Utilizing the Zebrafish Model to Determine Anti-Epileptic Properties of Mistletoe and Cannabis

by
Collin Dietrich

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Approved by

Advisor: Dr. Kristine Willett

Reader: Dr. Asok Dasmahapatra

Reader: Dr. Richard Buchholz
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ABSTRACT

Utilizing the Zebrafish Model to Determine Anti-Epileptic Properties of Mistletoe and Cannabis

Epilepsy affects around 50 million people in the world, therefore improving treatment efficacy and safety for epileptics is imperative. In this study we sought to screen the effectiveness and safety of cannabis constituents (delta-9-tetrahydrocannabinol and cannabidiol) and *Tapinanthus globiferus* extracts in treating epilepsy. We used a zebrafish model wherein seizures were induced by treatment with pentylenetetrazol. Our results showed that *Tapinanthus globiferus* dose-dependently reduced seizure activity, and no toxicities were seen at the concentrations used. In contrast delta-9-tetrahydrocannabinol and cannabidiol did not significantly reduce seizure activity and some toxicities were seen at the higher concentrations tested. We conclude that extracts of *Tapinanthus globiferus* show promise as anticonvulsants and further research is needed to identify the active constituents and their pharmacological properties.
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<table>
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<th>Description</th>
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<tr>
<td>DS</td>
<td>Dravet syndrome</td>
</tr>
<tr>
<td>SCN1A</td>
<td>voltage-gated sodium channel</td>
</tr>
<tr>
<td>THC</td>
<td>delta-9-tetrahydrocannabinol</td>
</tr>
<tr>
<td>CBD</td>
<td>cannabidiol</td>
</tr>
<tr>
<td>TG</td>
<td><em>Tapinanthus globiferus</em></td>
</tr>
<tr>
<td>IACUC</td>
<td>Institutional Animal Care and Use Committee</td>
</tr>
<tr>
<td>PTZ</td>
<td>pentylenetetrazol</td>
</tr>
<tr>
<td>MS-222</td>
<td>tricaine methanesulfonate</td>
</tr>
<tr>
<td>qPCR</td>
<td>Quantitative Real Time Polymerase Chain Reaction</td>
</tr>
<tr>
<td>DZP</td>
<td>diazepam</td>
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1. INTRODUCTION

1.1. Epilepsy

Epilepsy is a neurological condition that affects an estimated 50 million people worldwide and an estimated 2.4 million people are newly diagnosed each year (World Health Organization, 2017). It causes affected individuals to have seizures that can vary in frequency, duration and type. Causes of epilepsy include genetics, head trauma, brain conditions, certain infectious diseases, prenatal injury and certain developmental disorders. While some epilepsy causes are known, about half of the cases are idiopathic. Current treatments include medication, surgery, vagus nerve stimulation procedures and eating a ketogenic diet (Mayo Clinic, 2015). Pharmaceuticals, prescribed as a single medication or in combination with other medications, are the most common form of treatment for epilepsy and can have many side effects that increase in severity and incidence as more medications are used. Most people on anti-epileptic drugs will experience fatigue, dizziness, upset stomach and/or blurred vision. However, these drugs can cause much worse side effects, such as a decrease in cognitive function, bone loss, lowering white blood cell count, lowering platelet count, liver damage, pancreatic damage and rashes that cause skin to peel off (Schachter et al., 2013).

Dravet syndrome (DS) is a specific form of epilepsy that starts within the first year after being born and affects anywhere from 1:20,000 to 1:40,000 people. DS can
cause slowed mental and physical development, other neurologic conditions and musculoskeletal problems. By the age of 20, a person with DS has a mortality rate of 20%. Around 80% of DS cases are due to a mutation of the SCN1A gene that causes impaired voltage-gated sodium channels (Escayg & Goldin, 2010). The frequent seizures caused by DS are prolonged (> 5 minutes) and can be triggered by a slight increase in temperature, vaccines, illness, excitation and/or certain light. These seizures are also very resistant to medications, and if a SCN1A mutation is present, then some of the common anti-epileptic drugs will worsen seizures if administered. Due to the resistant nature of DS, treatment generally requires a combination of medications. The combination that shows the most success is valproic acid, clobazam and stiripentol. Stiripentol, however, is not approved by the FDA (Welborn, 2015). Given the side-effects and lack of effectiveness of anti-epileptic drugs for some portions of epileptics, research should be conducted to find treatments for all types of epilepsy that have less adverse effects and reduce seizures more effectively.

