

## Safety profile of *Matricaria Chamomilla*: insights from zebrafish embryo development toxicity testing

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In an alternative medicinal system, *Matricaria chamomilla* is used for various conditions like inflammation, higher cholesterol, wounds, diabetes and pregnancy-related issues. However, there is contradiction about the use of *Matricaria chamomilla* at the time of pregnancy. Therefore, the present study is designed to investigate the embryotoxic and developmental effects of *Matricaria chamomilla* using zebrafish embryos as a model. *Matricaria chamomilla*'s chemical profiling was done using GC-MS/MS analysis. Fertilized embryos were selected and exposed to 0.1% vehicle (alcohol) and test medicine *Matricaria chamomilla* (mother tincture, 6C and 30C) for 96 hrs. Normal control group embryo exposed to embryo media. All the embryos were observed for mortality, hatching, morphological parameters and heart rate till 96 hours. GC-MS/MS analysis revealed 80 phytochemical compounds in hydro-alcoholic extract of *Matricaria chamomilla*. It exerted a mild effect on hatching rate and showed mortality than any other group. Whereas, after the exposure of *Matricaria chamomilla*-6C and 30C, there were no changes observed in all the parameters. Overall, the result reveals that *Matricaria chamomilla*-6C and 30C (at high dilution) is found safe and indicated its safety at the time of pregnancy. However, further validation in higher animal models is recommended for proper clinical correlation.

**Keywords:** Pregnancy. *Danio rerio*. Development. Embryo. Teratogenic effect.

### INTRODUCTION

Chamomile (*Matricaria chamomilla* L.; M. chamo.), belonging to the family Asteraceae, is an annual plant native to Europe and Asia (Ortiz *et al.*, 2016). It possesses branched, erect, smooth stems and is also known as chamomile, Italian camomilla, German chamomile, wild chamomile and Hungarian chamomile (Mekonnen *et al.*, 2016). The main phytochemical constituents present in chamomile are sesquiterpenes, flavonoids, coumarins, and polyacetylenes (Singh, Khanam, Misra, 2011).

Medical system has used it for the treatment of

stomach aches, irritable bowel syndrome and insomnia. It also possess several activities like anti-inflammatory, bactericidal, anxiolytic, anti-mutagenic, cholesterol-lowering, wound healing, and antidiabetic properties (Amsterdam *et al.*, 2012; Hashempur *et al.*, 2015; Sharifi *et al.*, 2017). *Matricaria chamomilla* is known to contain flavonoids, including apigenin and quercetin, which has been identified as phytoestrogens (Piersen, 2003). Phytoestrogens are used to modulate estrogenic activity in the body, which may raise concerns about hormonal balance during pregnancy (van Duursen, 2017; Naji, Hossenzadeh Sahafi, Saffari, 2015). There are many studies available regarding the negative effects of M. chamo. in the case of pregnancy. A study in 2010 reported the regular use of M. chamo. resulted in a higher risk of preterm labor or miscarriage (Cuzzolin *et al.*, 2010). At 35 weeks gestation period, women of 32-year intake chamomile tea intermittently during

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their pregnancy, which results in premature constriction of the fetal ductus arteriosus (Sridharan *et al.*, 2009). According to reports, preterm births and lower birth weights have linked to regular chamomile consumption during pregnancy (Trabace *et al.*, 2015). Therefore it is advised not to take chamomile during pregnancy due to its unsafe and adverse perinatal outcomes.

In contrast to this, M. chamo. is also used in homoeopathic medicine systems for a number of diseases, such as various central nervous system disorders; stress, derangement of the cerebral functions, depression and other conditions. It is frequently used medicine for numerous symptoms in various diseased conditions (Samuel, 1880). In addition to this, at ultra-high dilution ( $10^{12}$  and  $10^{60}$ ) its use is also reported in the case of pregnancy for various symptoms like severe pains following labor, especially if they extend down the thighs, with great nervous excitement (Boericke, 1998). Though at high dilutions, chances of adverse effects are least or negligible however, a report of Posadzki *et al.*, (2012), alarming the scientist to check the safety of such preparations even at high dilution because they reported the adverse effects of homoeopathy in their systemic review. In addition to this, the WHO in 2009 also published a report and showed its concern about the risk evaluation of homoeopathic preparation at high dilution. The evidence regarding the safety of homoeopathic preparation of chamomilla (mother tincture and its potencies) in pregnancy cases has also not been found. Therefore, it is essential to conduct safety tests on the development of embryos to find out whether the homoeopathic preparation of chamomile has any effect on embryonic development or not. There will always be uncertainty about the use of homoeopathic chamomile preparation in pregnancy due to the lack of scientific proof and conflicts on this issue.

Due to the various advantages of the zebrafish model along with the following the principle of RRR, the zebrafish embryo model has become a prominent experimental model to assess the safety, toxicity, and efficacy of a number of drugs due to their rapid development, cost-effective, transparency in embryos

and highly-conserved genetic pathway between zebrafish and humans (Brannen *et al.*, 2013; Santoro 2014).

For rapid toxicological assessment for pharmaceuticals, the National Toxicology Program of USA is also trying to establish and validating of zebrafish as a model (Dach *et al.*, 2019). Besides, this model is also used by a number of scientific communities to evaluate the toxic effect of different chemical compounds as well as medicinal plants (Wang *et al.*, 2020; Chahardehi *et al.*, 2020; Sharma *et al.*, 2021a; Sharma *et al.*, 2021b). Therefore, the present study is designed to evaluate the embryotoxic and developmental effects of homoeopathic preparation of M. chamo. mother tincture and its potencies, i.e. 6C and 30C, using zebrafish embryos as a model.

## MATERIAL AND METHODS

### Collection of plant material

Fresh plant (whole part) of M. chamo. was collected, taxonomically identified, and authenticated, a voucher specimen was deposited at the Centre for Medicinal Plants Research in Homoeopathy (CMPRH), Emerald, Tamil Nadu, India (Voucher specimen No.: 9887).

