Original Article

Neuroprotective activities of three cannabinoids separated from confiscated cannabis in Thailand

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Abstract

Introduction: The female flower of *Cannabis sativa* L. (Cannabinaceae) contains many cannabinoids that possesses the psychological effects. Therefore, the scientific studies were still in dispute between usefulness and uselessness.

Method: The present study was designed to isolate the major cannabinoids compounds from confiscated cannabis and examine the neuroprotective activities using two models; serum deprivation method and co-administration of hydrogen peroxide assay.

Results: Three cannabinoids (CBD, CBN and THC) were isolated from the confiscated cannabis. CBD and CBN significantly protected the cultured neurons from the death caused by deprived serum under H_2O_2 induced oxidative stress. Significant difference was observed between the percentage of neuron viability when treated with 10 ng/ml (31.8 nM) CBD or 100 ng/ml (322 nM) CBN under oxidative stress conditions.

Conclusion: CBD was effective as a neuroprotective substance against neurodegenerative disorders such as Parkinson's and Alzheimer's diseases.

Keywords: *Cannabis sativa*, Cannabinoids, Neuroprotective

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Introduction

The term "*Cannabis*" encompasses all types of *Cannabis sativa* L. (Cannabinaceae) and also variants thereof, including *cannabis* chemovars or cultivars. *C. sativa* has many subspecies including the variants var. *indica* and var. *sativa*.[1] *C. sativa* naturally contains different amounts of various cannabinoids and non-cannabinoids compounds especially in female flowers. *C. sativa* has been used for a long time for medicinal and recreational purposes.[2] However, scientific evidences of the chemical constituents in *C. sativa* have led to FDA-approved medications that contain two active compounds tetrahydrocannabinol (THC) and cannabidiol (CBD).[2]

In Thailand, *C. sativa* or Ganja is classified as a psychoactive substance together with magic mushrooms and Kratom under the Narcotics Act B.E. 2522.[3] Since December 2018, Thailand became the first country in Southeast Asia to legalize medical cannabis in term of special access scheme (SAS).[4] Because *Cannabis* plant contains active substances that may help treat a range of illnesses and symptoms. Currently, there is a growing number of government sites possessing legalized cannabis for medicinal purpose. However, under uncontrollable circumstance, there may be some of confiscated cannabis for recreational purpose.

Therefore, the scientific studies were still controversy between harmful and helpful. This research was aimed to examine the neuroprotective effects of three cannabinoids which are the major chemical constituents of confiscated cannabis. The extraction was made by two methods 1) solvent extraction and 2) supercritical carbon dioxide extraction. The isolation of cannabinoids was accomplished to two methods 1) column chromatography and 2) preparative high performance liquid chromatography. The *in vitro* assays were determined to two assays; 1) viability assay using XTT reduction assay and 2) neuroprotective assay using serum deprivation method and co-administration of H_2O_2 assay reacted with P19-derived neurons.

Materials and Methods

Collection and Identification of *Cannabis* Plant

The leaf and female flower of confiscated *Cannabis* in Thailand, were kindly provided by the Narcotics Suppression Bureau, Royal Thai Police and legally authorized by Office of the Narcotics Control Board, Ministry of Public Health. The *Cannabis* plant materials were identified with the authentic specimens at the office of forensic science, Royal Thai Police. The voucher specimen (RSU-CSI-NK01) was kept in Department of Pharmacognosy, Rangsit University for future reference.

Extraction of *Cannabis* Plant material

The cannabis plant material was divided to extract by two extraction methods. Firstly, the leaf and female flower of *Cannabis* were ground using mortar and pestle and homogenized using a blender. One kilogram of the pulverized sample was extracted in 95% Ethanol and sonicated in ultrasonic bath for half an hour. The temperature of extraction was 45°C. After filtration with white linen, the filtrate was then evaporated to remove the solvent by vacuum rotary evaporator. Finally, the concentrated ethanolic extract (CEE) was obtained. Total yield of crude extract was 11%w/w of plant dry weight. Secondly, leaf and female flower of *Cannabis* was crushed, glided, and sieved through an 18-mesh sieve exceeding 1 mm. Two hundred fifty grams of fine sample was packed into a cellulose bag and placed in the extraction chamber. Then, the extractor using supercritical carbon dioxide as the whole solvent was set at designed temperature (40 °C) and pressure (200 MPa) for 90 min. Finally, the supercritical fluid extract (SCF) was obtained. Total yield of SCF extract was 0.15%w/w of plant dry weight. Since the SCF had very low yield, the concentrated ethanolic extract (CEE) was selected for further study on isolation of cannabinoids.

