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Industrial hemp decreases intestinal motility stronger than indian hemp in mice

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Abstract. – BACKGROUND: Indian hemp has shown beneficial effects in various gastrointestinal conditions but it is not widely accepted due to high content of tetrahydrocannabinol resulting in unwanted psychotropic effects.

AIM: Since industrial hemp rich in cannabidiol lacks psychotropic effects the aim of research was to study the effects of industrial hemp on intestinal motility.

MATERIALS AND METHODS: Animals were randomly divided in six groups (each group consisting of 6 animals): Control group, Cind group – receiving indian hemp infuse for 20 days, Cids group-receiving industrial hemp infuse for 20 days, M group – treated with single dose of morphine (5 mg/kg i.m.) Cind+M group – treated with indian hemp infuse and single dose of morphine (5 mg/kg i.m.), Cids+M – treated with industrial hemp infuse and single dose of morphine (5 mg/kg i.m.). On the 20th day of the study animals were administered charcoal meal, and were sacrificed 35 minutes after administration. Intestinal motility was estimated according to distance between carbo medicinalis and cecum in centimeters.

RESULTS: Decrease of intestinal motility in animals treated with indian hemp infuse was not significant compared to controls and it was smaller compared to animals treated with morphine (Indian hemp =15.43±10.5 cm, morphine = 20.14±5.87 cm). Strongest decrease of intestinal motility was recorded in animals treated with industrial hemp infuse, and it was significant compared to controls and morphine (industrial hemp = 26.5±9.90 cm, morphine = 20.14±5.87 cm; $p < 0.005$).

CONCLUSIONS: Although not completely without psychotropic activity cannabidiol could be a potential replacement for tetrahydrocannabinol. Since industrial hemp infuse rich in cannabidiol reduces intestinal motility in healthy mice cannabidiol should be further evaluated for the treatment of intestinal hypermotility.

Key Words:
Industrial hemp, Indian hemp, Intestinal motility.

Introduction

Hemp is one of the plants with the longest history of cultivation being cultivated for more than 6000 years. Fiber products were widely applied, but the knowledge about content of psychoactive substances and their misuse prohibited the cultivation of this plant[1]. Danger of misusing industrial hemp as a substitute for indian hemp prevented detailed research in therapeutic potential of industrial hemp.

Botanically, industrial hemp and marijuana-indian hemp (*Cannabis sativa* L.) belong, along with hop (*Humulus lupulus* and associated wild species), to the family Cannabaceae[2].

The genus *Cannabis* consists of only one species, namely *Cannabis sativa* L. But *Cannabis sativa* L. is often divided into sub-species or varieties, according to their composition of cannabinoids (namely tetrahydrocannabinol (THC)) or according to appearance. The content of the most important psychotropic compound, THC, is high in "indica" and low in the "sativa" (industrial) variety in which the THC content is restricted to a maximum of 0.3%. The ratio of the different cannabinoids is rather stable through genetic determination, but absolute content varies according to climate and other external factors[3-5].

Cannabis "indica" has shown beneficial effects in various gastrointestinal conditions that range from enteric infections and inflammatory conditions to disorders of motility, emesis and abdominal pain[6-8]. The reason why use of "indica" for such conditions in not accepted is high content of THC resulting in unwanted psychotropic effects[9,10].

Due to low content of THC, industrial hemp lacks unwanted effects in the brain. Predominant cannabinoid in industrial hemp is cannabidiol (CBD), and the CBD: THC ratio is > 2:1. Since there is data which suggests that CBD has certain

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effect on intestinal motility in experimental animals[11,12], the aim of our research was to study the effects of industrial hemp on intestinal motility.

Materials and Methods

Hemp

Both indian and industrial hemp were grown at the Institute of Field and Vegetable Crops, Backi Petrovac, Serbia. Hemp was harvested in the period of waxy maturation and was air dried until moisture content was below 10%. THC and CBD concentrations were determined at the Institute of Field and Vegetable Crops, Backi Petrovac by gas chromatography method recommended by United Nations[13].

Indian hemp (Cind) – genotype: "VIR SK" – Institute of Field and Vegetable Crops, Backi Petrovac – THC concentration 1.127%; CBD concentration 0.040%.

Industrial hemp (Cids) – genotype: "Novosadska" – Institute of Field and Vegetable Crops, Backi Petrovac – THC concentration 0.120%; CBD concentration 1.763%

Industrial Hemp (Cids) and Indian Hemp (Cind) Infuse

Both industrial and indian hemp infuse were prepared of 10 g chopped leaf, stalks and flower in one liter of boiling water. Infuse was left for one hour, afterwards it was strained and administered to the animals instead of water. Fresh amount of infuse was prepared every day.

