除草劑巴拉刈對成年斑馬魚運動與行為的影響

王瀅捷a、朱凱婗a、陳以惠a、陳麗文a、鐘國棟b國立台中教育大學科學教育與應用學系a陸軍專科學校b



The effect of paraquat on the movement and behavior of adult zebrafish
Ying-Chieh Wang^a, Kai-Ni Chu^a, Yi-Hui Chen^a, Li-Wen Chen^a, Kou-Toung Chung^b,
Department of Science Education and Applications, Taichung University of Education^a Army Academy
R.O.C.^b

實驗目的

巴拉刈近幾年被列為毒性藥品,目前巴拉刈的研究多以注射的方式打入斑馬魚的體內,但巴拉刈因易溶於水,且會在環境中流動及擴散,可能造成當地的水域汙染。因此本研究配製含有不同巴拉刈劑量的溶液,並將斑馬魚浸泡過巴拉刈劑量的溶液,或是否會因為浸泡過巴拉刈溶液而活動力下降。此外,有文獻指出長時間使用巴拉刈的農民,在罹患帕金森氏症的機率比未曾使用者高出2.5倍,因此本研究同時探討多少濃度的巴拉刈會誘發斑馬魚出現帕金森氏症中靜止性顫抖的症狀。

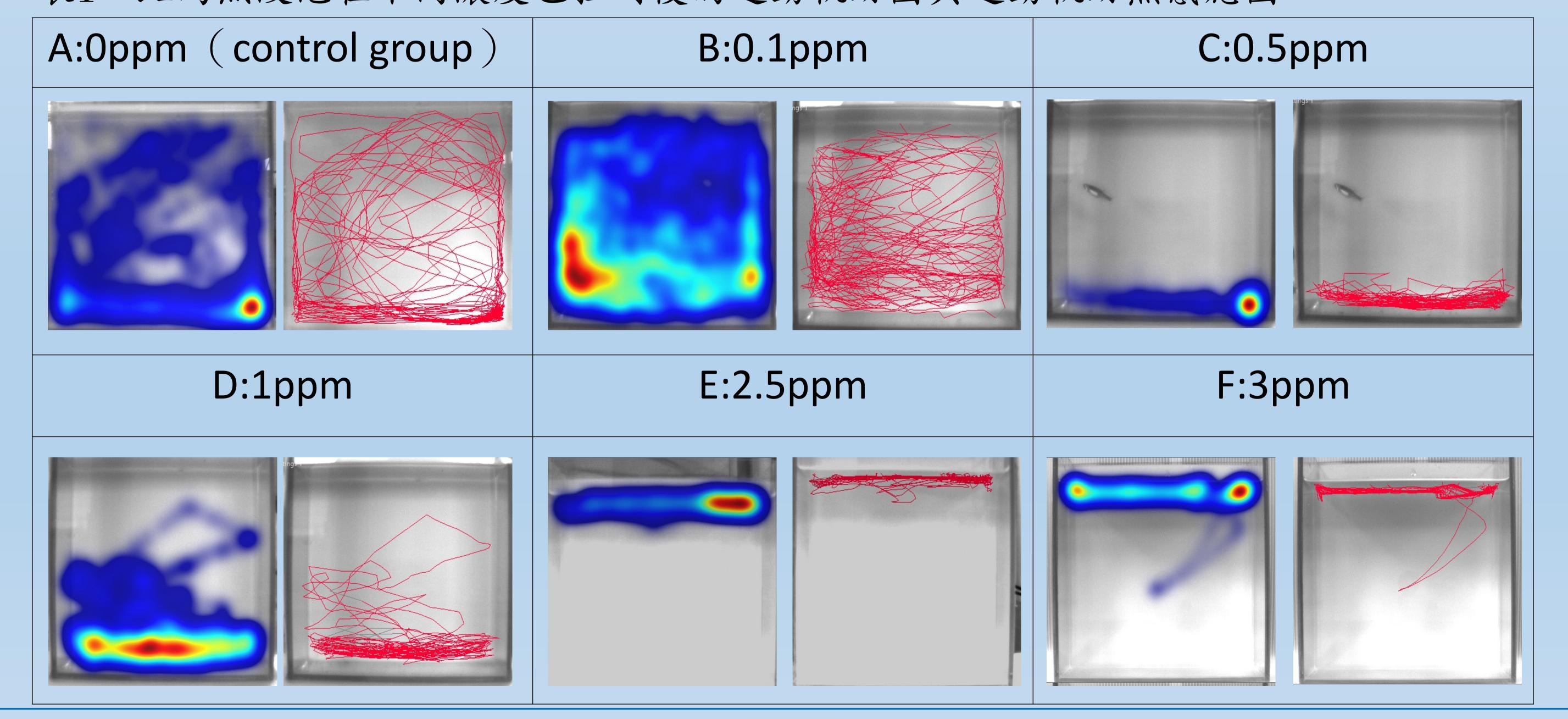
實驗方法

- 1. 本研究以10ppm, 5ppm, 3ppm, 2.5ppm, 1ppm, 0.5ppm, 0.1ppm, 共7種濃度的巴拉刈做實驗組,並以清水做對照,將魚浸泡在各濃度的溶液中持續4天(每缸6隻魚)。
- 2. 以動物研究追蹤分析系統(Noldus EthoVision XT 13)研究浸泡過巴拉刈的斑馬魚的運動路徑、運動行為,比較運動距離、速度、最大加速度、平均運動最大減速度的運動行為。