Isolation of *Cannabis* Crude extract

The brown viscous concentrated ethanolic extract (CEE 11 g) was transferred to a conventional glass chromatography with silica gel as the stationary phase using hexane: absolute ethanol (9:1) as the mobile phase. Four fractions were obtained (CES 1-4). Fraction CES1 was further subfractionated to give 5 extracts. The similar fractions were combined and stored at -20 °C until analysis and purification.

Separation Parameters

Preparative HPLC was performed with the Prep 150LC System using the methodology described in the previous study.[5] The mobile phase was gradient system starting with 90% methanol, increasing to 99% after running for 17 min, then decreasing to 90% methanol at time interval of 26 min, and maintaining to this condition until 35 min of experiment. The stationary phase was reversed phase using ACE 10 C18-AR column (25cm x 21.2 mm). Flow rate was scaled to 10 mL/min. The detector was YL9160S PDA at 210 nm wavelength. The fraction collectors were Foxy R1 and Foxy R2. The collection parameters were based on peak threshold within a window.

The biological assay on cultured P19-derived neuron

Cell culture

P19 cells were grown in alpha minimal essential medium (α-MEM) supplemented with 7.5% newborn calf serum (NCS), 2.5% fetal bovine serum (FBS), and 1% antibiotics-antimycotic solution in a 5% CO_2 humidified atmosphere, at 37 °C. Cells in monolayer cultures were maintained in exponential growth by subculturing every 2 days.[6]

Differentiation of P19 cells into P19-derived neurons

Exponentially grown cultures were trypsinized and dissociated into single cells. P19 cells (2×10^6 cells/mL) were then suspended in 10 mL α-MEM supplemented with 5% FBS, 1% antibiotics-antimycotic solution and 0.5 μM all trans-retinoic acid (RA) and seeded onto a 100-mm bacteriological culture dish. The cells formed large aggregates in suspension. After 4 days of RA treatment, aggregates were dissociated by 5-mL glass measuring pipette, re-plated on poly-L-lysine-pre-coated multi-well plates (multi-well plates were coated with 50 μg/mL poly-L-lysine dissolved in PBS for overnight and steriled under UV light for 30 min) at 7×10^4 cells/mL (150 μL/well in 96-well plate), in α-MEM supplemented with 10% FBS, and 1% antibiotics-antimycotic solution and incubated for 24 h. Cytosine-1-β-D-arabinoside or Ara-C (10 μM) was added at day one after plating and the medium was changed every 2-3 days. The differentiated cholinergic neuronal cells, P19 derived-neurons, were used after day 14 of the differentiation process[6-8]

Chemicals

P19 cell line ATCC CRL-1825 was obtained from American Type Culture Collection, USA. Alpha minimal essential medium (α-MEM), fetal bovine serum (FBS), newborn calf serum (NCS), and antibiotics-antimycotic solution were purchased from Gibco, USA. All trans-retinoic acid, cytosine-1-β-D-arabinoside, 1:250 porcine trypsin, poly-L-lysine (MW > 300,000), XTT (2,3-bis(2-methoxy-4-nitro-5-sulphonyl)-2H-tetrazolium-5-carboxanilide sodium), and phenazine methosulfate (PMS) were obtained from Sigma, USA. Dimethylsulfoxide (DMSO) cell culture grade and analytical grade methanol were purchased from Merck, Germany. 96-well plates were purchased from Corning, USA. The 100-mm Bacteriological culture dish was obtained from Hycon, USA.

Viability assay[9-13]

The assay was carried out on P19-derived neurons cultured in a 96-well plate. After 14 days of differentiation process, the P19SM (α-MEM supplemented with 10% FBS, 10 μM Ara-C, and 1% antibiotics-antimycotic solution) was removed and DMSO solutions of the sample, diluted with the P19SM were added to give the concentrations of 10,000, 1,000, 100, 10 and 1 ng/ml. The 0.5% v/v DMSO in

P19SM was used as control. The cells were incubated in a 5% CO_2 humidified atmosphere for 18 h at 37 °C. Then 150 μL of the medium was removed and 50 μL of XTT solution (1 mg/mL XTT in 60 °C α-MEM + 25 μM PMS) was added. After incubated at 37 °C for 4 hours, the 100 μL of PBS (phosphate buffer saline solution) pH 7.4 was added. The OD value was determined with microplate reader at 450 nm. The data were expressed as the mean ± SE (n = 3). The samples that significantly enhanced survival of cultured neurons higher than control will be further investigated for their neuroprotective ability.