### Preparation of homoeopathic drugs

The raw plant material was collected, sorted, washed, and powdered to various degrees of fineness. The hydro-alcoholic-preparation (mother tincture) was prepared as per standard protocol with 25 g of plant material of M. chamo. to prepare 100 ml of mother tincture ( $\phi$ ). The potencies were prepared by adding mother tincture with solvent (dispensing alcohol) in 1:9 ratio and “succussed”. The procedure was repeated to prepare the 6<sup>th</sup> and 30<sup>th</sup> potency of chamomilla (having dilution factors of  $10^{12}$  and  $10^{60}$  respectively) with the same above procedures as mentioned in Homoeopathic Pharmacopoeia of India (India, 1974).

### GC-MS/MS Analysis of Hydro-alcoholic Extract of *M. chamo.*

GC-MS/MS analysis of the sample was performed by using Trace 1300 GC Triplus RSH auto sampler TSQ 8000 Evo Mass Spectrometer (Thermo, USA) equipped with TG 5MS capillary column (30 m X 0.25mm), 0.25µm film thickness of stationary phase, 5% phenyl and 95% dimethyl polysiloxane. This was operated in split (split ratio 1:20) injection mode with an injector temperature of 80 °C. Helium was used as a carrier gas with a flow rate of 1.0 mL min<sup>-1</sup>. The GC oven temperature was programmed as follows: 65 °C (hold for 2 min), increased to 210 °C at a rate of 5 °C min<sup>-1</sup> (hold for 2.5 min) and finally reached to 300 °C (hold for 11min) at a rate of 12 °C min<sup>-1</sup>. Total run time was 53 min. The ion source and mass interface temperature were set at 230 °C and 300 °C, respectively. The mass spectrometer was operated at electron energy of 70eV. The samples were derivatized by using BSTFA (1%TMCS) as silylation agent. All the samples were analysed in full scan mode of mass in the range of m/z 40-600. 1µL of sample was injected into chromatographic system.

### Maintenance of fish and egg spawning

Adult zebrafish was locally purchased from a vendor and maintained in clean poly-sulphone tanks with UV treated, and filtered water with constant filtration and aeration. The pH of aquarium water was maintained between 7.0±0.2. The zebrafish facility environment was maintained with the temperature at 27–29 °C and a light/dark cycle of ~14/10 h, respectively. The environment of the zebrafish housing facility was according to the standard protocol (Westerfield, 1995). The protocol of the study was approved by IAEC (DDPR- CRIH/ Pharmacology/CPCSEA/IAEC/2018/006).

For breeding purposes, male and female zebrafish in a ratio of 1:2 were paired in breeding boxes a few hours before the onset of darkness on the day before the test. Spawning was triggered once the light was turned

on and was usually completed within 30 min on next morning. After spawning, the fish were returned to their home cage and the eggs were collected and placed in petri plates containing medium with the help of a stereo zoom microscope (DSRi-2, Nikon Corporation, India).

### Fish Embryo Acute Toxicity (FET) Test on Zebrafish

Zebrafish embryos exposed to the test medicine were carried out in 24-well plates according to OECD 236 guidelines on fish embryo toxicity tests with some modifications (OECD-236, 2006). Fertilized eggs were divided randomly into five groups namely: Water (N. control), dispensing alcohol (V. control), *M. chamo.*-mother tincture (ϕ), *M. chamo.*-6C, *M. chamo.*-30C and each group contained ten eggs in 24 well plates for further experiment and the experiment was performed in 3 independent replicates in a 24-well plate. The fish of group I were exposed to 2 mL of embryo media, and groups II, III, IV and V were exposed to 2 mL of embryo media containing 0.1% of dispensing alcohol, *M. chamo.*-ϕ, *M. chamo.*-6C and 30C respectively. The exposure was given in semi-static conditions at 10 embryos per group of exposure medium, and the test solutions were changed every day till 96 hours post fertilization (hpf). During the exposure period, dead embryos were removed immediately to avoid contamination. The parameters that were evaluated, egg coagulation, mortality, somite cell formation, tail detachment, eyes, otolith, heart-beat, blood circulation and skeletal deformities were recorded each day for 96 hpf (Table I). The parameters like eye diameter, body Length pericardial sac and yolk sac area were evaluated on 96 hpf. The malformation of the embryo body in each group for a period of 96 hpf was subsequently examined with the help of a stereo zoom microscope (SMZ-18, Nikon Corporation, India), and the images of embryos were captured with a camera (Nikon model no: DSRi2).

**TABLE I** - Morphological characteristics evaluated as measures for the normal development at different time points

Parameter	Time point for observation of normal development			
	24 hpf	48 hpf	72 hpf	96 hpf
Coagulation	√	√	√	√
Somite cell formation	√	√	√	√
Tail detachment	√	√	√	√
Otolith	X	√	√	√
Eye	X	√	√	√
Heartbeat	X	√	√	√
Blood circulation	X	√	√	√
Motility	X	X	√	√

#### Evaluation of Survival Rate

The survival rate of embryos was observed from the start of the exposure to 96 hpf. The following formula was used to calculate the survival rate of the embryos.

Survival Rate % = No. of live embryos or larvae / total number of embryos (10) X 100

#### Evaluation of Hatch Rate

The hatching of embryos was observed as a rupture in the chorion for the release of larvae. Zebrafish embryos of each group were observed for 96 hpf to determine their hatching rates using a stereo zoom microscope.

#### Evaluation of Heartbeat

The heartbeat was recorded at 48, 72 and 96 hpf after the treatment of M. chamo.-( $\phi$ ), 6C and 30C. The zebrafish cardiac ventricles were directly observed using a stereo zoom microscope to count the heartbeats. The heartbeat was recorded per minute with the help of a stopwatch.

#### Determination of Body Length

The image of the hatched larvae at 96 hpf was captured using a microscope camera (Nikon model no: DSRi2) and the body length was measured using imaging software (Nikon imaging software NIS-Elements BR-5.30).

