

Chemistry, Metabolism, and Toxicology of Cannabis: Clinical Implications

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Cannabis is one of the most widely abused substances throughout the world. The primary psychoactive constituent of cannabis, delta 9-tetrahydrocannabinol (Δ^9 -THC), produces a myriad of pharmacological effects in animals and humans. Although it is used as a recreational drug, it can potentially lead to dependence and behavioral disturbances and its heavy use may increase the risk for psychotic disorders.

Many studies that endeavor to understand the mechanism of action of cannabis concentrate on pharmacokinetics and pharmacodynamics of cannabinoids in humans. However, there is limited research on the chronic adverse effects and retention of cannabinoids in human subjects. Cannabis can be detected in body fluids following exposure through active/passive inhalation and exposure through breastfeeding. Cannabis detection is directly dependent on accurate analytical procedures for detection of metabolites and verification of recent use.

In this review, an attempt has been made to summarize the properties of cannabis and its derivatives, and to discuss the implications of its use with emphasis on bioavailability, limit of detection, carry over period and passive inhalation, important factors for detection and diagnosis.

Key words: Cannabis, Cannabinoids, Mental disorders, Tetrahydrocannabinol

Iran J Psychiatry 2012; 7:4: 149-156

Cannabis has been used for both recreational and medicinal purposes since several centuries (1-5). Hashish, a cannabis preparation, was found in Egyptian mummies (2). Marijuana, Hashish, Bhang and Ganja are the most widely used illicit drugs in the world. These psychoactive products are obtained from the plant Cannabis sativa (Indian hemp) and some of its subspecies. Cannabis is perceived as a recreational and harmless drug in some countries, even in leading medical journals and in some sections of the lay press (3). However, in most countries, it is categorized as a drug of abuse and its use is strictly prohibited (3,4). Marijuana comes from leaves, stems, and dried flower buds of the cannabis plant. Hashish is a resin obtained from flowering buds of the hemp plant (5). Most cannabis preparations are either smoked, or taken orally after mixing with other substances (See table 1). The prevalence of recreational use of cannabis has increased markedly worldwide, particularly among young people. A survey of schoolchildren in United Kingdom showed that more than 40% of 15-16 year old and up to 59% of 18-year-old students admitted to have abused cannabis at least once (6). India, which has a population of just over a billion, has 62.5 million alcohol users, 8.75 million cannabis users, two million who use opioids and 0.6 million who use sedatives or hypnotics. A majority of cannabis users in India start abusing cannabis in their adolescence and quit after

initial experimentation, while the rest develop dependence (7,8). Cannabis abuse among younger subjects is associated with poor academic performance and increased school dropout. Many studies have demonstrated that psychosis, violence, aggression, sexual encounters, accidents, and crime are closely associated with cannabis abuse (1-3). Cannabis use have been associated with conduct disorders, attention-deficit hyperactivity disorder (ADHD), and learning disorders. Evidence suggests that cannabis dependence in young people predicts increased risk of using other illicit drugs, under performance in school, and reporting of associated psychotic symptoms (7,8). Interestingly, the biomedical benefits of cannabis have also been recognized from millennia (5) and it has been shown to have therapeutic potential as an appetite stimulant, antiemetic and antispasmodic (9). Similarly, other clinical conditions where the potential use of cannabis has been suggested include epilepsy, glaucoma and asthma (10).

Chemical Components of Cannabis

The cannabis plant contains more than 421 chemicals of which 61 are cannabinoids (9,10) (Figure 1). Interestingly, more than 2000 compounds are produced by pyrolysis during smoking of cannabis (10,11) and they are represented by different classes of chemicals including nitrogenous compounds, amino acids, hydrocarbons, sugar, terpenes and simple fatty acids.

These compounds altogether contribute to the unique pharmacological and toxicological properties of cannabis. A list of the major cannabinoids present in cannabis is listed in table 2. Among the listed compounds, delta⁹-tetrahydrocannabinol (Δ⁹-THC) is considered as the most psychoactive component contributing to the behavioral toxicity of cannabis (11). The aim of the current review is to discuss the properties of cannabinoids, primarily Δ⁹-THC and its metabolites and the clinical implications thereof.

Pharmacological Actions of Cannabinoids

Δ⁹-THC has a tri-cyclic 21- carbon structure without nitrogen and with two chiral centers in trans-configuration (9). Δ⁹-THC is volatile viscous oil with high lipid solubility and low aqueous solubility and a pKa of 10.6. The metabolism of Δ⁹-THC is shown in figure (2). Δ⁹-THC is present in cannabis as a mixture of mono-carboxylic acids, which gets readily and efficiently de-carboxylated upon heating (9). It decomposes when exposed to air, heat or light (13) and readily binds to glass and plastic. Therefore, Δ⁹-THC is usually stored in basic or organic solvents in amber silicate glassware to avoid loss during analytical procedures (14).