Neuroprotective assay[9-14]

The assays were carried out on P19-derived neurons cultured in a 96-well plate and performed in triplicate.

Serum deprivation method:

The DMSO solution of the extracts diluted with the α-MEM supplemented with 10 μM Ara-C, and 1% antibiotics-antimycotic solution without FBS was added to give the final concentration of the extract at concentration that enhanced survival of cultured neurons higher than control. The 0.5% v/v DMSO in P19SM was used as control. The α-MEM supplemented with 10 μM Ara-C, and 1% antibiotics-antimycotic solution without FBS was used to give oxidative stress condition. The cells were incubated for 18 h at 37 °C. Cell viability was assayed by XTT reduction method. The data were expressed as the mean ± SE (n = 3) .[8-12] Quercetin at concentration of 1 nM was used as positive control.[14]

Co-administration of H_2O_2 assay:

The DMSO solution of the extracts diluted with P19SM was added to give the final concentration of the extract at concentration that enhanced survival of cultured neurons higher than control. The 0.5% v/v DMSO in P19SM was used as control. The 5 mM H_2O_2 in P19SM was used to give oxidative stress condition. The 5 mM H_2O_2 and the extracts in P19SM were added together in co-administration assay. The cells were incubated in a 5% CO_2 humidified atmosphere for 18 h at 37 °C. Cell viability was assayed by XTT reduction method. The data were expressed as the mean ± SE (n = 3). Quercetin at concentration of 1 nM was used as positive control.[14]

Statistical analysis:

Average viability of the neurons was statistically analyzed by Fisher's LSD to compare the statistical significance between the control or oxidative stress conditions and experimental groups. Differences were considered significant only when the *P*-value was less than 0.05.

Results

1. Separation of cannabinoids

Separation by preparative chromatography was based on the principle that different cannabinoids travel through a specific stationary phase at different speeds. The substances to be separated and purified were eluted and collected successively, as illustrated in Figure 1. The cannabis ethanolic extract contained three major cannabinoids (compounds A, B, C) which were identified as cannabidiol (CBD), cannabinol (CBN), and tetrahydrocannabinol (THC) (Figure 2) at retention time 10.97, 17.04, and 19.20 min, respectively as in previous study with the comparable authentic sample.[15]

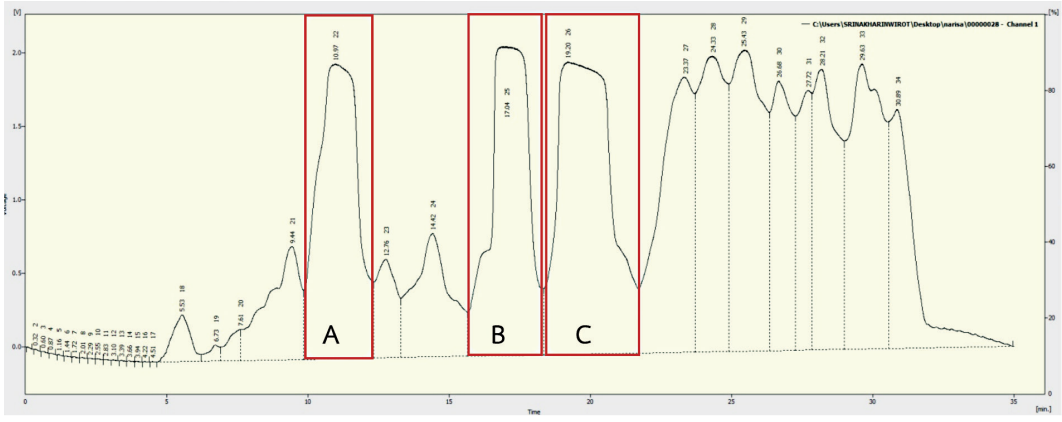
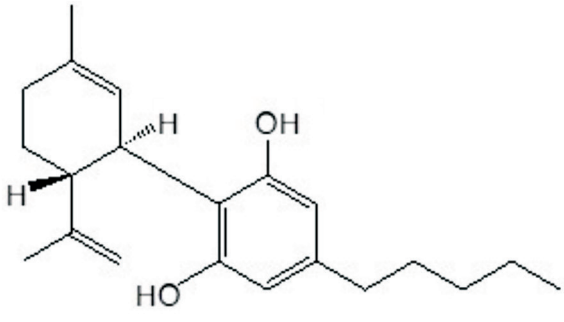
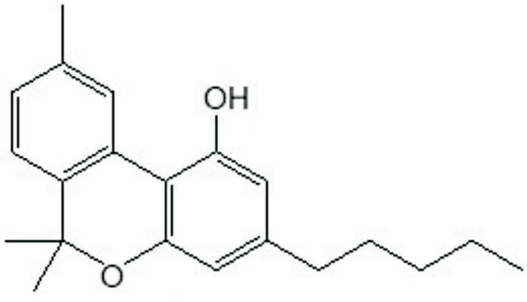