1.2. Cannabis

Delta-9-tetrahydrocannabinol (THC; Figure 1A) is a constituent of cannabis. It is psychoactive and is the reason for recreational cannabis abuse. In addition to its recreational use, it has uses as a therapeutic agent and is currently being utilized as an antiemetic and orexigenic pharmaceutical (Food & Drug Administration, 2004). THC can be produced synthetically or it can be extracted from the cannabis plant as an oil (Sharma, et al., 2012).
Cannabidiol (CBD; Figure 1B) is another constituent of cannabis that is similar to THC in structure but is not psychoactive. While it does not have significant psychoactive properties, CBD has shown promise as a therapeutic agent for many different ailments in preliminary studies. These include use as an anti-epileptic, an anti-inflammatory, a neuroprotective agent, an analgesic, an anti-tumor agent, an anti-psychotic, an anxiolytic, and a substance abuse treatment (Volkow, 2015). CBD can also be extracted as an oil from cannabis or created synthetically.

Cannabis use as a treatment for seizures is expanding but is hampered by its schedule I classification by the United States federal government. This means cannabis is not recognized as having an accepted medical use by the federal government, and it also makes research to determine its safety and effectiveness as a medicinal drug restricted (Drug Enforcement Administration, n.d.). However, 4 states plus D.C. allow recreational marijuana, 23 states allow medical marijuana, and 13 allow medical CBD, but the schedule I status of cannabis means that even in these states it can still be difficult to obtain the drug legally (Throckmorton, 2016). More research should be done on novel compounds to treat epilepsy so that effective and safe treatments can be more accessible.
to all people. That includes providing sufficient research on cannabis that would determine its effectiveness, safety, and mechanism of action. Determining the mechanism of action for cannabis and its constituents will allow research for new compounds that have similar mechanisms for use as a treatment and will not be as difficult to obtain.

1.3. *Tapinanthus globiferus*

*Tapinanthus globiferus* (TG) is a variety of mistletoe from the Loranthaceae family found in western Africa. It is a hemi-parasitic plant that grows on various types of trees such as the shea butter tree, the cocoa tree, the sweet orange tree, hog-plum tree, and rubber trees. These trees that TG uses as a host are economically valuable and so TG and the other African mistletoe varieties are seen as a nuisance by the farming community. While the farming community sees TG as a threat to their livelihood, it has beneficial applications as it has been traditionally used as a form of herbal medicine for multiple ailments including epilepsy, but there are no current publications on its effects on epilepsy (Adesina et al., 2013). A different variety of mistletoe (*Viscum album*), however, has been used by medical doctors to treat a patient with refractory childhood absence epilepsy, a drug-resistant version of epilepsy, and successfully eliminated her seizures (von Schoen-Angerer et al., 2015). As a traditional medicine, the leaves of TG are usually crushed up, soaked in either cold water or beer and then the liquid is consumed orally (Adesina, et al., 2013). Because TG has been used traditionally to treat epilepsy to some degree and other mistletoes have shown to be successful at treating epilepsy, we were
interested in determining which component of the plant is responsible for its anti-
convulsive properties.

