Research Article

The effects of hemp seed consumption on depression, anxiety and cognitive improvement based on machine learning

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Abstract

Background: Hemp seed (Cannabis sativa L.) is an annual herbaceous plant of the Cannabis genus that contains a large amount of protein, iron, and fatty acids, including linoleic, ω-linolenic, and γ-linolenic acid. These compounds are involved in a number of biological activities, including immunity enhancement, hypolipidemia, and inflammation reduction. Here, we investigated the antioxidant effects of hemp seed on human cognitive function.

Methods: The test was administered to 34 healthy volunteers aged ≥ 20 years. Participants were selected according to age and sex and were administered 10 g of hemp seed three times daily (30 g/day) for 45 days. The outcome measurements were recorded using a survey, computerized neurocognitive tests, and artificial intelligence.

Results: Survey analysis determined that both the Beck Anxiety Inventory and Beck Depression Inventory measurements decreased significantly after hemp seed consumption when compared to measurements taken before consumption (p < 0.05). Additionally, significant results were observed in the Stroop and Tower of London tasks (p < 0.05). The prediction performance for the antidepressant effect was 0.83 for the area under the curve in the random forest algorithm, which was superior to that of other machine learning methods.

Conclusion: These results suggest that hemp seeds have a beneficial effect on cognitive impairment.

Introduction

Hemp seeds are extensively used worldwide as a source of food and dietary supplements, with cannabidiol (CBD) nutritional supplements accounting for approximately one-third of the global 1.34-billion-dollar market for CBD [1-3]. They are rich in non-psychoactive yet biologically active cannabinoids, including cannabidiol (CBD), which exert potent anxiolytic, spasmylytic, and anticonvulsant effects, among others [4-8]. In addition to their potential as anxiolytic anticonvulsant drugs, hemp seeds are palatable and provide a valuable source of essential amino acids, fatty acids, minerals, vitamins, and fiber [9]. In particular, the hemp seed oil is composed of > 90% polyunsaturated fatty acids, which are known to have beneficial effects against cardiovascular diseases, cancer, and inflammatory conditions [10]. Not only do hemp seeds provide beneficial nutritional value but also cannabinoids present in these seeds interact with two G protein-coupled cannabinoid receptors (CB1 and CB2) and two endogenous ligands (anandamide and 2-arachidonoylglycerol) of the endocannabinoid system [11]. The endocannabinoid system is physiologically involved in...
the regulation of appetite, pain, mood, memory, inflammation, insulin sensitivity, and fat and energy metabolism and is involved in a wide variety of potential therapeutic implications for the treatment of pain, neuropsychiatric disorders, and neurological diseases, which may benefit from CB1 activation or antagonism [12].

The potential anti-anxiety effects of CBD have also been shown in preclinical models. In mice subjected to chronic unpredictable stress for 2 weeks, regular administration of CBD at doses of 30 mg/kg had potential anti-anxiety effects through CB1 cannabinoid receptors, evidence of which presented as high hippocampal anandamide levels associated with CB1-cell receptor activation [13]. Depression is one of the most common mental illnesses and causes severe difficulties and disabilities that affect the thoughts, emotions, behaviors, physical functions, and overall quality of life of those afflicted. Recently, the global prevalence of depression has increased due to the coronavirus pandemic, as well as other environmental and social stresses [14].

In Korea, reports suggest that the incidence of depression increases by 20% every year. The main causes of depression are imbalances and defects in monoamine-based neurotransmitters such as dopamine, serotonin, and norepinephrine [15]. The control of dopamine levels in the prefrontal cortex of patients suffering from depression involves the use of pharmacological treatments and natural substances. The prefrontal cortex is regulated by the neurotransmission of catecholamines and incorporates cognitive and emotional information. The upward adjustment of dopamine receptor D1 by low dopamine concentrations in the prefrontal cortex is a notable factor in depression [16]. Dopamine metabolites and receptors have been reported to affect mood and motivation in both cerebrospinal fluid and brain regions [17].

Antidepressants work by blocking the reuptake of certain neurotransmitters (norepinephrine, serotonin, and dopamine) that are transferred between neurons via synapses. The main antidepressants used are monoamine oxidase inhibitors, which enhance the function of monoamine transporters and increase norepinephrine and serotonin levels. Recently, selective serotonin reuptake inhibitors, such as fluoxetine (Prozac), which selectively acts on serotonin systems, have been used as an alternative to gold-standard treatments [18]. However, these drugs have negative side effects, including sexual dysfunction, vomiting, diarrhea, constipation, gastrointestinal disorders, loss of appetite, dry mouth, anxiety, and insomnia [19]. Therefore, many researchers are looking for natural antidepressants to use as an alternative to conventional drugs in the treatment of depression.