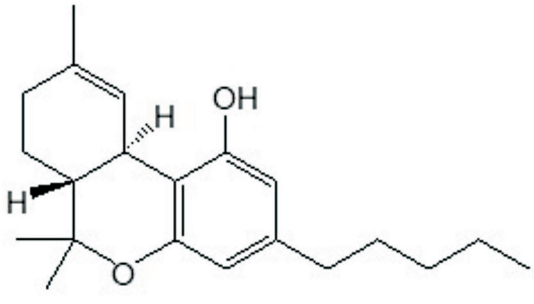
Figure 1 ChromScope preparative HPLC chromatogram showing collection simulation and three major com pounds A, B, C.



A = CBD



B = CBN

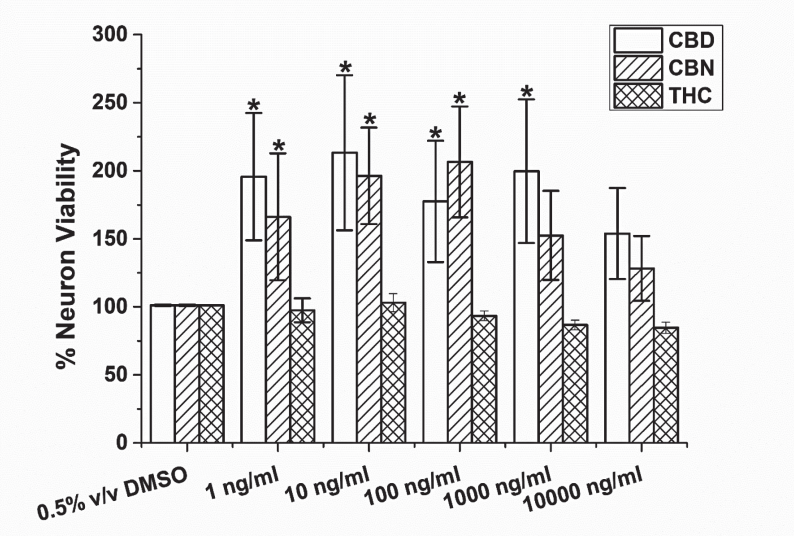


C = THC

Figure 2 Chemical structures of CBD, CBN and THC (compounds A, B and C, respectively)

2. Viability assay

P19-derived neurons culture was treated with 1-10,000 ng/ml of CBD, CBN, and THC. The neuronal viability was determined by XTT assay. The results were expressed as % cell viability ± SE. The concentrations tested at 1-1,000 ng/ml of CBD and 1-100 ng/ml of CBN significantly enhanced the viability of the neurons (% neuron viability of CBD ranged from 177.60 ± 44.55% - 213.29 ± 56.91% and % neuron viability of CBN ranged from 166.13 ± 46.61% - 206.53 ± 40.72%) which were higher than that of the control (% neuron viability of control was 101.15 ± 0.58%). However, THC at 10 ng/ml promoted viability of the neuron at 103.01 ± 6.67% but was not significant when compared to the control ($P < 0.05$). Therefore, only CBD at 10 ng/ml and CBN at 100 ng/ml which exhibited the highest % neuron viability at 213.29 ± 56.91% and 206.53 ± 40.72%, respectively, were selected for further investigation on their neuroprotective ability (Figure 3).