1.4. Zebrafish as a model

Zebrafish (*Danio rerio*) are small fish from the Indian subcontinent that have been
used as a model organism in research (Encyclopedia of Life, n.d.). They have a high
fecundity, mature quickly, share a majority of their genome with humans, have embryos
that develop outside of the parent organism and are easily observed under a microscope
while developing (Burke, 2016). All of these characteristics help to make zebrafish an
ideal candidate to be a research model. Furthermore, zebrafish have been shown to be an
effective model in the areas of epilepsy, chemical toxicity and development (Baraban et
al. 2005; Hill et al., 2005; Veldman & Lin, 2008). In addition to the ability to assess the
behavioral aspects of the seizures in zebrafish via the ViewPoint Zebrabox, *c-fos*
expression can be used to further confirm seizure activity. *C-fos* is a marker of neuronal
activity that is transiently induced in neurons during seizures (Sagar et al., 1988;
Dragunow and Robertson, 1988; Kiessling and Gass, 1993). Zebrafish can also be used as
a model that mimics DS by performing a SCN1A gene knockdown (Rosen et al., 2009).
The knockdown is performed using a morpholino that is antisense complementary to the
SCN1A gene. The morpholino is introduced to zebrafish embryos using microinjection.
This DS model has been used to identify both fenfluramine and clemizole as a promising
drug to treat this form of epilepsy (Zhang et al., 2015; Baraban et al., 2013).
1.5. Study Goals

In this study, we utilize the zebrafish model to address our three main goals. The first is to identify novel natural compounds that effectively reduce seizures; diazepam will be used as a reference drug to compare efficacy. The second is to determine the changes in gene expression associated with exposure to THC, CBD, or TG extracts. The third is to evaluate THC, CBD and TG extracts for developmental toxicities. We hypothesize that one or more of these compounds will show anticonvulsant properties (e.g. decreased large activity and c-fos gene expression) without causing overt toxicity.
2. METHODS AND MATERIALS

2.1. Zebrafish culture and egg collection

AB line wild-type zebrafish were purchased from Zebrafish International Resource Center (ZFIN, Eugene, OR) and raised under the approved IACUC protocol. The zebrafish were kept in the Aquatic Habitats ZF0601 Zebrafish Stand-Alone System (Aquatic Habitats, Apopka, FL) with each unit containing ~0.3 L of water per fish. The habitats contained zebrafish appropriate water (pH 7.0-7.6, 340 parts per million (ppm) Instant Ocean, Cincinnati, OH) in a climate (25-28°C) and light (14 hours of light and 10 hours of dark) controlled room. They were fed Gemma Micro 300 (Skretting Nutreco Company, Westbrook, ME) two times every day. The fish used for breeding did not have disease and were also of a healthy breeding age (4-18 months post fertilization). To prepare for egg collection, the fish were transferred to breeding tanks at a 1:1 ratio of males to females and were then left overnight to produce fertilized eggs.

After the lights turned on the following morning, fish were returned to their normal holding tanks. The eggs that had fallen through the protective grate to the bottom of the breeding tank were then collected by pouring the water from the breeding tanks through a sieve. The breeding tanks were rinsed and that water was then sieved again to ensure that every egg had been collected.
Unwanted debris and unfertilized/dead eggs were removed using a transfer pipette from the collected eggs. The cleaned eggs were then transferred to a petri dish containing zebrafish water (60 ppm Instant Ocean, pH 7.5) and placed in an incubator at 28°C for 5 days. Dead eggs were removed daily.