- 3. 透過拍攝影像觀察魚「靜止且下巴顫抖」之 症狀,來判斷巴拉刈是否會誘發帕金森氏顫 抖顫抖。

實驗結果

- 1. 實驗結果顯示低濃度的巴拉刈溶液就會使斑馬魚活動量降低,如:經浸泡過0.5ppm和 1ppm巴拉刈溶液的斑馬魚有長時間停留在水缸底部或角落的行為(如表1-C、表1-D)。而濃度2.5ppm及3ppm的巴拉刈中的斑馬魚多在水面(如表1-E、表1-F),推測是巴拉刈會對斑馬魚呼吸系統造成傷害,使斑馬魚無法正常在水中活動。
- 2. 實驗中,5ppm和10ppm巴拉刈溶液的斑馬魚在24小時內死亡率超過一半,而在2.5ppm和3ppm巴拉刈溶液的斑馬魚在4天中死亡率有33%,因此我們認為隨著巴拉刈溶入水中的濃度越高,斑馬魚的致死率也會增加。
- 3. 隨著巴拉刈的水域濃度增加,斑馬魚下巴顫抖的現象也會加劇。
- 4. 以上結果可知即使不是直接將巴拉刈打入斑馬魚體內,以浸泡巴拉刈的方式亦會使斑馬 魚活動立下降。

表1、斑馬魚浸泡在不同濃度巴拉刈後的運動軌跡圖與運動軌跡熱感應圖



結論

從以上結果可說明,若當巴拉刈污染水域生態時,可能會間接導致人類受到傷害, 更有機率罹患類帕金森氏症的症狀。

Effects of Sanleng on zebrafish embryos

周毅嘉、趙主淵、陳麗文國立台中教育大學科學教育與應用學系

YI JIA JHOU \ Zhu-yuan Zhao \ Li-Wen Chen
Department of Science Education and Applications, Taichung University of Education



