Pharmacokinetics of Capsule Formulation of Crude *Cannabis* Resin in Rats

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Abstract

As part of the development and evaluation of suitable delivery systems for crude Cannabis resin (CCR), the pharmacokinetics of a capsule formulation in rats after rectal administration was evaluated. After rectal administration of the capsule containing 50 mg of the active constituent, a half-life ($t_{1/2}$) of 10.83 ± 0.058 h was obtained. The elimination rate constant (k) and the time to reach peak plasma conc. (T_{max}) of CCR were respectively 0.064 ± 0.0012 (hour $^{-1}$) and 30 ± 0.01 (minutes) while the peak plasma concentration (C_{max}), area under the curve (AUC), volume of distribution (V_d) and clearance (CL) were 13.72 ± 0.01 µg ml^{-1}, 14.46 ± 0.003 µg ml^{-1} h^{-1}, 3.538 ± 0.0005 liters and 0.228 ± 0.0029 L h^{-1} respectively. These data suggest that CCR is bioavailable to a reasonable extent from the capsule formulation.

Keywords: Crude *Cannabis* resin, Pharmacokinetic parameters, Rectal administration, Albino rats

Introduction

There has been a renewed interest in the potential medical uses of *Cannabis* (*Cannabis sativa L,* also known as," hashish," "wee-wee," marijuana etc) in recent years, with opinions varying from country to country either for or against its reintroduction as a therapeutic agent. Reviews of medicinal and scientific evidence on its therapeutic use as in the treatment of multiple sclerosis (MS) (Grant, 2001), intra ocular pressure (Stevenson, 1998), acute post operative pain (Formukong *et al.,* 1988) and intractable pain (Lozano, 2001) as well as anorexia (Grotenhermen and Russor, 2002) have been reported. Health hazards associated with *Cannabis*-based medicines are largely as a result of the difficulty that physicians encounter in obtaining consistent dose from batches of plant material of varying potency (Gierienger, 1999). Consequently, patients suffer from ineffective (under) dose or the unwanted intoxication resulting from an over dose (Institute of Medicine Preface 1999). Although modern techniques have solved this problem of quality control in the *Cannabis* phytomedicinals through plant breeding and cultivation, the issue of narrow therapeutic window between the desired benefits and the usual unwanted psychic effects remains a challenge (Franjo, 2002). Delta -9-tetrahydrocannnabinol (Δ^9- THC) presently the most widely used *Cannabis*-based medicine, is taken orally (Brennesisen, 2002, McPartland and Pruitt, 1997). This oral route for THC is notoriously slow and unreliable, especially when compared with the non-conventional method of smoking which is a very efficient way of delivering the drug quickly and in a manner that allows flexible dose titration (Chukwu, 2002). Smoking, however, carries medical risk. In short term, the irritant effects of *Cannabis* smoke can lead to bronchitis; in the long term a far more serious hazard is the potential for increased risk of cancer of the lung, airway and mouth. The current challenge in the medicinal application of *Cannabis* is therefore the development of suitable dosage forms which would ensure the stability, and reasonable bioavailability of the drug. It is against this background that we have attempted in the present study to evaluate the pharmacokinetics of a trial capsule dosage form of crude *Cannabis* resin extract (CCR), administered via the rectal route.

Materials and Methods

Source and identity of plant materials: The fresh leaves of *Cannabis sativa L.* were collected from the Crude Drug and Research Unit of National Drug Law Enforcement Agency (NDLEA) in Enugu, Enugu State of Nigeria strictly for research purpose. It was authenticated by Mr. Ozioko of the Department of Pharmacognosy, Faculty of Pharmaceutical Sciences, University of Nigeria, Nsukka.

Preparation of plant extracts: Whole leaves of the plant (*Cannabis sativa*) were rinsed thoroughly in running tap water, sun-dried in open air for 48 hours and pulverized to coarse powder in a mortar. One kilogram (1 Kg) of the powdered leaves of *Cannabis sativa L.* was extracted with 1 litre of chloroform for 8 h using a soxhlet extractor (Gallenkamp, England). The crude chloroform extract was evaporated to dryness under reduced pressure, using a rotary evaporator (Gallenkamp, England) at an optimum temperature of between 40 ± 5°, to yield 173.25 g of crude resin tar.

