Speaking notes
Trustee Christopher
Richardson
June 11, 2015

Medical Marijuana Dispensary Public Hearing Statement
Vancouver School Board
June 11, 2015

As a partner of the City of Vancouver, the Vancouver Board of Education recognizes that the City has both the authority and the responsibility to enact measures to ensure that business is conducted appropriately in the context of health, safety and public interest. As the primary provider of education to children and youth in the city, the Board of Education has a responsibility to ensure that the safety and educational needs of those youth are taken into account whenever decisions are made that may impact them. Accordingly, the Board would be opposed to any action that could result in easier access to marijuana by children and youth, and requests that measures be put in place that would mitigate that possibility.

Although there are a variety of opinions about the impact of use of marijuana on adults, there is substantial and accepted medical evidence to indicate that use of marijuana by adolescents has significant and far-reaching harmful effects. Unfortunately, the recent proliferation of marijuana dispensaries, combined with inaccurate and harmful information available through the media, has 'normalized' the idea for many of our youth that marijuana use by youth is legal, routine, societally acceptable, and not harmful. We disagree.

In an article entitled *Adverse Health Effects of Marijuana Use* published in the New England Journal of Medicine in June 2014, a number of statements are made which clearly support the idea that marijuana use by adolescents is harmful. Among other things, the article states that:

- The regular use of marijuana during adolescence is of particular concern, since use by this age group is associated with an increased likelihood of deleterious consequences including:
    - altered brain development
    - addiction
    - poor educational outcomes, with increased likelihood of dropping out of schools
    - cognitive impairment, with lower IQ among those who were frequent users during adolescence
    - diminished life satisfaction and achievement
- Early and regular marijuana use predicts an increased risk of marijuana addiction, which in turn predicts an increased risk of other illicit drugs.
- During (adolescent) development periods, (the adolescent brain) is intrinsically more vulnerable than a mature brain to the adverse long-term effects of environmental insults, such as exposure to THC, the primary active ingredient in marijuana

In addition, an article in the Pediatrics: Official Journal of the American Academy of Pediatrics entitled *The Impact of Marijuana Policies on Youth: Clinical, Research, and Legal Update* published in January 2015 indicates that:

- The potential negative consequences of short and long term recreational use of marijuana in adolescents include:
    - Impaired short term memory, decreased concentration and attention span, decreased ability to problem-solve, all of which clearly interfere with learning
- Marijuana use during adolescence is associated with reductions in the odds of high school completion and degree attainment and increases in the use of other illicit drugs and suicide attempts
- The adolescent brain, particularly the prefrontal cortex areas controlling judgment and decision making, is not fully developed until the mid 20s
- The younger an adolescent begins using drugs, including marijuana, the more likely it is that drug dependence or addiction will develop in adulthood

This information is obtained from two of the most highly regarded scientific medical journals in the medical community, providing clear evidence that marijuana use amongst adolescents is, indeed, harmful.

As a result, the Board has concerns about the recent proliferation of marijuana dispensaries that have appeared in the city over the past two years. Some of these establishments are in close proximity to schools. We do not dispute that despite the best efforts of parents, school and district staff, and the support of community partners such as the Vancouver Police Department and Vancouver Coastal Health, youth choose and are able to access marijuana in a number of ways. We strongly believe, however, that

the answer to this problem is not to set up marijuana outlets near our schools. Unless very firm boundaries are set in place and enforced strictly, we have significant concern that these outlets could easily result in an increase in marijuana use by youth in the city.

Based on these concerns, the Board has a number of requests that we would ask council to consider as they make decisions regarding the proposed amendments to city by-laws:

1. **The Board would ask that a portion of any licensing fee be designated for educational purposes and youth addiction support services.** In collaboration with and through the support of our community partner, Vancouver Coastal Health, the district provides substance abuse education programming and support for students who struggle with drug-related behaviours. The current level of support, however, is already not able to meet the growing needs in this area. We would therefore ask that if the city is going to license these establishments, that you take steps to mitigate any potential increases in youth marijuana use by providing directly funded support to youth drug awareness and addiction support programs in the city.

2. **The Board would request that whatever by-law amendments are put in place, they be enforced strictly, particularly when they are initially enacted.** Should youth discover that some rules are not being enforced, they are extremely likely to take advantage of that fact. In particular, the Board would request that bylaws regarding access by minors to the proposed establishments be strictly enforced and that consequences to establishments that do not honour this be swift and meaningful.

3. **The Board would request that the city consider increasing the distance between licensed establishments and schools from the proposed 300 metres to a distance of 500 metres.** Although we understand that 300 metres appears to be the standard in other jurisdictions such as Washington and Colorado, it would be our belief that 300 metres is a very short distance between a school and a business selling substances that are illegal and harmful for youth consumption. Any measure that can be taken to make access more difficult for youth should be strongly considered.

4. **The Board would request that advertising to minors, either directly or indirectly, be strictly prohibited in all areas in and around the business.** It is requested that a 'zone' be established around the establishments in which advertisement is prohibited.

5. **The Board would request that, despite the very recent supreme court ruling enabling sales of forms of medical marijuana other than in dried product and oils, the City consider not allowing other products such as marijuana cookies and candies to be sold.** There is evidence to suggest that these products are attractive to children and youth, who may not understand what they are ingesting when it appears to be a cookie or candy. There is also concern about the delayed effect of the drug when it is consumed through edible products and potential for overdose.

6. **It is assumed that all establishments, including those currently in existence, will be subject to any and all new rules contained in the proposed by-law amendments.**

The Board understands and appreciates that the City has the responsibility to consider the views of all citizens, many of whom will hold a variety of views on the subject of marijuana use. The Board is not making a broad statement on the use of this drug. We do, however, wish to make it very clear that use of marijuana by youth is illegal, medically harmful and very likely to create barriers to life success. We therefore ask that you carefully consider the impact of any by-law amendments on this particular group of citizens before making any decisions that will negatively impact the current youth and future adult citizens of the City of Vancouver.

speaking notes
David Malmo-Levine
#28 June 11, 2015

Vancouver City Hall dispensary meetings – day two – June 11th, 2015

Speaker #28: David Malmo-Levine [redacted] s.22(1) Personal and Confidential

Good evening Mayor and Council.

My name is David Malmo-Levine. I'm an author, a dispensary owner, I help curate a museum of herbal medicine and I've been a pot activist for 23 years. I'm the youngest person to appear self-represented in front of the Supreme Court of Canada. I've done four and a half months in jail – one and a half months in maximum security - for "herb crimes".

I've had my life savings – in the form of a cannabis business – stolen by the VPD twice – in 1996 and 2008 – when they raided my Harm Reduction Club and my Herb School. I find it interesting that in neither of those cases there were any "Victim Impact Statements" issued.

I will probably lose my life savings again for a third time if you implement the excessive licensing fees or police background check elements of your regulations. And its not just me you might choose to hurt with your discrimination. Vancouver serves as a model for other cities around the world, especially with all things cannabis - your regulations could directly impact up to a half billion cannabis users, growers and dealers on planet earth.

I wrote a 24-study literature review of the latest information on cannabis and teens – that's the book I have provided. To nutshell my book, there's no evidence that cannabis use makes kids stupid or crazy – there's no uptick in psychosis rates or a dip in I.Q. rates in the general population - but there's a lot of evidence that cannabis laws result in dead kids from botched drug raids - like Daniel Possee who was killed by the RCMP in North Vancouver in 1992 over a half-ounce.

There's also evidence that stressed out kids are now forced to use Effexor instead of a less toxic and less dangerous herbal anti-depressant – cannabis – by brow-beating them with the latest reefer madness propaganda. I myself used cannabis as an anti-depressant and relaxant and performance enhancer starting at age 14, and it kept me from killing myself or doing something stupid as my hormones raged like a chainsaw on fire.

I also co-authored the two booklets I've provided you – a history of cannabis as a relaxant and anti-depressant – the two most-often cited uses of cannabis in the literature. The evidence seems overwhelming that all use is medical use – ever user is a medical user – and what we call "recreation" is actually preventive medicine – to prevent or mitigate stress and depression. Even the word recreation itself means "to restore to health", and recreation is prescribed to millions of people every year all over the world by doctors in the form of "R and R".

I think my job as a cannabis activist is to protect as many harmless growers, dealers and users from harm as possible. I want to get them all off the battlefield and give

them all a seat at the table of legalization. I also think it's essential that we don't replace an outright prohibition with a cartel, because I take fighting poverty very seriously and I didn't become a pot activist in order to get a front-row seat in the destruction of the last "inclusive to the poor" economy on earth. As the bible says – "The riches of the earth are for all." I don't want Harper's "millionaires only" mail-order model or your "upper middle class only" retail model – I want a medical cannabis model that will allow as many people as possible to participate in BC's largest economy.

You are relying on two anti-pot studies and a report by Dr. Daily to justify all your discriminatory proposals. One of your reports was written by Gabrial Nahas – the most discredited researcher in cannabis research history. The second is a non-commital study about cannabis and cancer that has a pile of studies that say the opposite, and Dr. Daily refuses to even discuss with me the counter-evidence to her "cannabis makes your kids crazy" hypothesis. None of your discrimination has an evidentiary basis.

As for your proposed regulations, I am in favor of keeping corporations out, and I would agree with any security regulations that also exist for pharmacies or alcohol retail shops, such as crash posts.

All the other regulations you have proposed are over-broad, and some are just designed to demonize the relaxed, hungry and happy people.

We don't need buffer zones because – like caffeine – cannabis doesn't harm teens just by the proximity of retail outlets to schools and community centers.

We don't need a ban on teens using cannabis – no politician has the right to second-guess a decision made by a teen, their parents and their doctor.

We don't need licensing in excess of what Tim Horton pays because we don't need any regulations in excess of what coffee shops adhere to.

We don't need a ban on medibles – standards for ingredients, dosing and child-proof packaging will address those concerns much more easily and effectively.

We don't need a ban on advertising or neon signs or delivery services – cannabis deserves to compete with the other stimulants, relaxants and euphorics such as alcohol, tobacco, pharmaceuticals and caffeine on an even playing field – or even with an advantage that reflects it's reduced risk.

We don't need criminal background checks. I believe Jesus said we should beat our swords into plowshares – he could have meant give the dangerous organized criminal people a way out of their criminal lifestyle and a stake in society. The more criminals are busy in their pot dispensaries, the fewer are out busy engaging in harmful activities.

To conclude, aside from excluding corporations, maintaining a strict parental permission policy and requiring crash poles, regulate cannabis as if it was caffeine, because even though the impairment levels of novice users are higher with cannabis, it is an undisputed fact that caffeine has more overdose deaths, over-use deaths and more severe withdrawal symptoms than cannabis does, and Vancouver teens now know this fact even if most adults still refuse to admit it.

