ELUCIDATION OF THE MECHANISMS OF TERATOGENICITY OF SOME COMMONLY USED MEDICINAL PLANTS

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ABSTRACT

An array of studies has proven the efficacy of numerous medicinal plants against ailments ranging from pain to microbial infection. However, their teratogenic potentials have not been well elucidated. One such ethnobotanical survey was used to determine the types of medicinal plants used by pregnant women in Lagos, Nigeria. The aim of this study is to determine the possible teratogenic effects on the cardiovascular and nervous systems and the mechanism of teratogenicity of these commonly used medicinal plants.

The aqueous extracts of some of the most commonly used plants (*Enantia chlorantha, Morinda lucida* and *Moringer olifera*) were prepared. Pregnant Wistar rats were treated with several doses (150-600 mg/kg) of these extracts at gestational day six and the litters were examined for any defects at gestational day 20. There was no significant change in the number of litters and their gross morphology. However, there was a significant increase in fetal resorptions, and the overall body weight and crown to rump length of the litters was affected in a dose dependent manner of which the direction is dependent on the plant species. Zebrafish embryos were also exposed to several concentrations (0-1.28 mg/ml) of the extracts between 4-144 hours post-fertilization. A dose dependent effect was observed in the developmental processes of the zebrafish resulting in embryo lethality, skeletal dysmorphogenesis and pericardial edema leading to the formation of a tubular heart. However, there was no significant change in locomotor activity of the eleutheroembryo at six days post-fertilization when there was no overt teratogenicity.

These research findings offer a basis for further investigation of the cellular and molecular mechanisms by which these extracts cause developmental defects in both zebrafish and rat whole embryo culture. These approaches remove the influence of maternal metabolism and other influences, and will provide valuable endpoints for exploration in pregnant mammals and newborns.

INTRODUCTION

Herbal medicine use had been on the increase for the management of various ailments, the World Health Organization (2007) estimates that about 65%-80% of the world's population uses traditional medicine as their primary form of health care. This has been primarily influenced by patients displeasure with conventional medicine safety and therapeutic outcome (Ernst et al., 2000). In addition, there is the common assumption that natural is better, hence, herbal medicines may seem to be an attractive natural option for women who want to avoid taking drugs during pregnancy.

Indeed, herbal medicines have profound therapeutic advantages ranging from their use as antibiotics, analgesic agent, anti-inflammatory agents and chemotherapeutic agents all of which are documented by various researchers.

However, despite these advantages, like any other substance, traditional medicines have the potential to be toxic.

Although there had been some research carried out on the toxicity profile of some herbal plants, there has been little to no work done on the teratologic effects of medicinal plants.

RESULTS

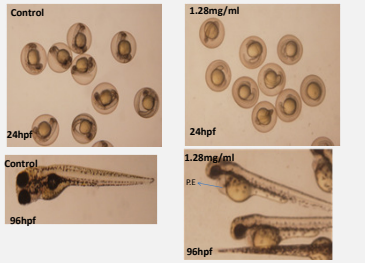

Figure 3: Overt toxic effects observed in wild type EK zebrafish embryos following treatment with *Enantia chlorantha*
There was a developmental delay at 24hpf at the highest dose and Pericardial edema (P.E) was observed at 96hpf.
24hpf Mag X 100 and 96hpf mag X 120

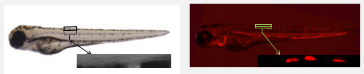

Figure 5: Location and visualization of the dorsal longitudinal anatomical vessel where the erythrocyte velocity was measured in 72HPF EK (left) and *Tg(gata1:dsRed)sd2/+(AB)*) Zebrafish (right)
Mag x 100 (upper) & x 200 (lower)

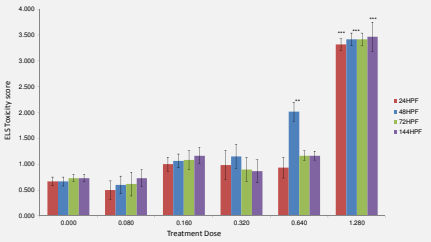

Figure 4: There was a significant increase in overt toxicity at the highest dose. Observed defects include developmental delays, pericardial edema, skeletal dismorphogenesis, and death. Result presented as Mean±SEM, *** P<0.001

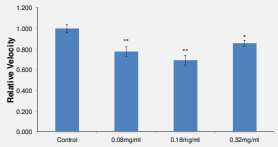

Figure 6: Relative velocity of the erythrocytes. The rate of travel of 12 erythrocytes was measured in each of 12, 72hpf *Tg(gata1:dsRed)sd2/+(AB)* zebrafish following treatment with *Enantia chlorantha.* *P<0.005 ** P<0.001

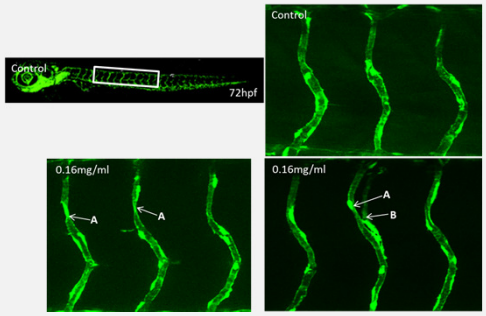

Figure 7: Images of the intersegmental vessels of 72hpf *Tg(Fli-1a:nEGFP)^{y7}/+(AB)* zebrafish following treatment with *Enantia chlorantha*. A = occlusion of the vessel. B = Bifurcation of the vessel.

The intersegmental vessels of the trunk are among the first angiogenic vessels to form in all vertebrates thus making it one of the important structures in the investigation of angiogenesis.

