Forensic Toxicology of Typical Drugs in Drug-Facilitated Sexual Assault

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Abstract. Drug-Facilitated Sexual Assault (DFSA) refers to sexual offense without consent when victim is under the effect of different drugs, especially ones that disable them to give consent (physical or psychological inhibition). Drugs are either added to victims' drinks or other products without their notice or consumed by the victims voluntarily. This review introduces γ-hydroxybutyrate (GHB) and cannabis, two of the most common drugs used in DFSA, including their drug mechanism, metabolism, forensic detection and treatment. Both GHB and cannabis influence mainly central nervous system, including GABA receptors and CB receptors, altering the victims' psychoactive functions, thus making them more vulnerable to sexual assault. The challenges found in forensic detection of drugs make the cases of DFSA underestimated and accusations difficult to confirm. More research on GHB's antidote, cannabis's metabolism mechanism is needed. Measures to ensure the forensic exams are done promptly for both victims' health and preserving evidence for legal purposes is needed as well, along with statistics on reported cases.

Keywords: Drug-facilitated sexual assault, drug-facilitated crime, GHB, forensic toxicology, cannabis, crime victim.

1. Introduction

There has been an alarming increase in the reports of drug-facilitated sexual assault (DFSA), defined as sexual offense without victims' permission or conscious consent while they are under the effect of drugs, such as incapacitation, loss of consciousness, hallucination [1]. It is usually portrayed as young people accepting drinks in entertainment venues, such as bars, raves and dance clubs, and later on losing their consciousness, becoming extremely susceptible to perpetrators [2]. DFSA occurs between both strangers and acquaintances. Surprisingly, 75% of acquaintance rapes can be potentially defined as DFSA. According to nationwide law enforcement, in a significant number of cases, victims are females and the perpetrators are males. Nevertheless, reported male victims have been increasing in proportion. As a study conducted by U.S. Department of Justice points out, the statistics of sexual assault have been finite, not to mention those of DFSA [3]. Possible factors contributing to the under-reporting of DFSA include victims' ignorance of being drugged, such as having a loss of memories, not reporting out of shame among other psychological factors, insufficient evidence of detected substance, and dishonesty because of victim's voluntary involvement in illegal drugs. Besides, few studies inspect the crime statistics of DFSA, further showing the lack of attention on this issue.

The easy access and cheapness of drugs used in DFSA settings facilitate the incident rate to some extent. Sometimes referred to as "date rape drugs," these psychoactive drugs alter the neurotransmission in brain, mainly the central nervous system (CNS), especially impacting GABA receptors and CB receptors, causing the victim to be an easy target to sexual assault. Alcohol is the most frequent substance used with or without drugs. Typical drugs include but are not limited to γ-hydroxybutyrate (GHB) and cannabis. Depending on the substance and dosage, the usual observed effects are nausea, dizziness, sudden change in body temperature, waking up with lost recent memories, etc [2].

This review focuses on the toxicology of the specific drugs mentioned above in the DFSA setting, including their background, drug mechanisms, metabolism and treatment, aiming to raise public awareness and caution on DFSA to prevent potential harm and provide information on the follow-up

after crime. This review also discusses forensic methods used to detect these drugs as an important measure of preserving DFSA evidence.

2. GHB

γ-hydroxybutyrate (GHB) is a type of psychoactive drug known for its use in DFSA. It is an organic compound produced endogenously within the CNS with a chemical formula of $C_4H_8O_3$. GHB is also called "divine water" in China (Shen Xian Shui), and widely known in western countries as a "club drug" and subsequently a "date rape drug" because of its use in DFSA, otherwise referred to as "fantasy, liquid ecstasy" for the symptoms it may cause [4]. GHB was initially used as a CNS inhibitor and anesthetic adjuvant for clinical use, but it was also used to treat problems related to narcolepsy, alcohol dependence and withdrawal, and a supplement to increase muscle mass [4, 5].

