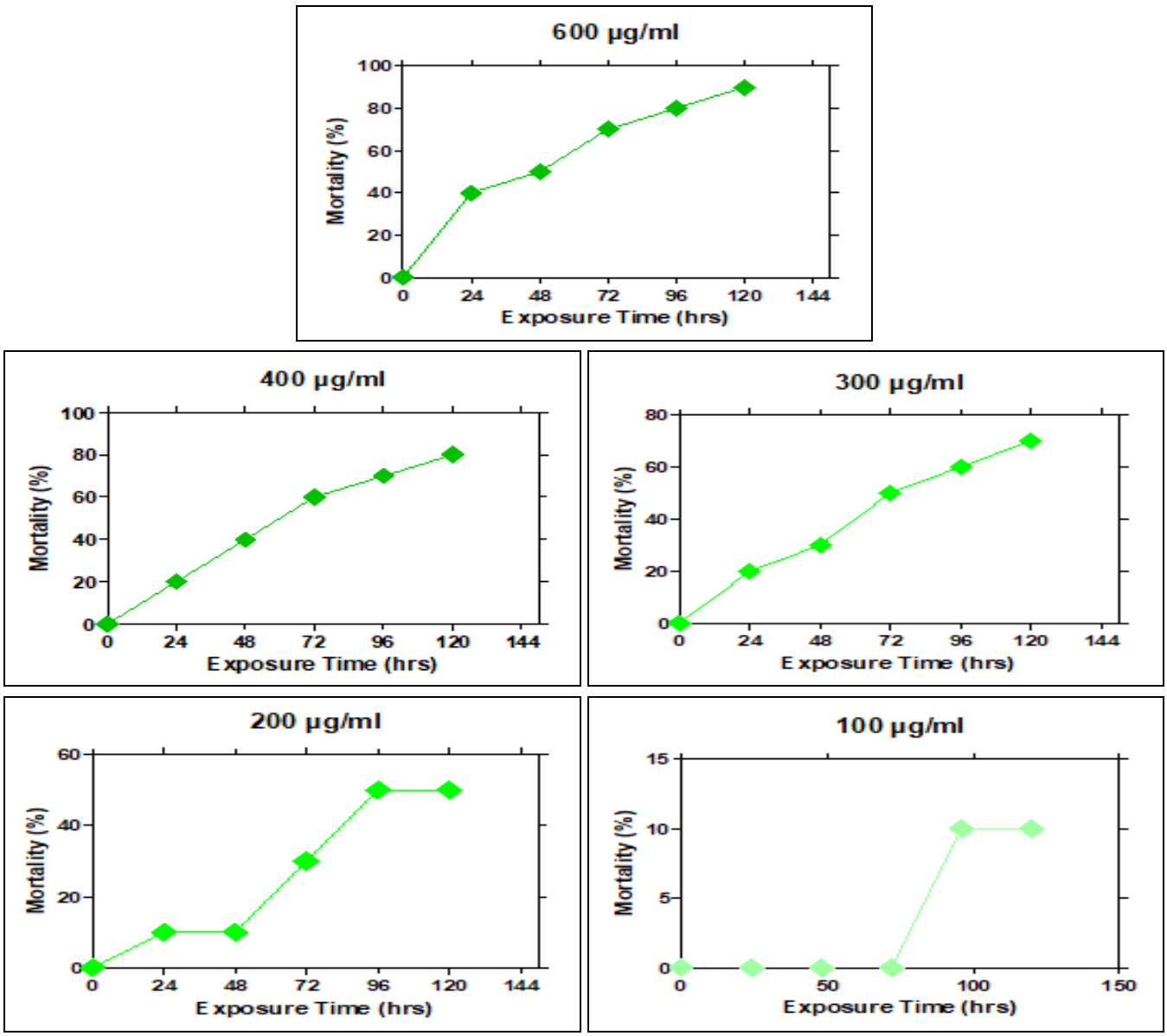
The lower the LC_{50} value the more toxic the substance. *Amaranthus viridis* (A1), *Aerva sanguinolanta* (A2), *Cynodon dactylon* (A3) have mean LC_{50} of 316.22080 µg/ml, 575.42800 µg/ml, 35486.000 µg/ml respectively at a time interval of 24 h on Zebrafish embryos. Among the three plant extracts *Amaranthus viridis* (A1) has the highest, *Aerva sanguinolanta* (A2) has medium, and *Cynodon dactylon* (A3) has the lowest toxicity on Zebrafish embryos.