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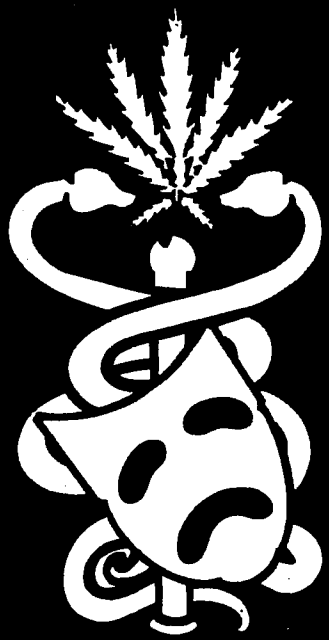
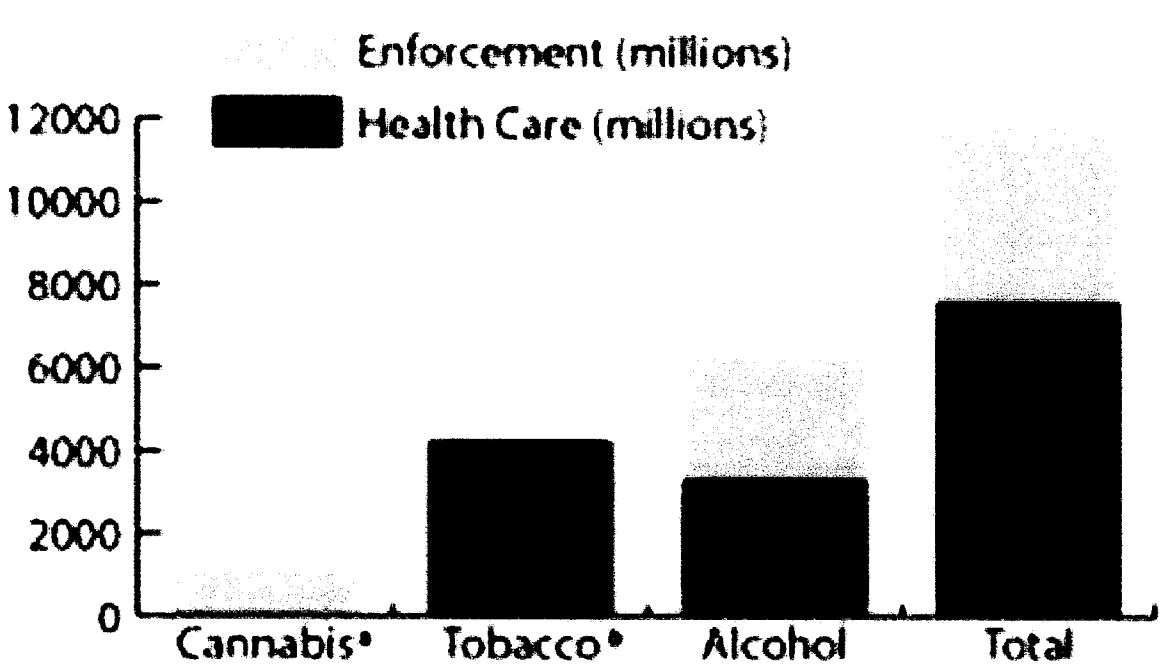


"A CHEERFUL HEART IS GOOD MEDICINE..." - PROVERBS 17:22

Fig. 2: Direct health and enforcement costs for cannabis, tobacco and alcohol, Canada 2002



Estimated Past-Year Users:

Health Costs / User:

Enforcement Costs / User:

Sources: Health Canada, n.d.; Rehm et al. 2006

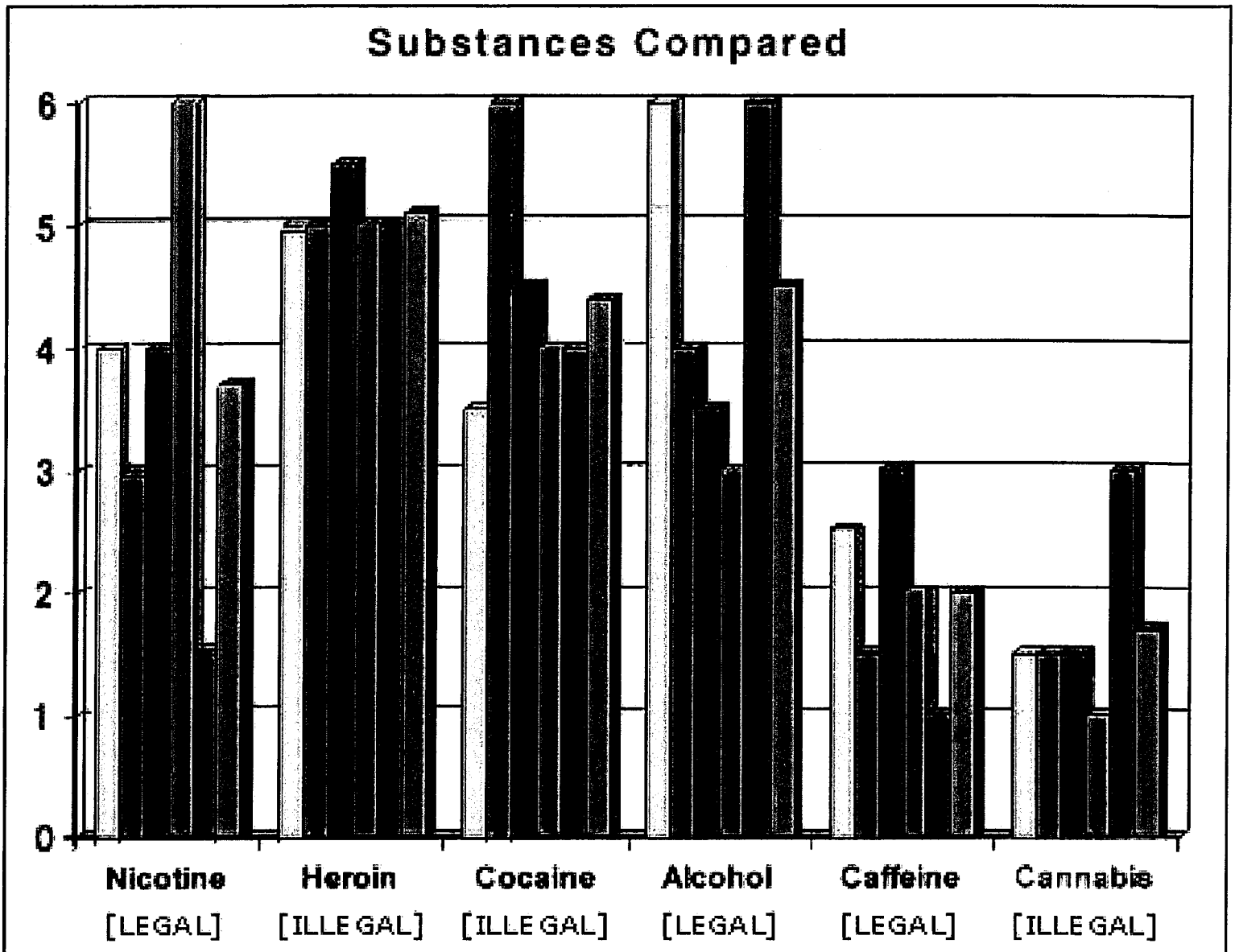


FIG. 15.1. Addiction ratings.
From Henningfield, Benowitz.
New York Times 1994

Submission
Paul Hunt #82
June 11, 2015

BRITISH COLUMBIA

NUMBER: S-50075

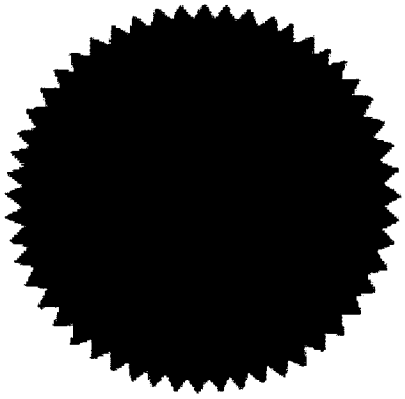
SOCIETY ACT

CERTIFICATE OF INCORPORATION

I Hereby Certify that

GREEN CROSS SOCIETY OF B.C.

has this day been incorporated under the *Society Act*



Issued under my hand at Victoria, British Columbia

on December 22, 2005

RON TOWNSHEND
Registrar of Companies
PROVINCE OF BRITISH COLUMBIA
CANADA

Alternative Health Care Application

Green Cross Society of B.C.

2145 Kingsway, Vancouver B.C. V5N 2T4

778.785.0370 778.785.0477

4296 Main Street, Vancouver B.C. V5V 3P9

604.875.6640 604.875.8600

www.greencrossofbc.org

Green Cross Society of B.C.

Code of Conduct

ATTENTION: The Green Cross Society of B.C. is a health facility. Our main focus is safety and the well-being of all members. There is a zero tolerance policy for any rules broken. Doing so will result in a review of your membership.

- ☐ No alcohol, tobacco, or other drugs will be tolerated. Please do not display or engage in loud, disruptive behaviour on GCS premises. Doing so will result in membership suspension or termination.
- ☐ All members MUST respect members and staff. This is a medical facility where many members are disabled. We focus on having a safe and comfortable environment for everyone.
- ☐ No sexual harassment, swearing, spitting, violence, or rude behaviors will be tolerated. Treat others how you want to be treated.
- ☐ Members must respect the neighborhood and the community. No medication is to be administerated outside the walls of the society other than your own home. Please be discrete and keep all medicine out of sight when leaving the premises.
- ☐ No member shall solicit to anyone or use the society for personal gain in any way. Re-selling or redistributing of any of the products/medicine will result in loss of membership as all products are sold for medical use only.
- ☐ Use of marijuana while driving may result in charges for driving under the influence. For your safety and others, please do not operate heavy machinery while using marijuana or marijuana products.
- ☐ Please do not loiter near the club.

Please sign below to acknowledge that you have read and are in agreement with all regulations and rules. Violations will result in immediate grounds for termination of membership.

Date: _____

Name:_____ Signature: _____

Green Cross Society of B.C.
Emergency Contact

Name:_____ Member Number:_____

Address:_____

Email Address:_____

Emergency Contact Name:_____

Emergency Contact Phone Number:_____

Allergies:_____

Special Medical Notes: _____

_____

_____

_____

Green Cross Society of B.C.

Practitioner's Statement:

Client Information:

First Name: _____ Last Name: _____ D.O.B.:_____

I am confirming that Mr/Mrs./Ms. _____

at the phone number: _____ has been diagnosed with _____

and is presenting symptoms of _____

☐ I recommend medical cannabis or other herbs to help my patient with his/her symptoms.

☐ This patient has reported that his/her symptoms are helped by cannabis and therefore, on the basis of my knowledge, he/she should have access to it.

☐ I do not recommend use because of the medical reasons stated below:

Medical: Please specify: _____

Legal: Please specify: _____

Other: Please specify: _____

Practitioner's Stamp

Practitioner's Signature: _____

Printed Name: _____

Date Signed: _____

Practitioner's Phone Number: _____

Practitioner's Address: _____

2145 Kingsway, Vancouver, B.C. V5N 2T4
4296 Main Street, Vancouver, B.C. V5V 3P9

778-785-0370 **778-785-0477**

RELEASE FORM FOR MEDICAL PRACTITIONERS:

Marijuana Medical Access Regulations

The Canadian Medical Protective Association recommends to member-physicians assisting patients in their application under the *Marijuana Medical Access Regulations* that they ask patient-applicants to sign a release from liability. The following form of release was developed and approved by the Canadian Medical Association:

I, _____ agree not to make any claim on complaint or commence any proceedings against **Dr(s).** _____ _____ in relation to the application process under the marijuana medical access regulations or my use of marijuana.

I release **Dr(s).** _____ from any and all actions, causes of actions, claims, complaints and demands for damages, loss or injury whatsoever arising directly or indirectly as a consequence of my application under the *Medical Marijuana Medical Access Regulations*, or my use of marijuana. This release from liability is to be binding on my heirs, executors, and assigns.

Signature of Applicant: _____ **Date:** _____

Signature of Witness: _____ **Date:** _____

ASSOCIATION MÉDICALE CANADIENNE
CANADIAN MEDICAL ASSOCIATION

GREEN CROSS SOCIETY OF B.C.

CONSTITUTION

1. The name of the Society is Green Cross Society of B.C.

2. The purposes are:

   a) To advocate for health care therapies and the right to access to those therapies which are alternative or complementary to those offered by public health care providers in British Columbia;

   b) To ensure a supply of natural products or remedies is available to members for medical purposes which meet or exceed the highest Canadian public health standards of quality, potency, and efficacy; tested to ensure such products or remedies meet these standards;

   c) To offer a non-judgmental approach to medical needs and where practicable, to provide compassionate access of the unmet needs of disabled and health- challenged members to alternative and complementary therapies and practices;

   d) To provide a warm, friendly environment for members to feel safe and as supported as possible;

   e) To receive and use whole medicines for medical uses as authorized;

   f) To educate the physically challenged, the medical community, health providers, politicians and the general public about the beneficial medical uses of complementary and alternative medicines, practices and therapies;

   g) To collaborate with Health Canada, BC Regional Health Authorities, and Municipal Governments to develop protocols and best practices for the research and personal utilization of cannabis as a medical adjunct or alternative application for conditions or diseases which do or may benefit from its use. Such considerations include, but are not limited to:
      (i) harm reduction protocols,
      (ii) addiction treatment or cessation programs,
      (iii) other medical or psychological conditions;

   h) To facilitate research into aspects of natural medicines including but not limited to, quality standards, potency, appropriate dosage levels, methods of administration and all other areas of interest to patient care, the medical community, patients and their governments;

   i) To support the widest available whole plant genetics as medical options for member needs and for research potentials;

j) To provide information as a result of the activities of the Society in support of legislation and enactments federally, provincially, and at the municipal level, to fairly and lawfully regulate the development, supply and availability of cannabis for medical purposes;

k) To raise funds and accept donations to facilitate the objectives of the Society;

l) To provide access to information and promote emerging protocols and practices regarding natural and complementary therapies;

m) To ensure that every member has the current information to facilitate their right to equal optimum health care from all available health providers;

n) To participate in the approval, control and regulation of growers and/or manufacturers of whole medicines or derivatives for medical purposes, to help provide infrastructure guidelines and resources to support other patient driven organizations in conjunction with government; i.e. municipal, provincial and federal governments, departments and agencies, and whole medicine manufacturers and suppliers;

o) To provide a supply of cannabis, for a time determined by the Society, for members or patients on prescription from their physicians or other recognized health professionals, upon confirmation of diagnosis of life-challenging illness, or other condition or disease for which cannabis has demonstrated or might be expected to be efficacious; or upon receipt of a copy of their files from other recognized compassionate care or medical societies and/or upon confirmation of membership in other organizations with similar standards of demonstrated medical need.;

p) To participate in and provide information, collaboration, personnel and resources for HIV/AIDS educational activities, support services, resource libraries and other activities and needs relating to the same;

q) To form alliances with organizations that share the Society's purposes or objectives.