Two hypotheses have been proposed to explain the mechanism of in vivo action of Δ⁹-THC. According to the first, Δ⁹-THC which is secreted as a glucuronide acts via non-specific interactions with cellular and organelle membranes in the brain supporting a membrane perturbation mechanism (15,16). The second hypothesis suggests that Δ⁹-THC interacts with specific cannabinoid receptors (10,17,18). Delineating a single mechanism of action is very difficult because molecular analysis has demonstrated Δ⁹-THC to act on several intracellular targets including opioid and benzodiazepine receptors, prostaglandin synthetic pathway, protein and nucleic acid metabolism (19,20). Further, cannabinoids inhibit macromolecular metabolism (21,22) in a dose-

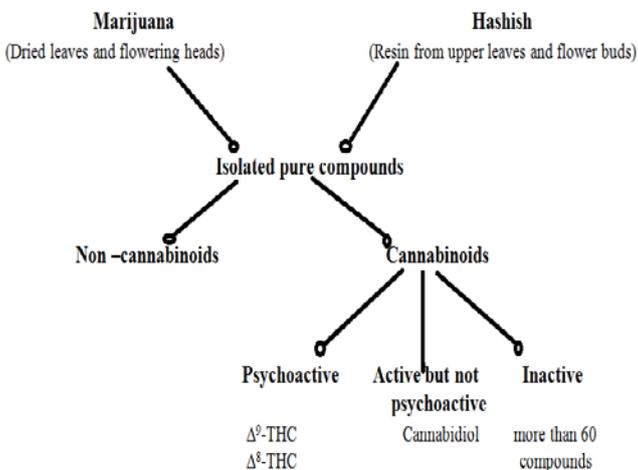


Figure 1. Chemical Components of Cannabis

dependent manner and have a wide range of effects on enzyme systems, hormone secretion and neurotransmitters (18,21-23). The evidence of numerous and diffuse in vivo effects support the non-specific interaction hypothesis for THC.

Cannabinoids exert various physiological effects by interacting with specific cannabinoid receptors (CB receptors) present in the brain and periphery (24). CB1 receptors in the brain (25) are particularly concentrated in anatomical regions associated with cognition, memory, reward, anxiety, pain sensory perception, motor co-ordination and endocrine function (26,27). CB2 receptors are localized to the spleen and other peripheral tissues (28). These receptors may play a role in the immune suppressive actions of cannabinoids. The physiological ligands for these receptors appear to be a family of anandamides (29) which are derivatives of arachidonic acid, related to prostaglandins. There is an endogenous system of cannabinoid receptors and anandamides, which normally modulate neuronal activity by its effect on cyclic-AMP dynamics and transport of Ca⁺⁺ and K⁺ ions (25,30-32). Although the physiological implications of these ligand-receptor interactions are not completely understood, it is suggested to be connected with opioids, GABAergic, dopaminergic, noradrenergic, serotonergic, cholinergic, glucocorticoid and prostaglandin systems (26,28,30,33). The many effects of exogenous cannabinoids derived from cannabis result from perturbation of this complex system, but the exact mechanism is not clear.

Behavioral and Physiological Effects of Cannabis

Cannabis is known to have behavioral and physiological effects (27-29). Behavioral effects include feeling of euphoria, relaxation, altered time perception, lack of concentration and impaired learning. Memory and mood changes such as panic and paranoid

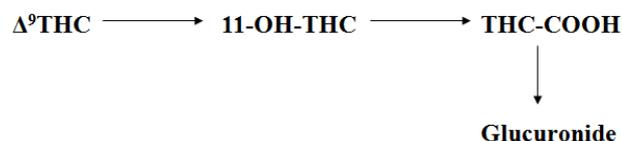


Figure 2. Metabolic route of Δ⁹-tetrahydrocannabinol (Δ⁹-THC), its primary active metabolite 11-hydroxy-Δ⁹-tetrahydrocannabinol (11-OH-THC) and the primary inactive metabolite, 11-nor-9-carboxy-Δ⁹-tetrahydrocannabinol(THC-COOH) 12.