ABSTRACT

In Chinese medicine, some medicines are effective in promoting blood circulation and removing blood stasis. This kind of medicine is classified as a kind of Chinese medicine in pregnancy taboos. Among them, the traditional Chinese medicine Sanleng is considered by scholars and experts to have the function of fighting early pregnancy. Therefore, we discuss its pharmacology through literature. We believe that the traditional Chinese medicine that promotes blood circulation and removes blood stasis will hinder pregnancy. The reason may be that when the embryo is affected by the drug, it will cause its death or deformity. Therefore, this research laboratory is to prove whether this hypothesis can be established. We will explore the development of zebrafish embryos at different stages, and how it will affect embryos and juveniles by injecting different concentrations of three-sided Chinese medicine. And record zebrafish eggs born after fertilization, according to different times: 0 hour, 6 hour, 12 hour, 24 hour, 48 hour (after 48 hours, record once every 12 hours until 120 hours), record the changes caused by different time and different concentrations of the drug, and cross-compared through the record sheet and taking photos of zebrafish. After waiting for the zebrafish to hatch, compare the differences in the zebrafish. The results are as follows: (1) Administering 4500ppm trigonal Chinese medicine at 0 hours will show that the hatching rate decreases and the mortality rate reaches more than 50%. (2) After 24 hours, the addition of three-sided Chinese medicine will delay the hatching of zebrafish, and we found that the heart envelope swells in the experiment. From the results of the above findings, we have further confirmed that the triangulation can indeed cause blood circulation and remove blood stasis, and will hinder pregnancy. The reason is that the embryo is affected by drugs and causes death or deformity. Therefore, the reason why this kind of traditional Chinese medicine can lead to lower hatching rate and death may be because the triangulation affects the development of the embryo's blood circulation system.

METHODS

Soak the embryos in different concentrations of the Sanleng solution for 120 hours continuously, and observe the development at 0, 3, 6, 12, 24, 36, 48, 60, 72, 84, 96, 108, and 120 hours after birth, and Recording in the form of images, image J for image analysis and SPSS for statistical analysis.

RESULT

- 1. Sanleng was added at 0 hours and observed for 60 hours. It can be found that about 10% of the 3000ppm group hatched 24 hours later than the control group; the hatching rate of the 4500ppm and 6000ppm groups decreased by 10% and 15%, respectively.
- 2. Embryos were fertilized at 0 hours by adding a Sanleng edge, and 96 hours of statistics, the mortality rate above 3000ppm reached 80%, and the half-lethal concentration was 2246ppm. From the above results, it can be seen that the Sanleng has an impact on the hatchability and mortality of the developing embryo.

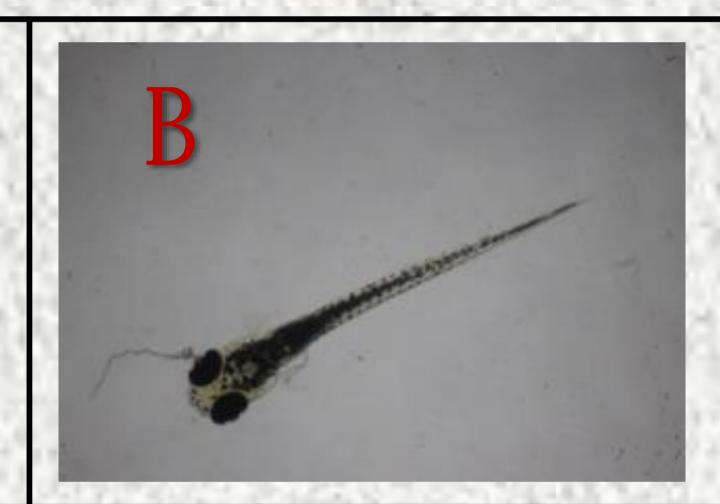
A Sanleng edge above 4500ppm causes a drop in hatchability and a drug concentration above 3000ppm causes more than 80% of deaths.

Future research direction

During the observation process, it was also found that the Sanleng may cause harm to the heart of zebrafish fry. Therefore, our laboratory will use molecular biology technology to do further research on the development and damage of Sanleng in the heart of zebrafish fry in the future.

FIGURES







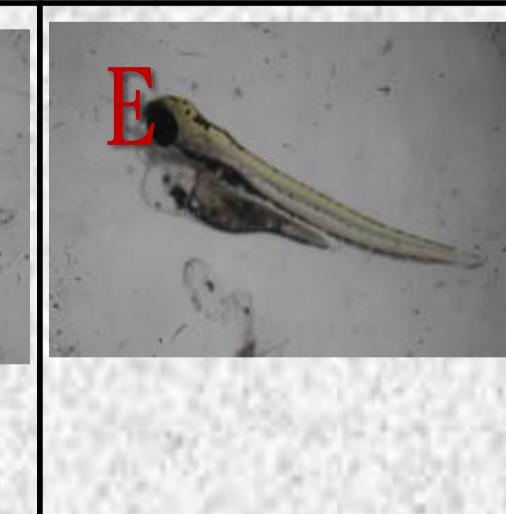


Fig1. The state of the fry after soaking in different concentrations of Sanleng for the first 120 hours (magnification: 40X)

