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Verification of the analgesic properties of natural compounds of cannabinoid origin in *Danio rerio* model of nociception

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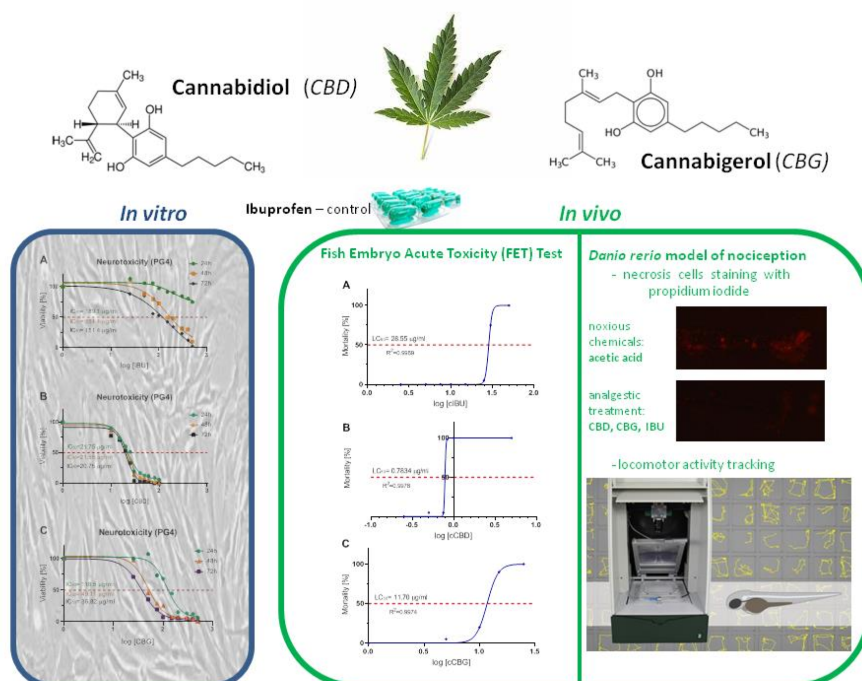

Abstract: The use of Cannabis sativa in human history dates back thousands of years, with various historical and cultural applications. However, at the beginning of the 20th century, many countries enacted regulations to criminalize and restrict the use of cannabis, leading to a significant reduction in research on its medical applications.

A novel approach to pain studies involves *Danio rerio*-based nociception models. These models use different methods to induce pain, with fish larvae often subjected to incubation in acetic acid solution, resulting in epidermal tissue damage. Nociceptive

responses are then observed by tracking fish movement.
Our research aimed to develop a simple and accessible *Danio rerio* (zebrafish) model of nociception to study the potential analgesic properties of CBD (cannabidiol) and CBG (cannabigerol) in comparison to the commonly known painkiller ibuprofen. This research seeks to contribute to our understanding of the potential therapeutic applications of cannabinoids in pain management.

Keywords: cannabidiol, cannabigerol, *Danio rerio* model of nociception

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Introduction

Proves of using *Cannabis sativa* reach thousands years back in human history. At the beginning of 20th century many countries put regulations in order to delegalize *Cannabis*. In consequence research on medical applications of *C. sativa* was highly restrained. 21st century brought interest of its properties back and emergence of movements for *Cannabis* legalisation. Medical and scientific environment is also part of this change as we observe peak in research on medical applications of cannabinoids in recent years. Clinical trials are mainly focused on THC and CBD as mostly studied phytocannabinoids but other *Cannabis* compounds also deserve attention of scientific community and more intensive research [1]. *Cannabis sativa* (from *Cannabaceae* family) is taxonomically complex species that can be divided according to chemical concentrations of certain metabolites. Phenotype I, the drug type, has the highest content of THC and CBD; phenotype II, intermediate, has CBD and THC present in lower concentrations; phenotype III, the fibre-type, with the least significant THC and CBD content [2].