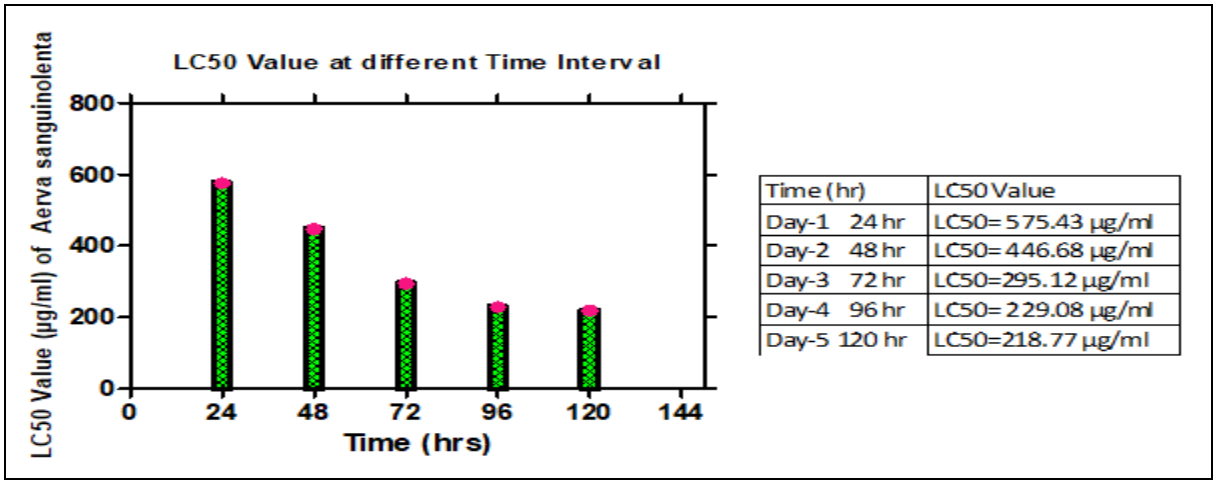
GRAPH 1: PERCENTAGE OF MORTALITY OF ZEBRA FISH EMBRYO FOLLOWING EXPOSURE TO SIX DIFFERENT CONCENTRATIONS OF *AMARANTHUS VIRIDIS* (A1) AFTER 24 h, 48 h, 72 h, 96 h AND 120 h TIME INTERVAL



GRAPH 2: LC_{50} OF ETHANOLIC WHOLE PLANT EXTRACTS OF *AMARANTHUS VIRIDIS* (A1) AFTER 24 h, 48 h, 72 h, 96 h AND 120 h TIME INTERVAL