Fig A; 0ppm; Fig B:1500ppm; Fig C:3000ppm; Fig Fig D:4500pp; Fig E:6000ppm

- 1.When the zebrafish's body length is 120 hours, the average fish length is 3.33mm (control)(A), 3.39mm (1500ppm)(B), 3.37mm (3000ppm)(C), 3.23mm (4500ppm)(D), 2.64 (6000ppm)(E).
- 2. Starting from the 60 hours, there was a significant difference between the control group and the fish length of 6000 ppm (p<.05).
- 3. The zebrafish was found to be obvious hydropericardium at 6000ppm in 120 hours.

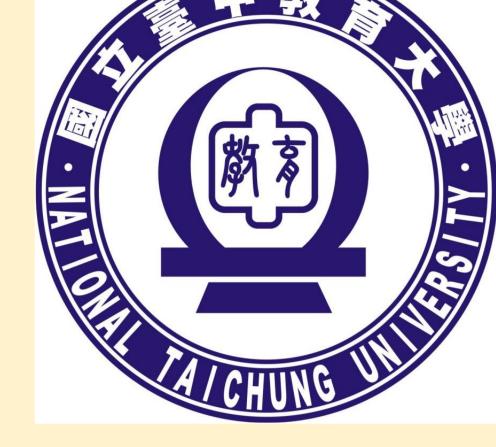
紅花對斑馬魚胚胎發育之影響

呂冠霆、吳驊恭、鄭域康、陳麗文 國立台中教育大學科學教育與應用學系

Effects of safflower on zebrafish embryos

Guan-Ting Lu ' Hua-Lung Wu ' Yu-Kang Cheng ' Li-Wen Chen

Department of Science Education and Applications, Taichung University of Education



摘要:

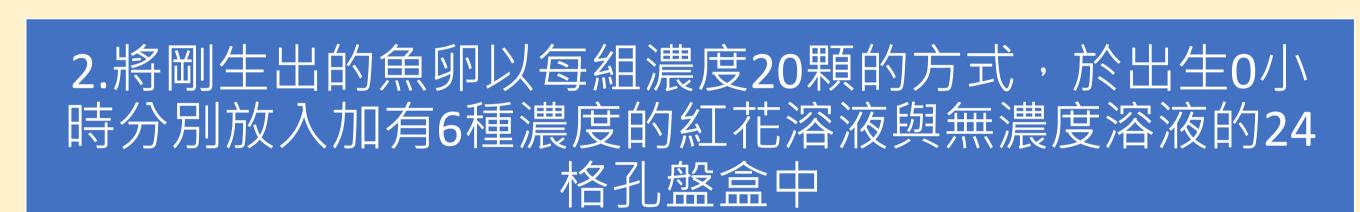
本研究透過配置0ppm、200-700ppm及1000ppm以上之中藥紅 花,進一步了解及觀察不同濃度之中藥紅花直接對斑馬魚胚胎 發育所造成的影響,以了解紅花不利於產孕以及造成胚胎發育 畸形的說法。我們發現::1.紅花於斑馬魚之LCD50(半數致死率) 濃度為730PPM。2.在0-700ppm的濃度區間,每增加100ppm, 斑馬魚約有1-1.5天的孵化延遲。 3. 紅花對斑馬魚胚胎發育的 影響包括腹部腫脹、卵黃囊未消化、心臟與心腔室腫脹、黑色 素不足、破殼困難、生長延遲、尾椎變形。

前言:

為驗證活血中藥材對生物胚胎發育所造成的影響,本研究選用 在中藥上有活血化瘀之功效的紅花,以及卵透明易觀察之斑馬 魚做為主要研究項目。

實驗流程與方法:

1.在200-700ppm的區間,配置6種濃度的紅花溶液並進 行離心,並另設Oppm做為對照組

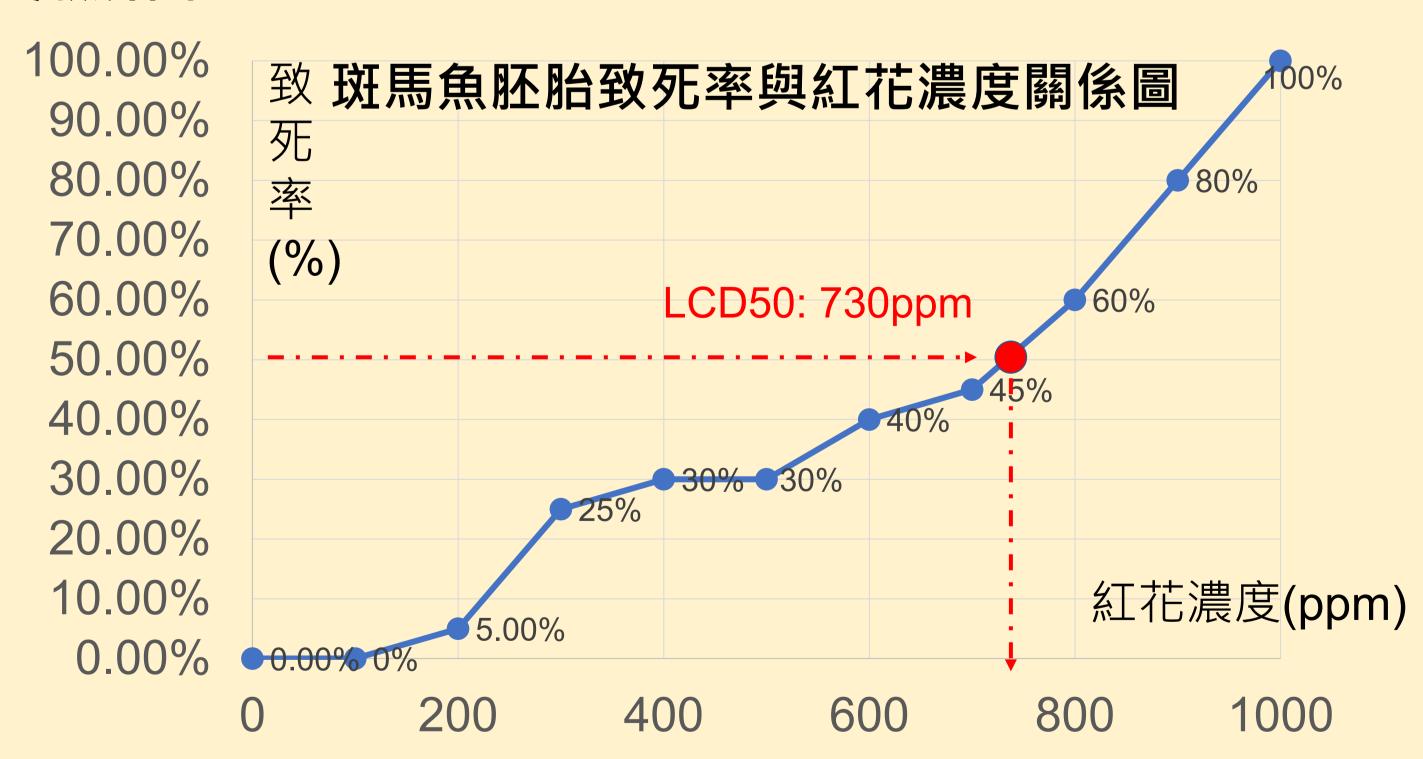


3.於出生的0、6、12、24、48、72小時進形觀察與時 程紀錄,為期7~12天,直至所有魚卵都已破殼孵化或

