Laboratory Studies

(O – 06)

Evaluation of nicotine-alcohol crosstalk through novel tank zebrafish swimming analysis

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Background: Co-abuse of nicotine and alcohol is a major health problem and is associated with high economical costs. Epidemiological research shows a greater prevalence of nicotine intake in people who use alcohol. Many researchers consider the early co-abuse of both drugs on the presumption that nicotine is likely to lessen the alcohol intoxication. However, there is a major disagreement on the nature of the crosstalk between alcohol and nicotine.

Objective: To evaluate the crosstalk between alcohol and nicotine, using the zebrafish as an animal model through the novel tank swimming activity.

Methods: Zebrafish were pre-treated with nicotine (0.5-1.5 mg/L) or alcohol (0.5-1.5% v/v) or a combination of both for 30 minutes. Novel tank zebrafish swimming videos were recorded using CCD cameras and video analysis using NIH Image J software.

Results: Swimming pattern in control showed 85% similarity. However, alcohol-treated fish produced differences in swimming patterns with more distribution towards the water air interface (top) of the tank in a dose-dependent manner. In contrast, nicotine-treated fish produced more bottom distribution. Co-treated fish showed only alcohol type effect, but nicotine type effect appeared only when the nicotine to alcohol ratio exceeded 1.5. Increasing the concentration of both alcohol and nicotine while keeping the ratio constant showed an increase in mortality when concentration in the ratio exceeded 1% v/v (alcohol) and 1 mg/L (nicotine).

Conclusion: Our investigations showed that alcohol could effectively mask the nicotinic effect. However, there was no reduction but an increase in alcohol intoxication and mortality in the presence of nicotine. Therefore, the effect of the combination of both alcohol and nicotine was different from that of either drug alone.

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Divergent effects of low dose delta-9-tetrahydrocannabinol on the compound action potential of the sciatic nerve of the toad (*Bufo marinus*)

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Background: Delta-9-tetrahydrocannabinol (Δ^9 -THC) is used topically to alleviate pain. We therefore investigated whether topical application of Δ^9 -THC would affect the amplitude and/or latency of the compound action potential (CAP) of the sciatic nerve of the toad, *Bufo marinus*.

Methods: Eighteen sciatic nerves were dissected from nine pithed adult toads and randomly assigned to three treatment groups (n = 6): 0.1 mg/mL Δ^9 -THC, 0.2 mg/mL Δ^9 -THC and 0.9% saline (controls). Recordings of the amplitude and latency of the CAP were obtained from each nerve trunk preparation, suspended on silver wire electrodes in a recording bath connected to a stimulator, amplifier and computerized recording system that utilized a PolyView software to convert the electrical signals to digital tracing on the computer. A current of 40V at 0.1 msec duration was applied intermittently over 30 minutes and used to compare the parameters of the CAP when 0.5 mL of drug or saline was topically applied to the nerves. Data were analysed using the Kruskal-Wallis equality-of-population rank test.

Results: 0.2 mg/mL Δ^9 -THC produced a non-significant increase in amplitude when latency to discharge decreased below, or increased above, 15 msec. In contrast, amplitude decreased linearly with increasing latency with 0.1 mg/mL Δ^9 -THC.

Conclusion: The biphasic increase in the response of the sciatic nerves to the higher dose of Δ^9 -THC supports reports of the dual effect of the drug. The decreased amplitude with delayed latency to discharge at the lower dose supports its use in pain management.

(0 - 08)

A comparison of the low dose effects of delta-9-tetrahydrocannabinol and amphetamine on conditioned place preference drug-seeking behaviour in mice

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Background: Delta-9-tetrahydrocannabinol (Δ^{9} -THC) produces both euphoric and dysphoric effects in animals. Reports on dosage that demonstrates Δ^{9} -THC addiction in animals remain unsettled. This study investigated the drugseeking effects of low doses of Δ^{9} -THC and amphetamine in the conditioned place preference (CPP) paradigm, a classical animal model of addiction.

Methods: Twenty-five albino mice (22–24 g) were exposed to a biased CPP paradigm that utilized a three-chambered box. The mice were individually placed in the neutral grey corridor of the box and allowed 15 minutes of exploration. The number of entries and the time spent in the black, white and neutral chambers were recorded. Twenty-four mice exhibited a minimum 80% preference for the black chamber. These mice were randomly assigned to four treatment groups: 0.9% saline (controls), 0.5 mg/kg Δ^9 -THC, 1.0 mg/ kg Δ^9 -THC and 5.0 mg/kg amphetamine sulphate (AMP). The mice were then exposed to an eight-day conditioning phase in which each mouse was treated on alternate days with 1 mL (intraperitoneal) of drug and confined to the white chamber or given saline (1 mL, intraperitoneal) and confined to the black chamber. The percentage means of time spent and the number of entries made to each chamber following a 10-minute exploration on the ninth day were compared using Student's t-test.