#### Determination of pericardial sac area and yolk sac area

The area of the pericardial sac and yolk sac was determined to quantify the severity of pericardial edema and yolk sac edema respectively (Prasch *et al.*, 2003). The pericardial sac and yolk sac area were outlined, traced and determined the area using imaging software (Nikon imaging software NIS-Elements BR-5.30).

#### Determination of eye diameter

To find out the effect of M. chamo. on the eye, the diameter of both eyes (left and right) was measured. The area of each eye was outlined, traced and the diameter was measured using imaging software (Nikon imaging software NIS-Elements BR-5.30).

#### Statistical Analysis

Results are expressed as mean  $\pm$  SEM. One-

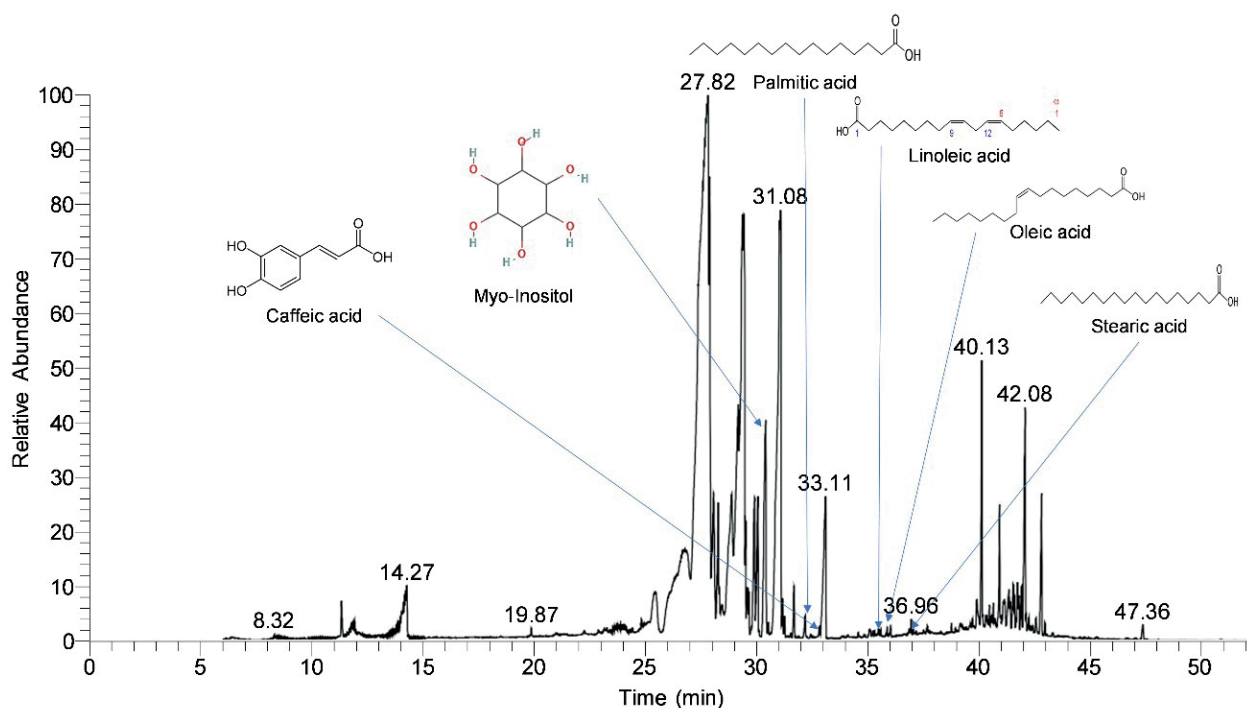
way ANOVA was used to determine the statistical significance and then *Tukey's post hoc* test was applied using the Graph Pad Prism ver.5. Differences were considered significant at  $p < 0.05$ .

## RESULTS

### Phytochemical constituents of *M. chamo.* through GC-MS/MS analysis

The GC-MS/MS analysis of the derivatized *M. chamo.* hydro-alcoholic extract, prepared according to HPL principles, revealed the presence of 80 phytochemical compounds. The main key phytochemicals among above compounds were primarily dominated by carbohydrates, organic acids, fatty acids, and other biomolecule groups (Table II). The carbohydrate profile was largely dominated by  $\alpha$ -D-lactose, D-allopyranose,

D-fructofuranose, trehalose, sucrose, and ribofuranose. Additionally, D-gluconic acid, a sugar acid derived from glucose, was also identified. These carbohydrates likely contribute to the plant's energy storage and structural integrity. The fatty acid profile of the extract included palmitic acid, linoleic acid, and stearic acid, which are commonly reported in the essential oil and extract of *M. chamo.* In addition to carbohydrates and fatty acids, various other compounds were identified in the extract. These included sugar alcohols like glycerol and dulcitol, sugar acids like arabinonic acid, and cyclic polyols like quinic acid. Myo-inositol, a carbocyclic polyol with potential biological activity, was also detected. Moreover, glycolic acid, an alpha hydroxy acid (AHA) known for its exfoliating and moisturizing properties, was also present. The chromatogram of *M. chamo.* having structure details of key fatty acids and carbocyclic sugar is depicted in Figure 1.