CASES JOURNAL

Case report

Open Access

Standardized natural product cannabis in pain management and observations at a Canadian compassion society: a case report

A Paul Hornby[1]*, Manju Sharma[2] and Bree Stegman[3]

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Abstract

An adult Caucasian male with excruciating pains following multiple traumas was monitored, daily, over one year while managing chronic pain by self-administering quantifiable amounts of natural cannabis. Tetrahydrocannabinol, Cannabidiol, and Cannabinol were all measured in tinctures, capsules, smoke-able product plus some baked goods, prior to their administration. By allowing standardization, the subject was able to develop a daily regimen of pain management that was resistant to a battery of most patent analgesics.

Introduction

The chronic pain resulting from a severe on-the-job injury is a frustrating experience both for the patient as well as the treating physician and leads to chronic dependence on opiate painkillers and anti-depressants. However the relief of pain may be less desired if quality of life of such individuals is poor. The physician and the patients are left with no option but to resort to alternative modes of therapy. Cannabis has been documented to be one of such measures, especially in advanced cases of cancer [1]. It holds an enormous potential as medicines derived from cannabis plant exhibit a phenomenon termed strain specific symptom relief. It has been documented to be of proven value in arthritis and multiple sclerosis; however no controlled clinical trial for its use in chronic intolerable pain is reported. Hence, this case report. The person involved in this study is a member of the Green Cross Society of British Columbia, which has Federal tax number to distribute cannabis for medical purposes. The Society provides natural product (cannabis, herbal medicine) to its qualified members.

Case presentation

The volunteer, a 33 year old Caucasian male, volunteer was selected from the membership based on his record keeping ability, the severity of his injury, plus his daily presence at the Society, allowing continuous monitoring. The subject kept detailed notes of his condition, including pain charts, medications and dietary habits, allowing comparison by study observers. The individual's note taking allowed an in depth review of his condition. The case described here is strikingly similar to four others of its type, run over the same year, with comparable observations and outcome.

Three years ago the subject (then, 33 years of age) was in good health and employed as a glacier, when he fell 28 feet onto concrete. As a result of this fall he suffered multiple injuries including C6 vertebral, skull, left olecranon, left hip, ankle and trochanteric fractures with multiple disc herniations. He underwent many surgical procedures for the management of these injuries and continued with pain of levels are 5/10 on a constant basis.

Cervical fracture impaired his ability of upper body to 75% and he was unable to read or work on computer. He had tension headache and tinnitus due to fractured skull. Shattered left elbow caused muscle spasms and pain at times. Left wrist pain was constant at level 8/10. It was burning at times, ice cold and had hammer like effect. Left hand was hard to use. He had back spasms 6/8 on both sides due to spine injury. He also had moderate degree of pain in left hip and ankle joints. Subsequent treatment resulted in, two years of physiotherapy, plus a long list of medications including: Arthrotec, Flexeril, ketorolac, Tylenol 3 with codeine, Naprosyn, Percocet, gabapentin, Marinol, Lyrica, Supradol, oxycodin and Oxycontin for pain. Doxepin, Imovane, Cipralex, trazadone, Elavil, Effexor XR for depression and HCTZ, Lipitor and ranitidine for a secondary hypercholesterolemia, the subject was unable to achieve satisfactory pain relief using these medications. And, he complained that the medications turned him into "a living dead person", unable to work, go to school and carry out normal daily functions.

HPLC

Chromatography, using a Hewlett-Packard (Agilent) 1090 Series II binary pumping system with a 79883a diode array detector, with primary absorbance at 219 nm, was conducted. Mobile phase at 1 ml/min was isocratic with 14% aqueous (1:25:974 phosphoric acid: acetonitrile: distilled water) and 86% organic phase (acetonitrile). The column was a Zorbax C18 reverse phase 4.6 mm × 25 cm. Samples were prepared by the method modified from Health Canada for the preparation of hemp samples for HPLC analysis [2]. Calibration curves were run for a number of commercial standards (Sigma) and averages made. 0.1 gram of dry (dryness determination appendix 1) cannabis was suspended in 10 ml THF and sonicated for 3 minutes then passed through a 0.45 micron nylon syringe filter into a 1 ml HPLC sample vial. Under these conditions suitable chromatography is achieved for the three most abundant cannabinoids present in the samples provided to the Society's members.

The total mean concentration of cannabinoids for 30 random samples taken at the Society was; CBD plus CBD-A (5.6 ± 3.1), CBN plus CBN-A (5.1 ± 3.2) & THC plus THC-A (172 ± 26). Review of the literature of cannabinoid concentrations found throughout the world shows dramatic variation, not only of THC but the other cannabinoids and their ratios [3]. The cannabis supplied by the Society's contracted growers is optimized for THC concentration through genetic selection of specific strains, growing conditions and fertilizers. Organic growing conditions are a priority. The high THC levels are preferred by the Society's membership for pain and tremor relief.

From January of 2007 to April 2008 we monitored the cannabis use of the participant case. We accumulated data on the amount of smoke-able, encapsulated, edible and tincture preparations consumed by the member, on a daily basis. His prescription record, physician's notes, urine (drug) tests, plus daily interviews were maintained and examined. Daily cannabis use totaling 10 g of natural product cannabis, translating to an average of 420-500 mg of THC, 40-80 mg of CBD and 20-60 mg CBN, was required to achieve a sufficient degree of pain management. Significant reductions in daily pain scores as well as improved sleep, muscle spasm and general quality of life were achieved. Although, by no means pain free, the patient is now able to do some part time volunteer work, go to the gym, and lead what resembles a normal life. He consumes 10-15 g of cannabis per day. He also finds benefit in a number of supplements: for chronic pain and depression, including, GABA (500 mg), L- Tyrosine (500 mg), L-Tryptophan (550 mg), DL- Phenylalanine (500 mg) and S-adenosyl methionine (liquid) 40 drops a day. For the breakthrough pain he used cannabis tincture at 10 mg THC/drop; 2 mg CBD/drop: 15-25 drops (as needed), which relieved intense pain, in a couple of seconds. He also used Volcano (vaporizer), 2-4 g a day. Recent medical examination showed all liver functions to be normal, including clearance of the hypercholesterolemia.

Discussion

The analgesic properties of cannabis are becoming well established in the literature [4]. However, it remains controversial as a medicine and even more so as a plant. The purpose of this case study was to observe the efficacy and usefulness of the standardized whole plant cannabis medicine. Indeed, the complexities of elucidating the efficacy of such preparations is a difficult task, yet the benefits of the natural product far outweigh the contrary in consideration of toxicity, efficacy and side-effects [5]. With regard to the latter, more frequently unwanted side effects from cannabis result from overdose than any other parameter. And, most frequently, this overdose results from oral ingestion of un-standardized baked goods (i.e. brownies). Overdose results in confusion, paranoia and fear that subsides after four to six hours, often into sleep. In no case, has it been observed to cause permanent physical or mental damage [6], but can often leave the

individual with extreme caution to repeating the event. The second most frequently observed un-wanted side effects arise from incorrect strain selection for the symptom. For example, a person seeking pain relief and also suffering from anxiety, chooses a strain containing high concentrations of CBN, with little comparative CBD and low THC, may experience increased anxiety, with little or no pain relief. Another important observation is that there is a genealogical factor in tolerance experienced by individuals of different ethnic backgrounds. Persons of Celtic descent (Scottish, Irish or Welsh) appear to be 3 to 5 times more tolerant to cannabis than persons of middle European or African descent. The person described in this study had a Scottish mother, which may explain the high THC levels required by him, but not by persons in similar studies but of different ethnic background.

Conclusions

The case reported here represents one of many observed at the Green Cross Society. With 70% of the members treating chronic pain the same phenomenon is observed over and over that people achieve a significant degree of pain management using standardized natural product cannabis. Often a better quality of life is attained with cannabis use only, or in conjunction with reduced opiate consumption. The subject in this study is nearly one year using only natural product cannabis plus supplements for his severe pain. He recently went through yet another two surgeries to back and hand using only cannabis for post-operative pain.

The roughly 4000 members of the Green Cross Society find similar benefit from standardized natural product cannabis medicine. To follow, will be publication of the Society's demographic data regarding use for various conditions such as arthritis, fybromyalgia, HIV/AIDS, and chronic pain, to name a few. A breakdown of the illnesses, what strains (cannabinoid profiles) is most effective, and at what dosages will be published at a later time.

List of abbreviations

CBD-A, Cannabidiolic acid; CBD, Cannabidiol; CBN-A, Cannabinolic Acid; CBN, Cannabinol; THC-A, Tetrahydrocannabinolic Acid; THC, Delta-9 tetrahydrocannabinol; THF, Tetrahydrofuran; HPLC, High Performance Liquid Chromatography; GABA, Gamma aminobutyric acid; HIV, Human immunodeficiency virus; AIDS, Acquired immunodeficiency syndrome.

Consent

Written informed consent was obtained from the patient for publication of this case report. A copy of the written consent is available for review by the Editor-in-Chief of this journal.

Competing interests

The authors declare that they have no competing interests.

Authors' contribution

PH was involved in data collection and analysis, plus manuscript draft preparation. MS, interpreted data and edited the manuscript. BS, took part in data collection, interpretation and write-up.

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Scientific Research

Cannabis Responsive Head Injury Induced Mutiple Disabilities: A Case Report

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ABSTRACT

In recent years cannabinoids and their derivatives have drawn renewed attention because of their diverse pharmacologic activities. We report here one such case, where all types of medical & psychiatric treatment failed to improve the symptoms; however cannabis use was able to bring back this patient to normal productive & meaningful life. The patient was a 47 year old left handed Caucasian had minor subdural hematoma at the posterior vertex and a minor focal subarachnoid haemorrhage following a physical assault. His impairments included cognitive slowing with decreased short term memory, organized skill & language deficit. His physical disabilities included spastic gait (hemiplegic), VII nerve palsy, mild cerebellar dysfunction, blurred vision and easy fatigue. He was unable to return to work or drive. In addition to cognitive deficit, right hemi paresis & dizziness; he had symptoms of anxiety & depression. Cannabis improved his gait and brought back lots of his memory. Within 6 months all his symptoms abated with use of cannabis and he started to look at cannabis as a real medicine. Slowly he also had improvement in cognitive functions, memory, vocabulary and his gait became increasingly better day by day with continued use of cannabis.

Keywords: Cannabinoids; THC; CBD; CBN

1. Introduction

Cannabis has been used for treatment in various types of malignancies. Its use in the management of disabilities following moderately severe traumatic brain injuries has not been reported. We report here one such case, where all types of medical & psychiatric treatment failed to improve the symptoms; however cannabis use was able to bring back this patient to normal productive & meaningful life.

2. Case Report

P Hunt, a 47 year old left handed Caucasian had minor subdural hematoma at the posterior vertex and a minor focal subarachnoid haemorrhage following a physical assault on 24th April 1997. He was hospitalized and had a Glasgow Coma score of 6. He remained unconscious for 3 days. He had post traumatic amnesia & was discharged on June 27th, 1997 with residual numbness in right hand & toes. He accidentally cut his finger, subsequently. He had occasional chocking with thin fluids. His major problem was cognitive disabilities. He had poor short term memories and was forced to use a memory book. He had some language problem along with blurry vision while watching TV. He also noted subtle changes in personality and felt less inhibited and more prone to outburst of laughing or tears. However he felt a bit more compassionate than before. He also complained of dry skin over face & scalp. Mr. Hunt was allergic to hay & codeine. He was a smoker & a social drinker. He had minimum family support & had received education up to 12th standard. He was running a small construction company before the assault.