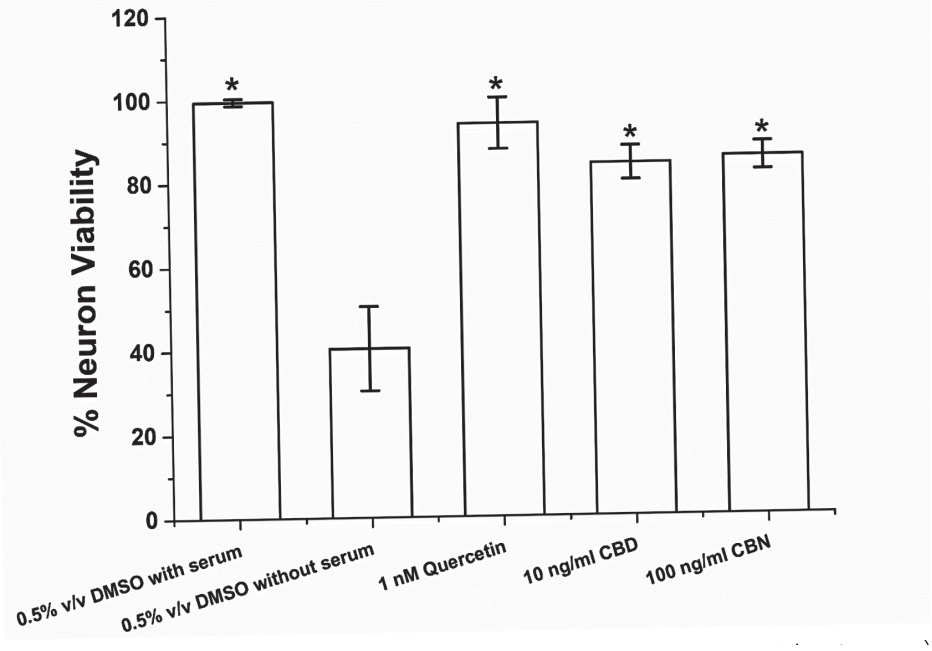


* $P < 0.05$ compare with control (0.5% v/v DMSO in P19SM)

Figure 3 P19-derived neurons cell viability in various concentration (1-10,000 ng/ml) of CBD (▭), CBN (▨) and THC (▩).

3. Serum deprivation method

P19-derived neurons culture was treated with 10 ng/ml (31.8 nM) of CBD and 100 ng/ml (322 nM) of CBN compared with toxic condition (0.5% v/v DMSO in α-MEM supplemented with 10 μM Ara-C, and 1% antibiotics-antimycotic solution without serum) and positive control (1 nM quercetin). Cell viability was assayed by XTT reduction method. Both compounds significantly protected the cultured neuron from death caused by oxidative stress due to serum deprivation (% neuron viability of CBD = 83.95 ± 4.03% and CBN = 85.32 ± 3.31%) when compared with the toxic condition (40.31 ± 10.10%) (Figure 4). The % neuron viability when treated with 1 nM quercetin (positive control) was 93.72 ± 6.13%. No significant difference was found between the % neuron viability when treated with 10 ng/ml (31.8 nM) CBD or 100 ng/ml (322 nM) CBN and 1 nM quercetin.



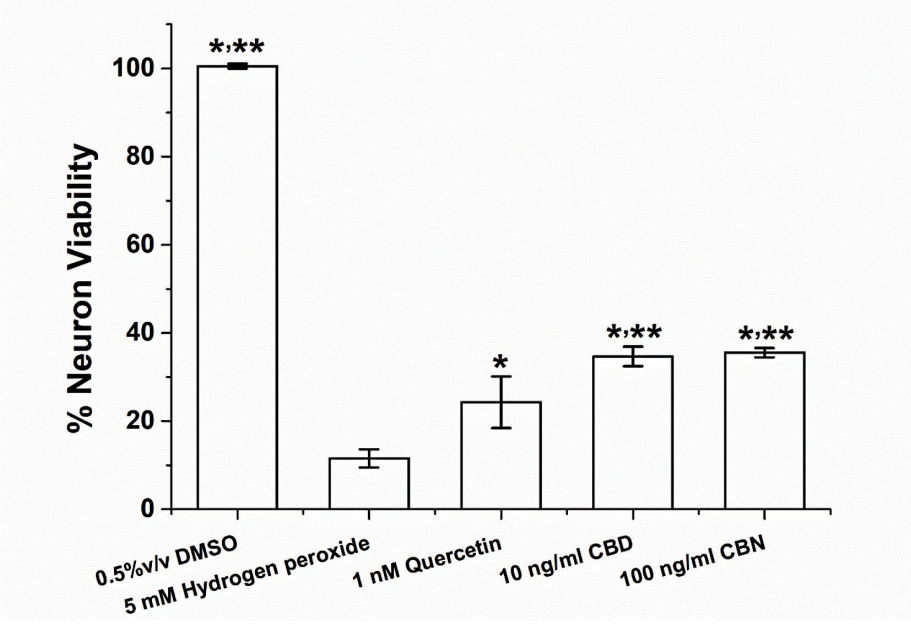
* $P < 0.05$ compare with toxic condition (0.5% v/v DMSO in α-MEM without serum)

** $P < 0.05$ compare with positive control (1 nM quercetin in α-MEM without serum)

Figure 4 P19-derived neurons cell culture viability in 10 ng/ml CBD, 100 ng/ml CBN compared with the positive control (1 nM quercetin) and toxic condition (0.5% v/v DMSO in α-MEM without serum).