Phytocannabioids, chemically characterised as aromatic oxygenated hydrocarbons, are agonists of CB_1 and CB_2 receptors. There are three classes of cannabinoids: (1) endogenous cannabinoids, derivatives of arachidonic acid, like anandamide (N-arachidonoylethanolamine, AEA) and 2-AG (2-arachidonoylglycerol); (2) phytocannabinoids – THC, CBD, CBC, CBG and others; (3) synthetic cannabinoids (e.g. dronabinol, nabilome - synthetic analogues of THC). In *Cannabis* plant CBs are synthesised from key precursor CBGA (cannabigerolic acid). In effect of subjecting dry *Cannabis* extract to high temperature, enzymatic reactions turn CBs precursors into compounds with much higher binding affinity to their receptors [3]. CB_1 and CB_2 are transmembrane proteins of class A family of G-protein

coupled receptors. CB_1 are localised mainly in brain tissue, muscles, lungs and gastrointestinal and immune system. CB_2 plays role in immune and cardiovascular systems. Due to its properties C. sativa is studied in therapy of chronic pain, as anti-emetic and appetite regulating drug but also neuroprotecting, anticancer and anti-inflammatory factor [4].

The capability to feel pain plays a crucial role in interaction and navigation in the environment. Ability to differentiate potentially hazardous and non-harmful factors is an inherent part of animal survival. Studies show that most species, including vertebrates as well as invertebrates, are capable of experiencing thermal, neuropathic, mechanical, and chemical types of pain. The ability to recognize potentially harmful environmental factors via receptors in epidermis, viscera and mucosae was called nociception [5].

Different methods of causing pain of varying intensity have been developed. Nociceptive response in rodents is most often measured with the heat tests, including tail flick, and hot plate test. In this model the heat intensity and animal exposure time is controlled. To avoid unnecessary distress and risk of burn, the animal is retrieved as soon as pain-related behaviour like tail flick, paw lick or paw withdrawal is observed. This test offers reproducible results, but is sensitive to analgesia induced by stress [6].

Since the nociception is observed not only in vertebrates, but also in invertebrates, *Drosophila melanogaster* based model of pain has been developed. Fruit fly larvae gently poked with a needle heated to ~42°C, or harshly with a needle in room temperature with a strength of ~50 mN exhibited behaviour described as "corkscrew-like rolling". Avoidance mechanisms have also been observed in adult flies. In the first test *Drosophila* was glued to a flask, received a piece of cotton to hold, and was heated with a laser beam to ~40°C. The time which passed to the drop of cotton was measured and considered as a redout of nociception. The other test included a flask heated to ~47°C with the hot plate, and the flies freely wandering on the bottom of the bottle. Avoidance behaviour in this model was a jumping response of the *Drosophila.* Nonetheless, the mechanism of nociception in insects is significantly simpler than in vertebrates, and, for now, flies-based models should be considered only as a clue to further research [7].

A novel technique in the studies of pain are zebrafish-based nociception models. Different methods of causing pain in adult *Danio rerio* have been developed. However, more popular are models based on fish larvae, because they are cheaper and simpler. In those procedures nociceptive response can be caused most often via incubation in Acetic Acid (AA) solution. AA leads to damage of epidermal cells, but does not affect deeper tissues. After incubation larvae are moved to recovery solution, and nociceptive response can be observed

by tracking fish movement. Epidermal tissue damage leads to reduced animal activity, the higher AA concentration was, the larva movement will be more decreased [8].

Nociception receptors in mammals are free nerve endings, and include two types of fibres: myelinated Aδ, and unmyelinated C. Aδ fibres are thicker and conduct nerve impulse faster than C-fibres. Nociceptive stimulation leads to pain described as "pricking" (Aδ), or "pressing" and "dull" (C fibres). Studies show that 30% of mammalian C-fibre are polymodal, and respond to thermal and mechanical, as well as chemical incentive, while Aδ are sensitive to mechano-thermal stimulation. After receptor activation, nerve impulses are going to the central nervous system, via spinal cord to the brain, where information is interpretate [9].