在觀察上我們主要分為三個面向

- 1. 紅花於斑馬魚胚胎上的LCD50(半數致死率)
- 2. 與0ppm對照組比較之生長歷程差異
- 3. 斑馬魚各類型畸胎的發生率與形態樣貌

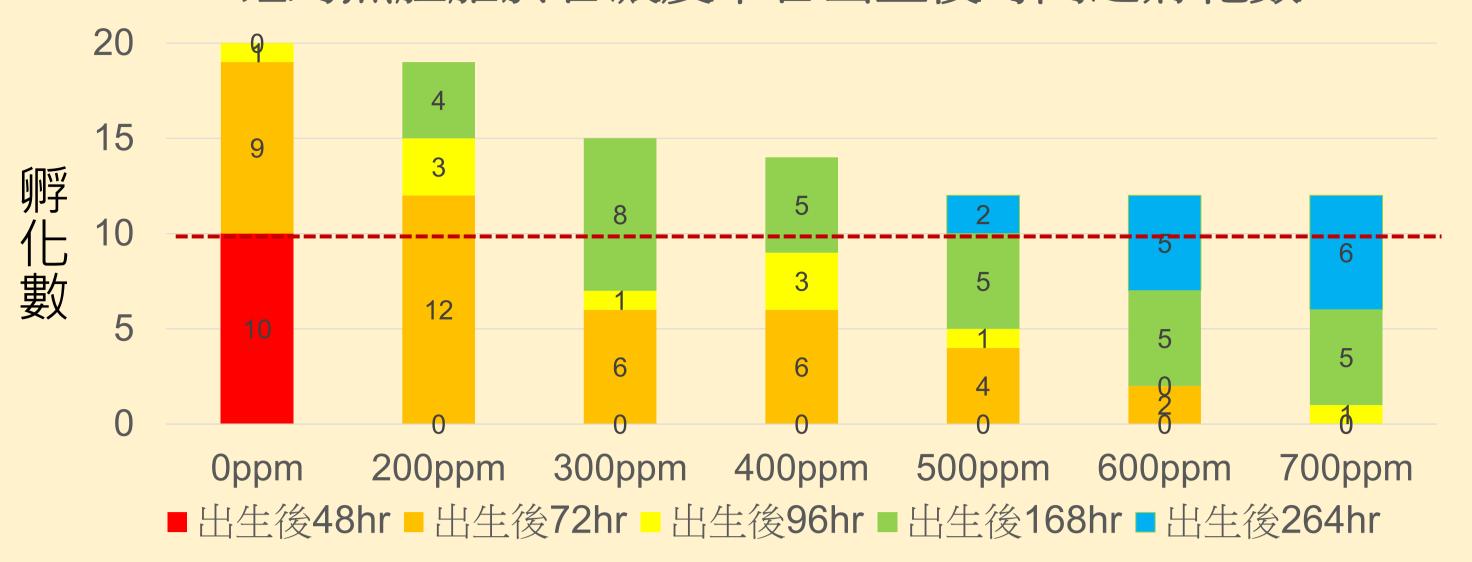
實驗結果:



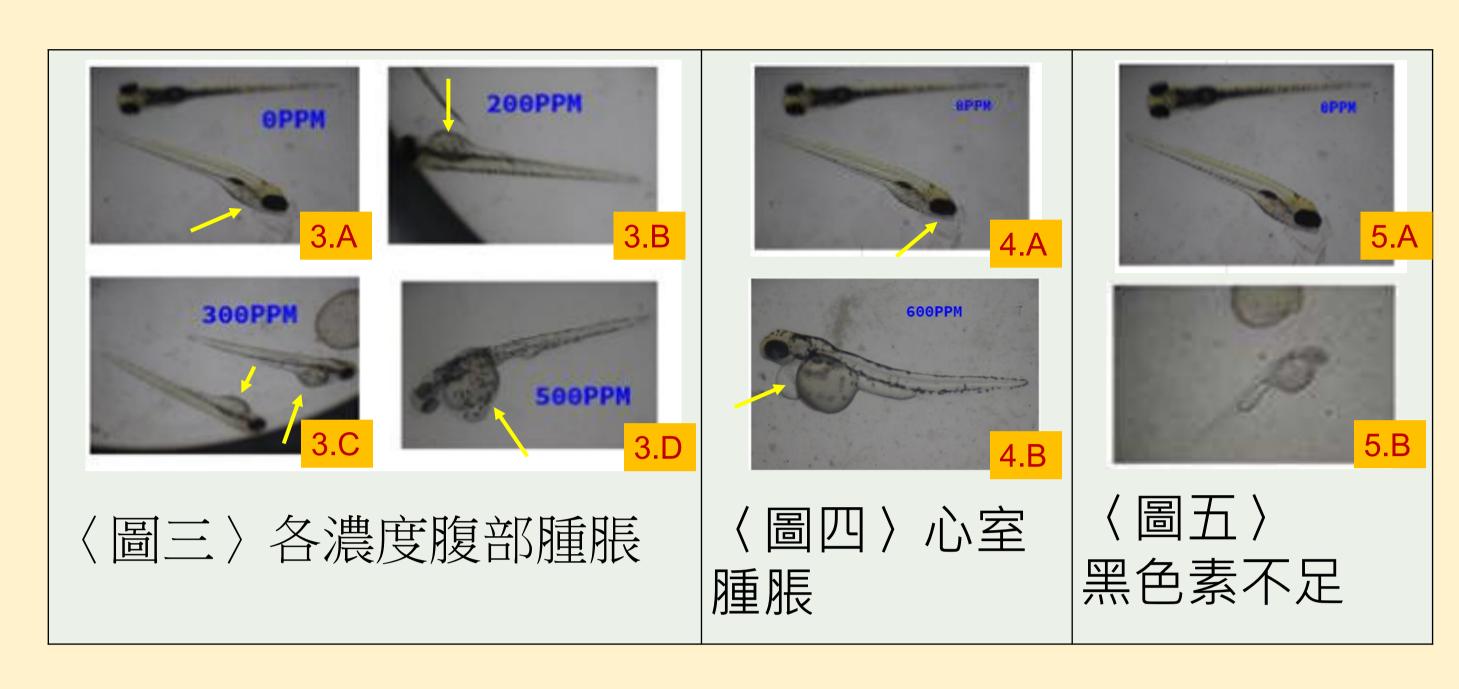
〈圖一〉斑馬魚於不同紅花濃度下之死亡率(n=20)

- 1. 紅花於斑馬魚之LCD50(半數致死率)為730PPM (圖一)
- 2. 據圖二中各紅花濃度的孵化率與紅色虛線處的半數孵化時間 統計(圖__)
- (1)隨著紅花濃度上升,斑馬魚的孵化率會隨之降低
- (2)隨著紅花濃度上升,斑馬魚的孵化所需時間會隨之增加
- (3)發現在0-700ppm的濃度區間,每增加100ppm斑馬魚約有 24-36小時的孵化延遲。
- 3.疑似卵黃囊未消化的腹部腫脹: (圖三)
- (1)在各個紅花濃度下,腹部腫脹之發生率均高於12/20
- (2)與Oppm無濃度之斑馬魚(圖3.A)相比, 在紅花濃度越高下的 斑馬魚,其腹部腫脹程度也越高(圖3.B 3.C 3.D)