4. Co-administration of H_2O_2 assay

P19-derived neurons culture was co-treated with 10 ng/ml (31.8 nM) of CBD or 100 ng/ml (322 nM) of CBN and 5 mM H_2O_2 compared with the toxic condition (5 mM H_2O_2). Cell viability was assayed by XTT reduction method. CBD and CBN significantly protected the cultured neuron from the death caused by H_2O_2 induced oxidative stress (% neuron viability of CBD = 34.66 ± 2.23% and CBN = 35.52 ± 1.06%) compared with the toxic condition, 5 mM H_2O_2 (11.54 ± 2.06 %) (Figure 5). The % neuron viability when co-treated with 1 nM quercetin (positive control) and 5 mM H_2O_2 was 24.27 ± 5.84%. Significant difference was found between the % neuron viability when treated with 10 ng/ml (31.8 nM) CBD or 100 ng/ml (322 nM) CBN and 1 nM quercetin.



* $P < 0.05$ compare with toxic condition (5 mM hydrogen peroxide)

** $P < 0.05$ compare with positive control (1 nM quercetin co-treated with 5 mM hydrogen peroxide)

Figure 5 P19-derived neurons cell culture viability in 10 ng/ml CBD or 100 ng/ml CBN co-treated with 5 mM hydrogen peroxide compared with the positive control (1 nM quercetin co-treated with 5 mM hydrogen peroxide) and toxic condition (5 mM hydrogen peroxide).

Discussions

The most important cause of nerve cell damage is the oxidative stress from many kinds of free radicals. Many neuroprotective compounds contain the chemical moieties that were related to their antioxidant activities. These compounds can also straightforwardly penetrate blood brain barrier and pass through the central nervous system (CNS). Prior studies demonstrated that the cannabinoids contained phenolic structures making them potent antioxidants that could protect neuron cultures from oxidative stress.[16-17] This research was performed to verify the hypothesis that both psychotic and nonpsychotic cannabinoids were helpful as the neuroprotective substances.

The major cannabinoids (CBD and THC) and the degraded cannabinoid (CBN) were isolated from the confiscated cannabis in this study and examined the *in vitro* neuroprotective assay using serum deprivation method and co-administration of H_2O_2 assay reacted with the P19-derived cholinergic neurons. Our result showed that CBD (10 ng/ml) possessed neuroprotective effect similar to CBN (100 ng/ml). Remarkably, CBD had ten times more potential neuroprotective property than CBN. Regarding to the structure activity relationship (SAR), the intrinsic molecular characteristics of these compounds were concerned. CBD contains two phenolic groups when CBN and THC contain only one phenolic group. These two phenolic groups could be oxidized consequently to two quinone groups and could reduce the oxidative stress from H_2O_2 free radicals. CBN showed the second competency since it contained a biphenyl moiety that presented rigidity to the molecule more than that of THC. The chemical reactions and their oxidative products have been described noticeably in Figure 6.