2.1. Physical Examination

A fully oriented pleasant man without distress. He was able to recall 3 out of 3 items after 5 minutes & was able to subtract serial 3 without difficulty. He did well with serial 7 but made gross errors when he reached 50.His forward digit span was 7 and abstraction was relatively intact. He had right supranuclear facial palsy. Extra ocular movements were full & visual fields were grossly normal. Sensations were intact. Tongue & palate were in midline. The tone was normal in upper & lower limbs & bulk was symmetrical. Power was grade V on left and it was IV plus on the right side. Posterior column sensations were intact. There was no subjective loss of pinprick in distal phalanges on right side. However he had patch pinprick loss in right foot. He was however able to differentiate between light touch & pinprick. Deep ten-

don reflexes were brisk bilaterally; however these were more on right side. Cerebellar functions were slightly impaired on right side. Romberg's test was negative, although there was increased swaying with closed eyes. Gait was consistent with spastic hemiplegic on right side. It was wide based & somewhat ataxic. There was decreased arm swaying on right side with flexor posturing. He was able to do heel-toe walk but was unable to perform tandem gait. He was able to stand on one leg with eyes closed. Therefore, his impairments included cognitive slowing with decreased short term memory, organized skill & language deficit. His physical disabilities included spastic gait (hemiplegic), VII nerve palsy, mild cerebellar dysfunction, blurred vision and easy fatigue. He was unable to return to work or drive.

During the outpatient program a number of issues were addressed which included, assistance of finances & physiotherapy for the gait, balancing & coordination exercises without much benefit. In October 1998 he was again hospitalized due to complaint of dizzy sensations (vertigo) which was thought to be central in origin for which he was advised compensation techniques. His physical & cognitive dysfunctions remain unchanged. He continued to have balancing difficulty as he was constantly unsteady & insecure on his feet. In February 1999, in addition to cognitive deficit & right hemi paresis, he had symptoms suggestive of depression & anxiety. He had insomnia & remained preoccupied with thoughts of his collapse of business and being cheated by his foreman. He had difficulty in talking and described it as "I freeze & cannot talk" especially when confronted with someone as he had difficulty in thinking quickly. He was therefore put on Effexor but he developed tachycardia & generalized weakness, therefore medication was discontinued.

2.2. Mental Status Examination

Mental assessment was carried out by the psychiatrist. A casually dressed middle aged Caucasian, looked pleasant, co-operative, accessible & reliable. His speech was normal rate and volume & was mainly goal oriented. He was preoccupied with the idea of collapse of his business. He described his mood being depressed & his effect was restricted to the element of sadness, although he was able to smile appropriately at times. In terms of suicidal ideation, he stated that it crossed his mind a couple of times but he was able to resist it. He denied any intent to hurt himself. There was no history of delusions or auditory or visual hallucinations. His insight and judgment seemed fair.

2.3. Rehabilitation & Follow Up

Therefore in addition to cognitive deficit, right hemi paresis & dizziness; he had symptoms of anxiety & depression. In order to calm his nerves, he was put on Zoloft 25 mg per day which was gradually titrated upwards till 200 mg/day. For his dizziness, vestibular rehabilitation program was instituted but he made little progress. He was also referred to Trauma Recovery Group for ongoing support, but in vain. Since he did not make any significant progress after his discharge from rehabilitation in 1997, therefore no further follow up were arranged for him. Therefore he weaned himself off of 150 and then to 200 mg Zoloft and Ativan but at the same time he started using Cannabis (with high CBD and low THC concentration). He started feeling that his vocabulary was returning and he could talk to people more easily. He smoked his whole life except after his injury in 1997 and about 1/2 year later when he joined the society who sold cannabis. He smoked quite frequently now. His daily consumption of this cannabis was 7 - 10 grams consumed as baked stuff and smoking. This strain of cannabis improved his gait and brought a lot of his memory back. Within 6 months all his symptoms abated with use of cannabis and he started to look at cannabis as a real medicine. Slowly he had improvement in cognitive functions and his memory, vocabulary and gait became increasingly better day by day. His former corporate knowledge returned and he remembered owning a medium sized construction company. His dizziness slowly abated and he could walk without fear of falling down as he felt more confident and steady. He utilized his previous knowledge of running the construction company and formed a non-profiting society for people with disabilities. Today, Paul is the Founder and President of the Green Cross Society of BC.

3. Discussion

The therapeutic properties of the hemp plant, *Cannabis sativa*, have been known since antiquity, but the recreational use of its euphoric and other psychoactive effects has restricted for a long time research on its possible pharmaceutical application. In recent years cannabinoids and their derivatives have drawn renewed attention because of their diverse pharmacologic activities. In the present case there were four major symptoms; cognitive dysfunction, spasticity, unsteadiness vertigo, blurred vision and anxiety with depression superadded to them. The purpose of this case study was to demonstrate the efficacy and usefulness of the standardized whole plant cannabis as a medicine. Indeed, it is a difficult task of elucidating the efficacy of such preparations.

A significant improvement in cognitive functions, decrease in muscular spasticity, unsteadiness, and dizziness, improvement in symptoms of blurred vision, anxiety, depression and general wellbeing were observed after the

use of this drug in the present case. Preliminary research on synthetic THC has been conducted in Tourette syndrome and it has been found useful in reducing tics & a trend towards improving cognitive functioning were reported during and after treatment [1]. A correlation between increase of cognitive functions and cannabinoids use has also been established in schizophrenics [2]. Central cannabinoid CB1 receptors have been found to be located at high concentrations in the output nuclei of the basal ganglia, in forebrain areas associated with higher cognitive functions, in the molecular layers of the cerebellum, hippocampal dentate gyrus, and other parts of the hippocampal formation [3]. After the discovery of the two specific molecular targets for THC, CB1 and CB2 [4], it became clear that most of the effects of marijuana in the brain and peripheral tissues were due to activation of these two G-protein-coupled cannabinoid receptors. Assuming an involvement of the CB1 receptor system in pathophysiology, it can be speculated that improved cognitive functions after cannabinoid treatment were because of a dysfunction in the cannabinoid receptor system following brain injury. Therefore influence of cannabinoids on cognitive processes might be different compared to healthy users. It has also been demonstrated that cannabinoids mediate increases in prefrontal noradrenaline, acetylcholine, and glutamate. In this context, it has been suggested that cannabis use may enhance executive functions and attention/processing speed in schizophrenia by stimulating prefrontal neurotransmission. These findings have important implications for the treatment of cognitive impairment. Functionally, the patient went from a state of virtual incapacitation to a dramatic improvement over the course of one year. The presence of (CBD) Cannabidiol and Cannabinol (CBN) appear to modulate the binding of Delta-9 Tetrahydrocannabinol (THC) to its receptor and thus alter the efficacy of the preparation. The cannabis which was used by this patient had high CBD and low concentration of THC.

Cannabinoids are found to have particular application as neuroprotectants, in limiting neurological damage following ischemic stroke & trauma. Non-psychoactive cannabinoids, such as cannabidiol, are particularly advantageous to use because they avoid toxicity. Neurogenerative properties of cannabinoids in adult brain cell have been established and new research indicates that the effect comes from non-psychoactive cannabinoids attaching to CB 1 receptors. It has now been shown that CBD in cannabinoids increases the formation of new nerve cells in brains of adult mice without impairing learning, while THC, the primary psychoactive component, has no effect on neurogenesis [5].

It has been found that CBD had anxiolytic and antidepressant properties, while THC analogues were shown to have anti-anxiety effect and potential of treating schizophrenia. Considering that the anxiolytic properties of CBD can be mediated by the activation of 5-HT1A receptors and that this modulation can induce antidepressant effects, this hypothesis has recently been tested using the forced swim test in mice & CBD was shown to be anxiolytic [6] and antidepressant [7]. It was concluded that cannabinoid receptors modulate a variety of brain functions including anxiety, fear, mood and target endocannabinoid system [8]. THC has other effects like relaxation, euphoria, altered space time perception; alteration of visual, auditory & olfactory sensations. The improvement in symptoms of anxiety, depression, spastcity & dizziness in the present case was therefore related to modulation of brain functions. Cannabinoids may, in the future, become an important option in the treatment of psychiatric symptoms and disorders. Due to the absence of psychoactive or cognitive effects, to its safety and tolerability, to the existence of clinical trials with positive results, and to its broad pharmacological spectrum, CBD is possibly the cannabinoid more likely to have initial findings translated into clinical practice. In particular, the results indicating that CBD has anti-psychotic and anxiolytic properties seem to be well established. These effects can be dependent on the cannabinoid dose and on genetic and individual factors that are not currently understood.

4. Consent

Written informed consent was obtained from the patient for publication of this case report. A copy of the written consent is available for review by the Editor-in-Chief of this journal.

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Scientific Research

In Vitro Anticancer Activity of Plant-Derived Cannabidiol on Prostate Cancer Cell Lines

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Abstract

Cannabinoids, the active components of *Cannabis sativa* Linnaeus, have received renewed interest in recent years due to their diverse pharmacologic activities such as cell growth inhibition, anti-inflammatory effects and tumor regression, but their use in chemotherapy is limited by their psychotropic activity. To date, cannabinoids have been successfully used in the treatment of nausea and vomiting, two common side effects that accompany chemotherapy in cancer patients. Most non-THC plant cannabinoids e.g. cannabidiol and cannabigerol, seem to be devoid of psychotropic properties. However, the precise pathways through which these molecules produce an antitumor effect have not yet been fully characterized. We therefore investigated the antitumor and anti-inflammatory activities of cannabidiol (CBD) in human prostate cancer cell lines LNCaP, DU145, PC3, and assessed whether there is any advantage in using cannabis extracts enriched in cannabidiol and low in THC. Results obtained in a panel of prostate cancer cell lines clearly indicate that cannabidiol is a potent inhibitor of cancer cell growth, with significantly lower potency in non-cancer cells. The mRNA expression level of cannabinoid receptors CB1 and CB2, vascular endothelial growth factor (VEGF), PSA (prostate specific antigen) are significantly higher in human prostate cell lines. Treatment with Cannabis extract containing high CBD down regulates CB1, CB2, VEGF, PSA, pro-inflammatory cytokines/chemokine IL-6/IL-8. Our overall findings support the concept that cannabidiol, which lacks psychotropic activity, may possess anti-inflammatory property and down regulates both cannabinoid receptors, PSA, VEGF, IL-6 and IL-8. High CBD cannabis extracts are cytotoxic to androgen responsive LNCaP cells and may effectively inhibit spheroid formation in cancer stem cells. This activity may contribute to its anticancer and chemosensitizing effect against prostate cancer. Cannabidiol and other non-habit forming cannabinoids could be used as novel therapeutic agents for the treatment of prostate cancer.

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Keywords

Prostate Cancer, Androgen Receptor, Cannabidiol (CBD), Anti-Inflammatory, CB1, CB2, Prostate Cancer Cell Lines

1. Introduction

Prostate cancer is the most commonly diagnosed malignancy in men and the second leading cause of cancer death in males. Most early tumours are androgen-dependent, thus depriving the tumour of androgens via surgical or medical castration [1] has proven to have significant effects at the initial stages of prostate cancer. Despite the early efficacy of androgen ablation, advanced prostate cancer is resilient to such treatments and eventually relapses into a hormone refractory (androgen-independent) disease, with devastating results on morbidity and mortality rates [2]. There is currently no curative treatment against hormone refractory prostate cancer (HRPC). The most effective treatment regime for patients with HRPC, docetaxel-based chemotherapy, can only improve the median survival time by 3 months [3]. Therefore, effective treatment strategies against metastatic HRPC are urgently needed. The current therapies are not only successful in targeting differentiated tumor cells but also sparing the putative cancer stem/progenitor cells [4]. Similar to normal stem cells, cancer stem cells (CSCs) are thought to be quiescent compared with mature cancer cells [5]. This property makes CSCs resistant to chemotherapeutic drugs, which mainly target actively replicating cells. In addition, two recent studies demonstrated that prostate CSCs are androgen independent [6] and may not respond to hormone ablation as mature tumor cells do. Owing to their ability to self-renew and differentiate, CSCs are capable of regenerating the heterogeneous tumor population (with both androgen-dependent and androgen-independent cells) after hormone ablation, which accounts for tumor relapse. Therefore, elimination of the bulk of frequently replicating tumor cells as well as the rare subset of slow, dividing stem-like cells that are responsible for tumor regeneration may represent a better therapeutic strategy in the treatment of prostate cancer.