**FIGURE 1** - GC-MS/MS chromatogram showing key metabolites of *M. chamo.*

**TABLE II** - Key phytochemicals identified in the *M. chamo.* using GC-MS/MS analysis

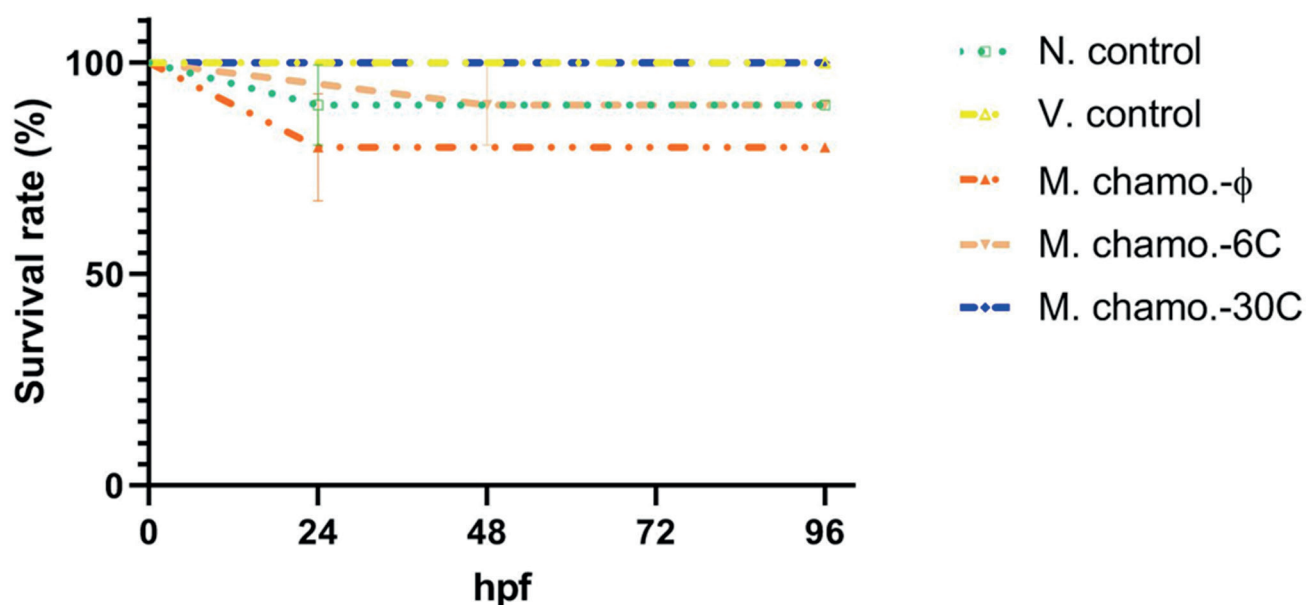
S. No.	Compound name	RT	Peak Area	Area %
1	Glycerol	14.27	1118189861	1.05
2	Ribofuranose	22.25	77053999.25	0.07
3	Arabinonic acid	23.27	79565645.38	0.07
4	D-Fructofuranose	27.89	5504693385	5.15
5	Quinic acid	28.45	177247688.3	0.17
6	D-Allopyranose	29.43	10150926781	9.49
7	Dulcitol	29.9	2573046023	2.41
8	Myo-inositol	30.41	5065360560	4.73
9	D-Lactose,	31.08	17956493295	16.78
10	D-Gluconic acid	31.27	179853669.5	0.17
11	Palmitic Acid:	32.19	387836092.5	0.36
12	Caffeic acid	32.83	115079903.6	0.11
13	Linoelaidic Acid	35.48	116004785.8	0.11
14	Octadecenoic acid (Oleic acid)	35.59	85213608.07	0.08
15	Stearic acid	36.02	168828751.9	0.16
16	Glycolic acid	37.68	131701017.5	0.12
17	Thymol glucoside	39.62	119468828.9	0.11
18	Sucrose	40.13	3299737312	3.08
19	Trehalose	42.07	4141370377	3.87



### Effect of *M. chamo.*- $\phi$ , 6C and 30C on survival rate

Every 24 hours, the survival rate was measured by optical monitoring and illustrated in Figure 2. Throughout the experiment, mortality stayed below 10% in the control group, which followed the validation

criteria of OECD 236. After the treatment with *M. chamo.*-6C and *M. chamo.*-30C, the survival rate was found normal and within the 10% criteria similar to the normal control group. On the other hand, exposure with *M. chamo.*- $\phi$  showed mild effect and showed 20% mortality at 24 hpf in embryos.

