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Detection of some Commonly Abused Substances in Postmortem Vitreous Humor and Abdominal Fluids using Dipstick Immunoassay Method

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ABSTRACT

Vitreous humor (VH) is a gelatinous substance in the posterior chamber of the eye that plays a mechanical role in the eyeball and is suitable for forensic toxicological analysis. Its resistance to rapid autolysis and putrefaction makes it a choice sample in postmortem investigation. Blood, urine, and other fluids become inaccessible and contaminated during the process of death. Determination of drugs or substances of abuse in postmortem cases is a challenge due to the rapid deterioration of the sample of choice, such as blood and urine. The high cost of estimation, the complex automation process, and the required workforce have further compounded the challenge of estimating drugs in a low-resource setting. This study was therefore designed to address these concerns by employing a semi-quantitative dipstick immunoassay method and cut-off extrapolation technique to estimate commonly abused substances such as cannabinoids and tramadol in a postmortem scenario using an animal model. The study used vitreous humor and abdominal fluids as samples of choice because of ease of collection and availability. Further, their resistance to early autolysis and putrefaction was an added advantage. The Mead equation was used to determine sample size, yielding four albino rabbits per paired group for the substances studied. The SPSS version 22 was used as the statistical package, and Student's t-tests were used to test whether the substances could be detected in the chosen matrices and to assess differences in concentrations. The study showed that the method could detect cannabinoids in vitreous humor but not in abdominal fluid, whereas it did not detect tramadol in either fluid. The findings have affirmed that in low-resource laboratories, postmortem toxicology of cannabinoids could be analyzed as a preliminary investigation to exclude or include cases of cannabinoid intoxication or death using the vitreous humor as a choice sample and the dipstick test as a technique. However, advanced methods such as high-performance liquid chromatography (HPLC) should confirm and validate dipstick results for admissibility in further scientific or legal use.

KEYWORDS

Cannabinoids, tramadol, postmortem, vitreous humor, abdominal fluid, forensic toxicology, drug detection

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INTRODUCTION

Postmortem toxicology of commonly abused drugs and substances of abuse is quite challenging due to the rapid deterioration and unavailability of body matrices and fluids used for detection[1]. The advanced sophistication of instrumentation and the technical know-how required further worsen this.

These gaps form the basis of this study, as the article sought a more durable and readily available sample that could detect commonly abused drugs and substances through an evolving procedure that could be accessed and used in a low-resource laboratory. Tramadol and cannabinoids are the chosen drugs and substances used for the study due to their prevalence. The fluids used were vitreous humor and abdominal fluids because they are readily accessible and resistant to deterioration.

Tramadol is a centrally acting synthetic opioid analgesic commonly used for the management of moderate to moderately severe pain. Its analgesic effect results from weak μ-opioid receptor activity and inhibition of serotonin and norepinephrine reuptake. Tramadol is metabolized mainly in the liver, with O-desmethyltramadol (M1) being an important active metabolite formed primarily through CYP2D6 activity[2]. Although tramadol has therapeutic benefits, misuse and excessive consumption can cause serious adverse effects, including nausea, dizziness, seizures, respiratory depression, dependence, and death. Its involvement in fatal intoxication has made tramadol an important substance in forensic toxicological investigations[3].

Cannabis sativa remains the most widely cultivated and abused illicit substance globally, and Nigeria is no exception. Year after year, the National Drug Law Enforcement Agency (NDLEA) has listed cannabis as the most frequently seized and most widely used psychoactive drug in the country. Its use cuts across age groups, educational levels, and social classes. The principal psychoactive constituent of the plant, delta-9-tetrahydrocannabinol (THC), produces the characteristic euphoria, altered perception, and cognitive effects that users seek[4]. Excessive use could lead to morbidity or mortality.

In forensic and postmortem toxicology, the accurate detection and, where possible, quantification of tramadol and cannabinoids is essential. Cases of impaired driving, sudden unexplained death, homicide, accidental injury, and workplace incidents often turn on whether a drug was present in the system at a relevant time[5].

Blood and urine have long been regarded as the gold-standard matrices for this purpose because they reflect circulating drug levels at the moment of sampling. This remains viable in antemortem, but not in postmortem. After death, however, the situation changes. Blood undergoes hemolysis, microbial invasion, and postmortem redistribution; drugs stored in solid organs or adipose tissue diffuse back into the bloodstream, sometimes elevating central blood concentrations far above the true ante-mortem value. The opposite can also occur. As a result, post-mortem blood concentrations may no longer reflect the concentration that existed while the person was alive.