Preparation of *Cannabis* capsule: The specific quantities of *Cannabis* resin, cornstarch, and lactose were mixed together for 10 minutes in a mortar. Predetermined volume of water was added to the above to form mucilage and triturated for 8-10 minutes. The damp mass formed was screened through a 1.7 mm stainless sieve and the resulting granules were dried at 55° – 60° for 1 hour in a hot air oven. The granules were further passed through a 1.0 mm stainless sieve, and thereafter shaken on

a 250 µm stainless steel sieve to separate fines from the coarse granules. The granules were filled into hard gelatin capsule no. 5 and stored in an airtight container. Each capsule contained an equivalent of 50 mg of CCR.

Table 1: Composition of the formulated capsule

Material	1 capsule	100 capsule
Cannabis crude extract (mg)	50	5000
Corn starch 15 % (w/w)	15	1500
Lactose (w/w)	22	2200
Gelatin 3 % (w/w)	3	30

Determination of pharmacokinetic parameters of *Cannabis* capsule formulation (*in vivo*) in rats: Ten Spraque-Dawely albino rats of both sexes (180-250 g, 3 months old) were obtained from the Faculty of Veterinary Medicine Animal House, University of Nigeria, Nsukka a week prior to the pharmacokinetic study. Prior to the test, the animals were fasted for 24 h and marked with indelible marker.

Ten rats were rectally administered with a single dose (whole capsule) of 50 mg of CCR formulation. A 0.4 ml volume of blood was collected from the retro-bulber plexus of the medial canthus of the eye of each rat by carefully inserting a microcapillary tube into the medial canthus of the eye to puncture the plexus and enable outflow of blood into heparinized vials. The collection of blood was carried out after 0, 0.5, 1, 2, 3 and 4 h of drug administration.

Analysis of blood samples: The collected blood samples, placed in heparinized vials were centrifuged at 3000 rpm for 10 minutes to separate the plasma from the serum. The total *Cannabis* constituents in the plasma were determined using UV spectrophotometer (Unico, USA) at a wavelength of 274 nm.

The pharmacokinetic parameters determined for each blood sample were: area under the time versus drug concentration curve (AUC), elimination rate constant (k), maximum plasma concentration (C_{max}), volume of distribution (V_d) and clearance (CL).

Data were expressed as the mean ± standard error of mean (SEM) by means of SPSS. Area under the curve (AUC) was calculated using the trapezoid rule.

Results and Discussion

Measurable cannabinoids concentrations were obtained from the plasma samples. Fig. 1 shows the cumulative plasma level-time curve of the crude *Cannabis* capsule formulation after rectal administration in rats. Peak plasma concentration occurred at approximately 30 minutes of administration. The result is similar to that obtained by Lemberger *et al*, 1970 and Garrett and Hunt, 1977a in dogs. The pharmacokinetic parameters are presented in Table 2. Figure 2 shows the semi-log plot of CCR plasma concentration from which $t_{1/2}$ and elimination rate constant (k) were extrapolated.

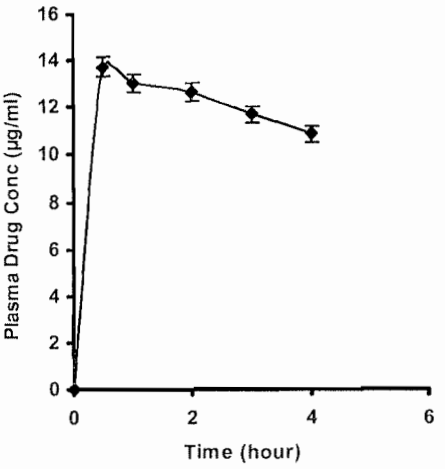

Fig. 1: Plasm level-time curve of CCR after rectal administration of capsule

Table 2: Pharmacokinetic parameters of *Cannabis* capsule formulation containing (50 mg active constituent) after rectal administration to rat

Parameters	Value
$t_{1/2}$ (hour)	10.83 ± 0.0058
k hour^{-1}	0.064 ± 0.0012
V_d (litres)	3.538 ± 0.0005
CL (L/Hour)	0.228 ± 0.0029
AUC (µg /mL/h)	1.446 ± 0.003
C_{max} (µg/mL)	13.72 ± 0.01
T_{max} (min)	30.00 ± 0.01