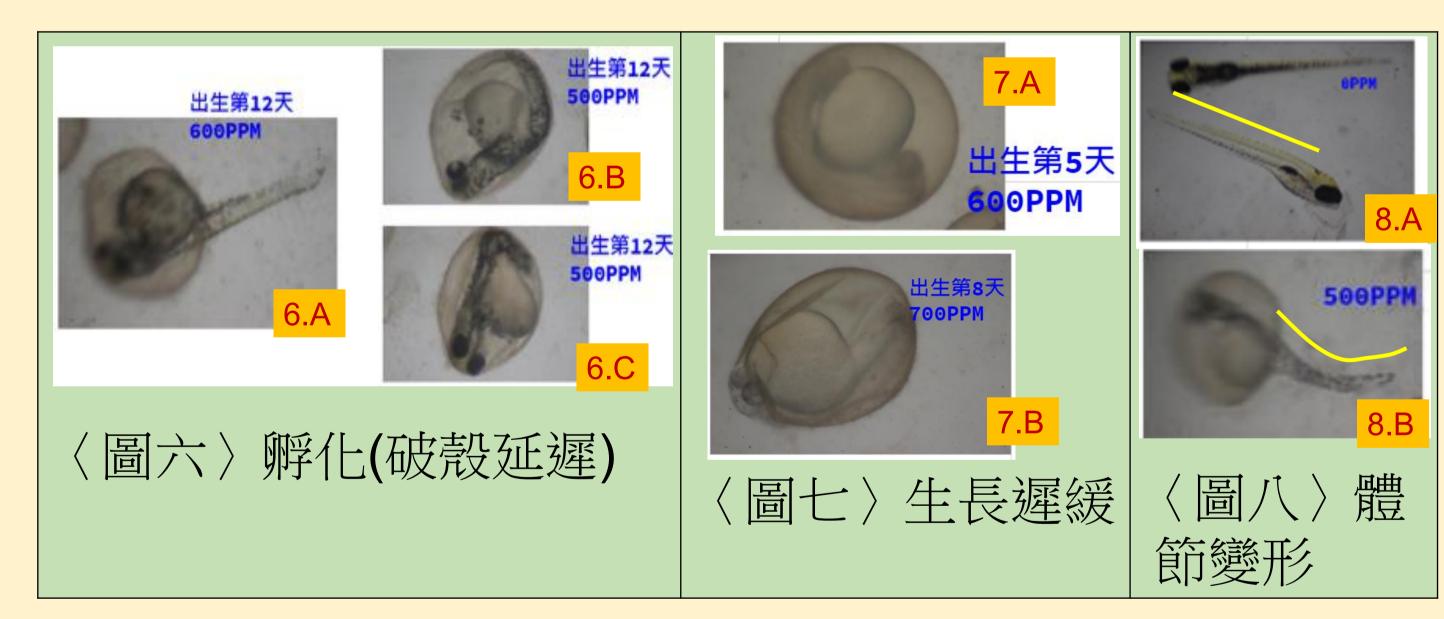
斑馬魚胚胎於各濃度下各出生後時間之孵化數



〈圖二〉斑馬魚於不同紅花濃度下之孵化數(率)



- 4.心腔室腫脹: (圖四)
- (1)在各個紅花濃度下,心腔室腫脹之發生率均高於10/20
- (2)與0ppm無濃度之斑馬魚(圖4.A)相比,在紅花濃度較高下的斑 馬魚(圖4.B),有明顯心室較大的情形。
- 5.魚體黑色素不足: (圖五)
- (1)在各個紅花濃度下,魚體黑色素不足之發生率均低於10/20
- (2)與0ppm無濃度之斑馬魚(圖5.A)相比,在高濃度500ppm下的斑 馬魚會有黑色素不足或眼睛、腹部側面、背脊至尾部均無黑 色素(圖5.B)的情形產生。



- 6. 孵化(破殼時間)延遲: (圖六)
- (1) 在各個紅花濃度下,孵化延遲的發生率均高於12/20
- (2)正常斑馬魚於出生72小時(3天)內即可順利脫殼,但在高紅花 濃度下之斑馬魚有延遲至第12天仍在卵內存活,但未破殼孵 化的情形(圖6.A 6.B 6.C)。
- 7. 生長遲緩: (圖七)
- (1)斑馬魚胚胎之生長遲緩,於高紅花濃度600ppm以上較易發生。
- (2)正常斑馬魚於出生24小時內可有部分體節與眼睛構造,但在 高濃度下之斑馬魚於出生第五天體節與眼睛仍未完全發育(圖 7.A 7.B) °
- 8. 體節變形(圖八)
- (1)斑馬魚之體節變形現象,於紅花濃度500ppm以上較易發生
- (2)與Oppm無濃度之斑馬魚(圖8.A)相比,高紅花濃度下之斑馬魚 有尾部呈外躬(8.B) 之體節彎曲情形。

實驗結論與建議:

從本研究的初步結果發現,紅花會造成斑馬魚胚胎之死亡、畸 胎及生長與孵化之延遲,且以上影響與紅花的濃度有關,進而 推斷活血化瘀中藥可導致生物胚胎發育上的各種影響。而在未 來則可根據斑馬魚各型畸胎、生長及孵化延遲的成因進行探討, 並了解其背後的生理與生化機制為何。