Table 1. Different Preparations of Cannabinoids (23)

No.	Form	Source	Methods of abuse
1.	Marijuana/Charas/ Ganja	Dried leaves, stalks, flower and seeds	Smoked as joint
2.	Bhang	Fresh leaves and stalk	Mixed with food items and consumed orally
3.	Hashish oil	Leaves, seeds, stem and flowers soaked in oil/solvent	Smoked as joint or consumed orally

Table 2. Cannabinoids and their Properties (11)

Psychoactive components	
Name	Effects
Δ^9 -tetrahydrocannabinol (Δ^9 -THC)	Main psychoactive component; causes psychological and behavioral effects
Δ^8 -tetrahydrocannabinol (Δ^8 -THC)	Less psychoactive than Δ^9 -THC.
Cannabinol (CBN)	Less powerful than Δ^9 -THC
11-hydroxy- Δ^9 -THC (11-OH-THC)	Liable for psychological effects of cannabis
Anandamide (arachidonylethanolamide)	Imitates activity of Δ^9 -THC and other cannabinoids that interact with cannabinoid receptors.
Non-psychoactive components	
Cannabidiol (CBD)	Lacks psychoactive properties has anticonvulsant action.
Cannabichromene	Not psychoactive
(-) Δ^8 -THC-11-oic acid	Not psychoactive has analgesic activity.

reactions have also been reported. Physiological effects include rapid changes in heart rate and diastolic blood pressure, conjunctival suffusion, dry mouth and throat, increased appetite, vasodilatation and decreased respiratory rate (31,32). Cannabis also affects the immune and endocrine system; and its abuse is associated with lung damage and EEG alterations (28,30,33, 34,35).

Cannabis Dependence and Tolerance

Cannabinoids appear to affect the same reward systems as alcohol, cocaine and opioids (34). Evidence for cannabis dependence is now available from epidemiological studies (6,8) of long-term users (58,59), clinical populations (75,77) and controlled experiments on withdrawal and tolerance (35,36,37,38). Tolerance to cannabis can occur in relation to mood, psychomotor performance, sleep, arterial pressure, body temperature, and antiemetic properties. The critical elements of cannabis dependence include preoccupation with its use, compulsion to use and relapse or recurrent use of the substance (39). Over 50% of cannabis users appear to have 'impaired control' over their use (40). Symptoms such as irritability, anxiety, craving and disrupted sleep have been reported in 61-96% of cannabis users during abstinence (36,41,42,43).

Psychiatric Conditions Associated with Cannabis Abuse

In addition to producing dependence, cannabis use is associated with a wide range of psychiatric disorders (44). While there is a clear relationship between the use of cannabis and psychosis, different hypotheses for the same have been propounded. One such, which describes psychosis occurring exclusively with cannabis use has limited evidence. There is strong evidence that cannabis use may precipitate schizophrenia or exacerbate its symptoms. There is

also reasonable evidence that cannabis use exacerbates the symptoms of psychosis (37).

Heavy cannabis (30-50mg oral and 8-30 mg smoked) use can specifically cause a mania-like psychosis and more generally act as a precipitant for manic relapse in bipolar patients (37,44, 45). It is possible that cannabis exposure is a contributing factor that interacts with other known and unknown (genetic and environmental) factors culminating in psychiatric illness (46). It is noticed that in many developed countries, persons with severe mental disorders are more likely to use, abuse, and become dependent on psychoactive substances especially cannabis as compared to the general population (47,48). The same phenomenon has not been established so far in India.

Pharmacokinetics of Cannabis

Δ^9 -THC which is highly lipophilic get distributed in adipose tissue, liver, lung and spleen (12,49,50). Hydroxylation of Δ^9 -THC generates the psychoactive compound 11-hydroxy Δ^9 -Tetra hydrocannabinol (11-OH-THC) and further oxidation generates the inactive 11-nor-9-carboxy- Δ^9 -tetrahydrocannabinol (THCCOOH). THCCOOH is the compound of interest for diagnostic purposes. It is excreted in urine mainly as a glucuronic acid conjugate (12). Δ^9 -THC is rapidly absorbed through lungs after inhalation. It quickly reaches high concentration in blood (51). Approximately 90% of THC in blood is circulated in plasma and rest in red blood cells. Following inhalation, Δ^9 -THC is detectable in plasma within seconds after the first puff and the peak plasma concentration is attained within 3-10 minutes (51-55). However, the bioavailability of Δ^9 -THC varies according to the depth of inhalation, puff duration and breath-hold. Considering that approximately 30% of THC is assumed to be destroyed by pyrolysis, the systemic bioavailability of THC is ~23-27 % for heavy users (18,56) and 10-14 % for occasional users (48,49).