2.1. Pharmacokinetics of GHB

GHB modulates the activity of dopamine and serotonin, as well as opioid systems, especially in the brain. When administered through oral path, GHB is quickly absorbed and reaches its peak serum concentrations no longer than 1 hour after intake [5]. Monocarboxylate transporters may help GHB pass the blood–brain barrier more easily. GHB is rapidly absorbed from the gut due to its low molecular weight, high hydrophilicity, and low affinity to plasma proteins [4]. Water content and the ratio of blood flow to tissue mass influence the speed and extent of GHB transport from the bloodstream to tissue compartments. Because GHB diffuses into total body water, this predicts that Vd in females should be lower than in males, albeit this has yet to be confirmed by experiment. Additionally, because the elderly and obese have less water per kilogram of body weight, Vd for GHB should be even lower. According to different dosage in humans and studies on recovery, only a small proportion of ingested GHB (between 2% and 5%) is eliminated intact in the urine. Because GHB is frequently abused or used by individuals who consume ethanol, there is a risk of a detrimental interaction between these two sedatives. Besides, subjects in the GHB and ethanol treatment condition reported more negative effects, with two suffering from hypotension and six vomiting.

The fact that less than 2% of a therapeutic dose of GHB is eliminated in the urine indicates that metabolism is the primary route of clearance [5]. The brain has multiple pathways for GHB metabolism, but there is little or no knowledge on the role of other tissues to GHB metabolism. A study points out that exogenously given GHB is extensively metabolized in the liver, with just a small percentage (less than 2%) eliminated unaltered in urine. After enormous doses are consumed and high plasma concentrations are obtained, the metabolizing enzymes become saturated with substrate [4]. Different enzymes in the cytosol and mitochondria metabolize GHB throughout the body. GHB dehydrogenase converts GHB to SSA in the cytosol, which can then be transported to the mitochondria or converted to GABA. Within the mitochondria, GHB transhydrogenase transforms GHB to SSA, which is associated with the conversion of ketoglutarate to D-2-hydroxyglutaric acid. In SSA dehydrogenase deficiency, GHB accumulates, implying that the transformation of GHB to SSA and entry into the Krebs cycle is the predominant metabolic pathway for GHB. Because GHB transhydrogenase, which converts GHB to SSA within the mitochondria, is unaffected by valproate, it is a minor mechanism for GHB metabolism. GHB metabolism has been studied mostly in brain homogenates and crude synaptosomal membranes. Mechanistic research in additional organs, such as the liver, kidney, and intestine, are required to elucidate GHB metabolism and metabolite dynamics.

2.2. GHB binds to GABA receptors

GHB is widely assumed to be the pharmacologically active molecule. There are three mechanisms for GHB that may take place [5]. First, GHB is converted to γ-Aminobutyric acid (GABA) and binds to GABAA but mostly GABAB receptors. GHB binds to four subtypes of the GABAA receptors in the brain with a high affinity but to GABAB receptors with rather low affinity. Second, GHB binds to GABAB and GHB receptors, where the transformation of GABA and GHB are reversible *in vivo*.

γ-butyrolactone (GBL) and 1,4-butanediol (1,4-BD) are GHB prodrugs metabolized by a calcium-dependent lactonase and two types of dehydrogenase, respectively (Fig. 1) [5, 6]. Lactonase is the enzyme that catalyses GBL, and alcohol dehydrogenase breaks 1,4-BD into 4-hydroxybutyraldehyde, which is ultimately transformed to GHB. Selective GABAB receptor antagonists were found to alleviate the inhibitory effects of GHB on hippocampus and thalamocortical neurons in later experiments. Third, the antagonist NCS-382 directly to GHB receptors on the ligands listed (Fig. 1) [6].

GHB exerts its physiological effects via binding to GHB receptor, which is a subset of GABAA receptors characterized by the α4, δ, and β1 subunits; [3H] GHB binding to the GHB receptor is pH-dependent, selective, and saturable, with optimum binding at pH 5.5 [7]. More binding sites were found in various tissues of rats, however their physiological functions are unknown. Sedation, hypothermia, respiratory depression, and death are among the toxicological consequences of GHB and its prodrugs GBL and 1,4-BD, which can be linked to agonism at GABAB receptors.