These changes are especially problematic in Nigeria. Logistical, infrastructural, and cultural factors frequently delay autopsies. Cold-chain storage is inconsistent outside major urban centers. By the time a blood sample reaches the laboratory, it may already be haemolysed, putrefied, or otherwise compromised. Under such conditions, the toxicologist is left with an unreliable matrix and, in some cases, no usable sample. Recent research has specifically demonstrated the forensic potential of vitreous humor for tramadol analysis[6].

Against this background, the present study examined whether THC or its metabolites, and tramadol, could be detected in the vitreous humor or abdominal fluid by employing the dipstick immunoassay method. To support this discussion, we used an animal model to prepare for a human model in our subsequent

studies. Studies using rabbits to investigate human diseases have been favorable, as noted by Esteves *et al.*[7]. Furthermore, similarities between human and rabbit vitreous humor have yielded favorable findings[8]. However, physiological and anatomical differences between humans and rabbits may affect the extrapolation of results, hence the need for further studies.

MATERIALS AND METHODS

Study location: Animal breeding and intoxication took place at the Department of Biochemistry Laboratory, Federal University Otuoke. Laboratory analysis was carried out at Eni-Yimini Laboratories Ltd., Yenezue-Gene Epie, Yenagoa, Bayelsa State, Nigeria. The study location mapping was adapted from similar studies on postmortem toxicology[9-13]. The study spanned from 15th of march to 30th August, 2026.

Research design/sample size: The study employed a quantitative experimental research design. Mead's resource equation was utilized for the calculation of sample size[14]. Mead's formula is presented below:

$$E = N-B-T$$

Where:

N = Total number of individuals or units in the study (minus 1)
B = Blocking component, representing environmental effects allowed for in the design (minus 1)
T = Treatment component, corresponding to the number of treatment groups (including the control group) being used (minus 1)
E = Degrees of freedom of the error component, and should be somewhere between 10 and 20

Using the above calculation, the value falls between 10 and 20, making the sample size of 20 suitable for the study.

The sample population consisted of control and treatment groups. The animals were divided into the following groups based on the degree of intoxication:

Control group: Four rabbits
Tramadol group 1: Four rabbits administered 500 mg of tramadol
Tramadol group 2: Four rabbits administered 1000 mg of tramadol
Cannabinoid group 1: Four rabbits were administered 500 mg of cannabinoids
Cannabinoid group 2: Four rabbits were administered 1000 mg of cannabinoids

Selection criteria: The albino rabbits used for the study were healthy, as confirmed by the university veterinarian. The study excluded animals that were unhealthy or disfigured. Similarly, vitreous samples found to be turbid were excluded from the analysis.

Ethical approval: Ethical approval for this study was obtained from the Directorate of Research and Quality Assurance (DR&QA), Federal University Otuoke, Bayelsa State, adopted from the Institutional Animal Care and Use Committee (IACUC), with Approval Number: AREC-2026-134, in accordance with the National Animal Welfare Act on the 5th of March, 2026. The mode of euthanasia for the rabbits was also included in the approval document.

Housing and nutrition: The rabbits were kept in a spacious and well-ventilated cage at room temperature, under a natural circadian rhythm, and allowed to acclimatize for 14 days. They were housed in standard cages and allowed access to feed (Top Feed Finisher Mash, Yenagoa, Nigeria) and water

ad libitum in the animal house. All the animals received humane treatment according to the criteria outlined in the Guide for the Care and Use of Laboratory Animals prepared by the National Institute of Health. Chloroform was used for the euthanasia to evade inflicting unnecessary pains on the animals used.

Procurement and acclimatization of experimental animals: Healthy adult rabbits were procured from the University of Port Harcourt Animal House and transported to the experimental facility. On arrival, the animals were housed in clean and adequately ventilated cages under appropriate laboratory conditions.

The rabbits acclimatized to the laboratory environment for 2 weeks before the experiment began. During acclimatization, the animals received adequate feed and clean drinking water. We regularly monitored their general health, physical activity, feeding behavior, and appearance. We excluded animals showing signs of illness or abnormal behavior.