2.2. Exposure procedures

At 120 hours post fertilization (hpf), the larvae were transferred to 96 well plates with one larva in each well. The larvae were chosen based on lack of deformities and the presence of an inflated swim bladder. The water in each well was removed and replaced with 150 µL of a dosing solution. TG extracts were provided from Dr. Jordan Zjawiony. CBD and THC were obtained from NIDA Drug Supply Program. The zebrafish (n=12/treatment/plate; 3 plates/treatment) were exposed to: control (0.05% DMSO), extracts from TG (0.008, 0.04, 0.2, 1, or 5 mg/L), THC (0.02, 0.09, 0.45, 2.23, or 11.2 µM) or CBD (0.003, 0.012, 0.06, 0.32, or 1.59 µM) and 25 µM (7 mg/L) diazepam (DZP). TG extracts that were tested were AF-Hx, AF-H2O, AF-Bu, AF.1.10.TG.9, AF.1.10.TG.14, AF.1.16.TG.8, AF.1.14.TG.4, AF.1.16.TG.3, AF.1.14.TG.2, AF.1.10.TG.3, AF.1.2.TG.2 H2O/MeOH, AF.1.2.TG.2 MeOH, AF.1.2.TG.2 ACE, AF.1.2.TG.2 CHCl3. Once the dosing was complete, the plates were placed back into the incubator and lightly covered with aluminum foil to protect the compounds from the light.
2.3. Seizure induction

Following a 24 hour exposure to the dosing solutions, the larvae were observed to determine if TG, THC, or CBD had any toxic side effects: a lack of response to touch, pericardial edema, yolk sac edema, a curved body axis and/or a non-inflated swim bladder. If any of these signs of toxicity were observed, then that larva was excluded from further analysis. To induce seizures, 50 µL of 20 mM pentylenetetrazol (PTZ; Sigma Aldrich) was added to each well, except for the first row which was the control, to yield a final concentration of 5 mM PTZ.

2.4. Viewpoint data collection and analysis

Following the addition of PTZ to induce seizures, the plate is placed in the ViewPoint ZebraBox and the larvae are acclimated for 5 minutes before behavior recording. The ViewPoint ZebraBox tracks larval movement for 15 minutes with the lights on at 100% with a threshold of 27 and small and large activity parameters set at 5-9 and >9 mm/s, respectively. The ZebraBox software creates an excel sheet that includes the duration each larva spends in the inactive, small, and large movements over the 15 minute duration. Movement that meets the large activity requirements is indicative of seizure activity and was the data that was used to test for a compound’s effectiveness as an anticonvulsant. Therefore, PTZ significantly increases the duration of large activity compared to control and a compound with anticonvulsant properties would significantly decrease the PTZ-induced large activity compared to PTZ alone and ideally would not be
significantly increased compared to control. Larvae were then euthanized with buffered MS-222, placed in RNAlater and stored at -80°C

The data collected from the ViewPoint ZebraBox was analyzed using GraphPad Prism 5.0 (La Jolla, CA). The data sets were first checked for normality using a Kolmogorov-Smirnov test. If the data passes the Kolmogorov-Smirnov test for normality, then statistically significant differences compared to PTZ was determined using a t-test. If the data did not pass the Kolmogorov-Smirnov test for normality, then a Mann-Whitney test was used for analysis. All tests used $p \leq 0.05$ for determination of statistical significance ($n=22-36$ larva/treatment). Each data set is given as the mean ± the standard error.

2.5. RNA extraction, reverse transcription and qPCR

To determine changes in gene expression caused by the addition of the compounds, the mRNA expression of c-fos (higher expression is associated with more epileptic activity) is measured using qPCR. The RNA was isolated using TRIzol (Ambion) and then purified by using an RNeasy Mini Kit (Qiagen) according to manufacturer’s protocol. The purified RNA collected was reverse transcribed to cDNA using TaqMan Reverse Transcription reagents (Applied Biosystems). The abundance of c-fos gene expression was normalized to 18S housekeeping gene expression and determined using qPCR with SYBR Green in a GeneAmp 7500 Sequence Detection System (Applied Biosystems). The primers used in this qPCR are c-fos forward: 5'-CAC CGA TAC ACT GCA AGC TGA A-3', c-fos reverse: 5'-CAG GTT GGC GAT GTC GTT
CT-3’, 18S forward: 5’-TGG TTA ATT CCG ATA ACG AAC GA-3’, 18S reverse: 5’-CGC CAC TTG TCC CTC TAA GAA-3’ (Corrales et al., 2014a). Amplification efficiencies of c-fos and 18s were determined using the formula \( E = 10^{\frac{-1}{s}} - 1 \) and were between the accepted range of 78-106% (Higuchi et al., 1993). The linearized 2^{-\Delta Ct} values from the qPCR were analyzed using a t-test (p<0.05; n=3 pools/treatment; 10-12 larva/pool) and fold change relative to control was plotted \( 2^{-\Delta\Delta Ct} \).
3. RESULTS