Previous research has identified that hemp seeds have an antidepressant effect and can induce cognitive improvements and behavioral changes in vertebrates. Therefore, in this study, we investigated the effects of hemp seed consumption on antidepressant ability and cognitive improvements in the general population. Furthermore, the findings were based on artificial intelligence (AI) analysis of the correlation between hemp seed consumption and antidepressant ability and cognitive enhancement.

**Methods**

**Participant selection**

The participants selected for this study were those with normal or corrected vision, without brain damage or history of brain injury, without learning/linguistic problems, without claustrophobia who did not overwork/drink on or the day before the study, and who could participate in the experiment.

**Inclusion criteria, exclusion criteria and recruitment method**

The inclusion criteria for participants were as follows: right-handed, no history of mental illness, learning or speech impairment, normal or corrected vision, native speaker of Korean, good reading and writing skills in Korean, and able to use PCs and mobile devices.

The exclusion criteria for participants were as follows: those with vision problems, such as color blindness, coloration, and dry eye syndrome; subjects with physical constraints on their PC and mobile use; those with a history of mental illness and learning/language disabilities; and those with brain damage and a history of brain damage.

Recruitment of participants was outsourced to the Korea University community site, Gopas and external part-time job recruitment sites to enlist participants to volunteer in the survey and online research. Participants were contacted individually through the use of e-mail and mobile phone messages. In addition, an official letter was sent to the public agency responsible for the recruitment of participants to seek cooperation from the designated company or school and to recruit participants using public announcements, bulletin boards, etc. Later, we sent experimental portal (site) links to participate in surveys and online research through separate means, such as e-mail and mobile phone messages.

**Calculating the number of participants required for study design**

The number of participants included in this study was 31 adults aged 20–60 years, and online-based surveys and cognitive tasks were conducted for all. The rationale for the calculation is as follows: to measure the response time and error rate using cognitive tasks and to secure statistical reliability, 100 cognitive tasks (behavior) and questionnaires were randomly conducted for each experiment [20]. For the significance of the experiment, the effect size formula that
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affects the power (Co) with 0.05 significance and a minimum verification power of 80 bases is as follows:

\[ \delta = \frac{\mu D}{\sigma} \]  
(\( \delta \): effect size, \( \mu D \): differences in a mean between experimental and control groups, \( \sigma \): variance of means).

Simulation results based on the number of participants (including brainwave and brain imaging studies) require at least 20 experimental participants in one study to achieve statistically significant results [21]. This work compares pre-middle-post results with an in-subject design but assigns 25 people to each of the four groups, divided by age (20-39 years, 40-60 years) and gender (male and female), for post-validation. The reason for assigning 25 people is that, in addition to 20 for statistical stability, 25 people will be assigned to each group to conduct post-validation according to pre-middle-post result details (age, gender) to enhance verification.

Hemp seed administration

The pre-middle conditions of 1.5 months (45 days) from the start of the experiment were the control conditions, and the middle-post conditions of 1.5–3 months (45 days) were the experimental conditions. The values before and after each condition were compared with the within-design. There was no treatment under the control conditions, whereas, under the experimental conditions, the participants were required to take 10 g of hemp seed three times a day for 45 days (30 g/day).

Survey development

A survey was also conducted to compare the results with those of the CNT program. The questionnaire used was of four types: Perceived Stress Scale (PSS), Patient Health Questionnaire (PHQ-9), Burnout Assessment Tool (BAT), and Beck Depression Inventory (BDI). The PSS was developed by Cohen, et al. [22] and is a measure of how the surveyor generally perceives and interprets stress over the course of a month. Despite exposure to the same stressors, there can be a difference in the degree of stress experienced by individuals. Thus, the PSS can be considered appropriate for assessing the degree of stress an individual feels compared to other techniques that measure only objective stress situations. The Patient Health Questionnaire (PHQ) is a self-report test designed to screen for depression and evaluate its severity. In this study, we used the Korean version of the PHQ-9, which was translated and studied domestically by Han, et al. [23], to confirm its validity and reliability. It consists of a nine-symptom checklist that corresponds to the diagnostic criteria for DSM-IV major depression disorders and examines how often these problems were experienced over a 2-week period. The response was evaluated on a 4-point scale of “not at all”, “several days”, “more than half of the days”, and “nearly every day”, with scores ranging from 0 to 27. Sums of scores > 10 were considered as having a major depressive disorder [24].