The therapeutic properties of the hemp plant, *Cannabis sativa*, have been known for many years, but the recreational use of its psychoactive effects has restricted its possible pharmaceutical application. In recent years cannabinoids and their derivatives have drawn renewed attention because of their diverse pharmacologic activities such as cell growth inhibition, anti-inflammatory effects, and tumor regression (Guzman *et al.* 2003) [7]. Cannabinoids have been shown to induce apoptosis in gliomas [8], PC-12 pheochromocytoma [9], CHP100 neuroblastoma [10], and hippocampal neurons [11] *in vitro*, and most interestingly, regression of C6-cell gliomas *in vivo* [12]. Plant-derived cannabinoids, especially cannabidiol, are shown to be potent inhibitors of prostate carcinoma both *in vitro* and *in vivo* [13]. Induction of apoptosis by cannabinoids in prostate and colon cancer cells may be phosphatase dependent [14]. Our case studies confirmed cannabinoid (CB) efficacy in reducing muscle spasticity in multiple sclerosis [15], pain levels over a 12-month period [16] and Cannabis responsive head injury induced multiple disabilities [17].

To date, cannabinoids have been successfully used in the treatment of nausea and vomiting [18], two common side effects that accompany chemotherapy in cancer patients. Nevertheless, the use of cannabinoids in oncology might be somehow underestimated since increasing evidence exist that plant, synthetic, and endogenous cannabinoids (endocannabinoids) are able to exert a growth inhibitory action on various cancer cell types. However, the precise pathways through which these molecules produce an antitumor effect has not been yet fully characterized, also because their mechanism of action appears to be dependent on the type of tumor cell under study. It has been reported that cannabinoids can act through different cellular mechanisms, e.g., by inducing apoptosis, cell-cycle arrest, or cell growth inhibition, but also by targeting angiogenesis and cell migration [7] [19]. Furthermore, the antitumor effects of plant, synthetic and endocannabinoids can be mediated by activation of either CB1 [20] or CB2 receptors or both [21] [22]. After the discovery of the two specific molecular targets for THC, CB1, and CB2 [23], it became clear that most of the effects of marijuana in the brain and peripheral tissues were due to activation of these two G-protein-coupled cannabinoid receptors. However, evidence is also accumulating that some pharmacological effects of marijuana are due to *Cannabis* components different from Tetrahydrocannabinol (THC). Indeed, *C. sativa* contains at least 400 chemical components, of which 66 have been identified to belong to the class of the cannabinoids [23]. The main limitation of the possible future use of THC in oncology

might be represented by adverse effects principally at the level of the central nervous system, consisting mostly of perceptual abnormalities, occasionally hallucinations [24]. However, most non-THC plant cannabinoids seem to be devoid of direct psychotropic properties. In particular, it has been ascertained that cannabidiol is nonpsychotropic [25] [26] and may even mitigate THC psychoactivity by blocking its conversion to the more psychoactive 11-hydroxy-THC [27]. Moreover, it has been recently found that systematic variations in its constituents (*i.e.* cannabidiol and cannabichromene) do not affect the behavioral or neurophysiological responses to marijuana [28]. Finally, it has been also shown that, unlike THC, systemic administration to rats of cannabigerol does not provoke poly-spike discharges in the cortical electroencephalogram during wakefulness and behavioral depression [29]. These and other observations reinforce the concept that at least cannabidiol, cannabigerol, and cannabichromene lack psychotropic activity and indicate that for a promising medical profile in cancer therapy, research should focus on these compounds, which so far have been poorly studied with regard to their potential antitumor effects. By keeping this goal in mind, we decided to investigate the antitumor properties of plant cannabis. We screened two distinct chemically characterized Cannabis extracts (enriched in high cannabidiol and low THC), where the presence of nonpsychotropic cannabinoids along with THC has been reported to mitigate the potential side effects of the latter compound in clinical trials [27].

Despite research efforts, little is known about the prostatic cancer stem cells which were suggested to play an important role in tumor initiation, progression, and chemoresistance [6]. Because CSCs are believed to contribute to chemoresistance, we reasoned that the chemosensitizing effect of cannabis may be mediated through targeting of prostate CSCs. In this study, we show that cannabis extract treatment significantly down regulates protein expression of prostate CB1 and CB2. Meanwhile, Cannabis treatment not only suppresses the spheroid formation ability of prostate cancer cells but also down regulates pro-inflammatory cytokine IL-6 and chemokine IL-8, decreases secreted protein and mRNA expression of prostate-specific antigen and VEGF levels. Our overall findings support that cannabidiol (CBD) may possess anti-inflammatory, anti-CSC effects, and down regulate both cannabinoid receptors CB1 and CB2, leading to their anticancer and chemosensitizing effect against prostate cancer.

2. Materials and Methods

2.1. Chemicals and Reagents

Cannabinoid reference standards for THC, CBD and CBN were purchased from Sigma. For extractions, HPLC grade methanol, formic acid and acetonitrile were purchased from Fischer scientific. All reagents were at least of analytical grade. Stem cell reagent with supplements was purchased from Stem Cell technology, Vancouver. ELISA kits for PSA were purchased from (Roche Diagnostics PSA, Indianapolis, IN).

2.2. Pre-Treatment of Cannabis Samples in the HPLC Method

The cannabis extracts were kindly provided by Green Cross society of BC, Vancouver. Plant material samples were dried for 24 h in a 35˚C forced ventilation oven. Crumbly samples were then ground and mixed. 200 mg of this fine powder was weighed in a flask and extracted with 10 mL of a methanol by agitation during 30 min. The extract was filtered and diluted hundred times with methanol for running on HPLC equipment. Under these conditions suitable chromatography is achieved for the three most abundant cannabinoids present in the samples.

2.3. HPLC Equipment and Chromatographic Conditions

Fifty samples of cannabis were analyzed by HPLC, out of which two were selected with high CBD and low THC. All chromatographic runs were carried out using a Waters 2695 HPLC with a 996 Photodiode array Detector. Full spectra were recorded in the range 228 - 300 nm. Chromatographic separations were achieved using a Waters XTerra® MS C18 analytical column (2.1 × 150 mm column). Equipment control, data acquisition and integration were performed with Empower Pro 2.0 software. The mobile phase at 1 ml/min. consisted of a mixture of water, Acetonitrile and 1% Formic acid. Initial setting was 55% acetonitrile (v/v), which was linearly increased to 85% acetonitrile over 30.5 min, then increased to 95% over next 2 min and 100% over next 0.5 min. After maintaining this condition for 2.5 min, the column was set to initial condition in 0.5 min and re-equilibrated to 55% for another 4.5 min. The total runtime was 40 min. Flow-rate was set to 0.3 ml/min, the injection volume was 10 µl. All experiments were carried out at 30˚C. Calibration curves were run for a number of com-

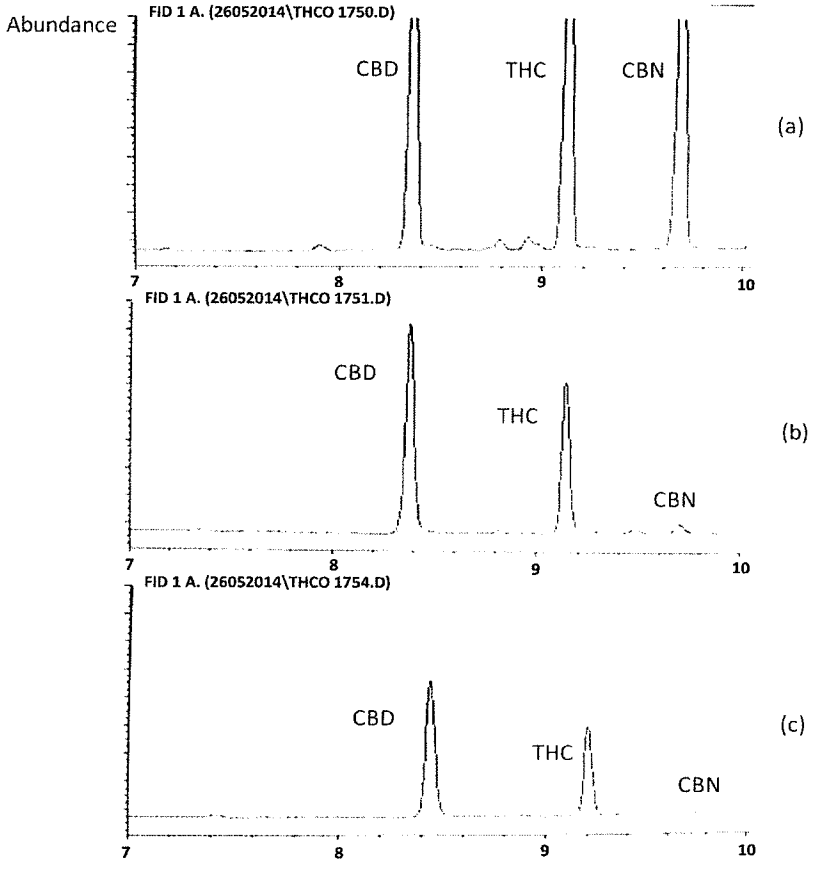
mercial standards (Sigma). Two cannabis extracts with high CBD and low THC were also run on 5890 series 11 Gas chromatograph from Hewlett Packard. Typical chromatograms with peaks showing THC, CBD and CBN are shown in **Figures 1**(a)-(c).

2.4. Cell Lines and Culture Conditions

LNCaP, DU145, PC3, BPH parental and PNT1B parental cells were obtained from American Type Culture Collection (Manassas, VA). PC3, DU145, BPH parental and PNT1B parental cells were cultured in DMEM supplemented with 5% fetal bovine serum. LNCaP were maintained in RPMI 1640 medium (Invitrogen, Carlsbad, CA) supplemented with 5% fetal bovine serum (Invitrogen). Primary cultures of human dermal fibroblasts (Lonza, Walkersville, MD, USA) were cultured in DMEM supplemented with 5% fetal bovine serum.All cell types were kept at 37°C in a 5% CO_2 environment.

2.5. Treatment of Cells

Cannabis (dissolved in DMSO), was used for the treatment of cells. For dose-dependent studies, cells were treated with cannabis at final concentrations of 0.5 - 7 µl/ml of medium (**Figure 4** for concentration of THC,



Samples	Retention time in Minutes		
	CBD	THC	CBN
(a)	8.362	9.125	9.681
(b)	8.377	9.139	9.691
(c)	8.444	9.204	9.768

Figure 1. Example of chromatogram and retention times of the compounds (CBD, THC and CBN) (a) Standard, (b) and (c) Herbal cannabis samples.

CBD and CBN in μg/μl). We also included a control treated with 0.5 - 7 μl/ml of DMSO. It was established that DMSO had no effect on the growth of the spheroids for 24 or 48 hours.

2.6. Cell Viability Assay

Cytotoxic profiles of high CBD extracts were assessed using the MTS™ [(3-(4, 5-dimethylthiazol-2-yl)-5-(3-carboxymethoxyphenyl)-2-(4-sulfophenyl)-2Htetrazolium) and an electron coupling reagent (phenazinemethosulphate; PMS)] cell viability assay (Promega, Madison, WI, USA). Briefly, 5000 cells seeded in wells of 96 well plates (Corning, NY, USA) were allowed to attach overnight, exposed to 0.5 to 7.0 μl/ml cannabis extract for 24 h. To make sure that the vehicle was not affecting cell functions, dimethyl sulfoxide (DMSO) (Sigma) was added to control experiments. The tetrazolium dye was added to each well of the plate and incubated for a further 4 h. Optical density was then measured at 490 nm using a ELX 808 Automated Microplate Reader (Bio-TEK® Instruments, Winooski, VT, USA). All treatments were performed in triplicate. The percentage change in viable cell mass was presented as the OD_{490} ratio between the untreated and treated cells at the indicated concentrations.

2.7. Lipopolysaccharide-Stimulated Cytokine-Related Factorprofiling

Dermal fibroblasts were grown in 12 well plates (Corning) to produce confluent monolayers in DMEM. LPS was added to the culture media at 1 μg/ml for 24 h ± 45 or 20 μg/ml CBD cannabis extract. Conditioned media was harvested for ELISA tests. In data not shown, we observed that medium alone, with or without an equivalent volume of DMSO, and cell-free supernatant derived from control, uninfected cells exhibited indistinguishable levels of basal cytokine/chemokine production.

2.8. Enzyme-Linked Immunosorbent Assays

Sandwich ELISAs for IL-6 and IL-8 (RayBiotech, Norcross, GA, USA) were performed on culture supernatants from the indicated treatments according to manufacturer's instructions. Standard curve was constructed with supplied standards to allow conversion of OD_{450} absorbance readings of experimental samples to pg/ml. All samples were assayed in triplicate.