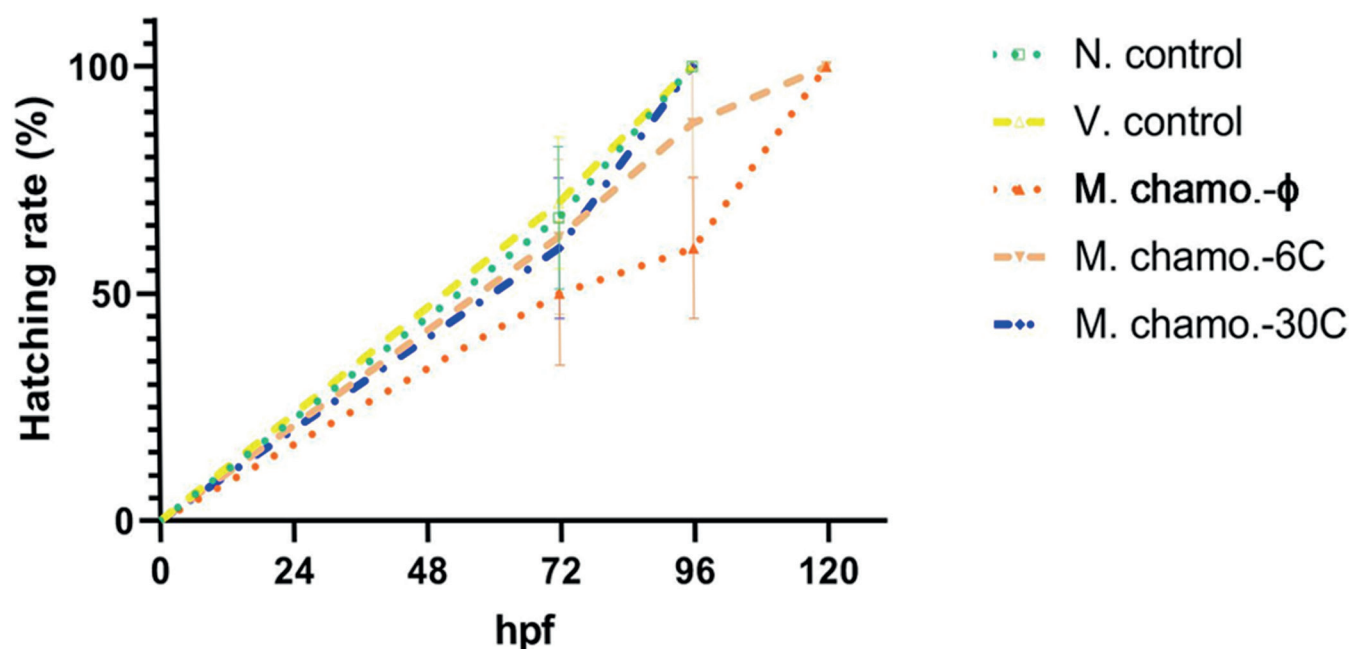


**FIGURE 2** - Effects of *M. chamo.*  $\phi$ , 6C and 30C on survival rate at 24, 48, 72 and 96 hpf.

### Effect of *M. chamo.*- $\phi$ , 6C and 30C on hatching rate

Zebrafish embryos exposed to *M. chamo.* showed delayed hatching at 6C and 30C in terms of the hatching rate represented in Figure 3. Hatching rates at 48 hpf were 50% in the *M. chamo.*-30C group and 72% in the *M. chamo.*- $\phi$  and 6C group. At 96 hpf, 100% hatching

was achieved in normal control, vehicle control and *M. chamo.*-6C groups, whereas approx. 60% hatching was found in *M. chamo.*- $\phi$  group, which was increased in *M. chamo.*-30C exposure i.e., 87%. The 100% hatching rate was recorded in the *M. chamo.*  $\phi$  treatment group at 120 hpf, which is comparable to the results seen in the control and vehicle control groups.



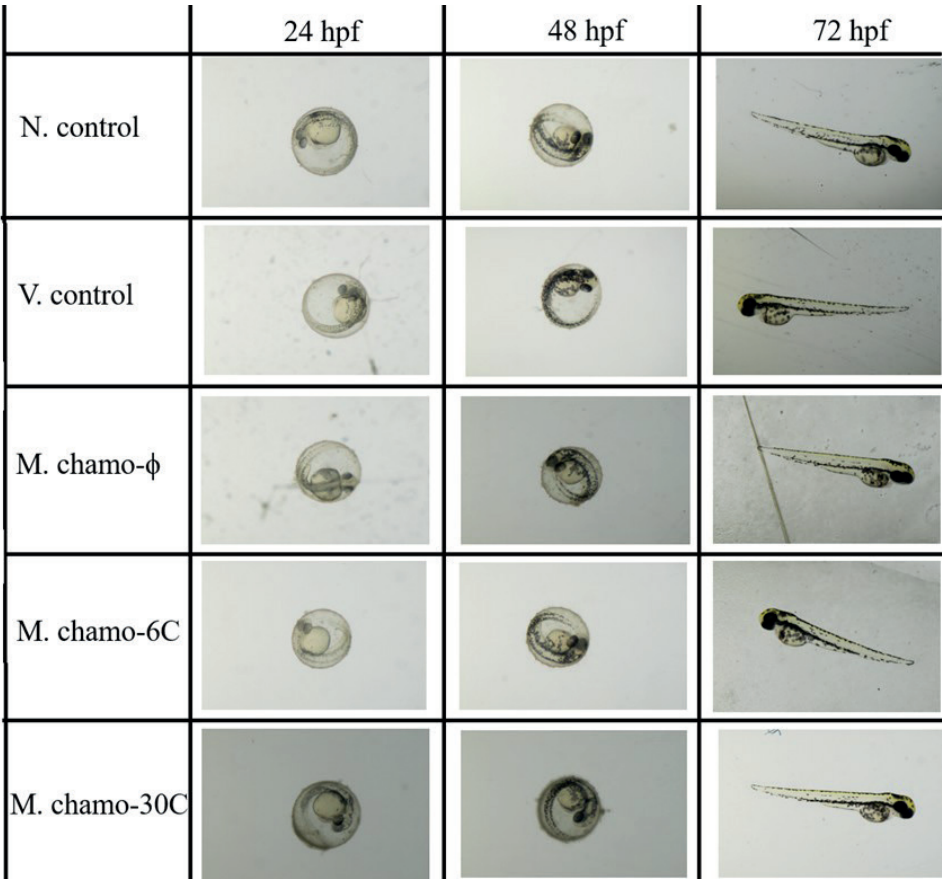
**FIGURE 3** - Effects of M. chamo.  $\phi$ , 6C and 30C on hatching rate at 24, 48, 72 and 96 hpf.