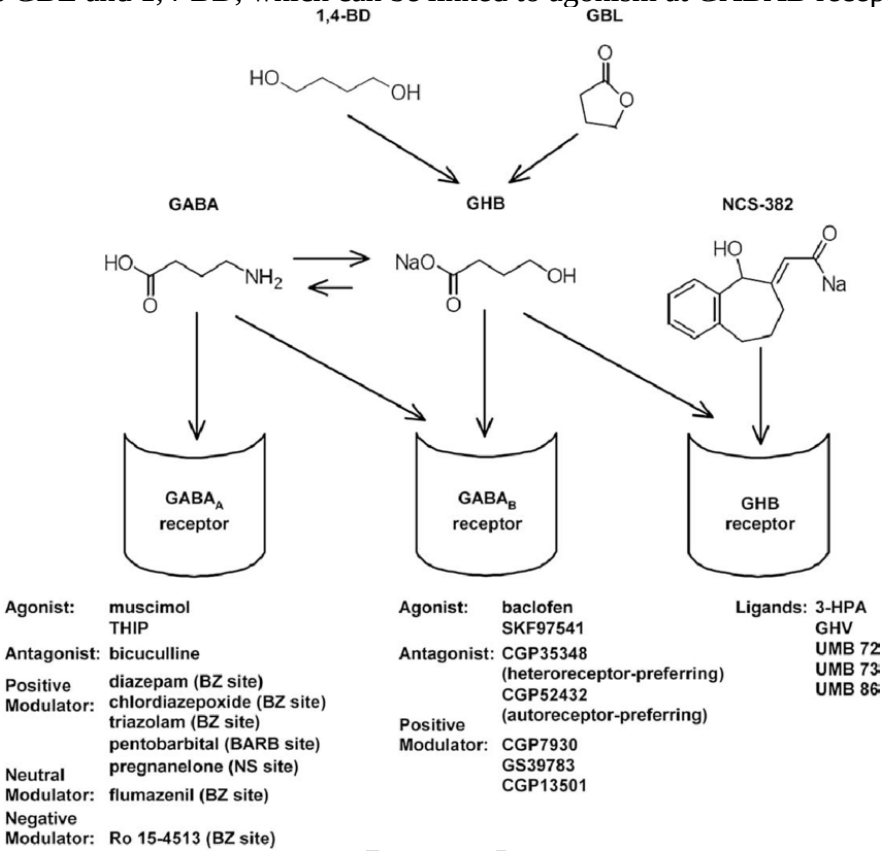


Figure 1. Possible pharmacological mechanisms of GHB [6]. Both GBL and 1,4-BD, as GHB's prodrug, are later metabolized into GHB. GHB and GABA's formation is interchangeable, and GABA can bind with both GABAA receptor and GABAB receptor while GHB can bind with GABAB and GHB receptor. NCS-382 is a selective antagonist for the GHB receptor.

2.3. Symptoms of GHB Abuse, Detection and Treatment

The symptoms are not considered specific, so they can be easily confused with other poisonings caused by CNS inhibitors [5]. Some effects include sedation, euphoria, decreased intelligence, increased libido and amnesia [2]. There is a dose-related effect as lower doses cause symptoms such as euphoria and few signs of intoxication, but higher doses may cause dizziness, headache, nausea, vomiting, speech impairment and amnesia [7]. Recreational use is increasing, with both chronic and acute intoxication leading to morbidity and even death, often used with alcohol and stimulants [2]. Long-term recreational use can cause tolerance and withdrawal reactions, with heavy users requiring use with an interval of 1-3 hours and tolerance can develop rapidly [7]. However, the exact toxicity of GHB is not yet decided due to its fast metabolism, difficult analysis and the fact that it pre-exists

in human body. There is no effective antidote for acute intoxication yet. Even though benzodiazepines are considered an antidote, GHB withdrawal syndrome that is resistant to its therapy is very common and the recommended approach is to use barbiturates or switch to titration instead, but relapse rates within 3 months of detoxification treatment are as high as 60%.