Job burnout refers to a state of psychological exhaustion that occurs when exposed to long-term stressors and, thus, appears to be a failure to cope with psychological resources [25]. In this study, the Korean version of the BAT, which was developed by the Schaufeli Research Team [26], a developer of the Maslach Burnout Inventory and a leading researcher in the field of exhaustion, was used as the BAT (K-BAT). The BDI is a measure of depression that is currently used in clinical practice. It was developed by Beck [27] to measure the type and extent of depression based on clinical depression symptoms and is widely used worldwide.

CNT program (http://ntnnt.net/group/nnt)

In this study, the participants used six types of CNT programs: finger tapping (FT), emotional picture perception, n-back test, Balloon Analogue Risk Task (BART), Tower of London task (TOL), and Stroop.

First, in FT, two to eight cards with human facial expressions were displayed simultaneously and the facial expression on each card was either negative or positive. If the expressions on the cards were all the same, the participant was required to press a designated button; conversely, if the expressions on the cards were different from each other, the participant was required to press an alternative button. The inspection was conducted in this order of implementation after the performance of the task description and sufficient practice guidelines [28,29].

Second, in the emotion perception experiment, the study participants were simultaneously shown two to eight cards with human faces on them. The facial expressions on each card were either negative or positive. If the facial expressions on the cards were the same, the participant was required to press the “Same” button, whereas if the expressions were different, the “Different” button was pressed. The inspection was conducted in the order of this implementation after the task description and sufficient practice implementation [30-32].

Third, in the n-back test, the participants were presented with a sequence of characters on a screen one by one. If the current character was deemed to be the same as the previous character (one, two and three dictionaries), the space key on the keyboard was pressed before the character disappeared. The response time and noon rate were also recorded. The inspection was conducted in the order of this implementation after the task description and sufficient practice implementation [33].

Fourth, in the BART, the study participants sat in front of a computer monitor with balloon-shaped stimuli present on the screen, and participants were required to click the button below the balloon. Clicking the button caused the balloon to
increase in size, and there were no restrictions on how many
clicks the participants could use once the balloon reached a
particular size, it burst and the feedback of “popped” alerted
the participant that the balloon had burst. Before the balloon
burst, the participants could press the “Stop” button on the
left side of the screen to choose not to make the balloon
larger. In this case, the participants would receive feedback
informing them that money had accumulated as the balloon
increased in size. Blue, red, and yellow balloons were in the
fast-, medium-, and slow conditions, respectively, and the
participants recognized this association as they went through
the task [34].

Fifth, in the TOL, the study participants were required to
use their computer mouse to move images of green, red, blue,
and yellow discs placed in various sequences and locations
to the same shape as the target image on the computer screen.
The participants could only move one disk at a time and only
select the top disk. The aim was to move the disk in a minimal
number of steps. The inspection was conducted in the order of
this implementation after the task description and sufficient
practice [35,36].

Finally, in the Stroop task, a +-shaped starting point was
presented in the center of the screen, and letters or numbers
were presented up to three digits in length shortly after the
starting point appears. The letters or numbers presented were,
for example, 1 MM and ZZZ. The task is to enter the number,
not the letters or numbers presented, and inspection was
conducted in the order of this implementation after the task
description and sufficient practice implementation [37,38].

AI analysis

Many of these features involve simple noise or a high
correlation with each other, although various quantitative
features can be extracted from hemp consumption for
antidepressant datasets. Therefore, feature selection is
important for building a prediction model with specific
features. To minimize the computational cost, unselected
features were not used in the prediction model. Among
cognitive functionality features of hemp consumption,
significant features for predicting antidepressant factors
were selected with a random forest regressor using Python
Software Foundation, version 3.6, with the Scikit-learn
package (https://github.com/scikit-learn/scikit-learn). We
analyzed five machine learning algorithms, namely, random
forest, adaptive boosting, decision tree, linear support vector
machine, and logistic regression, using Python 3.6, for a
prediction model with significant factors of antidepressant
functional bioactive compound features from hemp seed
intake datasets. To train the five machine learning algorithms,
20 repeated 10-fold stratified cross-validations were applied
to improve the stability of performance.

Statistical analysis

Data analysis was performed using SPSS software
(Statistics Package for Social Science, Ver. 20.0). To analyze
the difference in the averages of the samples, one-way ANOVA
was conducted, and significant differences were determined
by Duncan’s multiple range tests at the level of $p < 0.05$. In
addition, five algorithms in independent datasets were
compared using a machine learning model with Delong’s
test [xx] using open-source R software (version 3.5.1; R
Foundation for Statistical Computing, Vienna, Austria).
Statistical significance was set at $p < 0.05$.