After acclimatization, we assigned the four rabbits to control and treatment groups. The control rabbit received no tramadol or cannabinoid, while the treatment groups were administered designated doses of tramadol and cannabinoid.

Experimental procedure: After the two-week acclimatization period, the experimental procedure was commenced. The control rabbits were not exposed to either tramadol or cannabinoid, while other treatments were administered varying concentrations of the drugs and substances used. However, both the control and treatment groups received the same water and diet.

Tramadol groups 1 and 2 received 500 and 1000 mg of tramadol, respectively, whereas cannabinoid groups 1 and 2 received the same concentrations. The tramadol tablets and cannabinoids were individually dissolved in water (10 mL) before oral administration using a 5 mL syringe. We sourced the cannabinoid from dried leaves, weighed 500 mg and 1000 mg, dissolved them in 50 mL of water, centrifuged, and extracted the supernatant for oral administration.

After administration, we closely observed the experimental rabbits for physical and behavioral responses. After death, we collected samples from the animals and prepared them for the screening tests. After administration, the animals died between 6 and 10 hrs and were left for 12 hrs before sample collection, to mimic a real-world scenario of drug-induced death discovery.

Collection of samples: We collected vitreous humor and abdominal fluid samples from the rabbits after death. Appropriate precautions were taken during sample collection to minimize contamination and maintain specimen integrity. We transferred the samples into a clean, appropriately labeled plain container for the corresponding group.

Coe[15] method for vitreous humor collection was employed. Briefly, using a 5 mL syringe and a needle, a scleral puncture was made on the lateral canthus, and the total extractable vitreous humor was aspirated from the eye. On average, we collected 1.0 mL of vitreous humor from each rabbit eye. We used only crystal-clear liquid free of tissue contaminants and fragments. However, we collected abdominal fluid by inserting a syringe into the abdominal cavity and aspirating the contents. On average, we extracted about 1.5 mL of aspirate from the abdominal cavity. After collection, we centrifuged the samples at 1200 rpm for 10 min at room temperature and extracted the supernatant for laboratory analysis for both the vitreous and abdominal fluids. We processed the samples immediately and analyzed them within 5 hrs post-collection; however, during collection, we kept the samples refrigerated.

Laboratory methods and procedures: Semi-quantitative screening for tramadol and cannabinoids (9-THC) was performed with a commercial dipstick and an immunochromatographic screening device manufactured by ACRO BIOTEC, Inc., USA, under the brand name JusCheck with a Ref No.: DOA-1144 and Lot No.: DOA23030001ACO. We tested vitreous humor and abdominal fluid samples separately from the control rabbit and the treatment groups. We tested each sample in duplicate using two kits. We did not note discordant duplicate results during analysis.

The results were interpreted according to the manufacturer's instructions after 10 min of observation. The control line confirmed that the test functioned correctly, whereas the test line determined the result. Record a negative result when both the control line and test line appear. Record a positive result when the control line appears, and the test line is absent. A test showing no control line was considered invalid, regardless of whether the test line appeared, and required repetition using a new test device. We performed the analyses at the end of each sample, making it a non-simultaneous analysis, to avoid timing errors.

Statistical analyses: Data were analyzed using the Statistical Package for Social Sciences (SPSS) program (SPSS Inc., Chicago, IL, USA; Version 18-21) and Microsoft Excel. Qualitative results were reported as positive or negative after summing duplicate results across groups. The Student's t-test was used to compare the extrapolated matrix data from the kit cut-off values. The t-test was used to compare the control and treatment groups.

RESULTS

Table 1 shows that the dipstick method detected cannabinoids in vitreous humor and not in abdominal fluids in the treatment groups. In contrast, the study did not detect tramadol in any matrix used. However, Table 2 and 3 show a quantitative comparison indicating a significant increase in cannabinoids and tramadol in vitreous humor compared with abdominal fluids, as indicated by the cutoff values.