The aquatic environment is relatively stable. Water is a high-efficient thermal isolator, and quickly dilutes most of noxious chemicals, thus fishes developed less C-fibres than mammals, and may have lower capacity to experience nociception. The amount of C-fibres is different among aquatic species. Studies show that despite a small number of C-fibres, teleost fish have polymodal Aδ fibres, which mirror its function. Aδ are myelinated, so nociceptive signalling may be faster in fish, than in mammals. *Danio Rerio* larvae are responsive to stimulation with CO_2-infused water, heat, and acetic acid. Interpretation of incentive is similar to mammals, taking place in the brain and the nerve impulse is conducted via spinal cord [9]. In zebrafish development of nociception begins in late embryogenesis. Dechorionation occurs between 48 to 72 hpf (hours post fertilization). At this stage larvae usually lay on side, and their movement is limited to spontaneously darting forward, or circling around. Fish respond to light, sound, touching, or water flow, and are more active in dark than in light. Between 96 and 120 hpf swim bladder begins to inflow, and larvae start to swim. 5 days old fish are no longer feeding via yolk sac, and start actively hunting for food, which may be connected with development of their vision. After 120 hpf, due to production of melatonin, fish become more active in light than in dark [10]. In response to noxious stimuli, in adult zebrafish avoidance behaviour, as well as changing movement activity, can be observed, while in larvae only changes in activity have been reported. Freezing may be connected with the strategy of predator avoidance. However, it remains unclear if lack of avoidance behaviour in larvae is survival strategy, or just caused by inability to swim as efficiently as adults, or whether larvae interpret noxious and stressful stimuli as extensively as older fishes [11].

Anaesthetics are developed with nociception models. According to regulations, preclinical trials should be performed on mammals. However, to reduce the number of used rodents, animals with potentially lower capacity to experience pain are useful in preliminary research. The interpretation of nociceptive stimuli in zebrafish larvae is still debatable, but it

is believed to be less noxious than in terrestrial vertebrates. Nonetheless, some scientists claim that nociception is developed faster than in 5 days, and protection age should be decreased. To get a clear answer on that hypothesis, further research should be performed.

The novelty and scientific application of the paper can be considered in two areas. Firstly, as a new research on expand of medical application of cannabinoids. The paper emphasizes the medical and scientific community's increased attention to cannabinoids, particularly CBG and CBD, as promising compounds with various potential medical applications. Secondly, it presents a development of a modern tool for screening the analgesic properties of new substances. The novel use of *Danio rerio*, as a cost-effective and relatively simple model for studying nociception is highlighted, making it a valuable tool for preliminary research. The main goal of the research was to develop the model of nociception to study potential analgesics properties of CBD and CBG in comparison to ibuprofen, commonly known and used pain killer. This research focus addresses the need for alternative analgesics and pain management strategies.

In summary, this paper combines scientific advancements and innovative research using zebrafish to investigate the potential therapeutic properties of cannabinoids. It contributes to the growing body of knowledge surrounding the medical applications of *Cannabis sativa* compounds and offers a new avenue for pain research using *Danio rerio* experimental model.

Results and discussion

To evaluate analgestic potential of studied cannabioids-orgin compounds, their cytotoxicity versus neuronal cell line has been checked first to determine their neurotoxicity and compare them to well known and commonly used drug, ibuprofen (IBU). Astrocyte cell line PG-4 (S+L-), that was isolated in 1980 from the brain of a normal embryo, has been used. This cell line was deposited by KJ Dunn and is widely used in neuroscience research. In neurological research, astrocytes are a type of glial cell that play an important role in supporting and regulating neuronal function. While neurons are traditionally the primary focus when studying pain, it's now well-recognized that glial cells, including astrocytes, also play a crucial role in modulating pain signaling and processing in the central nervous system [12].

