CAFFEINE’S EFFECT ON ZEBRAFISH

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11TH GRADE
CENTRAL CATHOLIC
PURPOSE

• To determine if caffeine has an effect on survivability and heart rate (HR) of zebrafish
CAFFEINE

• Naturally occurring substance, \( \text{C}_8\text{H}_{10}\text{N}_4\text{O}_2 \)
  • Bitter, white purine compound
  • Similar chemical structure to adenine and guanine
• Most commonly used stimulant worldwide
CAFFEINE EFFECTS

- Increases blood pressure, respiration rate and mental alertness
- Toxic effects include
  - Tachycardia
  - Dysrhythmias
  - Seizures, coma and death
CAFFEINE EFFECTS

• Stimulates oxidative stress in human osteoblasts
• Oxidative stress may lead to mitochondrial malfunction
• Caffeine may independently induce apoptosis
ZEBRAFISH, *DANIO RERIO*

- Genome similar to humans
  - 70% of protein-coding human genes are related to zebrafish genes
  - 84% of genes associated with human disease have a zebrafish counterpart
- Widely used in study of vertebrate development and physiology
ZEBRAFISH, *DANIO RERIO*

- Embryonic stages easy to observe given transparency
- Small, easy to maintain
- Quick developmental period
**ZEBRAFISH**

Adult, ~2.5cm long
CAFFEINE & ZEBRAFISH

• Angiogenesis disrupted by caffeine in zebrafish
• Caffeine antagonizes adenosine receptors (ADRs)
• ADRs play vital role in angiogenesis
HYPOTHESIS

• Null
  • Caffeine will not have a significant effect on survivability and heart rate of zebrafish

• Alternative
  • Caffeine will have a significant effect on survivability and heart rate of zebrafish
MATERIALS

- Zebrafish embryos
- Distilled water
- Instant Ocean
- Petri dishes
- Floor lamp
- Incubator
- Powdered caffeine

- Scientific scale
- 10mL measuring cup
- Pipettes
- Microscope
- Thermometer
- Stopwatch
PROCEDURE

• Zebrafish embryos obtained commercially (Carolina Biological)

• Caffeine solutions prepared in three concentrations – 0.1mg/mL, 0.5mg/mL and 2.5mg/mL

• Embryos transferred to labeled petri dishes with pipettes
PROCEDURE

• Embryos in stock solution transferred to petri dishes and used as control
• Petri dishes stored in incubator at 28.5 degrees Celsius
• Light/dark cycle of 14/10 hours per day
PROCEDURE

• Observations made at repeating time intervals for 96 hours

• Embryos evaluated for
  • Viability
  • Heart rate

• Heart beat counted for 15 seconds and multiplied by 4, resulting in beat per minute (bpm) data points
ZEBRASFISH IMAGES

control

[0.1mg/mL]

[0.5mg/mL]

[0.1mg/mL]

[2.5mg/mL]
ZEBrAFiSH SURViVAL

Specimen number

Time, hours

control

0.1mg/mL

0.5mg/mL

2.5mg/mL
SURVIVAL ANALYSIS

• Kaplan-Meier log rank testing shows statistically significant difference in survival between the four study groups

• p value < 0.001

• Time frame – 96 hours
SURVIVAL ANALYSIS

• No survival difference between control and [0.1mg/mL] group; p=0.299

• Statistically significant difference, p<0.001, between
  • Control and [0.5mg/mL] group
  • Control and [2.5mg/mL] group
CONCLUSION

• Null hypothesis rejected

• Alternative hypothesis accepted – caffeine had a significant effect on zebrafish survivability
  
  • Specifically, increasing concentrations of caffeine led to decreased survivability of zebrafish embryos
HEART RATE RESULTS

• At least 65% of subjects had HR measured at five different time points

• Mean HR similar across time:
  • Control 128.9 – 145.8 bpm
  • [0.1mg/mL] 194.7 – 203.3 bpm
  • [0.5mg/mL] 65.5 – 73 bpm
HEART RATE ANALYSIS

• ANOVA testing of all HR values
• 170 different HR data points
  • 68 from control group
  • 64 from [0.1mg/mL] group
  • 38 from [0.5mg/mL] group
HEART RATE ANALYSIS

SUMMARY

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ANOVA

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$F > F_{crit}$ with $P$-value $< 0.00001$; three groups of HR data are not all equal.
AVERAGE HEART RATE

beats per minute (bpm)

control [0.1mg/mL] [0.5mg/mL]

134.6 197.7 68.5
HEART RATE ANALYSIS

• ANOVA does not specify where the difference lies between the HR groups

• Post hoc Dunnett testing done to compare
  • Control to [0.1mg/mL] – p value <0.001
  • Control to [0.5mg/mL] – p value <0.001
HEART RATE ANALYSIS

• Caffeine concentration of 0.1mg/mL increased HR compared to control
• Caffeine concentration of 0.5mg/mL decreased HR compared to control
• Both changes occurred at a statistically significant rate
CONCLUSION

• Null hypothesis rejected

• Alternative hypothesis accepted
  – caffeine had a significant effect on survivability and heart rate of zebrafish
CONCLUSIONS

• Increasing concentrations of caffeine led to decreased zebrafish embryo survival, compared to control

• Statistically significant results found with 2 of 3 test solutions
  • [0.1mg/mL] – not significant
  • [0.5mg/mL] and [2.5mg/mL] - significant
CONCLUSIONS

• Caffeine affected zebrafish heart rate in a statistically significant fashion

• Compared to control
  • [0.1mg/mL] caffeine solution increased heart rate
  • [0.5mg/mL] caffeine solution decreased heart rate
INTERPRETATION

• Caffeine at the lowest concentration had the expected stimulant effect, increasing heart rate while not affecting survival

• Increasing caffeine concentrations led to decreases in both survival and heart rate
  • Oxidative stress, mitochondrial malfunction
  • Apoptosis
  • Disruption of angiogenesis
  • Some combination of all 3 factors?
LIMITATIONS AND EXTENSIONS

- Limitations
  - Time interval between measurements
  - Zebrafish availability
  - Inexperience in zebrafish care

- Extensions
  - Different caffeine concentrations
  - Longer observation period
  - Different stimulants – nicotine, pseudoephedrine?
BIBLIOGRAPHY


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