- Morphine – Sigma-Aldrich (5 mg/kg i.m.).
- Carbo medicinalis emulsion (0.4 g carbo medicinalis + 0.2 g gummi arabicum in 10 ml of olive oil – 0.6 ml per mice orally administered).

Laboratory Animals and Procedures

Experiments were carried out on 8-12 weeks old male NMRI-Haan mice, body weight 23-25 g, bred in the Department of Pharmacology, Toxicology and Clinical Pharmacology, Faculty of Medicine, Novi Sad, Serbia. Animals had free access to water or infuse, and food with 12-h successive light and dark periods.

Laboratory animals were under human care in accordance with the criteria given in the "Guide for the Care and Use of Laboratory Animals"[14]. The study was approved by the Animal Ethics Committee of the University of Novi Sad.

The animals were randomly divided into test and control groups as follows (each group consisting of 6 (six) animals):

1. Control group (Co): animals fed with standard food and water;
2. Cind group: animals fed with standard food and indian hemp infuse *ad libitum* for 20 days;
3. Cids group: animals fed with standard food and industrial hemp infuse *ad libitum* for 20 days;
4. M group – animals treated with single dose of morphine (5 mg/kg i.m.);
5. Cind+M group – animals pretreated with indian hemp infuse *ad libitum* for 20 days, and single dose of morphine (5 mg/kg i.m.) on day 20;
6. Cids+M – animals pretreated with industrial hemp infuse *ad libitum* for 20 days, and single dose of morphine (5 mg/kg i.m.) on day 20.

On the 20th day from the beginning of the study all animals were orally administered charcoal meal (0.6 ml emulsion – 0.4 g carbo medicinalis and 0.2 g gummi arabicum in 10 ml of olive oil). Animals were sacrificed 35 minutes after charcoal meal administration. Intestinal motility was estimated according to distance between carbo medicinalis and cecum in centimeters. All animals were without fed for 24 hours before charcoal meal administration.

Statistical Analysis

The level of significance between the groups was assessed with the Student's *t*-test for small independent samples using MedCalc 9.2.0.1. All data are expressed as mean ± standard deviation (SD). A value of $p < 0.05$ was considered to be statistically significant.

Results

Effect on intestinal motility in mice treated with morphine (M) (5 mg/kg i.p.), indian hemp infuse (Cind) and industrial hemp infuse (Cids) is shown in Table I.

Morphine (5 mg/kg i.p.) significantly decreased intestinal motility compared to control group (M = 20.14±5.87 cm, Co = 10.85±1.63 cm; $p < 0.005$). Decrease of intestinal motility in group of mice treated with indian hemp infuse was not signifi-

Table I. Distance of the charcoal meal from cecum (cm) in control group (Co), group treated with single dose of morphine (M) (5 mg/kg i.p.), indian hemp infuse (Cind), and industrial hemp infuse (Cids).

Group	Distance of charcoal meal from cecum (cm) (X ± SD)
Co	10.85 ± 1.63
M	20.14 ± 5.87[a]
Cind	15.43 ± 10.5
Cids	26.5 ± 9.90[b]

[a]$p < 0.005$ compared to control group (Co); [b]$p < 0.005$ compared to control group (Co).

cant compared to control and it was smaller than decrease in group of animals treated with morphine (Cind = 15.43±10.5 cm, M = 20.14±5.87 cm). Strongest decrease of intestinal motility was recorded in group of mice treated with industrial hemp infuse, and it is significant compared to control group (Cids = 26.5±9.90 cm, M = 20.14±5.87 cm; $p < 0.005$).

Effect of pretreatment with indian (Cind+M) and industrial hemp (Cids+M) before morphine administration is shown on Table II.

Discussion

An increasing number of articles have shown that cannabinoids may reduce intestinal motility *in vivo* through activation of CB_1 and CB_2 receptors. 9-THC, the main cannabinoid in indian hemp activates two $G_{i/o}$-coupled membrane receptors, named CB_1 and CB_2 receptors in the gut. Activation of these receptors results in reduction of smooth muscle contractility, ascending neural contractions and peristalsis in gastrointestinal tract[15-17].