GHB's half-life in urine is short so symptoms of acute intoxication may last for no more than a few hours [5]. Some point out that its colorless and odorless properties make it not readily detectable, thus more preferred for crime purposes. However, the short half-life can lead to less detection than actuality, so the timing of sample collection is important, in which blood samples are collected optimally within 8 hours and hair sampling within 12 hours [9]. Hair can be used to detect past GHB exposure based on comparisons of GHB concentration in different locations, but there is no consensus on a universal standard for the ratios determining GHB exposure, and it usually takes one month to collect the hair and perform the segment analysis. Others that can be used for sampling include saliva vitreous humor, breast milk and brain medulla [5]. Because vomiting is common, additional samples for testing should be provided. Avoiding external contamination of hairs requires special cleaning procedures and cutoff concentration to distinguish passive exposure from exogenous ingestion [4]. Treatment is essentially supportive, with observation and ensuring that gastric contents are not inhaled.

3. Cannabis

Cannabis is one of the most famous illicit drugs especially in marijuana form, referred to as "weed" frequently as well. Its most influential psychoactive components are tetrahydrocannabinol (Δ9-THC) and cannabidiol (CBD), contributing to most of its psychiatric and hallucinogenic effects [10]. Based on the statistics of drug prevalence in reviewed DFSA cases in the US, cannabis was found in 10.9% in 2026 of the positive cases, ranking as the second place behind alcohol (67%) [11, 12, 13, 14]. Although it is frequently presented in DFSA cases, some argue that it is unlikely to be secretly added to the victim's drink or other consumption products, as its usual forms can be distinguished quite easily [5]. It implies that it is more likely the victim consciously consumes the drug and later become at a disadvantage to the perpetrator. In a study conducted by Kloft and their colleagues, false memories were found related to cannabis intoxication [15]. Based on the Deese/Roediger–McDermott (DRM) paradigm adopted to test intoxicated participants with false memories, it is found that the acute cannabis intoxicated participants showed more vulnerability to generating suggestion-based false memories actively (Table 1). The memory test was done again one week afterwards, in which the intoxicated participants show higher false memory rate than the placebo group, suggesting that the memory impairment brought by cannabis intoxication could be long-lasting.

3.1. Pharmacokinetics of Cannabis

Δ9-THC is lipid soluble and binds with plasma protein tightly [16]. Being the principal psychoactive element that binds to cannabinoid receptors in the brain, it causes stimulant, hallucinogenic, or sedative effects that are dose and time dependent. It can cause a wide range of neurological and ophthalmological, cardiovascular, and gastrointestinal symptoms and signs. CB-1 and CB-2 are the two cannabinoid receptors that have been identified. The sites where CB-1 receptors frequently appear are assumed to intensify cannabis' impairing function on cognition and motor. A difference in mechanism occurs when there is a different route of administration, demonstrated by the following table. When taken into the system from the lungs, Δ9-THC is quickly transported cross the body to areas of high permeality [17]. This allows intensely imposed pleasure and the potentiality of abuse.

Table 1. Means from DRM and misinformation parameters (rates in proportion) [15].

	Cannabis Condition (Immediate)	Placebo Condition (Immediate)
DRM		
n	63	64
True recognition (old)	0.68 (0.02)	0.68 (0.02)
False alarms (critical)	0.62 (0.02)	0.56 (0.03)
False alarms (related)	0.42 (0.03)	0.27 (0.03)
False alarms (unrelated)	0.40 (0.03)	0.16 (0.02)
Net accuracy	0.68 (0.08)	0.75 (0.13)
Misinformation eyewitness		
n	32	32
Presented	0.78 (0.02)	0.78 (0.02)
Suggested	0.19 (0.04)	0.08 (0.02)
Nonsuggested	0.06 (0.02)	0.01 (0.01)
Misinformation perpetrator		
n	31	32
Presented	0.68 (0.03)	0.65 (0.03)
Suggested	0.31 (0.05)	0.23 (0.03)
Nonsuggested	0.08 (0.02)	0.03 (0.01)