Results

Analysis of survey

Analysis of the PSS/PHQ score showed no significant
differences in the PSS and PHQ scores when hemp seed
was consumed (data not shown). In contrast, the BAI score
significantly changed depending on whether hemp seed was
consumed $[F(2, 62) = 8.007, p = .001]$, and post-hoc analysis
revealed that the BDI score significantly decreased after hemp
seed consumption $[t (1, 32) = 2.930, p = .006]$ (Figure 1). In
addition, the BDI score significantly changed depending
on whether hemp seed was consumed $[F(2, 62) = 10.184,
p = .001]$, and post-hoc analysis revealed that the BDI score
was significantly reduced after hemp seed consumption
$t (1, 32) = 3.237, p = .003$ (Figure 1). The BAI and BDI
values obtained for depression and anxiety levels decreased
with hemp seed consumption, indicating that hemp seed
consumption positively affects depression and anxiety. Hemp
seed consumption is considered to play a role in alleviating
depression and anxiety, which are critical psychological vital
signs that are deeply related to an individual’s mental health
status.

Analysis of CNT program

The results of the FT test showed no significant
difference between the reaction time and tapping interval depending
on hemp seed consumption. However, the interval between
towels appeared to shorten with hemp seed consumption.
These results are significant in demonstrating the
improvement in motor function with hemp seed consumption
(data not shown).

![Figure 1: Pre: initial data, mid: data before hemp seed consumption, post: data after hemp seed consumption.](https://doi.org/10.29328/journal.afns.1001047)
The emotion perception experiment showed that positive bias had a greater effect than negative bias \( F(1,32) = 3.572, \quad p = 0.068 \). No significant difference was observed with hemp seed consumption. Although the emotion perception experiment did not show significant differences when hemp seed was consumed, further analysis is needed given that there was a trend towards positive bias in the data (Figure 2).

In addition, the results of the n-back test showed no significant difference in either the reaction time or the accuracy rate depending on hemp seed consumption (Figure 3). These results are likely due to hemp seed consumption having little influence on the n-back task, which measures working memory.

The BART results showed no significant difference in the number of successful acquisitions of yellow balloons with moderate burst probabilities and blue balloons with low burst probabilities (Figure 4). However, the number of successful acquisitions of orange balloons, which had a low probability of bursting, did not differ between the pre- and middle-conditions, and the number in the post condition tended to increase. Two-way ANOVA revealed significant differences in balloon color. \( F(1,33) = 262.060, \quad p = .001 \). It was observed that the data changed over time, so further studies will be necessary to analyze additional data.

The TOL test results showed significant differences between hemp seed consumption and planning and execution capabilities [planning capabilities: \( F(2,66) = 4.185, \quad p = 0.019 \); execution capabilities: \( F(2,66) = 4.136, \quad p = 0.020 \)] (Figure 5). This suggests that the cognitive ability to execute tasks and to formulate pre-execution plans improves with hemp seed consumption.

The results of the Stroop task were highly significant \( F(1,33) = 221.106, \quad p = .001 \), and changes in the two scores were significant \( F(2,66) = 3.244, \quad p = 0.045 \). The results showed that promotion and inhibition function was more positive after than before hemp seed consumption (Figure 6).
AI analysis

We selected features using each dataset and analyzed the diagnostic performance of the prediction model. Eleven features were selected using the random forest regressor model from the datasets, including n-back, TOL, BART, Stroop, FT, and emotion perception. The three most important features among those selected were n-back, TOL, and Stroop. In addition, we analyzed the antidepressant effects of hemp seed consumption by using five classifier models. Figure 1 and Table 1 show the receiver operating characteristic curve (ROC) curves, AUCs, and P-values for each model. Figure 7(b) shows a box plot comparing five machine learning methods for diagnostic performance, and Figure 7(a) is an ROC analysis using selected features for predicting the antidepressant task. Among the five methods with significant features from the datasets of hemp seed consumption and its features, random forest had the highest diagnostic performance for predicting antidepressant effect (AUC: 0.83) when using significant features, whereas the lowest diagnostic performance was a decision tree (AUC: 0.52). There was a significant difference between the AUC of the random forest and that of the two models (decision tree and linear support vector machine) ($p < 0.05$) (Table 1).