Table 1: Comparison of some postmortem abused substances in the vitreous humor and abdominal fluid

Rabbit ID	Vitreous humour	Abdominal fluid
C1	Negative	Negative
C2	Negative	Negative
Tramadol group 1	Negative	Negative
Tramadol group 2	Negative	Negative
Cannabinoids group 1	Positive	Negative
Cannabinoids group 2	Positive	Negative

Table 2: Comparison of tetrahydrocannabinol (THC) in vitreous humor and abdominal fluid

Grouping (n = 4)	Vitreous humour (ng/mL) Mean±SD	Abdominal fluid (ng/mL) Mean±SD	t-test	p-value
Control 1	0.00±0.00	0.00±0.00	-	-
Control 2	0.00±0.00	0.00±0.00	-	-
Group 1	59.00±0.05	0.300±00.1	22.34	0.02
Group 2	70.00±0.04	1.09±00.16	34.09	0.01

Table 3: Comparison of tramadol in vitreous humor and abdominal fluid

Grouping (n = 4)	Vitreous humour (ng/mL) Mean±SD	Abdominal fluid (ng/mL) Mean±SD	t-test	p-value
Control 1	0.00±0.00	0.00±0.00	-	-
Control 2	0.00±0.00	0.00±0.00	-	-
Group 1	1.00±0.03	0.100±00.9	34.01	0.05
Group 2	2.10±0.02	0.05±0.20	8.02	0.01

DISCUSSION

The study revealed that the dipstick immunoassay method could detect cannabinoids (THC/metabolites) in vitreous humor but not in abdominal fluid (Table 1). The positive dipstick results in vitreous humor of the treated rabbits are consistent with the broader forensic literature showing that some xenobiotics can cross into the ocular compartment[16]. The blood-ocular barrier restricts entry of many molecules, but molecular traffic still occurs, especially for lipophilic molecules such as THC[17]. Detection of THC in vitreous humor using dipstick immunoassay in postmortem circumstances has further enriched the literature on the suitability of vitreous humor in postmortem instances, as blood or other fluids are not suitable due to autolysis and putrefaction. Furthermore, the method is not sophisticated and can be used in low-resource laboratory settings, making it a suitable screening test in forensic toxicology.

Consistently negative abdominal fluid results may reflect lower concentrations in that matrix, differences in distribution rate, or concentrations below the dipstick immunoassay cut-off[18]. Another plausible reason is the rapid autolysis and putrefaction in the intestine[19]. Fermentation can degrade the bioactive components of cannabinoids into byproducts that the dipstick could not detect. This finding shows that abdominal fluid is not suitable for cannabinoid estimation using the dipstick method.

This study also compared tramadol detection in vitreous humor and abdominal fluid samples from experimental rabbits following acute tramadol administration (Table 1 and 3). The results showed negative tramadol reactions in both fluids by dipstick, but negligible extrapolated cutoff values. This contrasts with Vasileiou *et al.*[6], which used GC-MS and reported a very low concentration in the vitreous compared with blood, though in an antemortem scenario.

The negative results obtained in both fluids should be interpreted as non-detection by the dipstick test rather than confirmation that tramadol was completely absent from the samples. This indicates that the method employed is not suitable for detecting tramadol in this matrix; however, sophisticated methods such as LC-MS/MS or GC-MS could be employed to advance the discourse[20].

The findings also highlight the importance of specimen selection and analytical methodology in forensic toxicology. Blood is commonly regarded as an important specimen because it reflects systemic drug exposure and is routinely used for quantitative toxicological analysis. However, postmortem redistribution, decomposition, hemolysis, and microbial activity can alter blood drug concentrations[21]. Vitreous humor, by contrast, is relatively protected from several of these changes and can therefore provide complementary information, particularly when blood is unavailable or unsuitable for analysis, especially in postmortem investigation[22,23].

The study also shows the limitations of using a qualitative dipstick method for forensic comparison of biological specimens. While the method has advantages such as simplicity, rapidity, and relatively low cost, it does not provide an exact concentration. Therefore, it was not possible to determine the concentration ratio between abdominal and vitreous humor or establish a quantitative correlation between the two specimens. This limitation matters because comparing biological matrices in forensic toxicology often aims to determine how closely drug concentrations in one specimen correspond to those in another. These limitations also open avenues for further research.

CONCLUSION

This study compared detection of cannabinoids and tramadol in postmortem vitreous humor and abdominal fluid using a dipstick immunoassay method. The study showed a positive reaction for cannabinoids in the vitreous, but a negative reaction for tramadol in both vitreous and abdominal fluids. This indicates that the method is suitable for cannabinoid detection in postmortem conditions. This dipstick could be used as a screening test for cannabinoid detection in a low-resource laboratory setting.

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