Our study revealed (Fig. 1), that CBD exhibited the most neurotoxic properties, with toxicity independent on the duration time (IC_{50} within range 21.75-20.75 μg/ml), while toxicity of CBG towards glial cells was found comparable to ibuprofen (IC_{50} within range

189.1-151.4 µg/ml) after 24 hours of incubation (IC_{50} 118.5 µg/ml) with significant increase in neurotoxicity after longer incubation (up to 36.82 µg/ml after 72h).

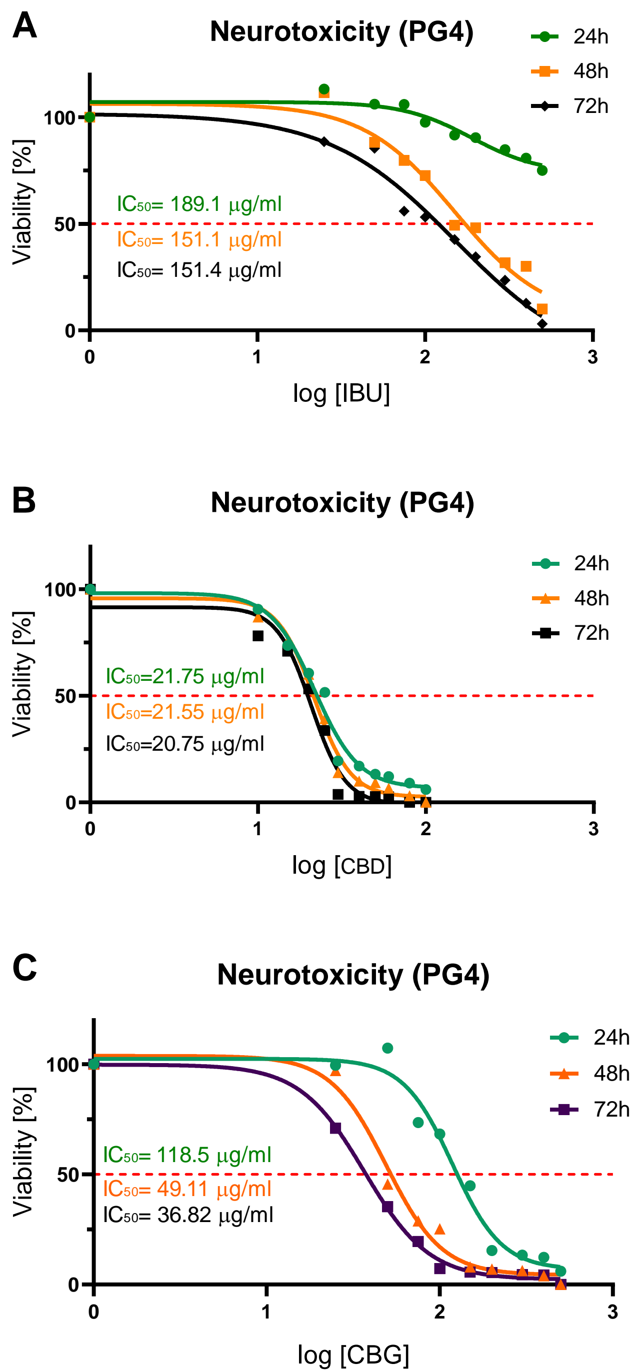

Fig. 1. Cytotoxicity results for IBU (A), CBD (B) and CBG (C).

