Alexandria Journal of Medicine (2014) 50, 241–251

HOSTED BY

Alexandria University Faculty of Medicine

Alexandria Journal of Medicine

http://www.elsevier.com/locate/ajme

Protective and therapeutic effects of cannabis plant extract on liver cancer induced by dimethylnitrosamine in mice

Neveen Abd El Moneim Hussein [a,*], Mervat Abd El-Fattah El-Toukhy [a], Amany Hussein Kazem [b], Mahmoud El-Said Ali [c], Mohammad Abd El-Rahman Ahmad [d], Hossam Mahmoud Rashad Ghazy [a], Ahmed Mohamed Gamal El-Din [a]

[a] *Applied Medical Chemistry, Applied Medical Chemistry Department, Medical Research Institute, Alexandria University, Alexandria, Egypt*
[b] *Department of Pathology, Medical Research Institute, University of Alexandria, Alexandria, Egypt*
[c] *Toxicology Department, Forensic Science College, Naif Arab University for Security Sciences, Saudi Arabia*
[d] *Clinical Pathology Department, Medical Military Academy, Alexandria, Egypt*

Received 26 December 2013; accepted 19 February 2014
Available online 23 May 2014

KEYWORDS

Hepatocellular carcinoma;
Cannabis;
mTERT;
Caspase-8

Abstract Hepatocellular carcinomas will emerge as a major form of malignancy in the coming decades. When these tumors are in advanced stages, few therapeutic options are available. Therefore, it is essential to search for new treatment modalities to fight this disease.

Aim: Evaluate the possible protective and therapeutic effects of Cannabis extract on dimethylnitrosamine (DMNA)-induced hepatocarcinogenicity in mice.

Methods: Seventy-five male mice were divided into five groups of 15 each: group I mice received corn oil only as the control group; group II mice were injected intraperitoneally with DMNA (10 μg/kg body weight) weekly for 12 weeks; group III mice were pretreated orally with cannabis extract (0.5 ml/kg body weight) every other day for two weeks before the injection of DMNA, and continued until the end of the experiment (12 weeks); group IV mice were treated orally with cannabis extract every other day simultaneously with DMNA injection and continued until the end of the experiment; group V mice were treated orally with cannabis extract every other day after receiving the last intraperitoneal injection of DMNA. A real time PCR was used to quantify telomerase reverse transcriptase and caspase-8 m-RNA expression level.

Abbreviations: HCC, hepatocellular carcinoma; hTERT, human telomerase reverse transcriptase; hTR, human telomerase RNA; CND, cannabinoids; Δ^9THC, tetrahydrocannabinol; CBD, cannabidiol; DMNA, dimethylnitrosamine.

* Corresponding author. Address: Applied Medical Chemistry Department, Medical Research Institute, Alexandria University, 165 El Hadra Avenue, Alexandria, Egypt. Tel.: +20 122 3667426.
E-mail addresses: neveen39@yahoo.com (N.A.E.M. Hussein), medhatanwar@gmail.com (M.A.E.-F. El-Toukhy), amanikazem@hotmail.com (A.H. Kazem), mahtox2007@yahoo.com (M.E.-S. Ali), mohammad_rhman1963@yahoo.com (M.A.E.-R. Ahmad), Semo_abdo@yahoo.com (H.M.R. Ghazy), dr.biochemist@ymail.com (A.M.G. El-Din).

Peer review under responsibility of Alexandria University Faculty of Medicine.

http://dx.doi.org/10.1016/j.ajme.2014.02.003

Results: As compared to the control group, mTERT mRNA expression level was significantly increased in group II. The gene in groups (III, IV, and V) was insignificantly higher than the control group but it was significantly decreased as compared to group II. The caspase-8 mRNA expression level was significantly decreased in all groups as compared to the control group. As compared to group II, caspase-8 mRNA level was significantly increased in group III.

Conclusion: The protective effect of cannabis extract is more pronounced in group taking cannabis before DMNA. Cannabinoids might exert their anti-tumor effects by the direct induction of apoptosis and can decrease telomerase activity by inhibiting the expression of the TERT gene. Coordination between inhibition of telomerase activity and induction of apoptosis might be a potential therapeutic agent for cancer treatment.