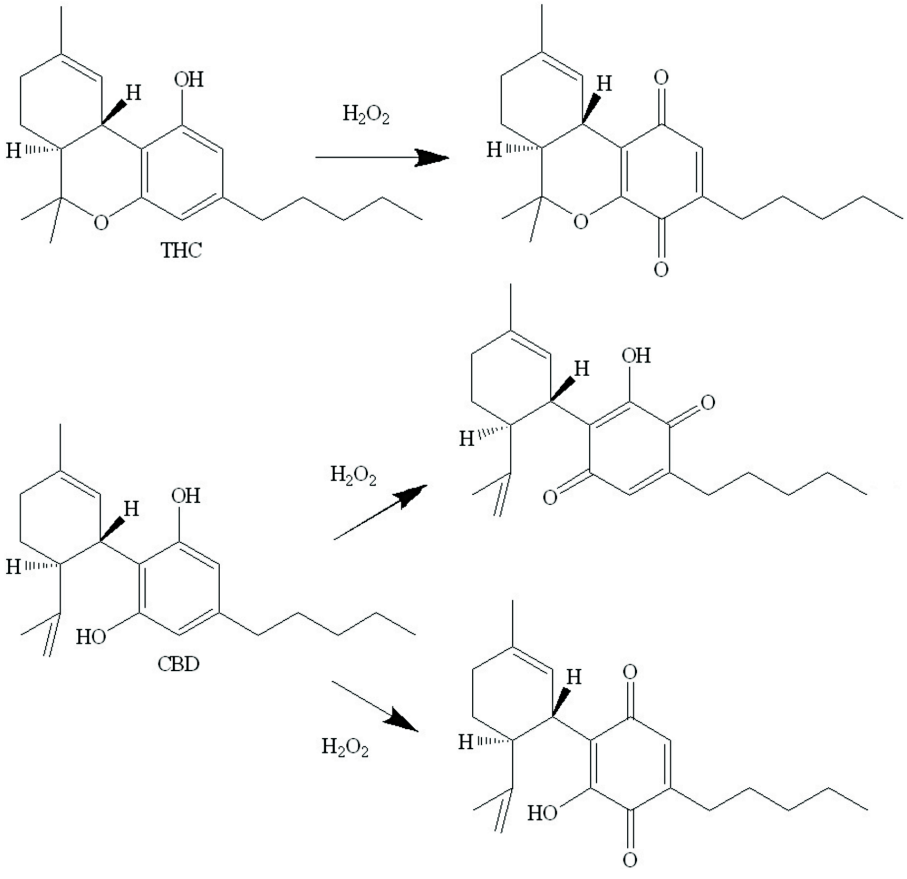


Figure 6 Oxidative products of THC and CBD by H_2O_2 free radicals.

Our study results complement data from the latest study[18]. Although CBD showed both neurotoxic and neuroprotective effects on hippocampal neurons in the *in vitro* setting, the use of low-concentrated (5 μM) CBD, not causing toxic effects on the neurons, significantly rescued the neurons from the oxidative stress by H_2O_2, confirming its neuroprotection capability.

However, further research is now required in order to find out the agents responsible for other *in vivo* activities as well as the molecular mechanisms of other cannabinoids involved in their activities and toxicities.

It can be concluded this study supported the neuroprotective effects of *C. sativa* female flower especially CBD, and warrants further study of its potential in medicinal purpose for neurodegenerative disorders, in particular, Parkinson's and Alzheimer's diseases.

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Potential conflict of interests

None.

References

1. *Cannabis sativa* L. The Plant List Website. http://www.theplantlist.org/tpl1.1/record/kew-2696480. Published 2013. Accessed May 26, 2020.
2. The United Nations Office on Drugs and Crime (UNODC). Booklet 5 Cannabis and Hallucinogens. United Nations, June 2019.
3. Bureau of Narcotics Control. *Narcotics Act* B.E. 2522. Ministry of Public Health, Bangkok, Thailand;1979.
4. Bureau of Narcotics Control. Narcotics Act (No. 7) B.E. 2562, Ministry of Public Health, Nontaburi, Thailand;2019.
5. Zivovinovic S, Alder R, Allenspach, M.D., et al. Determination of cannabinoids in *Cannabis sativa* L. samples for recreational, medical, and forensic purposes by reversed-phase liquid chromatography-ultraviolet detection. *J Anal Sci Technol*. 2018;9:27.
6. Jones-Villeneuve EMV, McBurney MW, Rogers KA, Kalnins VI. Retinoic acid induces embryonal carcinoma cells to differentiate into neurons and glial cells. *J Cell Biol*. 1982;94:253-262.
7. MacPherson PA, McBurney MW. P19 embryonal carcinoma cells: a source of cultured neurons amenable to genetic manipulation. *Methods* 1995;7:238-252.
8. Jones-Villeneuve EMV, Rudnicki MA, Harris JF, McBurney MW. Retionic acid-induced neuronal differentiation of embryonal carcinoma cells. *Mol Cell Biol*. 1983;3:2271-2279.
9. Tadtong S, Kanlayavattanakul M, Lourith N. Neuritogenic and neuroprotective activities of fruit residues. *Nat Prod Commun*. 2013;8:1583-1586.
10. Supasuteekul C, Nonthitipong W, Tadtong S, Likhitwitayawuid K, Tengamnuay P, Sritularak B. Antioxidant, DNA damage protective, neuroprotective, α-glucosidase inhibitory activities of flavonoid glycoside from leaves of *Garcinia gracilis*. *Rev Bras Farmacogn*. 2016;26:312-320.
11. Tadtong S, Chatsumpun N, Sritularak B, Jongbunprasert V, Ploypradith P, Likhitwitayawuid K. Effects of oxyresveratrol and its derivatives on cultured P19-derived neurons. *Trop J Pharm Res*. 2016;15:2619-2628.
12. Supasuteekul C, Tadtong S, Putalun W, Tanaka H, Likhitwitayawuid K, Tengamnuay P, Sritularak B. Neuritogenic and neuroprotective constituents from *Aquilaria crassna* leaves. *J Food Biochem*. 2017;e12365.
13. Puksasook T, Kimura S, Tadtong S, Jiaranaikulwanitch J, Pratuangdejkul J, Kitphati W, Suwanborirux K, Naoki Saito N, Nukoolkarn V. Semisynthesis and biological evaluation of prenylated resveratrol derivatives as multi-targeted agents for Alzheimer's disease. *J Nat Med*. 2017;71:665-682.
14. Tangsaengvit N, Kitphati W, Tadtong S, Bunyapraphatsara N, and Nukoolkarn V. Neurite outgrowth and neuroprotective effects of Quercetin from *Caesalpinia mimosoides* Lamk. on cultured P19-derived neurons. *Evid Based Complement Alternat Med*. 2013;1-7
15. Saingam W, Sakunpak A. Development and validation of reverse phase high performance liquid chromatography method for the determination of delta-9-tetrahydrocannabinol and cannabidiol in oromucosal spray from cannabis extract. *Revista Brasileira de Farmacognosia*. 2018;28:669-672.