Next stage of our experiment was to check toxicity of examined substances on tested model organism, i.e. *Danio rerio* embryos. Therefore Fish Embryo Acute Toxicity (FET) Test according to modified guidelines of OECD (Organisation for Economic Co-operation and Development), test no. 236 was implemented to evaluate toxicity CBD, CBG and IBU. The

Fish Embryo Toxicity test is a valuable tool in medical science and toxicology for assessing the potential harmful effects of chemicals and substances on developing fish embryos. This test primarily serves to evaluate the potential toxicity of pharmaceutical drugs. Before a new medication is approved for human use, it must undergo rigorous testing to ensure its safety. FET tests help in assessing the drug's potential impact on the early development of fish embryos, which can provide insights into its safety for use in humans. FET tests are considered a more humane alternative to traditional animal testing methods, such as tests conducted on mammals. Using fish embryos reduces the need for animal experimentation, aligning with ethical concerns related to animal welfare. Moreover, due to low costs FET tests can also be used as a screen platform to study high number of potential therapeutic compounds. Researchers can expose fish embryos to various substances to identify those that may have a positive impact on embryonic development. This can lead to the discovery of compounds with potential medical applications [13].

After 5 days of zebrafish embryo incubation, LC_{50} (Lethal Concentration for 50 % of larvae) has been defined as: IBU at 28.55 µg/ml, CBD at 0.7834 µg/ml, and CBG at 11.700 µg/ml. The results of toxicity tests are presented on Fig. 2. They were important, to set concentrations of examined substances for the proper experiment on nociception model. As an optimal non-toxic concentration during 40 min of incubation was chosen 0.5 – 1.0 µg/ml of CBG and CBD, and 5.156 – 10.314 µg/ml of IBU.

The applied experimental model was based on chemical nociception assays, where nociceptive responses were induced by applying noxious chemicals to zebrafish (acetic acid). Zebrafish larvae five days post fertilisation (dpf) were placed in solutions of 0.15%, 0.25% and 0.5% of acetic acid (AA) and their responses (such as swimming) were observed and AA-induced injuries were quantified.

Propidium iodide is a common dye used in flow cytometry. This chemical compound has an ability to stain necrotic cells. However, it is also useful in visualizing non-apoptotic dead cells in fluorescent microscopes. To define damage of the external tissue made by AA and protective properties of therapeutic, propidium iodide staining has been performed. Results have been evaluated by examination of the damage of the caudal fin, and are presented on Fig. 3.