Mag whole fish: X 100; intersegmental vessels X 200

METHODOLOGY

Plant Extraction

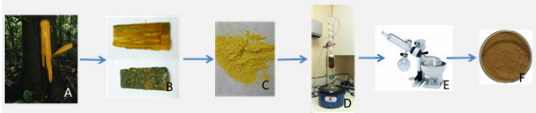

Figure 1: Plant extraction process; A= de-barking of the Plant (*Enantia chlorantha*), B = air drying the bark, C = powdered form of the dried bark after grinding, D = soxhlet extraction where the powdered plant is placed in the thimble where the heated solvent acts on it and extracts its constituent E = Rotary Evaporator used to recover the solvent and also dry the extract F = plant extract.

Animals

Wild-type (EK) zebrafish (stock originally purchased from EkkWill Waterlife 17 Resources, Ruskin, FL) and transgenic strains (*Tg(gata1:dsRed)sd2/+(AB); Tg(Fli-1a:nEGFP)^{y7}/+(AB)*) were reared in 28 °C dechlorinated, filtered municipal water (DFMW) in the NIEHS Children's Environmental Health Sciences Core 19 Center (CEHSC) animal facility. The fish were maintained at 14/10 hours light/dark cycle fed live Artemia twice daily and flakes once daily.

Erythrocyte flow rate

72 hour post-fertilization (hpf) *Tg(gata1:dsRed)sd2/+(AB)* zebrafish were anaesthetized with 0.1% buffered tricane and placed on a heated 22 ˚C stage for Image collection on the Zeiss LSM510 confocal microscope using Aim 4.2 software and 20x lens. RFP was excited with 561nm laser and images were collected using LP575nm emission filter. Images were collected in the wide field format using time lapse module at 33msec per frame for a total of 1000 frames. The rate of blood cell travel through 50-100 micron of the dorsal longitudinal anatomical vessel was measured using the Aim 4.2 software and the relative velocity was calculated as an estimation of flow rate.

Morphological analysis of vascular development

Images of 72hpf GFP positive, *Tg(Fli-1a:nEGFP)^{y7}/+(AB)* larva fixed in 4% paraformaldehyde were acquired digitally with an ORCA-ER camera mounted to a Zeiss inverted microscope with epi-fluorescence capabilities. The GFP filter cube on the microscope was used to acquire the GFP fluorescent signal. Digital images from the region caudal to the anal fin were acquired with bright field DIC optics with a 40x objective. 8-10 segments were imaged in each fish per group.

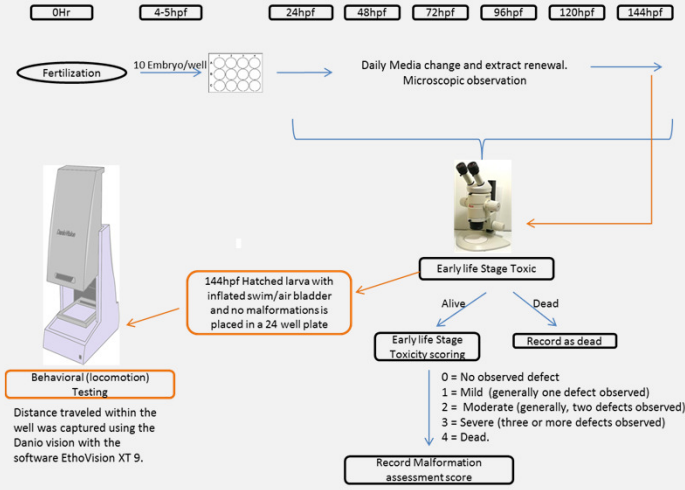

Figure 2: Embryo and larva Assessment work flow for Early life stage toxicity screening and Locomotion assay.

RESULTS

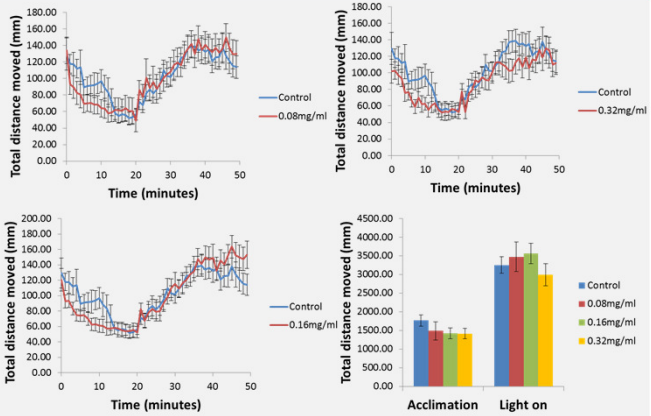

Figure 8: Locomotion assay showing no significant change in the distance moved by the 144hpf zebrafish larva after being exposed to 20minutes of darkness for acclimatization and then having light on for 30 minutes.

CONCLUSION

Zebrafish embryos treated with *Enantia Chlorantha* showed early life developmental delay at the highest dose (1.28mg/ml) tested which was not used in further study.

Exposure of transgenic zebrafish *to Enantia Chlorantha* produced an effect on cardiovascular function, as indicated by the relative velocity of erythrocytes.

There was as an effect on angiogenesis in transgenic zebrafish treated with *Enantia Chlorantha* observed from the morphological examination of the intersegmental vessels.

Zebrafish embryos treated with *Enantia Chlorantha* show an apparent change in erythropoiesis of which quantification is ongoing.

Zebrafish larva locomotion was not affected by treatment with Enantia Chlorantha.

Further study on the molecular mechanism underlying the observed effects are on going.

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