3.1. Large activity post-PTZ exposure

The results for THC are shown in Figure 2A. None of the THC concentrations tested showed a significant decrease in large movement compared to 5 mM PTZ. The results for CBD are shown in Figure 2B. None of the CBD concentrations tested showed a significant decrease in large movement compared to 5 mM PTZ.

![Figure 2: THC (a) and CBD (b) seizure activity. Zebrafish larval behavior was analyzed using the Viewpoint Zebrabox (15 min recording with 100% light) to record duration of large activity (> 9mm/sec), following a 24 hr exposure (120-144 hpf) to natural compounds, induced by 5 mM PTZ to determine if these natural compounds have anticonvulsant properties (n=29-35 per treatment; Student t-test; p≤0.05).](image-url)
Eleven different fractions of TG extracts were tested and two of these fractions (AF.1.10.TG.9 and AF.1.10.TG.14) showed a significant decrease in large movement compared to 5 mM PTZ. Figure 3A shows that AF.1.10.TG.9 significantly decreased large movement in both the 1 and 5 mg/L concentrations by 29 and 41%, respectively, compared to PTZ alone. Figure 3B shows that AF.1.10.TG.14 significantly decreased large movement in the 5 mg/L concentration by 22% compared to PTZ alone. The 1 mg/L concentration for AF.1.10.TG.14 was almost statistically significant as well. AF.1.16.TG.8, AF.1.14.TG.4, AF.1.16.TG.3, AF.1.14.TG.2, AF.1.10.TG.3, AF.1.2.TG.2 H₂O/MeOH, AF.1.2.TG.2 MeOH, AF.1.2.TG.2 ACE, AF.1.2.TG.2 CHCl₃ extracts from

Figure 3: AF.1.10.TG.9 (a) and AF.1.10.TG.14 (b) seizure activity. Zebrafish larval behavior was analyzed using the Viewpoint Zebrabox (15 min recording with 100% light) to record duration of large activity (> 9mm/sec), following a 24 hr exposure (120-144 hpf) to natural compounds, induced by 5 mM PTZ to determine if these natural compounds have anticonvulsant properties (n=22-36 per treatment; Student t-test; p≤0.05).
TG did not show any significant differences from PTZ. AF.16.TG.3 data is shown in Figure 4 as an example of one of the fractions that did not show anticonvulsant activity.

Figure 4: AF.1.16.TG.3 seizure activity. Zebrafish larval behavior was analyzed using the Viewpoint Zebabric (15 min recording with 100% light) to record duration of large activity (> 9mm/sec), following a 24 hr exposure (120-144 hpf) to natural compounds, induced by 5 mM PTZ to determine if these natural compounds have anticonvulsant properties (n=28-35 per treatment; Student t-test; p≤0.05).

3.2. Gene Expression

Expression of the c-fos gene as determined by qPCR was performed for AF.1.10.TG.9. The results are shown in Figure 5 expressed as the fold increase compared to the control group. PTZ did significantly induce c-fos expression as compared to control. However, there were no significant differences in expression between PTZ only and the treatment groups. We hypothesized a reduction in the 1 and 5 mg/L AF.1.10.TG.9 and DZP groups, but there was actually a slight increase. Expression of c-fos was also
determined for both CBD and THC. The results in Figure 6 show a dose-dependent increase in \textit{c-fos} expression for both THC and CBD.