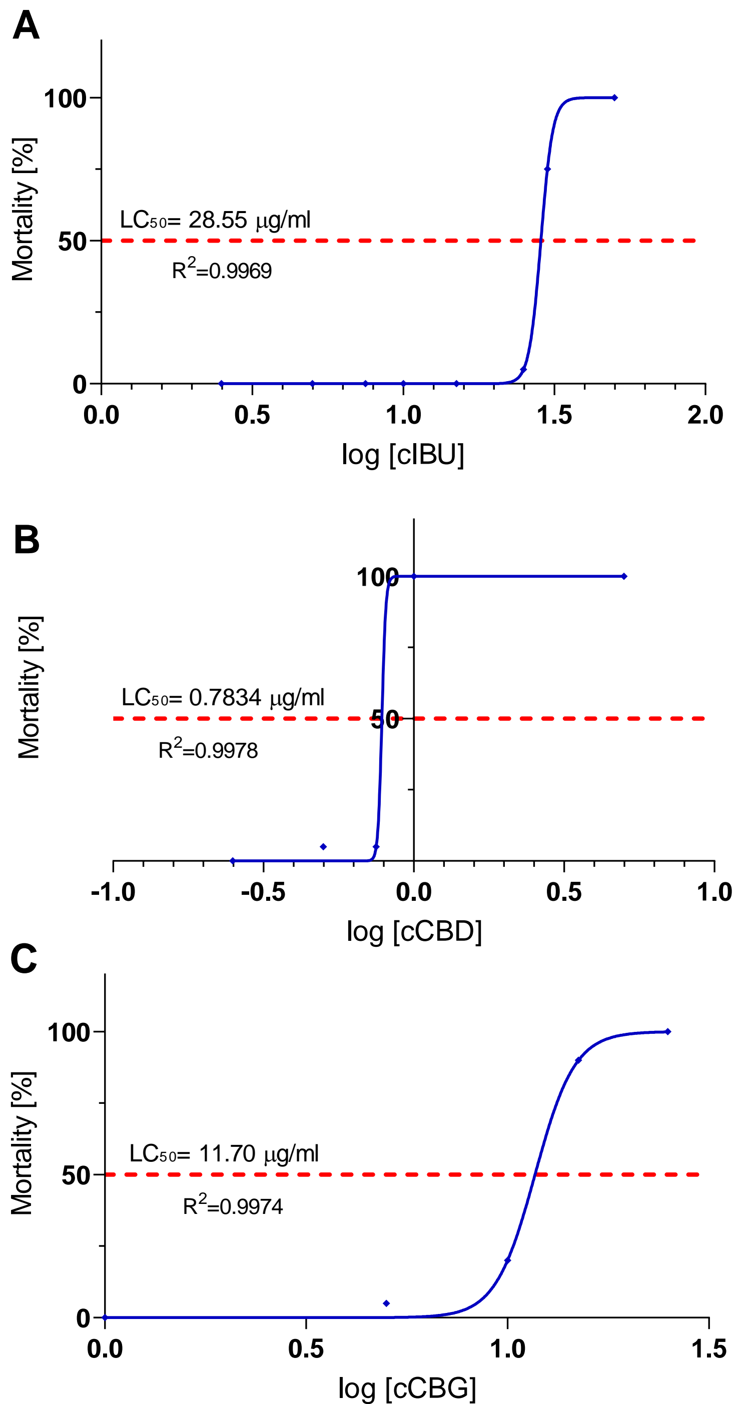

Fig. 2. Fish Embryo Toxicity test results for IBU (A), CBD (B) and CBG (C).

Experiment shown, that the damage of caudal fin of the larvae incubated in 5.156 µg/ml solution of IBU was lower than the control group in all three AA concentrations. In 10.314 µg/ml IBU solution group, propidium iodide staining showed significant reduction of the necrosis cells area. In both CBD and CBG 0.5 µg/ml groups decrease of damage was not observed, but in the groups incubated in 1.0 µg/ml cannabinoids, the level of caudal fin

damage was significantly lower than in the control group, with higher protection in the CBD group. However, the mechanism of protection was not examined in this investigation.

	AA 0.15%	AA 0.25%	AA 0.5%
cc			
IBU 25µM			
IBU 50µM			
CBD 0.5µg/ml			
CBD 1.0µg/ml			
CBG 0.5µg/ml			
CBG 1.0µg/ml			

Fig. 3. Acetic acid concentration-dependent damage of zebrafish larvae incubated, or not incubated in examined therapeutics.

In the first phase of the activity test, the impact of AA, IBU, CBD and CBG on zebrafish larvae behaviour compared to E3 control group has been evaluated. The mobility of fish incubated in 0.15% and 0.25 % AA was statistically significantly lower compared to E3 control, and in 0.5 % AA reduced even more than in less concentrated AA (Fig. 4**.** A, B), thus 0.5 % AA was not used in further research. In the control group of therapeutics, mobility of larvae in IBU was comparable to E3 control, and lower in cannabinoids with higher reduction in CBG than in CBD. Among therapeutics only in CBG statistically significant reduction in larvae locomotor activity has been observed (Fig. 4. C, D). Due to one variable (therapeutics), statistical significance has been determined with one-way ANOVA test.