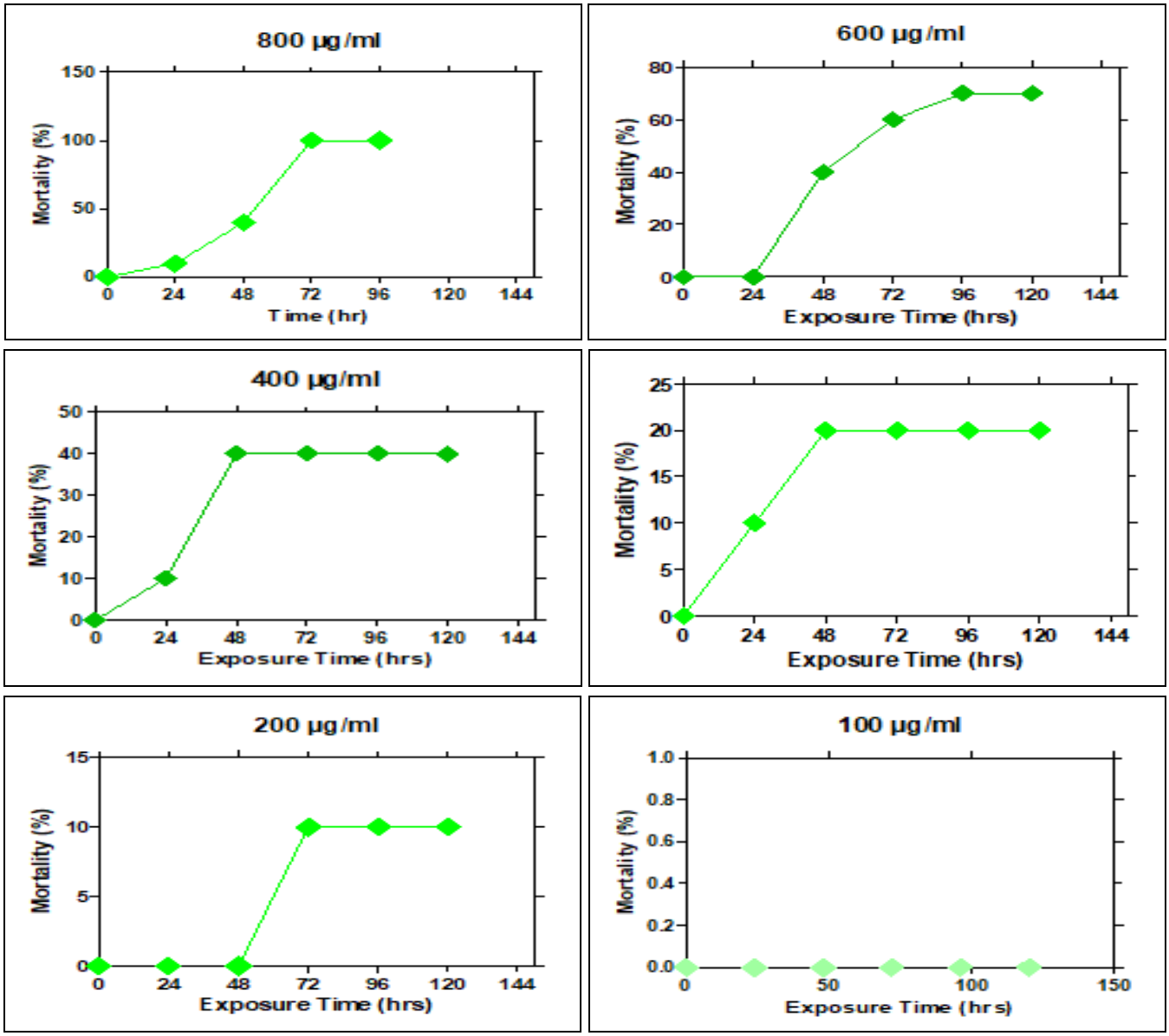


GRAPH 3: PERCENTAGE OF MORTALITY OF ZEBRA FISH EMBRYO FOLLOWING EXPOSURE TO SIX DIFFERENT CONCENTRATION OF *AERVASAN GUINOLENTA* (A2) AFTER 24 h, 48 h, 72 h, 96 h AND 120 h TIME INTERVAL