### Effect of M. chamo.- $\phi$ , 6C and 30C on morphological characteristics of zebrafish embryos

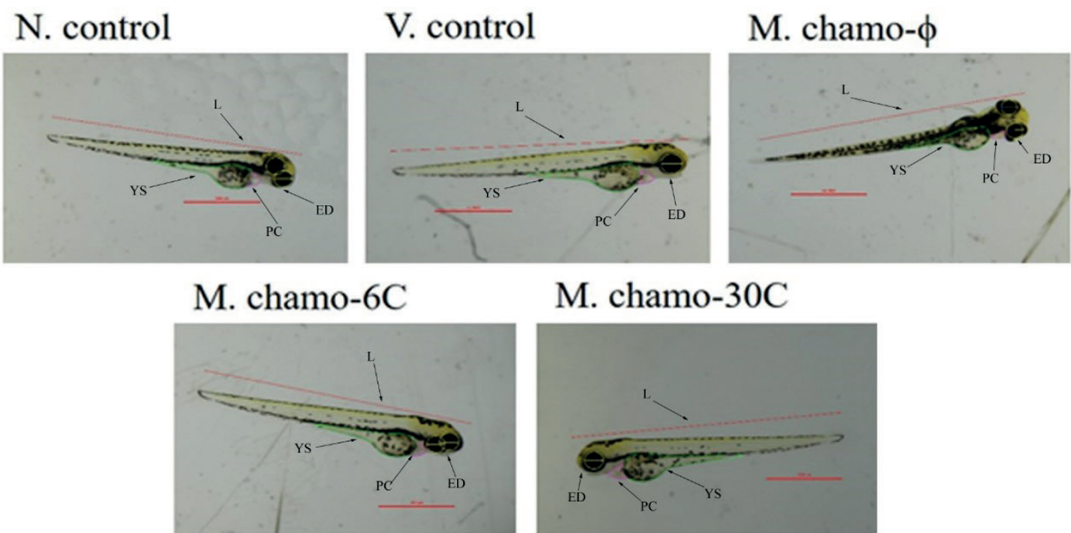
At 24, 48 and 72 hpf, the embryos were incubated with different M. chamo. potencies ( $\phi$ , 6C and 30C). In the present study, the N. control group exhibited normal embryonic development, similar to a previous study (Kimmel, Ballard, 1995). Figure 4-5 demonstrates that exposure to M. chamo.- $\phi$ , M. chamo.-6C and M. chamo.-30C had no deleterious effects on the development of zebrafish and found similar morphological characteristics as a normal control group. The sharp-edged somite formation and tail detachment were observed in all the groups at 24 hpf. An evaluation of the morphological features showed that all study groups had a circular yolk sac, a straight spine, a normal body form and heart rate, and pigmentation on the body and eyes. No coagulation and organ malformation occur during the study period. The effect of exposure to M. chamo.- $\phi$ , M. chamo.-6C and M. chamo.-30C in larvae at 96 hpf on various parameters like length (Figure 6a), eye diameter (Figure 6b), yolk sac area (YS; Figure 6c), pericardial area (PC; Figure 6d) and heart rate at 48, 72

and 96 hpf shown in Figure-7. Statistical analysis in all the mentioned parameters (larvae length, eye diameter, YS and PC) showed that there was no significant ( $p \geq 0.05$ ) difference observed within the groups as compared to the normal control group (Figure 5a-5d). Heart rate was constant throughout the experiment after continuous 96-hour exposure and found same as the normal (E-3 media) control group (Figure 7). From 48 hpf to the end of the experiment (96 hpf) blood circulation was exist and visible under the microscope in all the zebrafish in all groups. From 24 hpf onward the otolith started appearing and at the end of the exposure i.e., 96 hpf, the otolith was fully developed and seen in all the groups. The tail formation was found normal, straight line in all the larvae of the groups. There was no bending or kinking of the tail observed among the larvae throughout the study. The notochord was found normal, and extended from the cranium to the cauda parts in all the larvae among the groups. No abnormality was observed in the notochord structure after exposure to M. chamo. Overall, no physical changes were found in all the groups, after the microscopical observation.