2.9. Quantitative Real-Time PCR for mRNA Expression of CB1, CB2, PSA, AR and VEGF

Total RNA was isolated from LNCaP cells using RNeasy kit according to the vendor's protocol. The ratio of optical densities of RNA samples at 260 and 280 nm was consistently >1.8. cDNA was synthesized using SuperScript First-Strand synthesis system for RT-PCR(Invitrogen). CB1/CB2 receptors, VEGF, AR and PSA were amplified using real-time PCR system ABI-PRISM 7900 (Applied Biosystems, Foster City, CA). Primers were designed as follows: CB1 receptor forward, 5'-CTCACAGCCATCGACAGGTA-3'; reverse, 5'-CGCAGGTCCTTACTCCTCAG-3'; CB2 receptorforward, 5'-TGCTCTGAGCTTTTCCCACT-3'; reverse, 5'-GGGCTTCTTCTTTTGCCTCT-3'. 18Srna forward, 5'-CGATGCTCTTAGCTGAGTGT-3'; reverse, 5'-GGTCCAAGAATTTCACCTCT-3'; VEGF, forward, 5'-TCTTCAAGCCATCCTGTGTG-3; and reverse, 5'-ATCTGCATGGTGATGTTGGA-3'. Androgen receptor (AR) forward, 5'-AAGACGCTTCTACCAGCTCACCAA; reverse, 5'-TCCCAGAAAGGATCTTGGGCACTT; PSA forward, 5'-ACTCACAGCAAGGATGGAGCTGAA; reverse, 5'-TGAGGGTTGTCTGGAGGACTTCAA.

The cycler was programmed with the following conditions: a) initial denaturation at 94°C for 2 minutes, followed by 35 cycles of b) 94°C for 40 seconds; c) annealing of the primer template at 58°C for 40 seconds; and d) extension at 72°C for 40 seconds.Target gene expression was normalized to 18Srna levels in respective samples as an internal standard and relative transcript quantity was calculated using the ΔΔCt method of Applied Biosystems [30]. Each assay was performed in triplicate.

The calculating equation for ΔΔCt is as follows:

$$\text{Fold change} = 2_T^{-\Delta\Delta C} \tag{1}$$

$$2_T^{-\Delta\Delta C} = \left[\left(\text{CT gene of interest} - \text{CT internal control}\right) \text{ sample A} - \left(\text{CT gene of interest} - \text{CT internal control}\right) \text{sample B}\right] \tag{2}$$

2.10. ELISA for PSA

The human PSA ELISA kit was used for the quantitative determination of PSA levels in culture medium on a Cobas e 411 immunoassay analyzer (Roche Diagnostics PSA, Indianapolis, IN), according to the manufacturer's instruction. This kit uses a technique of quantitative sandwich immunoassay for determination of PSA with an estimated sensitivity of 1 ng/ml PSA antigen.

2.11. Quantification of Apoptosis by Flow Cytometry

The cells were grown at density of 1×10^6 cells in 100 mm culture dishes and were treated with Cannabis extract (0.5 - 7 µl/ml) for 24 hours. The cells were trypsinized, washed with PBS and fixed with ice cold 70% ethanol. Cells were subsequently stained with a propidiumiodide (PI) staining buffer (0.1 mg/ml RNase A, 0.05% Triton-X-100 and 50 µg/ml of PI in PBS) for 1 hour. Samples were analyzed by flow cytometer and BD FACSDiva Software.

2.12. Spheroid Formation Assay

Briefly, cells were trypsinized, washed with 1% PBS and re-suspended in DMEM with 5% FBS. Five hundred cells were added to each well of a 6-well plate (Ultra low Cluster plate, Corning NY 14831). Cells were grown in Stem cell medium with proliferation medium supplemented with heparin and hydrocortisone from Stem cell Technology, Vancouver. Fresh medium with the above supplements was added every week. Cannabis extract (20 µg/µl - 60 µg/µl CBD) was added to the medium and the number of spheroids was counted at the Day 14 of the assay. Each experiment was repeated in triplicate, and each data point represents the mean and SD.

2.13. Statistical Analysis

MTS assay: Differences in cell viability, expressed as the OD ratio between treated and untreated cells at the indicated concentrations, were calculated using a Student's two-tailed t-test assuming equal variances.

Enzyme-linked immunosorbent assays: Results were compared using a one-way analysis of variance followed by a Bonferroni post-test comparing only the pairs of interest. All analysis of variance p-values were significant, and the post-test results are shown in the respective tables.

3. Results

3.1. Expression of Cannabinoid Receptors in Normal and Prostate Cancer Cells

We first compared the expression levelsof both cannabinoid receptors CB1 and CB2 in Prostate epithelial cells (PrEC) and a series of human prostate cancer cells. The data shown in **Figure 2** revealed that expression of both CB1 and CB2 receptors was significantly higher in prostate cancer cells, CB1, 23 fold for LNCap, 90 fold for DUI45, 570 fold for PC3 as compared with normal prostate cells BPH1 and PNT1B PrEC cells (**Figure 2(a)**). CB1 for LNCaP was further upregulated by 841 fold when stimulated with androgen R1881 (**Figure 2(b)**). CB2, 105.8 fold for LNCap, 45 fold for DUI45, 71 fold for PC3 as compared with normal PrEC cells (**Figure 2(c)**). CB2 for LNCaP was further upregulated by 1683 fold when stimulated with androgen R1881 (**Figure 2(d)**).

3.2. Effect of Cannabis Extract on Cell Viability of PrEC (BPH and PNT1B), LNCaP and PC3 Cells

To evaluate the cell viability response of cannabis extract on PrEC, LNCaP and DU145 cells, MTS assay was employed. Data in **Figure 3(a)** & **Figure 3(b)** show that treatment of PrEC cells with cannabis extract (20 - 80 µg/ml CBD) for 24 hours had no effect on cell viability. However, treatment of LNCaP and PC3 cells with similar doses of cannabis extract in a dose-dependent manner significantly decreased the viability at 24 hours (**Figure 3(c)** & **Figure 3(d)**).

3.3. CB1 and CB2 Receptor Activation Signals Growth Inhibition in LNCaP Cells

To study the possible implication of CB1 and CB2 receptors in cannabis extract-induced cell death, mRNA expression of the two receptors were quantified by real time PCR. Treatment of LNCaP cells with cannabis extract

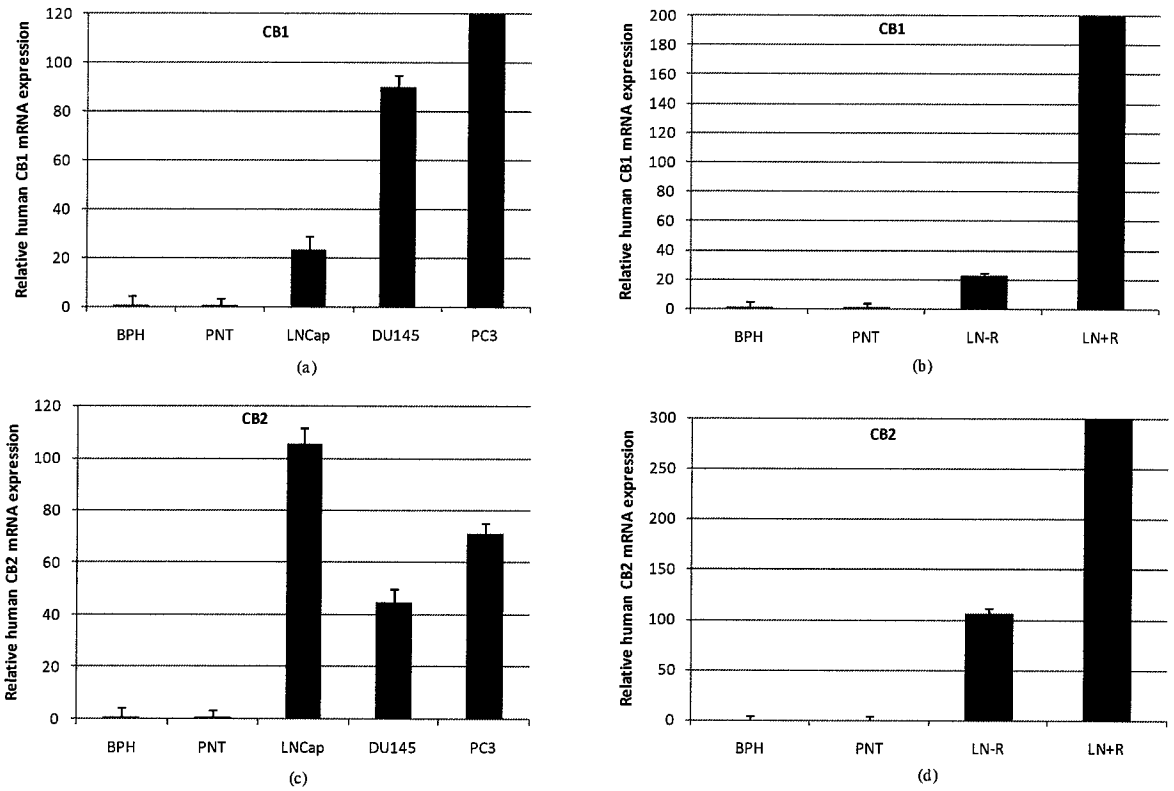


Figure 2. CB1 & CB2 Expression are increased in Prostate cancer cells. Expression of CB1 and CB2 in human prostate epithelial cells and prostate cancer cells. mRNA expression of CB1 and CB2 was determined in androgen dependent LNCaP with and without R1881, androgen independent DU145 and PC3 prostate cancer cell lines. Expression of CB1 and CB2 mRNA was up regulated in PCa cell lines as compared to BPH1 and PNT1B prostate epithelial cell lines.

(20 - 70 µg/mlCBD) for 24H down regulated expression of CB1 and CB2.

These data suggest that both CB1 and CB2 receptors may be involved in cannabis extract mediated growth inhibition and apoptosis (**Figure 4(a)** & **Figure 4(b)**).

3.4. Effect of Cannabis Extract on Androgen Receptor and PSA in LNCaPCells

Androgens are involved in the development and progression of prostate cancer where androgen receptor is assumed to be the essential mediator for androgen action. In the next series of experiments, we determined the effect of cannabis extract on mRNA expression of androgen receptor. In a dose-dependent study, we found that treatment of LNCaP cells with cannabis extract resulted in a marked decrease in androgen receptor expression (**Figure 5(a)**). Studies have also shown that modulation in androgen receptor leads to alteration in androgen-responsive genes [31]. PSA is an androgen-responsive gene and is regarded as the most sensitive biomarker and screening tool for prostate cancer in humans [32]. The dose-dependent effect of cannabis extract on LNCaP cells showed a significant decrease in PSA mRNA expression at 20, 40, 60 and 70 µg/ml CBD concentration when assessing at 24 hours post-treatment (**Figure 5(c)**). We next examined the effect of cannabis extract on secreted levels of PSA in LNCaP cells. Employing ELISA technique, we found that treatment of LNCaP cells with cannabis extract resulted in a dose-dependent decrease in the secreted levels of PSA by 57.9%, 49%, 33.5%, 24.9% and 17.1% at 20, 40, 60 and 70 µg/ml CBD concentrations, respectively (**Figure 5(d)**). From these data, it seems that the decrease in LNCaP cell growth was concomitant with a decrease in androgen receptor mRNA expression as well as a decrease in secreted PSA level.