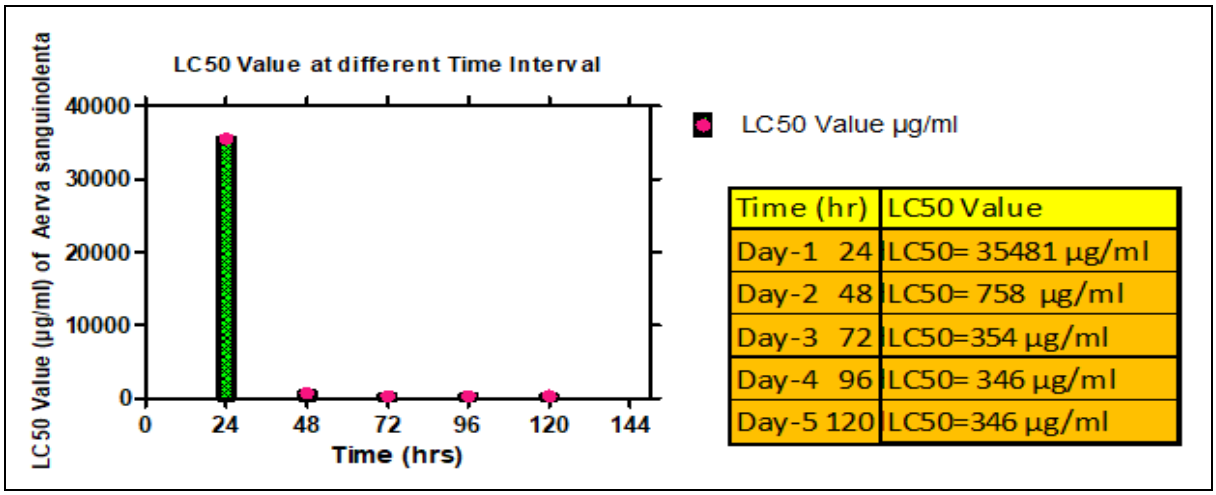


Time (hr)	LC50 Value
Day-1 24 hr	LC50= 575.43 µg/ml
Day-2 48 hr	LC50= 446.68 µg/ml
Day-3 72 hr	LC50=295.12 µg/ml
Day-4 96 hr	LC50= 229.08 µg/ml
Day-5 120 hr	LC50=218.77 µg/ml

GRAPH 4: LC_{50} OF ETHANOLIC WHOLE PLANT EXTRACTS OF *AERVASAN GUINOLENTA* (A2) AFTER 24 h, 48 h, 72 h, 96 h AND 120 h TIME INTERVAL



GRAPH 5: PERCENTAGE OF MORTALITY OF ZEBRA FISH EMBRYO FOLLOWING EXPOSURE TO SIX DIFFERENT CONCENTRATION OF *CYNODON DACTYLON* (A3) AFTER 24 h, 48 h, 72 h, 96 h AND 120 h TIME INTERVAL



Time (hr)	LC50 Value
Day-1 24	LC50= 35481 µg/ml
Day-2 48	LC50= 758 µg/ml
Day-3 72	LC50=354 µg/ml
Day-4 96	LC50= 346 µg/ml
Day-5 120	LC50=346 µg/ml

GRAPH 6: LC_{50} OF ETHANOLIC WHOLE PLANT EXTRACTS OF *CYNODON DACTYLON* (A3) AFTER 24 h, 48 h, 72 h, 96 h AND 120 h TIME INTERVAL

TABLE 2: COMPARISON OF LC_{50} VALUES (µgm/ml) AT 24 h TIME INTERVALS IN TERMS OF {MEAN (SD)} AMONG THE THREE GROUPS A1, A2, A3 USING ANOVA TEST (AT 24 h)

Group	N	Mean	Std. Deviation	F value	P value
A1	10	316.22080	0.004541	1905190202	<0.001**
A2	10	575.42800	0.019322		
A3	10	35486.000	2.5385		
Total	30	12125.882	16800.8230		

(p <0.05 - Significant*, p < 0.001 - Highly significant**)

	A1	A2	A3
A1	-	<0.001**	<0.001**
A2	<0.001**	-	<0.001**
A3	<0.001**	<0.001**	-

(Tukey's post hoc analysis)

TABLE 3: COMPARISON OF LC_{50} VALUES (µgm/ml) AT 48 h TIME INTERVALS IN TERMS OF {MEAN (SD)} AMONG THE THREE GROUPS A1, A2, A3 USING ANOVA TEST (AT 48 h)

Group	N	Mean	Std. Deviation	F value	P value
A1	10	288.4100	0.01826	136734.944	<0.001**
A2	10	446.6750	0.01581		
A3	10	757.4000	3.53396		
Total	30	497.4950	198.14751		

(p <0.05 - Significant*, p < 0.001 - Highly significant**)

	A1	A2	A3
A1	-	<0.001**	<0.001**
A2	<0.001**	-	<0.001**
A3	<0.001**	<0.001**	-

(Tukey's post hoc analysis)

TABLE 4: COMPARISON OF LC_{50} VALUES (µgm/ml) AT 72 h TIME INTERVALS IN TERMS OF {MEAN (SD)} AMONG THE THREE GROUPS A1, A2, A3 USING ANOVA TEST (AT 72 h)

Group	N	Mean	Std. Deviation	F value	P value
A1	10	239.8180	0.02201	5251.153	<0.001**
A2	10	294.1260	3.15738		
A3	10	355.2000	3.01109		
Total	30	296.3813	47.99880		

(p <0.05 - Significant*, p < 0.001 - Highly significant**)