Table II. Distance of the charcoal meal from cecum (cm) in control group (Co), and in mice treated with single dose of morphine (M) (5 mg/kg i.p.), mice pretreated with indian hemp infuse before morphine administration (Cind+M), and mice pretreated with industrial hemp infuse (Cids+M) before morphine administration.

Group	Distance of charcoal meal from cecum (cm) (X ± SD)
Co	10.85 ± 1.63
M	20.14 ± 5.87[a]
Cind+M	19.5 ± 10.45
Cids+M	33 ± 7.35[b,c]

[a]$p < 0.005$ compared to control group (Co); [b]$p < 0.001$ compared to control group (Co); [c]$p < 0.05$ compared to Cind+M group.

In our experiment indian hemp infuse, rich in THC, decreased intestinal motility compared to control (Co = 10.85±1.63 cm, Cind = 15.43±10.5 cm) but the decrease was not significant. Decrease was also weaker than the effect of morphine, which reduces peristaltic activity by binding to μ opioid receptors in gut[18,19] (Co = 10.85±1.63 cm, M = 20.14±5.87 cm; $p < 0.005$). Since THC might have certain activity on μ opioid receptors[20] we also studied possible interaction of indian hemp infuse and morphine. However, indian hemp infuse did not change morphine effect on intestinal motility (Cind+M = 19.5±10.45 cm, M = 20.14±5.87 cm) suggesting that the effect of indian hemp was not mediated through μ opioid receptors.

On the contrary, infuse prepared from industrial hemp, rich in CBD, significantly decreased intestinal motility compared to controls (Co = 10.85±1.63 cm, Cids = 26.5±9.90; $p < 0.005$). Decrease was stronger then in group of animals treated with single dose of morphine (Cids = 26.5±9.90 cm, M = 20.14±5.87 cm; $p < 0.05$). Synergistic activity with morphine was also recorded since the effect of morphine in animals pretreated with industrial hemp infuse was stronger than in group of animals treated with single dose of morphine (Cids+M = 33±7.35 cm, M = 20.14±5.87 cm). The exact mechanism of this interaction is to be determined in further research.

Since the content of THC is low in industrial hemp and since the absorption of THC from the alimentary tract after oral administration of industrial hemp infuse is low (around 6%,[21]) it can be presumed that the CBD, main cannabinoid constituent in industrial hemp is responsible for the effect on intestinal motility.

Cannabidiol itself has a wide pharmacological profile (anti-anxiety[22], anticonvulsant[23], neuroprotective[24], antinociceptive[25], anti-ishaemic[26], vasodilatatory[27], anticancer[28], anti-inflammatory[29] activity in rodents *in vivo*, decrease of body weight gain[30], antinauseous activity[31], appetite regulation[32]), but the effects of CBD in the digestive tract are largely unexplored.

Early studies showed that CBD did not modify gastric emptying and small intestinal transit in mice and rats[33,34]. However, there is also data which suggests that CBD has certain effect on intestinal motility. Capasso et al[11] shown that CBD reduces motility in the experimental model of intestinal ileitis. Jamontt et al[12] found on rat model

of colitis, that THC and CBD not only reduces inflammation but also lowers the occurrence of functional disturbances, and that the combination of CBD and THC could be beneficial therapeutically, via additive or potentiatig effects.

The exact mechanism of activity of CBD is not clear[9]. Several mechanisms of action were proposed, including diffuse targets on the endocannabinoid system[35], enhancement of adenosinergic signaling[36], agonism of 5HT1a serotoninergic receptors[37] and TRPV1 vanilloid receptors[35]. Cannabidiol weakly binds to CB_1 and CB_2 receptors and inhibits the uptake and hydrolysis of anadamide, an endocannabinoid ligand[35].

Conclusions

In addition to stronger decrease in intestinal motility, advantage of industrial hemp and CBD is lack of effects such are sedation and cognitive dysfunction. Nonselective cannabinoid receptor agonists, like THC, beside activation CB_1 and CB_2 receptors in the gut also activate brain CB_1 receptors, resulting in sedation, cognitive dysfunction and psychotropic effects. Due to its poor binding to CB_1 and CB_2 receptors CBD lacks effects such are sedation and cognitive dysfunction. Although not completely without psychotropic activity CBD could be a potential replacement for THC.

Since industrial hemp infuse rich in cannabidiol reduces intestinal motility in healthy mice, and since cannabidiol reduces intestinal hypermotility in mice with terminal ileitis cannabidiol should be considered as a good candidate to be further evaluated for the treatment of intestinal hypermotility.

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