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3.3. Metabolism of Δ9-THC

Without categorizing the routes of administration, more than 80 metabolites were found for Δ9-THC [17]. The structures of some metabolites have not yet been found, though these chemicals may play a role in the drug's overall efficacy. Phase I metabolism includes oxidation reactions involving allylic and aliphatic hydroxylation. Δ9-THC is rapidly converted to various phase I metabolites by the CYP450, 2C9, 2C19, and CYP3A4 isoenzymes. P450 isoenzymes vary significantly between species, resulting in a distinct pattern of cannabis hydroxylation. Δ9-THC-COOH, 11,40,50trisnor-9, 30-carboxy-Δ9-THC, and 11-nor-9-carboxy-Δ9THC glucuronide are the most dominant metabolites in urine as acid derivatives [18]. Δ9-THC-COOH is found to be helpful for diagnosing. It is primarily eliminated in urine and it can be used as a biomarker for up to 25 days to indicate the frequent usage of marijuana cigarette for its long half-life. In phase II, the metabolism process could be detoxifying but activating as well [17]. uridine 50-diphosphoglucuronosyltransferase (UGT) promotes the process of metabolizing Δ9-THC and the phase I metabolites into ester glucuronide (O-ester glucuronide), further hydrolyzed into Δ9-THC, 11-OH-Δ-9-THC and Δ9-THC-COOH in the intestines [19]. Eventually, O-ester glucuronide and 11-OH-Δ9-THC are the most frequently detected metabolite found in urine and feces, respectively [20].

CBD, the other major component of cannabis, is degraded in the liver and intestine by CYP450, CYP2C19 and CYP3A4, UGT1A7, UGT1A9, and UGT2B7 isoforms, resulting in hydroxylated and

carboxylated metabolites [4, 17]. As a result of the suppression of CYP3A and CYP2C enzymes, CBD decreased barbiturate metabolism and increased barbiturate-induced sleep time length in mice. CBD also inhibited phenazone hepatic metabolism. CBD was also found to stimulate hepatic CYP3A, CYP2B, and CYP2C in other studies. In humans, CBD was later discovered to prevent the metabolism process of THC, explaing why taking CBD before THC enhances the effects of THC.

3.4. Detection and Treatment of Cannabis Intoxication

Acute cannabis toxicity includes both psychological effects such as euphoria, relaxation, time distortion, lack of inhibitions, and physical symptoms when consumed in excess, including tachycardia, conjunctival injection, impairment in cognitive, short-term memory tasks, etc [14]. Supportive treatment of acute intoxication is implemented along with observrion on patients' vitals and related symptoms [17]. Detecting cannabis use requires electrocardiogram, serum toxicology panel and urine drug screen, in which urine screening needs to be done as soon as possible to facilitate the process. 3 to 5 days after the intake of cannabis, Δ9-THC, Δ9-THC-COOH could still be found in the urine samples. Standard urine drug screens have a lower detectionr range, depending on the individual test. Up to one month after last use, long-terms cannabis users or individuals with a hgher body fat content may facilitate the detection of Δ9-THC-COOH in urine. Δ9-THC is the only compound that could be detected in standard confirmatory tests of urine, blood, or serum using gas chromatography-mass spectrometry, indicating a good marker for cannabis ingestion.

4. Conclusion

The understanding on drugs used in DFSA settings, especially GHB and cannabis, are far from enough to ameliorate the issue of DFSA. GHB is an endogenous psychoactive drug that binds with GABA receptors and has a rather quick metabolism process. However, the relapse of GHB withdrawal syndrome caused by long-term recreational use, and GHB has not yet found its ideal antidote, as the existing ones all have paradox effect to some extent. Although 80 metabolites for cannabis were found, there are still other ones associated that have not been affirmed yet. A thorough understanding of how it is metabolized would provide insights for treatment of acute cannabis intoxication in terms of facilitating excretion. Both GHB and cannabis may induce short-term memory loss or loss of consciousness along with other negative effects that alter psychological functions, adding challenge to the victims when self-reporting potential DFSA. In addition, patients who have taken cannabis are more susceptible to suggestive false memories. Although the short half-life of GHB poses challenges to forensic detection as well, both GHB and cannabis stress the importance of promptness when a forensic exam is needed. The exam typically involves urine sampling, blood sampling, etc. When the exam exceeds the optimal timing, it is suggested to do a hair segment analysis, although it can be confusing in the case of GHB and time demanding. These significantly complicate the situation when collecting evidence against the perpetrators. Therefore, countermeasures towards DFSA should be preventive. Besides the emphasis on future research, more data of DFSA cases is needed in order to understand the epidemiology to predict future trends specific to this DFSA crime.

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