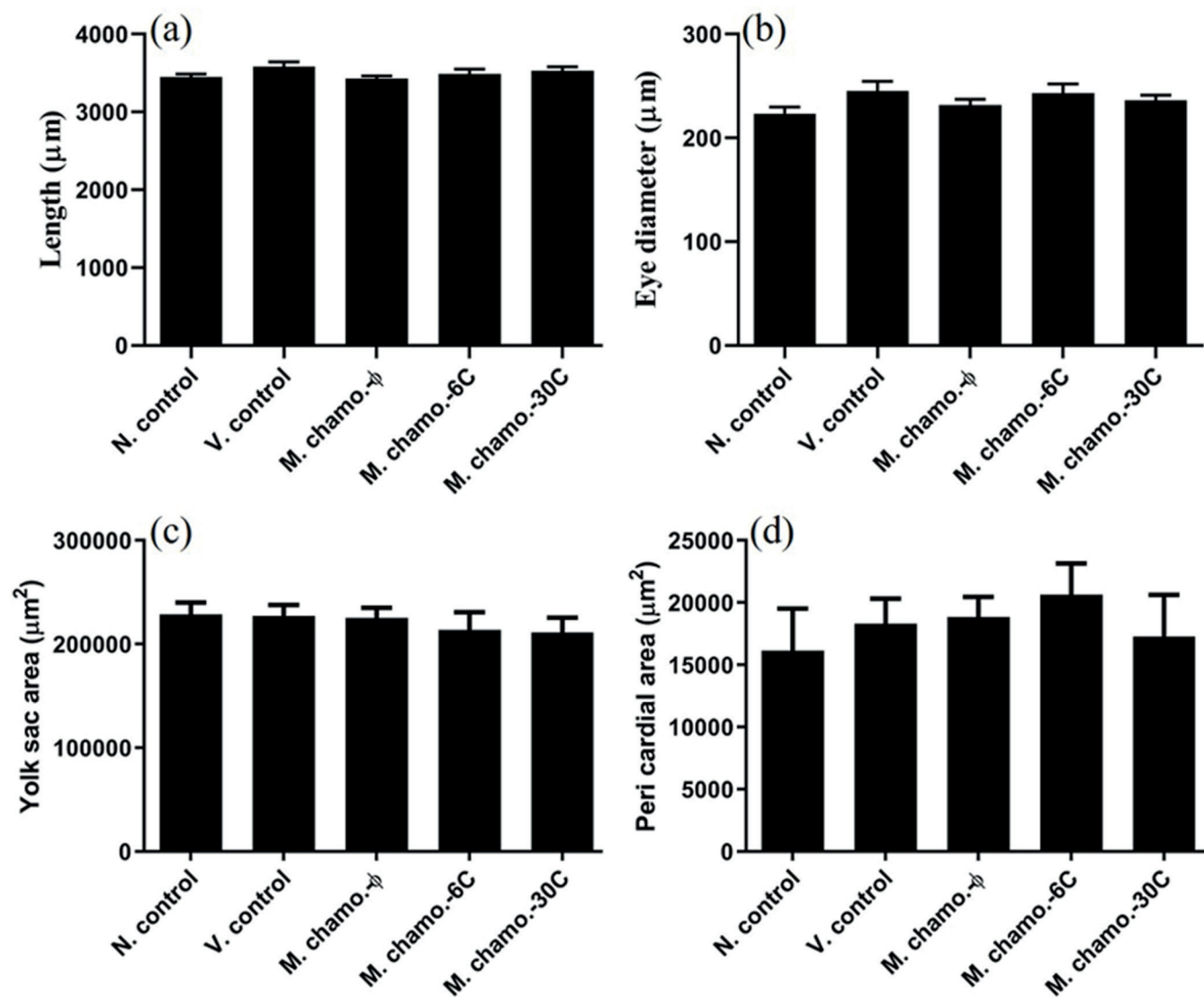


**FIGURE 4** - Morphological characteristics assessed as a measure for zebrafish embryo development effect of M. chamo. φ, 6C and 30C at different time points.

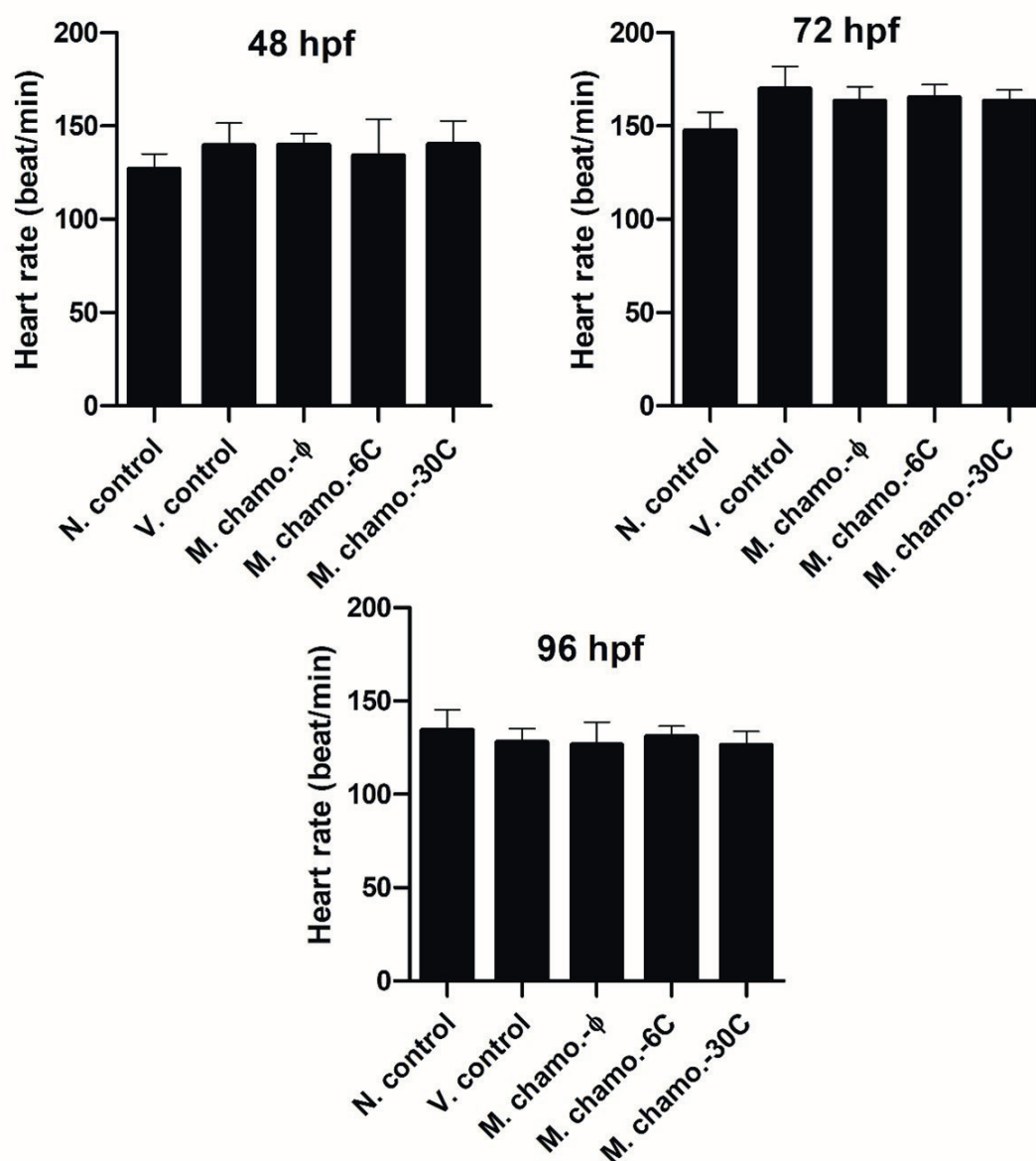


L: Length; YS: Yolk sac; PC: Peri cardial; ED: Eye diameter

**FIGURE 5** - Larval image at 96 hpf where red colour indicates the length, yellow colour indicates eye area, Green colour for yolk sac area and pink colour for the pericardial area of zebrafish larvae.



**FIGURE 6** - Effects of *M. chamo.*  $\phi$ , 6C and 30C on length (a), eye diameter (b), yolk sac area (c), pericardial area (d) of the zebrafish larvae after 96 hpf. All values are Mean  $\pm$  SEM (One-way ANOVA followed by *Turkey's post hoc* test).



**FIGURE 7** - Effects of *M. chamo.* φ, 6C and 30C on heart rate of embryos at 48, 72 and 96 hpf. All values are Mean ± SEM, (One way ANOVA followed by *Turkey's post hoc* test).