Maximum Δ^9 -THC plasma concentration was observed approximately 8 minutes after onset of smoking, while 11-OH-THC peaked at 15 minutes and THC-COOH at 81 minutes. This Δ^9 -THC concentration rapidly decreases to 1-4 ng/mL within 3-4 hour (57).

In comparison to smoking and inhalation, after oral ingestion, systemic absorption is relatively slow resulting in maximum Δ^9 -THC plasma concentration within 1-2 hours which could be delayed by few hours in certain cases (56, 58). In some subjects, more than one plasma peak was observed (52, 59). Extensive liver metabolism probably reduces the oral bioavailability of Δ^9 -THC by 4-12% (53). After oral administration, maximum Δ^9 -THC plasma concentration was 4.4-11 ng/mL for 20 mg (50) and 2.7-6.3 ng/mL for 15 mg (58, 60). Much higher concentration of 11-OH THC was produced after ingestion than inhalation (56, 58). Following assimilation via the blood, Δ^9 -THC rapidly penetrates in to fat tissues and highly vascularized tissues including brain and muscle resulting in rapid decrease in plasma concentration (61, 63). This tissue distribution is followed by slow redistribution of it from the deep fat deposits back into the blood stream .

It should be noted that the residual Δ^9 -THC levels are maintained in the body for a long time following abuse. The half- life of it for an infrequent user is 1.3 days and for frequent users 5-13 days (64). After smoking a cigarette containing 16-34 mg of Δ^9 -THC, THC-COOH is detectable in plasma for 2-7 days (57, 65). A clinical study carried out among 52 volunteers showed that THC-COOH was detectable in serum from 3.5 to 74.3 hours. Initial concentration was between 14-49 ng/mL (65). This was considerably less than the THC-COOH detection time of 25 days in a single chronic user (66).

Metabolism and Elimination of Δ^9 -THC

Δ^9 -THC is metabolized in the liver by microsomal hydroxylation and oxidation catalyzed by enzymes of cytochrome P450 (CYP) complex. The average plasma clearance rates have been reported to be 11.8 ± 3 L/hour for women and 14.9 ± 3.7 L/hour for men (59). Others have determined approximately 36 L/hour for naïve cannabis users and 60 L/hour for regular cannabis users (12).

More than 65% of cannabis is excreted in the feces and approximately 20% is excreted in urine (58). Most of the cannabis (80-90%) is excreted within 5 days as hydroxylated and carboxylated metabolites (67). There are eighteen acidic metabolites of cannabis identified in urine (68) and most of these metabolites form a conjugate with glucuronic acid, which increases its water solubility. Among the major metabolites (Δ^9 -THC, 11-OH-THC, and THCCOOH), THCCOOH is the primary glucuronide conjugate in urine, while 11-OH-THC is the predominant form in feces (51, 69). Since Δ^9 -THC is extremely soluble in lipids, it results in tubular re-absorption, leading to low renal excretion of unchanged drug. Urinary excretion half-life of

THCCOOH was observed to be approximately 30 hours after seven days and 44-60 hours after twelve days of monitoring (69, 70). After smoking approximately 27 mg of Δ^9 -THC in a cigarette, 11-OH-THC peak concentration was observed in the urine within two hours in the range of 3.2-53.3 ng/mL, peaking at 77.0 ± 329.7 ng/mL after 3 hours and THCCOOH peaking at $179.4 \text{ ng/mL} \pm 146.9$ after 4 hours (71, 72).

Detection and Analysis of Cannabinoids by Different Analytical Techniques

Measurement of cannabinoids is necessary for pharmacokinetic studies, drug treatment, workplace drug testing and drug impaired driving investigations (73). Because of increasing use of cannabis, developing a whole range of efficient testing methods has become essential. Cannabinoids can be detected in saliva, blood, urine, hair and nail using various analytical techniques, including immunoassays (EMIT®, Elisa, fluorescence polarization, radioimmunoassay) (74). Various chromatographic techniques such as Thin Layer Chromatography (TLC) (Foltz and Sunshine, 1990), High Performance Thin layer Chromatography (HPTLC) (72), Gas Chromatography-Mass Spectrometry (GC-MS) (79), high performance liquid chromatography-Mass Spectrometry (HPLC- MS) (79) are reliable in detection and quantitation of various cannabis metabolites .

Urine is the preferred sample because of higher concentration and longer detection time of metabolites in it. Moreover, urine can easily be sampled. Apart from cut off concentration, sensitivity and specificity of assay other factors like route of administration, amount of cannabinoids absorbed, body fat contents rate of metabolism and excretion, degree of dilution and time of specimen collection also influence detectability of Δ^9 -THC and its metabolites (12, 72).