Results: Mice treated with AMP exhibited a significant place preference (p < 0.01) for the drug-paired, white chamber. Mice treated with 0.5 mg/kg Δ^9 -THC showed an equal preference for both chambers (p > 0.05), whereas mice treated with 1.0 mg/kg Δ^9 -THC exhibited a non-significant place aversion to the white chamber.

Conclusion: The CPP to Δ^9 -THC was highly dose-dependent.

(0 - 09)

The *in vitro* effect of delta-9-tetrahydrocannabinol and cannabidiol on blood viscoelasticity and P50 of the oxygen haemoglobin dissociation curve

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Background: Recently, increasing interest has been focussed on the medicinal properties of cannabis. The main

biological activities of cannabis are believed to be due to the presence of several compounds known as cannabinoids. Delta-9-tetrahydrocannabinol (THC) and cannabidiol (CBD) are two of the main cannabinoids present in the cannabis plant. The effects of THC and CBD can be modulated by each other.

Objective: To investigate the effect of different concentrations of THC and CBD, separately and in combination, on blood viscoelasticity and P50 of the oxygen haemoglobin dissociation curve.

Methods: Blood samples were collected from 10 healthy, adult non-smokers. Blood viscoelasticity was measured using the BioProfiler while the P50 was determined with the Hemox Analyser using different concentrations (0, 2.5, 25 and 100 ng/mL, respectively) of CBD and THC.

Results: There was a decrease in P50 (shift to the left) with increasing concentrations of both CBD and THC. All concentrations of CBD, except 2.5 ng/mL, differed significantly from the control (p < 0.05). However, the decrease in P50 with THC was not statistically significant. In contrast, blood viscoelasticity increased with increasing concentrations of both CBD and THC separately. However, the change in viscoelasticity was statistically significant for CBD but not THC. The combination of CBD and THC showed statistical significance difference (p < 0.05) from the control for both P50 and blood viscoelasticity.

Conclusion: The changes observed with increasing concentrations of CBD and THC suggest that these cannabinoids have increased affinity for oxygen and cause an increase in blood viscoelasticity.

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Microbiological profile of isolates from high vaginal swabs collected, over two years, at the Microbiology Lab, University Hospital of the West Indies

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Background: High vaginal swabs are used for the purpose of screening and diagnosis of vaginal infection. The results of cultures and sensitivity profiles are useful in guiding aetiologic management of vaginal infections as well as identifying patients who may benefit from antimicrobial prophylaxis as with maternal group B streptococcal (GBS) infection in pregnancy.

Objective: To determine the organisms isolated from cultures of specimens from high vaginal swabs (HVS) sent to the microbiology laboratory at the University Hospital of the West Indies (UHWI). **Methods:** A retrospective assessment of culture results of all HVS submitted to the microbiology laboratory at the UHWI between May 1, 2015 and May 31, 2017 was conducted. Results were subjected to basic statistical analysis. **Results:** A total of 2145 HVS were submitted over the time period, representing 3.7% of all samples. Only 754 (35%) of the samples yielded positive cultures. Yeast (*Candida albicans* (69%) and Non-*Candida albicans* (16%) species) were the most frequently isolated organisms, being found in 85% of the positive cultures. The most common bacterial isolate was GBS and was found in 42% of the samples.

Conclusion: The findings suggested that the prevalence of GBS may be quite high in the local population and indicated the need for investigation into the need for a national obstetric screening programme.

(O – 11) E-liquid induces zebrafish embryo toxicity

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Background/Objective: Studies have shown that pure nicotine has a lesser negative impact on health and behaviour compared to tobacco. Based on this, there have been many pure nicotine delivery products on the market (e-cigarettes, nicotine patches and gum). The e-cigarette, which mimics tobacco smoking, has increased in popularity drastically over the past few years. E-liquid has been shown to induce cellular apoptosis; this had not been tested on zebrafish development. Therefore, we assessed the embryo toxicity induced by e-liquid and different forms of nicotine (pure nicotine, e-liquid nicotine and tobacco extract nicotine).

Methods: Sets of eight 3 hpf (hours post fertilized) zebrafish eggs were exposed to varying concentrations of e-liquid or different forms of nicotine continuously for a period of 24 hours. The changes during development were recorded using webcam microscopy, recording the timing of death and the number of dead embryos.

Results: The nicotine itself showed a high degree of embryo toxicity, with e-liquid nicotine and tobacco extract nicotine synergistically increasing embryo toxicity. E-liquid at low concentrations did not induce embryo toxicity. However, at higher concentrations (approximately four times that in e-liquid nicotine), time and concentration-dependent embryotoxicity was observed, similar to that of the nicotine. In contrast, the tar content of tobacco reduced the rate of embryo development by decreasing the movement of embryotic plates.

Conclusion: Short-term use of e-cigarettes may not be harmful, but the prolonged use of these products could be more detrimental than tobacco smoke due to the accumulation of the toxic e-liquid.