## DISCUSSION

Fetal development is a highly organized process in which complex modifications occur in the specified time, and molecular as well as cellular modifications can manifest throughout the entire organism. It is essential to evaluate the teratogenic and embryotoxic effects of medicinal plants on fetal development must be assessed because several herbal formulations are made from

toxic sources or have teratogenic effects. These types of formulations are becoming more popular in the global healthcare system without having their toxicological profiles disclosed. The present study was designed to investigate whether the exposure of *M. chamo.* showed toxic effects at the early life stage in the embryos of zebrafish.

Overall, the results of this GC-MS/MS analysis provided valuable insights into the chemical

constituents of *M. chamo*. The identification of a wide range of carbohydrates, fatty acids, and other bioactive compounds supported the traditional use of chamomile for various health conditions and suggests potential avenues for future research and development of chamomile-based therapies (Milovanovic *et al.*, 2023; Dai *et al.*, 2022).

Zebrafish are popular models for testing lethality and teratogenicity due to their advantages over other models (Zon, Peterson, 2005; Brannen *et al.*, 2010). Due to the similarity in genetics and physiology of zebrafish embryos and humans, this model gains attention for researching developmental toxicity and using to predict how people may react to different chemicals (Jayasinghe, Jayawardena, 2019; Chahardehi *et al.*, 2020; Cassar *et al.*, 2020). Understanding chamomile's safety profile in zebrafish embryos offers useful information on possible impacts on human development and health (Jarque *et al.*, 2020; Song *et al.*, 2021). There are number of studies available regarding the use of herbal medicines, their benefits, and the risks associated with being a mother. Studies have shown that *M. chamo*. administration during pregnancy can be safe and effective in decreasing vomiting and depressed reflexes (Modares *et al.*, 2012). Additionally, positive effect is found on spontaneous abortion and histopathological changes in the placenta and liver associated with STZ diabetes in pregnant female rats after the administration of *M. chamo* (Namjooyan *et al.*, 2011). On the other hand, frequent usage of chamomile during pregnancy is associated with an increased risk of kidney enlargement, cardiac abnormalities, miscarriages, and preterm labor (Cuzzolin *et al.*, 2010; Sarecka-Hujar, Szulc-Musioł, 2022). Regular use of the third trimester is also associated with a lower birth weight and a higher risk of preterm birth (Trabace *et al.*, 2015; Artur *et al.*, 2017). Chamomile use during pregnancy may be harmful and have adverse effects on the fetus, causing significant controversy in the literature. Therefore the present study is designed to investigate the embryotoxic and developmental effects of *M. chamo*. using zebrafish embryos. So far, our work

has shown the first source of evidence regarding *M. chamo*. and its teratogenic effects as a natural botanical drug utilizing the zebrafish bioassay.

The present study revealed that *M. chamo*.- $\phi$  (the highest concentration) showed a mild effect of delaying hatching rate and mortality in zebrafish embryos as shown in a previous study which showed the toxic effect of *M. chamo*. in larvae of *Gulex pipens* L (Gayar, Shazli, 1968). In contrast to this, the present study revealed the ultra-high dilution of *M. chamo*. i.e. 6C and 30C (having dilution factors of  $10^{12}$  and  $10^{60}$  respectively) were found safe in case of both mortality as well as hatching rate after 96-hour exposure to zebrafish embryos.

It has been reported that the fundamental body structure of zebrafish is formed at 24 hpf, the hatching occurs between 48 and 72 hpf, and the major organ organogenesis and larval development occur at 96 hpf (Kimmel, Ballard 1995). For evaluating the effect on the development of organisms in early-life stages, the exposure to 96 hpf along with these time points was considered using morphological techniques. Direct or microscopic observation of morphological changes can be done using a variety of model species, including *Daphnia magna* and zebrafish, whose embryos are completely transparent (Mu *et al.*, 2013; Toumi *et al.*, 2015). In the present study, morphological methods were used to examine the impact of *M. chamo*. on the early life stages of zebrafish development. Based on these findings, *M. chamo*. not caused early-life developmental abnormalities in zebrafish, including somite cell formation, tail detachment, yolk sac, pericardial region, eye size, otolith, blood circulation, and tail formation. These all parameters were found normal in all the treatment groups. In addition to these parameters, the heart is the first organ to develop and function in vertebrates (Wang *et al.*, 2006). It is complex, morphogenetic, and functional elements that interact to build mature organs (Matrone *et al.*, 2015). Similarly, cardiac deformities may lead to aberrant growth or even death. It has been reported that significant heart deformity and malfunction caused by acrylamide in zebrafish embryos, led to a compromised cardiovascular

system. (Huang *et al.*, 2018).

According to the obtained results, no abnormalities in larvae, including yolk sac edema, pericardial edema, and heart rate were observed after the 96-hour exposure of *M. chamo.* to zebrafish. The obtained results showed that *M. chamo.* at high dilution (6C and 30C) exhibited neither lethal nor developmental toxicity in zebrafish. Taken all together, it can be hypothesized that the consumption of *M. chamo.* 6C and 30C at the time of pregnancy could be found safe and may be used for pregnancy-related complications. The present study on chamomile safety using the zebrafish embryo model enhances our understanding of its potential benefits and risks, providing evidence-based recommendations for clinical practice and public health. However, research using larger animal models is recommended to verify and validate the impact of *M. chamo.* as well as molecular level analysis may be required in the future.

## CONCLUSIONS

This present research has shown that 0.1% *M. chamo.* mother tincture showed a mild effect on mortality and hatching rate. In contrast to this the 6C and 30C potencies (high dilution) of *M. chamo.* found safe with no effect on the hatching rate. Additionally, there were no morphological changes that occurred in the early life stage of zebrafish, like effect on eyes, length, area of the yolk sac and pericardial, notochord, otolith, somite cell, etc., after the exposure of *M. chamo.* Since this herbal medicine is usually consumed at the time of pregnancy-related issues, the present study indicates that *M. chamo.* at high dilution could be safe at that time. However, to prove that *M. chamo.* is safe for expectant mothers, a thorough toxicity assessment on a higher animal model together with a molecular investigation would be conducted.

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