16. Marsicano G, Moosmann B, Hermann H, Lutz B, Behl C. Neuroprotective properties of cannabinoids against oxidative stress: role of the cannabinoid receptor CB1. *J Neurochem*. 2002;80:448-456.
17. El-Remessy AB, Khalil IE, Matragoon S, et al. Neuroprotective effect of (-)Delta9-tetrahydrocannabinol and cannabidiol in N-methyl-D-aspartate-induced retinal neurotoxicity: involvement of peroxynitrite. *Am J Pathol*. 2003;163:1997-2008.
18. Kim J, Choi JY, Seo J, Choi IS. Cannabis and Cannabinoid Research. ahead of print. http://doi.org/10.1089/can.2019.0102.

บทคัดย่อ

ฤทธิ์ปกป้องเซลล์ประสาทของแคนนาบินอยด์สามชนิดที่แยกได้จากกัญชาของกลางในประเทศไทย

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บทนำ: ช่อดอกตัวเมียของกัญชา มีสารประกอบเคมีจำพวกแคนนาบินอยด์หลายชนิด บางชนิดออกฤทธิ์ต่อจิตและประสาท การศึกษาทางวิทยาศาสตร์ยังมีข้อถกเถียงกันระหว่างประโยชน์และโทษของกัญชา

วิธีการศึกษา: งานวิจัยนี้ แยกสารประกอบเคมีสำคัญจากกัญชาของกลาง จากนั้นนำไปทดสอบฤทธิ์ปกป้องเซลล์ประสาทที่เพาะเลี้ยงในสภาวะเครียดที่เกิดจากการเหนี่ยวนำด้วยวิธีงดอาหารซีรัมและการเติมไฮโดรเจนเปอร์ออกไซด์

ผลการศึกษา: แคนนาบินอยด์สามชนิด คือ CBD, CBN และ THC แยกได้จากกัญชาของกลาง CBD และ CBN แสดงฤทธิ์ปกป้องเซลล์ประสาทที่เพาะเลี้ยงในสภาวะเครียดที่เกิดจากการเหนี่ยวนำด้วยวิธีงดอาหารซีรัมและการเติมไฮโดรเจนเปอร์ออกไซด์ โดยแสดงความแตกต่างอย่างมีนัยสำคัญต่อการมีชีวิตรอดของเซลล์ประสาท ความเข้มข้นของ CBD 10 นาโนกรัมต่อมิลลิลิตร (31.8 นาโนโมล) และ CBN 100 นาโนกรัมต่อมิลลิลิตร (322 นาโนโมล)

สรุปผลการศึกษา: CBD แสดงผลการปกป้องเซลล์ประสาท ในโรคที่เกิดจากความเสื่อมของเซลล์ประสาท เช่น โรคพาร์กินสัน และอัลไซเมอร์

คำสำคัญ: กัญชา, แคนนาบินอยด์, ฤทธิ์ปกป้องเซลล์ประสาท