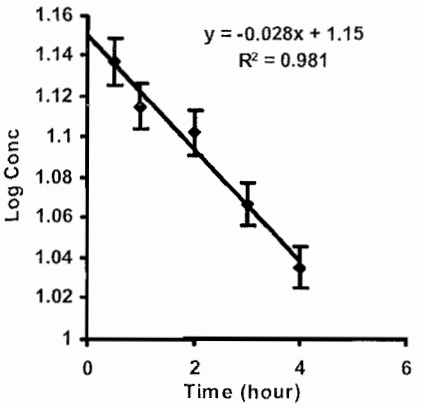

Fig. 2: A semi- log plot of CCR plasma concentration against time

However, from the table, values of the T_{max} indicated fast absorption of the resin, a value which differs significantly from that obtained by Lemberger *et al.*, 1970 and Garrett and Hunt 1974 who obtained values of 3 to 7 minutes after smoking or i.v administration of cannabinoids in both man and animal. The maximum concentration (C_{max}) of the drug was found to be 13.72 ± 0.01 µg mL^{-1}, and the

half life ($t_{1/2}$) was 10.83 ± 0.0058 hours. An average $t_{1/2}$ of 14.2 to 26.0 hours has been reported for *Cannabis* in animals and man respectively by Hunt *et al*, 1980. The $AUC_{0-4\ hour}$ indicated that *Cannabis* was excreted within 1.446 ± 0.003 hours. This is also reflected in a relatively low elimination rate constant (k) value obtained to be 0.0064 h^{-1}. The value of 0.228 ± 0.0029 L h^{-1} obtained for the clearance of the drug further proves a relatively slow elimination of the drug following rectal administration.

Although the pharmacokinetics of cannabinoids have been studied in both man and animals, there has been no quantitative evaluation of rectal administration of *Cannabis*. Pharmacokinetic parameters of cannabinoids in man and animals using various analytical techniques have been reported following i.v. infusion (Lemberger *et* al., 1970; Lemberger *et al.*, 1971a; Lemberger *et al.*, 1971b; Lemberger *et al.*, 1972a; Lemberger *et al.*, 1972b; McCallum *et al.*, 1976; Wall, 1971; Wall *et al.*, 1972; Wall *et al.*, 1976; Wall *et al.*, 1979; Perez-Reyes *et al.*, 1976; Agurell *et al.*, 1973) smoking (Lemberger *et al.*, 1972b; McCallum *et al.*, 1976; Agurell *et al.*, 1976 and Hunt and Jones, 1980) and oral administration (Lemberger *et al.*, 1972b; McCallum *et al.*, 1976).

Though little information is available regarding the plasma level of cannabinoids after different routes of administration, cannabinoids (THC) has been measured for over 30 minutes (i.v infusion) using 5.0 mg of the drug (Wall, *et al.*, 1972). In general, the plasma level measured in this work differed from other published data. As evident from Figure 1, fast absorption followed by a biphasic plasma decay curve can be observed during the first 4 hours with little individual variation. This route of administration of the capsule evidently is responsible for the relatively low bioavailability and slow onset of action of the CCR. In addition, the rectal route of administration affects absorption thereby altering the concentration of the cannabinoids present in plasma. The value obtained for the volume of distribution (V_d) of *Cannabis* capsule is within the range reported in man and animals (Lemberger *et al.*, 1972a; Lemberger *et al.*, 1972b; McCallum *et al.*, 1976; Wall, 1971; Wall *et al.*, 1972). Though the value of V_d seems small for such a lipophilic substance, it is close to the expected plasma volume of approximately 2.5 to 3.0 Litres in 70 kg man and animals (Truitt, 1971). This may be due to the fact that THC (a cannabinoid) which is the principal active constituent in *Cannabis* is a tissue protein bound substance. In addition, it has been reported that different values of V_d may be obtained when different vehicles are used for drug administration (Truitt, 1971; Hunt *et al.*, 1980; Barnett *et al.*, 1982; Fehr and Kalant, 1974 and Law, 1978). It should also be noted that initial disposition events are complicated by this protein binding. As cannabinoids infuse, there will be a competition between their tissue uptake and plasma protein uptake.

In animals, the liver is not the only *Cannabis* metabolizing organ; the lung also metabolizes *Cannabis* in the form of THC, but has only a fraction of the liver's intrinsic clearance. Based on these, if one assumes that in man the liver is the primary metabolizing organ and the hepatic blood flow is approximately 1500 ml min^{-1}, then the observed total blood clearance value of cannabinoids average 1216-1461 ml min^{-1} which implies that *Cannabis* metabolism may be hepatic blood flow limited. Secondly, the low systemic availability of cannabinoids may be due to not only high first-pass effect due to extensive uptake in the liver but also to chemical breakdown. The result of this study is however, in line with plasma profile reported by other workers (Lemberger *et al.*, 1972b; Agurell *et al.*, 1973).

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