Discussion

The Korean society is gradually becoming an aging society with an increasing rate of chronic diseases presenting in adulthood, such as memory deterioration, cognitive function decline, dementia, senile stress depression, arteriosclerosis, diabetes, and arthritis [39-43]. Therefore, self-medication is becoming increasingly important in maintaining a healthy lifestyle, and the selection of functional foods is one of the major efforts required to sustain it [44,45]. The health functional food market generated a global revenue of approximately US$ 143.2 billion by the end of 2019 and was projected to reach US$ 639.4 billion by 2020 [46].

The purpose of this study was to confirm the antioxidant effects of hemp seed as a functional food that affects psychological state and cognitive function [5,7,8]. With the outcome of AI analysis for the PSS/PHQ score, hemp seed consumption did not show any effect on the outwardly expressed psychological states, such as social phobia or aggression. However, both the BAI and BDI values were significantly reduced, suggesting that hemp seed consumption had a positive effect on depression and anxiety caused by the inward psychological state. This was observed among various individuals. In addition, there were observed changes in the statistical analysis of cognitive ability according to hemp seed consumption using six cognitive tests (balloon analog risk task, n-back task, emotion perception task, Stroop task, FT task, and TOL). In the case of emotional perception and FT, there were no significant differences based on hemp seed consumption; however, some positive improvement in motor function was observed, with a positive bias shown for depression measurement. In particular, the Stroop task and TOL data analysis showed a significant difference before and after hemp seed consumption. The Stroop task functions to identify facilitation and inhibition, which are two of the many cognitive functions of humans. Among the cognitive functions tested, the executive function of executing the task and the ability to establish a plan before execution were found to act faster. The Stroop task analysis showed that the change in score after hemp seed consumption was significant and confirmed that the functions of facilitation and inhibition in executive functions were further improved after hemp seed consumption.

Table 1: AUCs of five machine learning models using significant features acquired from random forest regression analysis for antidepressant and increasing cognitive function through hemp seed consumption.

<table>
<thead>
<tr>
<th>Machine learning models using significant features from the result of antidepressant and cognitive function dataset by hemp seed consumption</th>
<th>Prediction for antidepressant effect</th>
<th>AUC</th>
<th>$p$ - Value*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Random forest</td>
<td>0.83</td>
<td>(0.73, 0.94)</td>
<td>Reference</td>
</tr>
<tr>
<td>Adaptive boosting</td>
<td>0.74</td>
<td>(0.53, 0.94)</td>
<td>0.776</td>
</tr>
<tr>
<td>Decision tree</td>
<td>0.52</td>
<td>(0.36, 0.68)</td>
<td>0.0002</td>
</tr>
<tr>
<td>Linear support vector machine</td>
<td>0.70</td>
<td>(0.58, 0.82)</td>
<td>0.024</td>
</tr>
<tr>
<td>Logistic regression</td>
<td>0.74</td>
<td>(0.68, 0.86)</td>
<td>0.327</td>
</tr>
</tbody>
</table>

Note: Numbers in parentheses are 95% confidence intervals.

* $p$ - value was acquired from comparison with the reference standard using the Delong method.
seed consumption. For the TOL task, an effect on planning ability and executive function was found after hemp seed consumption. This indicates that planning ability was faster and executive function was significantly faster after hemp seed consumption. In particular, this study demonstrated the potential ability of AI in predicting antidepressant effects. The selected features (n-back, TOL, BART, Stroop, FT, and emotion perception, using the random forest regressor) were very important for developing this model. This strategy, based on machine learning, can be used as a biomarker in clinical practice.

Conclusion
This study has some limitations. First, the sample size was too small to generalize the model. In order to overcome the limited sample size, the development of machine learning algorithms is required. Second, the study population was limited to a single hospital. In the future, we intend to collect data from various patients to predict antidepressant effects and analyze improvements after hemp seed consumption. If datasets are collected from multiple sites, we will attempt to predict antidepressant effects using deep learning. Consequently, it could be predicted that hemp seed exerts a positive effect on cognitive ability based on the observed results and interpretation via statistical and AI analysis. It may also be desirable to utilize AI for analyzing or predicting significant relations in the early phase of study to consider further evaluation during execution.

Ethics approval and consent to participate
Participants signed a consent form before participating in the study. The study was approved by Korea University’s Institutional Review Board (KUIRB-2020-0137-01).

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Authors’ contributions
JB. J, YW. C, BJ. H and SH. H conceived and designed the study. SY. K, SY. C, and KH. H analyzed the data and wrote the original manuscript. YW. C performed the statistical analyses. SH. H reviewed and edited the manuscript. All authors have read and agreed to the published version of the manuscript.

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(Depression Questionnaire)

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