3.5. Effect of Cannabidiol on VEGF

Because VEGF is a marker for angiogenesis, blocking the angiogenic process may represent a promising way of

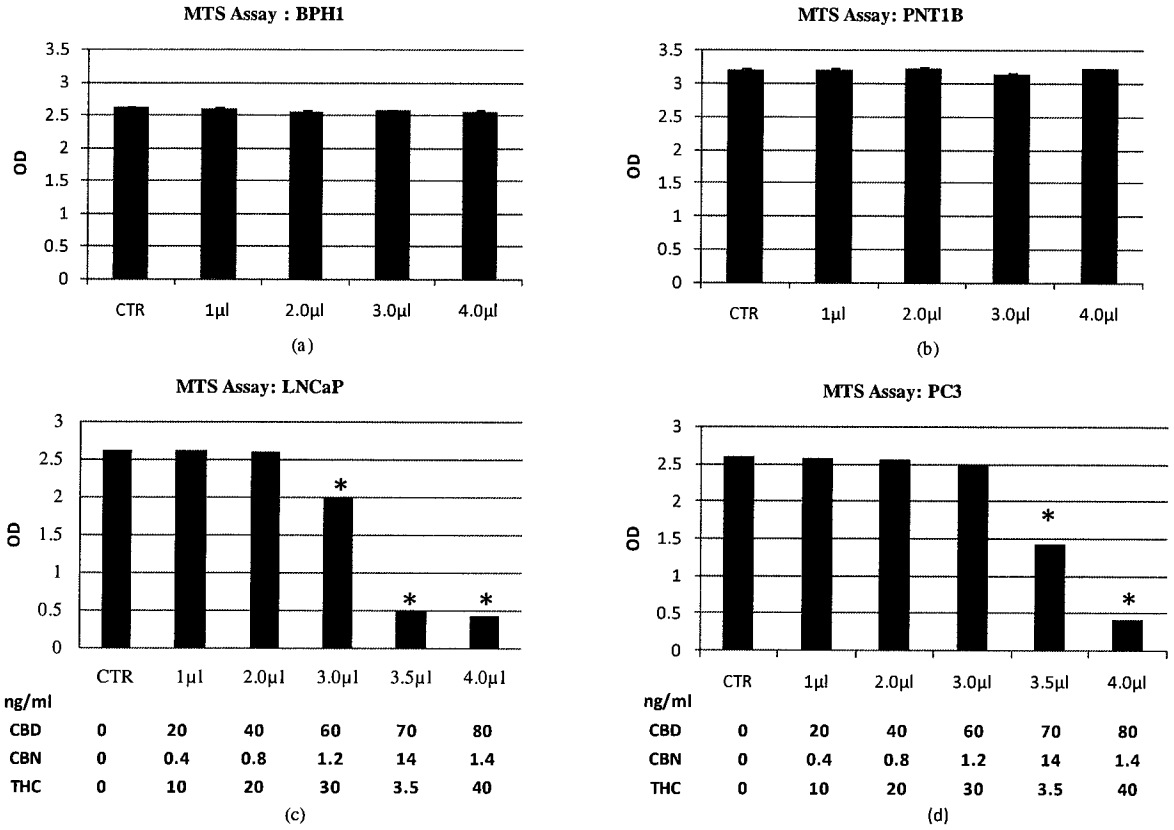


Figure 3. Cannabinoids are selectively cytotoxic to PCa. Effect of cannabis extract on cell viability: A, and B, effects on the viability of PrECs A & B, LNCaP (c) and PC3 (d). As detailed in Materials and Methods, the cells were treated with cannabis extract in different doses for 24 hours, and their viability was determined by MTS assay. Columns, means; bars, ± SE of three separate experiments; $^{*}P < 0.001$ compared with control (0 CBD).

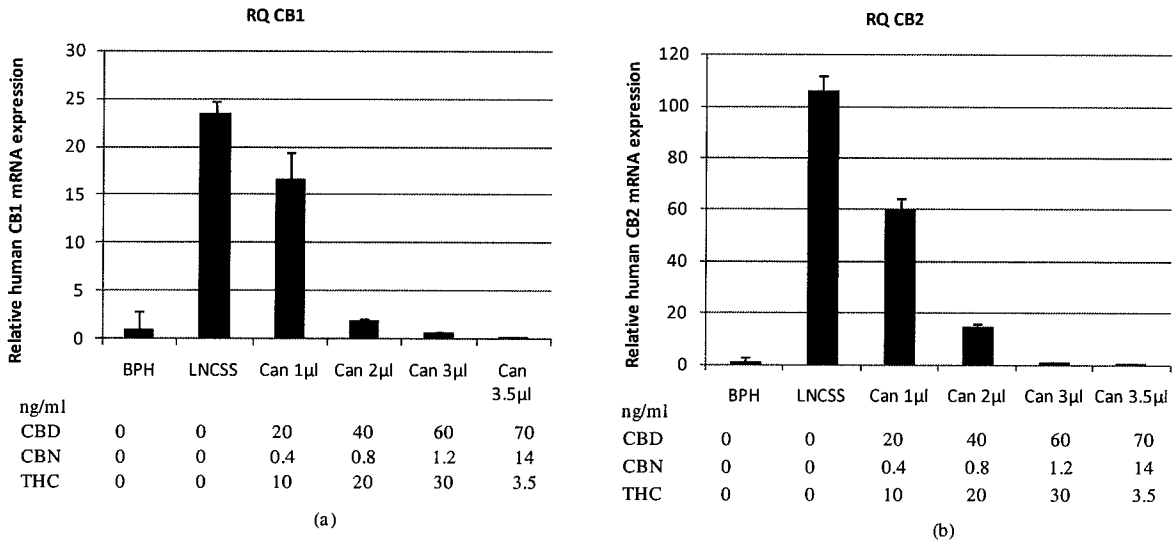


Figure 4. Evidence for cytopathic effects of cannabinoids on PCa cell lines. Effect of cannabis extract on mRNA expression of cannabinoid receptors CB1 and CB2 in human prostate epithelial cells and prostate cancer cells. Dose dependent effect of cannabis extract on mRNA expression of CB1 and CB2 was determined in LNCaP cells by quantitative real time-PCR from representative experiments repeated thrice with similar results. Relative levels of expression normalized to the mRNA level of 18 Srna. The fold change in LNCaP cells were compared with PrEC.

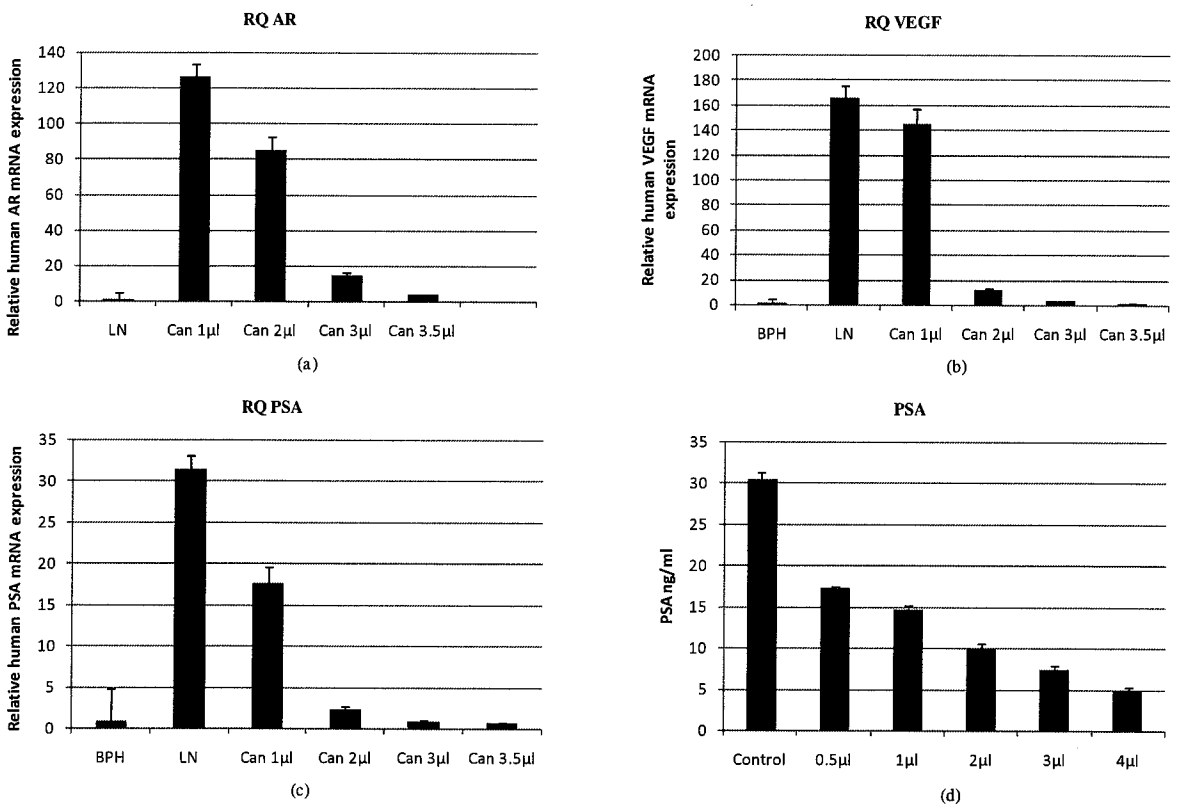


Figure 5. Evidence for cytopathic effects of cannabinoids on PCa cell lines. Effect of cannabis extract on mRNA expression of androgen receptor,VEGF and PSA in LNCaP cells. As detailed in Materials and Methods, the cells were treated with DMSO alone or with specified concentrations of cannabis extract in DMSO and then harvested. Dose dependent effect of cannabis extract on mRNA expression of androgen receptor (a), VEGF (b) and PSA (c) in LNCaP cells was determined by quantitative real time-PCR from representative experiments repeated thrice with similar results. (d) Effect on secreted levels of PSA. Cells were treated with different doses of cannabis extract for 24 hours and then harvested. The PSA levels were determined by ELISA as described under Materials and Methods.

treating the tumor. Studies have shown that androgen regulates VEGF content in prostate cancer [33]. As cannabis extract treatment resulted in a decrease in androgen receptor expression, the effects on VEGF were also determined. It was observed that cannabis extract treatment resulted in a dose dependent decrease in VEGF mRNA expression (**Figure 5(b)**).

3.6. Effect of Cannabis Extract on Apoptosis of LNCaP Cells

We next assessed whether the cell growth inhibitory effect of cannabis extract was associated with induction of apoptosis. We quantified the extent of apoptosis by flowcytometric analysis of the cells labeled with Propidium iodide (PI). LNCaP cells were treated with cannabis extract (20 - 60 µg/ml CBD, 10 - 30 µg/ml THC and 0.4 - 12 µg/ml CBN) for 24 hours. As shown by the data in **Figure 6(a)**, cannabis extract treatment of LNCaP cells resulted in 10.1%, 25% and 42% of apoptotic cells at a dose of 20, 40 and 60 µg/ml CBD respectively. Whereas the induction of apoptosis was almost negligible at the lowest dose (20 µg/ml CBD) used, the highest dose employed (60 µg/ml CBD) resulted in a massive induction of apoptosis. Cannabis extract treatment of PC3 cells resulted in 21%, 21% and 39.4% of apoptotic cells at a dose of 20, 40 and 60 µg/ml CBD respectively (**Figure 6(b)**). Both LNCaP and PC3 showed G0/G1 cell cycle arrest of cells at 40 and 60 µg/ml CBD treatment.

3.7. Anti-Inflammatory Properties of High CBD Cannabis Extract on Dermal Fibroblasts

Prior to assessing anti-inflammatory properties of the cannabis extract, the ability of LPS to induce secretion of key cytokine-related factors (IL-6 and IL-8,) was assessed in dermal fibroblast cultures. After 24 h of LPS

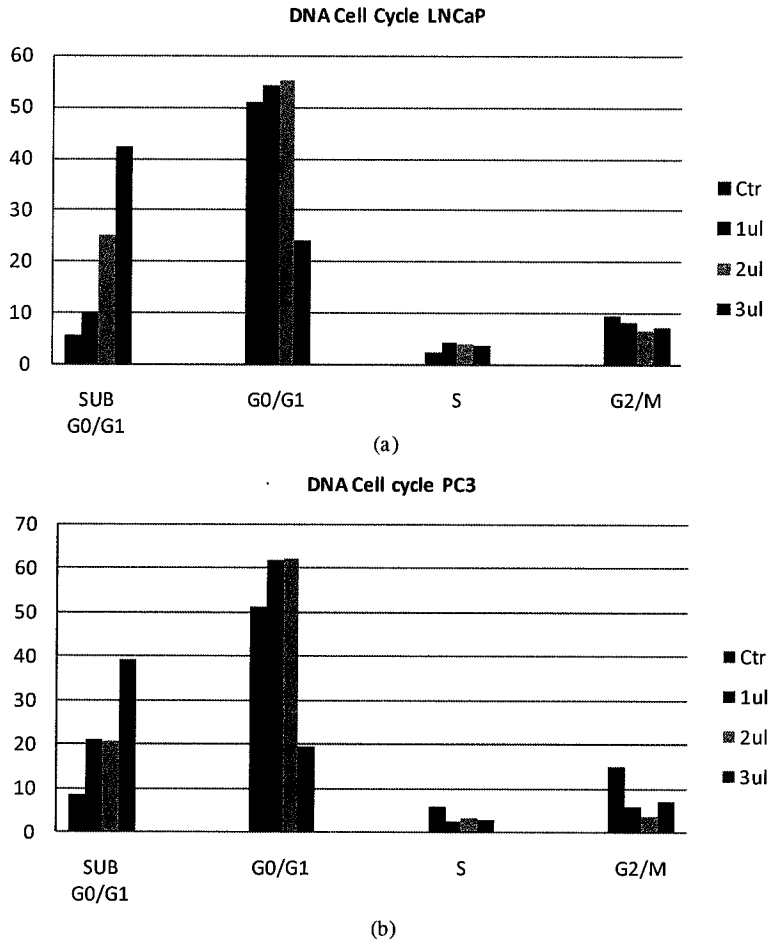


Figure 6. Quantification of apoptosis by Flow Cytometry. Effect of cannabis extract on apoptosis in LNCaP cells. (a) and (b) Quantification of apoptosis in LNCaP and PC3 cells by flow cytometry. The cells were treated with cannabis extract (20 µg/ml of CBD) for 24 hours. The cells were trypsinized, washed with PBS and fixed with ice cold 70% ethanol. Cells were subsequently stained with a propidium iodide (PI) for 1 hour. Samples were analyzed by flow cytometer and BD FACS Diva Software and apoptosis rate in PCa cell lines were calculated as the percentage of cells in sub G0/G1 and Go/G1 phase. Data from representative experiments repeated thrice with similar results.

stimulation, dermal fibroblast conditioned media contained substantially elevated levels of IL-6 and IL-8 (**Figure 7(a)** & **Figure 7(b)**). As an assessment of potential anti-inflammatory properties of cannabis extract, we observed that accumulation of the indicated cytokines in conditioned media of the LNCaP cells was significantly suppressed by 20 µg/ml CBD cannabis extract treatment. MTS assays performed on LNCaP cells at the end of the experiment indicated that none of the LPS or cannabis extract treatment combinations affected cell viability relative to vehicle controls (data not shown). Additional control tests showed that the cannabis extract did not interfere significantly in any of the ELISA steps.