The cut off value for detection of cannabinoids recommended by the Substance Abuse and Mental Health Services Administration (SAMHSA) and European threshold of 50 ng/mL was found to be consistent with recent or heavy cannabis abuse (51, 73). Lower concentrations of THCA can be associated with occasional use, carry over period or probable cannabis exposure (70). Immunoassay is adopted as a preliminary method in the drug testing program (78). However, false negative and false positive results occur from structurally related drugs that are recognized by the antibodies or occasionally artifacts such as adulterants affecting pH, detergents and other surfactants (73, 77). For this reason, any positive result using immunoassay must be confirmed by chromatographic techniques (75, 78). Cannabis has a long half-life in humans (67 days) (57). In chronic cannabis users, it is particularly difficult to determine whether a positive result for cannabis represents a new episode of drug use or continued excretion of residual drug (62). Algorithmic models have been devised to determine whether THC levels represent new use or the

carry-over from previous use (62, 64). However, these models are not very accurate in discriminating new use and carry-over in chronic users (66).

Interactions of Cannabis with other Drugs of Abuse

Interaction of chronic marijuana with other drugs of abuse has not been studied in detail. It has been demonstrated that there is no cross-tolerance between LSD and Δ^9 -THC (80, 81). Studies are required to understand and compare the abuse potential of marijuana in isolation and in combination with other drugs and its adverse effects on performance (80, 81). A unique opportunity is available in India for studying various facts of cannabis uses since it is possible to locate people that are abusing cannabis continuously in one form or other over several years, both in isolation, or in combination with other drugs. In such subjects, the pharmacology and the interaction of cannabis with other drugs can be studied (26, 82, 83).

Effects of Indirect Cannabis Exposure

There can be indirect exposure to cannabis through passive smoking. It has to be noted that from approximately 50% of Δ^9 -THC that survives pyrolysis during the smoking, a major portion (16-53%) is delivered to the smoker, while a lesser amount (6-53%) is released into the air as side stream (57). A passive inhaler in the proximity of the smoker is involuntarily subjected to inhalation of Δ^9 -THC smoke. In a study, five drugs-free male volunteers with a history of abuse and two marijuana naïve subjects were inactively exposed to the side stream of marijuana smoke (81). Analysis of Δ^9 -THC concentrations in that study confirms that the detection time increased according to the passive dose. The Δ^9 -THC absorbed by the passive smoking depends on several features related to the condition under which passive inhalation took place (viz. environment, duration, Δ^9 -THC content, number of smoked joints). There is now a consensus that positive results due to passive inhalation are possible (84).

In an interesting study from Pakistan, major metabolites of cannabis were found in the milk of cows which had grazed upon naturally growing cannabis vegetation (73). Children fed on such milk showed metabolites of cannabis in the urine, suggesting passive consumption through milk (83).

Δ^9 -THC is secreted into human breast milk in moderate amounts (85, 86, 87). A feeding infant would ingest 0.8% of weight adjusted maternal intake of one joint (86-88). Another point to be considered is that cannabis has an effect on the quality and quantity of the breast milk (85, 86). It could inhibit lactation by inhibiting prolactin production via direct action on the mammary gland. However, this data has not been confirmed in human subjects. Clinical and pharmacokinetic data indicate that cannabis use is dangerous during breast-feeding for the child (89). Δ^9 -THC can accumulate in human breast milk and infants exposed to marijuana

through their mother's milk will excrete Δ^9 -THC in their urine during the first 2-3 weeks (88). Due to the intake of cannabis or other drugs (psychotropic, antiepileptic) by mothers, infant children depending on breast-feeding might exhibit physiological effects such as sedation or reduced muscular tone and other adverse effects (85).

Conclusions

The recreational use of cannabis among youth has increased worldwide over the past few decades. Despite the demonstration of some bio-medical applications, cannabis abuse is associated with different disease conditions including probable risk of developing psychiatric disorders. Hence, there have been significant efforts to identify the toxic factors in cannabis and establish the role of component causes that underlie individual susceptibility to cannabinoid-related psychotic disorders. Secondly, it has necessitated the development of efficient methods to identify and quantify various cannabis metabolites from different body fluids. While immunoassay is adopted as a preliminary test, advanced chromatographic techniques are used for confirmation. Research in the future should focus on the molecular changes induced by acute and long-term exposure to cannabis and the contribution of individual psychoactive components.

Conflict of interest

All the authors declare that they have no conflicts of interest.

Contributors

PS conducted the literature searches and wrote the first draft of the manuscript. All authors contributed to and have approved the final manuscript.

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