1. Introduction

Hepatocellular carcinoma (HCC) is one of the most common solid tumors and the third leading cause of cancer-related death worldwide.[1] Its prognosis remains reserved, with a 5-year survival rate of $<5\%$.[2] Hepatocarcinogenesis is a multi-step process involving different genetic alterations that ultimately lead to malignant transformation of the hepatocytes.[3,4] One of the molecular events that underlie the multigenetic process of hepatocarcinogenesis is the activation of human telomerase reverse transcriptase (hTERT) which is normally suppressed in most human somatic tissues after birth.[5,6]

The replicative potential of eukaryotic cells is regulated through specialized DNA structures called telomeres, which cap the ends of the chromosomes. Telomerase is a ribonucleoprotein complex composed of two essential components: a catalytic subunit with reverse transcriptase activity (hTERT) and RNA subunit with human telomerase RNA (hTR). In the adult organism, telomerase expression is restricted to a few cell types, most notably germ cells and stem/progenitor cells. Telomerase has been a target of increasing interest because high telomerase activity is one of the mechanisms that sustain the unlimited growth of cancer cells.[7,8] The hTERT gene encodes the catalytic subunit of telomerase, which mediates pleiotropic effects, including the regulation of senescence and proliferation and plays an important role in carcinogenesis.[9]

Programed cell death (apoptosis) is a potent mechanism that limits the expansion of tumor cells by triggering their suicide, while defects in apoptosis underpin both tumorigenesis and metastasis.[10] Caspase-8 belongs to the caspase family of proteases and plays a key role in the regulation of apoptosis during normal development as well as in adult life. Since signaling via the death receptor (extrinsic) pathway critically depends on caspase-8, the distribution of caspase-8 expression or function may contribute to human diseases.[11]

Cannabis (bhang, ganja, charas, hashish, kif, marijuana etc.) is one of the popular plants among common people since time immemorial due to its various uses and abuses. The hemp plant Cannabis sativa produces unique compounds known as cannabinoids (CND).[12] The most important cannabinoids found in the cannabis plant are tetrahydrocannabinol (Δ^9THC) and cannabidiol (CBD). Cannabinoids have been shown to be effective in the treatment of nausea and vomiting associated with cancer chemotherapy, anorexia and cachexia seen in HIV/AIDS patients, as well as neuropathic pain, and spasticity in multiple sclerosis.[13,14]

Therefore, the aim of the present study was to evaluate the possible protective and therapeutic effects of Cannabis extract on dimethylnitrosamine-induced hepato-carcinogenicity in mice through studying TERT m-RNA, caspase-8 m-RNA gene expression levels, alanine transaminase (ALT), aspartate transaminase (AST) and gamma-glutamyl transpeptidase (γ-GT) activities.

2. Materials and methods

2.1. Preparation of plant extract

Cannabis sativa plant (the flowering tops of plants) was obtained by permission from the Public Prosecutor. One hundred gms of dry cannabis plant were minced into very small pieces and boiled with water for 5 min. The boiling water was discarded and the minced plant was left to dry at room temperature. Extraction was carried out by boiling the dried minced plant with 100 ml of ethyl alcohol for 10 min followed by filtration. The filtrate was transported into a specific apparatus used to evaporate cannabis residue by heating at specific temperatures (180–220 °C) to obtain cannabinoids free of some carcinogenic compounds (such as benzopyrene, benzene, toluene and naphthalene). The residue was heated up to 180 °C, the vapor was collected in petroleum ether (for trapping hydrocarbons) and then discarded. On raising the temperature from 180 °C to 220 °C the resulting vapor (contains cannabinoids) was received in methyl alcohol (trapping agent).[15]

Mass spectroscopy coupled with gas chromatography (GC/MS)[16] was used as a method for detection of the received cannabinoids using column hp 5, capillary 30 m, GC type is Agilent 6890N, mass is Agilent 5973 N. Temperature program: start of 50 °C for 3 min, then temperature was increased at a rate of 50 °C/min up to 280 °C for 25 min. Injection temperature = 250 °C, detector temperature = 280 °C, injection volume = 3 µl and wavelength λ = 50–550 M/Z (M = molecular weight, Z = charge). Methyl alcohol was evaporated and the cannabinoids' residue was dissolved in 100 ml of corn oil until time of use.