Figure 5: AF.1.10.TG.9 \textit{c-fos} expression. Expression of \textit{c-fos} was determined by qPCR for each dosing group of the zebrafish larvae. The p-value above each bar shows that group compared to control using a Student t-test. (p ≤ 0.05; n=3 pools/treatment; 10-12 larvae/pool)

Figure 6: THC (a) and CBD (b) \textit{c-fos} expression. Expression of \textit{c-fos} was determined by qPCR for each dosing group of the zebrafish larvae. The p-value above each bar shows that group compared to control and the p-value above the horizontal bars shows that group compared to PTZ using a Student t-test. (p ≤ 0.05; n=3 pools/treatment; 10-12 larvae/pool)
3.3. Toxicity

Figure 7 shows examples of deformities that are indicative of exposure to a toxic substance including pericardial edema, yolk sac edema, non-inflated swim bladder, etc. Figure 8A shows that CBD had a low incidence of mortality and physical deformities, however, at 1.59 µM it caused abnormal, erratic swimming patterns. Figure 8B shows that THC had a very high incidence of mortality at the 11.2 µM concentration. THC had a low incidence of erratic swimming and physical deformities. None of the compounds from TG showed toxicity at any concentration tested.

**Figure 7: Zebrafish morphology.** A normal zebrafish is shown in A and B along with labels to show what areas are observed for deformity. The left picture in C shows a close lateral view of a normal zebrafish while the right picture shows a close lateral view of a deformed zebrafish with deformities labeled. Any significant deviation from A and B is considered a deformity that is indicative of toxicity. (Corrales et al., 2014b)
Figure 8: CBD (a) and THC (b) toxicity. Zebrafish larvae were given different concentrations of either CBD (0.003, 0.012, 0.06, 0.32, 1.59 µM), THC (0.02, 0.09, 0.45, 2.23, 11.2 µM), DZP (25 µM), or weren’t given any drug as a control group. Larvae were checked for signs of toxicity following the dosing. n=24-52
4. DISCUSSION

The goal of this study was to use zebrafish to screen a series of compounds and extracts for anti-seizure activity. Compounds or extracts that are effective may ultimately prove to be useful in the treatment of epilepsy. We hypothesized that cannabinoids or mistletoe extracts would have anticonvulsant properties. However, the two compounds tested from cannabis, CBD and THC, did not show much promise in reducing PTZ-induced seizures according to the View Point data. Cannabinoids have shown mixed results in treating generalized seizures in other animal studies (Devinsky, et al., 2014). This could possibly be due to different experimental designs such as length of treatment and the variety of routes of administration in these studies. There is anecdotal evidence that THC and CBD are useful in people who suffer from DS and other similar forms of drug resistant epilepsy. The seizure induction trials in this study used a zebrafish model that utilized PTZ to induce seizures that represent a general form of epilepsy. This model does not represent the DS class of seizures, so while THC and CBD did not look like a viable seizure treatment in this study for general seizures, further research should be done with zebrafish DS models to determine their effects on this specific type of epilepsy (Gupta, 2013).

In contrast to the cannabis compounds, the results from the activity monitoring for TG showed that two of the eleven extract fractions could be useful in reducing seizures. AF.1.10.TG.9 appeared to be the more effective of these two fractions; it reduced PTZ-
induced seizure activity at the 1 and 5 mg/L concentration. AF.1.10.TG.14 only showed significant reduction at the 5 mg/L concentration; however, 1 mg/L had a p value of 0.051. It is possible that if more trials are done that the 1 mg/L concentration would reach statistical significance. While no studies have been done specifically on TG for epilepsy, there are reports with different varieties of African mistletoe that have suggested anti-seizure activity. *Tapinanthes dodoneifolius* completely eliminates PTZ-induced seizures in Wistar rats at a dose of 500 mg/kg methanolic leaf extract (Uthman et al., 2015). A methanol extract from the stem of *Viscum capense* also reduced PTZ-induced seizures in albino mice at concentrations of 50-100 mg/kg (Amabeoku et al., 1998). *Globimetula braunii* extract prevented PTZ seizures in 83.33% of the mice given 150 mg/kg of an ethyl acetate fraction (Aliyu et al., 2014). This suggests that there may be a similar compound in all of these different varieties of mistletoe that causes a dose-dependent reduction in seizures. If the hypothesized similar compound could be identified and isolated in mistletoe, then it could possibly be even more effective and/or synthetically replicated. These studies also support that the dose of the TG extracts should be increased in future trials to see if higher concentrations will continue to show a greater reduction.