	A1	A2	A3
A1	-	<0.001**	<0.001**
A2	<0.001**	-	<0.001**
A3	<0.001**	<0.001**	-

(Tukey's post hoc analysis)

TABLE 5: COMPARISON OF LC_{50} VALUES (µgm/ml) AT 96 h TIME INTERVALS IN TERMS OF {MEAN (SD)} AMONG THE THREE GROUPS A1, A2, A3 USING ANOVA TEST (AT 96 h)

Group	N	Mean	Std. Deviation	F value	P value
A1	10	223.8690	0.01792	4635.548	<0.001**
A2	10	228.1730	2.84012		
A3	10	347.8000	4.89444		
Total	30	266.6140	58.50090		

(p <0.05 - Significant*, p < 0.001 - Highly significant**)

	A1	A2	A3
A1	-	0.017*	<0.001**
A2	0.017*	-	<0.001**
A3	<0.001**	<0.001**	-

(Tukey's post hoc analysis)

TABLE 6: COMPARISON OF LC_{50} VALUES (µgm/ml) AT 120 h TIME INTERVALS IN TERMS OF {MEAN (SD)} AMONG THE THREE GROUPS A1, A2, A3 USING ANOVA TEST (AT 120 h)

Group	N	Mean	Std. Deviation	F value	P value
A1	10	190.5460	0.01506	8802.438	<0.001**
A2	10	218.7660	0.02503		
A3	10	347.8000	4.89444		
Total	30	252.3707	69.67877		

(p <0.05 - Significant*, p < 0.001 - Highly significant**)

	A1	A2	A3
A1	-	<0.001**	<0.001**
A2	<0.001**	-	<0.001**
A3	<0.001**	<0.001**	-

(Tukey's post hoc analysis)

DISCUSSION: Most plant extracts are generally recognized as safe, but many a time it is a misleading perception, and adverse events sometimes occur after consuming herbal products. An adverse effect may cause from several factors including the side effects of active compounds, contamination or substitution with toxic herbs, heavy metal contamination, *etc*. leading to liver, kidney and lung failure, high blood pressure, heart attack and stroke have been reported [8, 9, 10, 11, 12]. Plant phytochemicals have a significant role in the plant's defense mechanism and also important for their unambiguous physiological action in the human body. Bioactive compounds are many times toxic in high doses. Pharmacology is basically toxicology at a lower dose, and toxicology is just pharmacology at a higher dose [13].

In the present study, among the three plant extracts *Amaranthus viridis* ethanolic extract showed the highest toxicity in Zebrafish embryos with a mean LC_{50} of 316.22080 µg/ml. Arsirapol S *et al*., found out the 24 h LC_{50} value of the methanolic extract of *Amaranthus viridis* on guppy fish to be ca.947mgL($^{-1}$)(r^2=0.95). No mortality is observed at higher concentrations for thirty seconds [14]. K.J. Umar *et al.,* found out that feeding the albino rats with 75% spiny Amaranthus leaves decreased their body weight when compared to the control group. The results showed that spiny Amaranthus leaves have a relatively low or no toxicity [15]. In the present study, *Amaranthus viridis* ethanolic extract showed no mortality at 100µg/ml but showed 100% mortality Zebrafish (Danio rerio) embryos with 800µg/ml when exposed for 24 h.

Similarly, Affy Mataphouet Emmanuel in a toxicological study on rats, found out that the dose 200 and 400 mg/kg of methanolic extract of *Amaranthus viridisis* safe; in contrast, a dose of 600 mg/kg, revealed signs of toxicity when administered for 12 days [16]. In an earlier study by the present author's qualitative phytochemical evaluation of ethanolic whole plant extract of *Amaranthus viridis* (A1) revealed the presence of Alkaloids as phytoconstituent [17]. The toxic effect of a high dose of *Amaranthus viridis* (A1) extract on zebrafish embryo could be attributed to its alkaloid content. Alkaloids are among the largest groups of secondary metabolites and are low-molecular-weight nitrogen-containing compounds. It is widely accepted that the main role of alkaloids in plants is toxicity against predators and pathogens. The same toxic properties observed in the plant defense scenario can often be used in prospection for new drugs [18].