3.8. Cannabidiol Inhibits Prostasphere Formation of LNCaP Cells under Non-Adherent Culture Conditions

The ability to form prostaspheres in non-adherent culture is one of the characteristics of prostate CSCs (Reynolds *et al.* 1992) [34]. To confirm that cannabis extract treatment can inhibit prostate CSC properties, prostasphere formation of LNCaP cells was studied in the presence or absence of cannabis extract. 500 hundred LNCaP

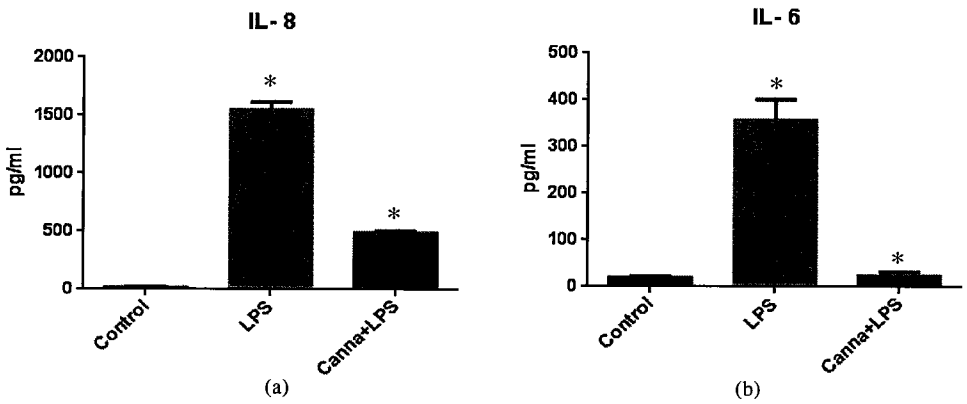


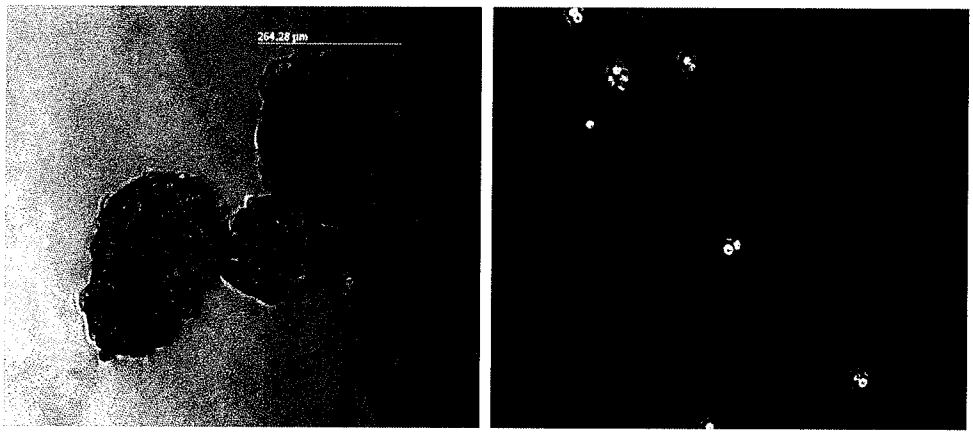
Figure 7. Cytokines/chemokine: Dermal fibroblasts Treatment with Cannabis extract 24 h. Suppression of lipopolysaccharide (LPS)-stimulated accumulation of sentinel cytokines/chemokines by cannabis extract in dermal fibroblasts. Dermal fibroblasts were grown in 12 well plates (Corning) to produce confluent monolayers in DMEM with 5% fetal bovine serum. LPS was added to the culture media at 1 μg/ml for 24 h ± 45 or 20 μg/ml CBD cannabis extract. Accumulation of IL-6 (a) and IL-8 (b), was determined from conditioned media by enzyme-linked immunosorbent assay. Cytokine/chemokine accumulation was compared with levels of LPS-stimulated samples, expressed as pg/ml, $^{*}p < 0.001$.

cells were seeded onto non-adherent 6-well plates and treated with either cannabis extract or vehicle for 14 days. Images of the prostaspheres were captured under the microscope. The number of prostaspheres formed was counted at the end of 14 days. The cannabis extract treatment (20 μg/ml CBD) efficiently suppressed the spheroid formation ability of LNCaP cells. LNCaP cells spheroids in the vehicle measured 150 μm to 250 μm diameter after 14 days. The cells treated with cannabis extract did not form spheroid and appeared as single or group of 2 or 4 cells after 14 days (**Figure 8(a)** & **Figure 8(b)**).

4. Discussion

Prostate cancer is the most frequently diagnosed cancer in western males [35]. The progression of most of the cancers including prostate cancer may be a result of defects in cell cycle and apoptotic machinery. Thus, the agents which can modulate apoptosis in cancer cells may be useful in the management and therapy of cancer. Hence, there is a need to develop novel targets and mechanism-based agents for the management of prostate cancer. One of the most exciting and promising areas of current cannabinoid research is the ability of these compounds to control the cell survival/death decision [7]. In this study, we found that compared with PrEC cells, the expression levels of both cannabinoid receptors CB1 and CB2 were significantly higher in human prostate cells LNCaP, DU145, PC3 (**Figure 2**). These data suggest that CB1 and CB2 receptors could be targets for treatment options for prostate cancer. We also found that cannabis extract treatment of LNCaP and PC3 cells resulted in a decrease of cell viability as determined by MTS assay at varying doses (**Figure 8(c)** & **Figure 8(d)**), suggesting the involvement of both CB1 and CB2 in the anti-proliferative action of cannabinoids. Treatment of LNCaP cells with varying doses of cannabis extract resulted in down regulation of mRNA expression of CB1 and CB2 implicating cytopathic effect of cannabis extract on PCa cells (**Figure 4**). Apoptosis is a physiological and discrete way of cell death and is regarded to be an ideal way of cell elimination. In this study, we also observed an increase in apoptosis of LNCaP cells by treatment with cannabis extract and this was confirmed by flow cytometry (**Figure 6(a)** & **Figure 6(b)**). The observation could be useful for the management of human prostate cancer.

Androgens are essential for the growth, differentiation, and functioning of the prostate as well as in increasing prostate cancer development [36]. The over expression of androgen receptor in prostate cancer may promote cell growth. Hence, elimination or reducing the androgen receptor in prostate cancer should help in treating this neoplastic disease. We further studied the effect of cannabis extract on androgen receptor mRNA expression and its subsequent effect on PSA production. Our results indicate that cannabis extract treatment significantly decreases androgen receptor mRNA expression (**Figure 5(a)**) in LNCaP cells. PSA is an androgen receptor (AR)-regulated serine protease produced by prostate epithelial cells [37], and is the most widely employed



(a) (b)

Figure 8. Effect of cannabis extract on LNCaP prostaspheres formation: Spheroid formation assay was performed with cells treated with cannabis extract or vehicle. 500 LNCaP cells were seeded onto non-adherent 6-well plates and treated with either cannabis extract or vehicle for 14 days. The number of prostaspheres formed was counted at the end of 14 days. (a) Typical LNCaP prostaspheres generated after 14 days and image of the prostaspheres was captured under microscope, 10× magnification. LNCaP prostaspheres in the vehicle measured 150 µm to 264 µm diameter after 14 days. (b) Note that prostaspheres are reduced to single or group of 4 cells in the cannabis extract treated group. The cannabis extract treatment efficiently suppresses the spheroid formation ability of LNCaP cells.

marker in the detection of early prostate cancer. Therefore, agents which could reduce PSA levels may have important clinical implications for prostate cancer. Earlier studies reported that PSA is primarily regulated by androgens [38]. Increased PSA level is used extensively as a biomarker of prostate diseases including prostatitis, benign prostatic hypertrophy, and prostate cancer. It is reported that in LNCaP cells, androgens regulate PSA glycoprotein expression and mRNA via androgen receptor [39]. Our studies show a significant decrease in intracellular mRNA (**Figure 5(c)**) as well as secreted levels of PSA (**Figure 5(d)**) by cannabis extract treatment of cells, suggesting that cannabinoid receptor agonists may be exploited to prevent prostate cancer progression.

VEGF is a ubiquitous cytokine that regulates embryonic vasculogenesis and angiogenesis. Normal prostate epithelium expresses low levels of VEGF, whereas increased levels are reported in prostate carcinoma [40]. Studies have shown that cannabinoid treatment markedly reduced the expression of VEGF in gliomas, the most potent proangiogenic factor and also of angiopoietin 2, which contributes to the angiogenic process by preventing vessel maturation [41]. Our results showed that treatment of LNCaP with cannabis extract inhibits VEGF mRNA expression in LNCaP cells (**Figure 5(b)**).

Chronic inflammation has been linked to various steps in tumor formation, including cellular transformation, proliferation, invasion, angiogenesis, and metastasis [42]. Among the inflammatory cytokines, interleukin 1 (IL-1), IL-6, IL-8, and IL-10 have been reported as present in the prostate cancer cells [43], indicating the significance of these inflammatory factors in prostate cancer progression. Therefore, controlling inappropriate inflammation would appear to be one strategy that might help control cancer progression. Prior to assessing anti-inflammatory properties of cannabis extract, the ability of LPS to induce secretion of key cytokine-related factors (IL-6 and IL-8) was assessed in dermal fibroblasts. After 24 h of LPS stimulation, dermal fibroblast conditioned media contained substantially elevated levels of cytokine IL-6 and chemokine IL-8 (**Figure 7(a)** & **Figure 7(b)**). Our results show that accumulation of the indicated cytokines in conditioned media of dermal fibroblasts was significantly suppressed by cannabis extract treatment confirming potential anti-inflammatory property of the high CBD cannabis extract preparation that may be considered to target chronic inflammation in prostate cancer.

Prostate cancer cells are in general highly resistant to common chemotherapeutic agents and the presence of CSCs is suggested to contribute to chemo resistance. The ability to form spheres in non-adherent, serum free conditions is a key property of stem cells (Reynolds *et al.* 1992) [34]. In this study, prostate cancer cell line LNCaP was able to form spheroids in non-adherent culture, suggesting the presence of cancer stem-like cells within these cell lines. Because prostaspheres are enriched with CSCs [44], the inhibitory effect of cannabis ex-

tract on prostasphere formation supports that high CBD and low THC cannabis extract may be a potent agent in targeting or eliminating prostate cancer stem-like cells *in vitro* (**Figure 8**).

Recently, cannabinoids have received considerable attention due to their diverse pharmacologic activities such as cell growth inhibition, anti-inflammatory effects, and tumor regression. Our results suggest that treatment of androgen-responsive human prostate carcinoma LNCaP cells resulted in a decrease in intracellular and secreted levels of PSA, with concomitant inhibition of androgen receptor, cell growth, and induction of apoptosis. The data also demonstrate that cannabis extract is capable of significantly suppressing the expression of specific pro-inflammatory cytokine/chemokine in human dermal fibroblast cells. The study explains usefulness of LPS-stimulated *in vitro* systems for the evaluation of the anti-inflammatory properties of plant extracts using this methodological approach [45] [46]. To conclude, the data presented in this paper provide further support for the concept that traditional medicines, such as cannabis, can be valuable additions to the modern therapeutic armamentarium, and non-habit-forming cannabinoid agonist(s) which lack psychotropic activity may be used for the management of prostate cancer.

Conflict of Interest

There is no conflict of interest to be declared.

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