Gene expression analysis was performed on the most promising potential therapeutic from TG (AF.1.10.TG.9), THC, and CBD. The expression of *c-fos* in the brain is hypothetically indicative of seizure activity, so if seizures increase or decrease, *c-fos* expression should as well (Baraban et al., 2005). Our results showed an unexpected dose-dependent increase in *c-fos* expression for all three of the compounds. While THC and CBD did not show any seizure reduction, AF.1.10.TG.9 did. Therefore, we
hypothesized to, but did not see, a dose-dependent decrease in $c$-$fos$ expression for AF.1.10.TG.9. However, we did not measure the $c$-$fos$ expression of just the brains of the larvae, but a pool of 10-12 whole larvae (only three biological replicates; n=3). It could be that AF.1.10.TG.9 did decrease $c$-$fos$ expression in the brain, but this was masked by increased expression in the other tissues. Also, $c$-$fos$ gene expression has been shown to increase with administration of other forms of anesthesia in rats, so the MS-222 might have affected the results as well (Bullitt, 1990). All treatment groups did receive the same amount of anesthesia, so any overall increase should be the same for each group reducing its significance. Future studies should be done with AF.1.10.TG.9 in larger animal models so that changes in $c$-$fos$ expression of the brain alone can be measured or by *in situ* hybridization whole mounts on individual zebrafish larvae.

Another advantage of the zebrafish assay is that toxicity can be assessed on each organism prior to behavioral analysis. The highest THC concentration of 11.2 $\mu$M caused death in over half of the zebrafish, but the rates of other deformities remained fairly low at concentrations between 0.02 and 2.23 $\mu$M. In contrast, CBD caused less than 10% of death or physical deformities at any of the concentrations tested. It did, however, cause 25% of the zebrafish to engage in abnormal swimming patterns at 1.59 $\mu$M. The reason for the erratic swimming is not clear, but it could due to an adverse interaction with the nervous systems and the signals to the musculature or with the musculature directly. This is consistent with research that links marijuana with adverse effects on brain development and physical deformation (Thomas, 1975; Volkow et al., 2014). THC has also been shown
to be lethal to Fischer rats in high enough doses but the toxic concentration varies depending on the route of administration (Rosenkrantz et al., 1974).

Importantly for future drug development, TG did not show any toxicities or adverse effects at the concentrations tested. This result is very significant in that the most ideal treatment would cause minimal side effects. Further testing should be done to determine the highest concentrations of TG extracts that can be considered safe. Additionally, longer term exposures should be conducted to determine if acute or chronic exposures cause toxicities.

The overall results from this study indicate that TG could be a potential source of effective treatment for epilepsy that may be less likely to cause side effects and toxicities. While cannabis did not show any effectiveness for treating general seizures in this study, further studies should be conducted utilizing a DS model to determine its efficacy in reducing drug-resistant epilepsy seizures. TG could potentially be useful in treating drug-resistant varieties of epilepsy as well, so further research using DS models should be conducted.

Because AF.1.10.TG.9 was not toxic at the highest concentration tested and PTZ-induced seizure activity was not reduced completely to control levels, higher concentrations of this extract should be tested and may be more effective. Furthermore, chemical analysis of the constituents of this fraction can be determined. Individual chemicals of the extract can then be tested and may prove to be more effective alone than the AF.1.10.TG.9 mixture. If these compounds are deemed effective and safe, then they should be scaled up to mammal trials and eventually epilepsy clinical trials.
5. REFERENCES