Among the three plant extracts *Aerva sanguinolanta* (A2) showed moderate toxicity with a mean LC_{50} 575.42800 µg/mlat a time interval of 24 hours on Zebrafish (Danio rerio) embryos. A. Mangala Gunatilake *et al*., in their study, using rat animal model, demonstrated that the administration of dried *Aervasan guinolenta* at a concentration of 25g/200 ml and 100g/200ml for a period of one month did not have a negative impact on the renal function of rats [19]. B. Kayode S. *et al*., found out the LD_{50} of aqueous extract of *Aervasan guinolenta* on albino rats for oral and intraperitoneal acute toxicity tests were 22.62 g/kg and 0.432 g/kg respectively [20]. In the present study *Aervasan guinolenta* (A2) ethanolic extract caused no mortality at 100µg/ml but showed 80% mortality of Zebrafish (Danio rerio) embryos with 800µg/ml when exposed for 24 h.

C. Annie Shirwaikar *et al*., found out that the ethanol extract of the entire plant of *Aerva sanguinolenta* possesses significant nephroprotective activity and minimal toxicity in cisplatin- and gentamicin-induced acute renal injury in albino rats of either sex [21]. In an earlier study by the present author, qualitative phytochemical evaluation of ethanolic whole plant extract of *Aerva sanguinolanta (*A2) revealed presence of Flavonoid as phytoconstituent [17]. Flavonoids are widely-distributed polyphenolic secondary metabolites with diverse biological activities in plants and benefit human health as protective dietary agents [22]. Flavonoids have the capacity to act as anti-oxidants [23]. The minimum toxic effect of a high dose of *Aerva sanguinolanta* (A2) extract on a zebrafish embryo could be attributed to its flavonoid content.

In the present study *Cynodon dactylon* (A3) ethanolic extract caused no mortality of Zebrafish (Danio rerio) embryos at both 100µg/ml (lowest concentration) and 800 µg/ml (highest concentration) when exposed for 24 h. Heikham Chandani Devi *et al*., in 2017 studied the hepatoprotective activity of aqueous extract of *Cynodon dactylon* at 200 mg/kg body weight and 400 mg/kg body weight against paracetamol-induced liver damaged albino rats and found out that there is a significant reduction in the values of SGOT, SGPT, and ALP [24].

Arun K. Yadav, Purobi Nath in an *in-vitro* assay, tested Cynodondactylon whole plant extract at 10, 20, and 40 mg/ml concentrations against adult *Hymenolepis diminuta* (hymenolepididae), a zoonotic tapeworm. They found out that the LD_{50} of the extract was estimated to be greater than 2000 mg/kg [25]. Whereas in the present study LC_{50} value for zebrafish embryo when exposed to ethanolic whole plant extracts of *Cynodon dactylon* (A3) after 24 h time interval is found to be 35486.000 µg/ml, which is the highest among all the three plant extract thereby with least toxicity on Zebrafish (Danio rerio) embryos. In an earlier study by the present author, qualitative phytochemical evaluation of ethanolic whole plant extract of *Cynodon dactylon* (A3) revealed the presence of Alkaloids, Flavonoids, Saponin Glycoside, Carbohydrate and Protein as phytoconstituents [17]. The least toxicity induced by the extract to the fish embryos may be attributed to an individual or synergistic action of phytochemicals found in this plant over the period of exposure [26].

Current safety and toxicology testing for preclinical studies comprise of *in-vitro* and *in-vivo* models. Typically, these tests are time-consuming, expensive, and required a large number of animals. Potentially, zebrafish (Danio rerio), an emerging model in the early drug discovery and toxicological screening offers several advantages on physiological, biological, and molecular alteration. Its low maintenance, high fertility, ex utero development, and transparent eggs offer clear visualization in all stages of organogenesis monitoring [27, 28, 29].

CONCLUSION: *Amaranthus viridis* (A1), *Aerva sanguinolenta* (A2), *Cynodon dactylon* (A3) have LC_{50} of 316.22 µg/ml, 575.43 µg/ml, 35481µg/ml respectively at a time interval of 24 h, so a concentration below these concentrations of the respective plant extracts was considered safe to be consumed by Zebrafish embryos. LC_{50} values of three whole plant ethanolic extract A1, A2, A3 with six different concentrations each on Zebrafish embryos will aid in determining the therapeutic dose of the respective plant extracts.

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