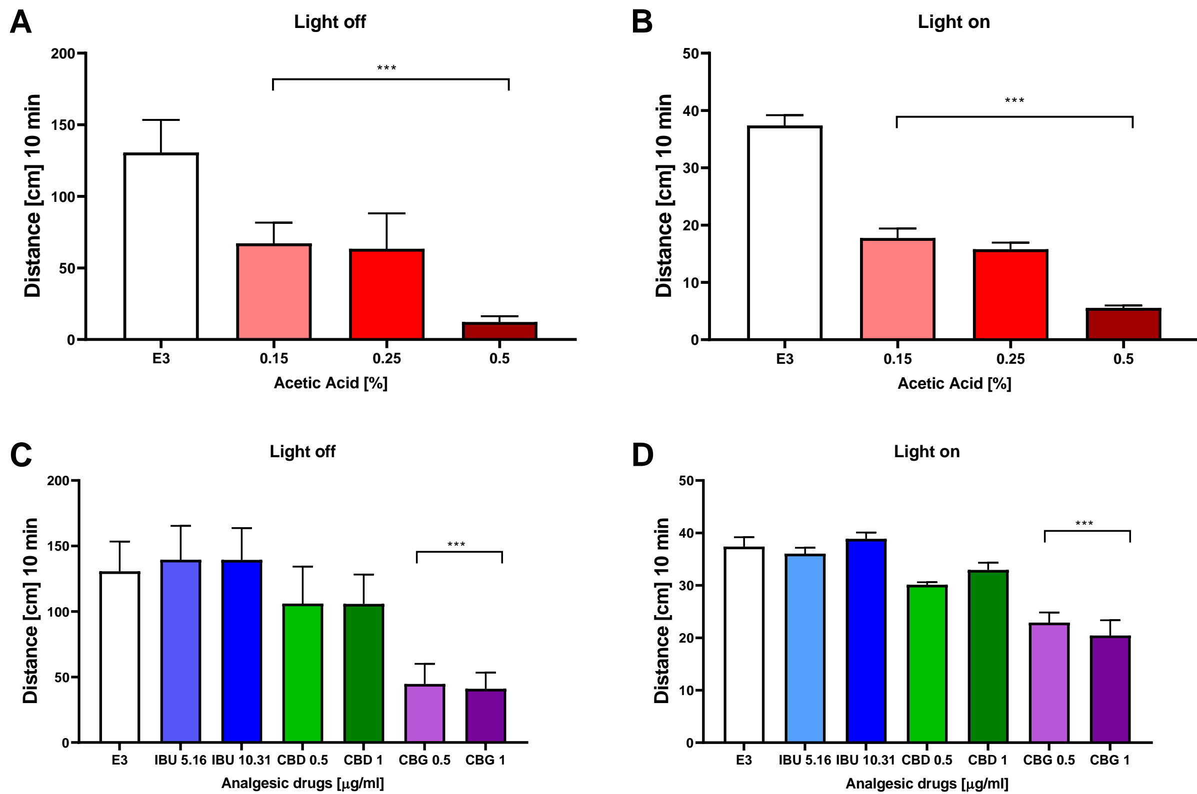

Fig. 4. Comparison the covered distance of zebrafish larvae incubated in AA, IBU, CBD and CBG with animals without incubation (E3). Statistical significance has been marked by *** for p < 0.001 vs E3 control group, post-hoc Tukey test.

Second phase of this test included comparing behavioural alterations in groups preincubated in therapeutics and then in AA, with groups incubated in AA without preincubation. In the preincubated groups, both IBU and CBD lead to statistically significant increase in larvae activity compared to fish without preincubation. In light and dark IBU and dark CBD groups (Fig. 4. A – C) mobility was higher in more concentrated therapeutics in both 0.15 % and 0.25 % AA. The same dependence was observed in the CBD light group (Fig. 5. D). However, in the CBD dark group (Fig. 5. C) increased activity was comparable in both CBD concentrations. The larvae in groups preincubated in 0.5 µg/ml CBG (Fig. 5. E, F) were insignificantly more active, but preincubation in 1.0 µg/ml CBG (Fig. 5. E, F) lead to statistically significant decrease in mobility when the light was off in both AA concentrations, compared to groups without preincubation (Fig. 5. E). Due to two variables (AA concentration and therapeutics), in the 2nd phase statistical significance has been determined with a two-way ANOVA test. Experiments also showed that zebrafish larvae are more active in dark, than in light, and behavioural alterations of animals preincubated in CBD were more significant in the phase of test performed in light.

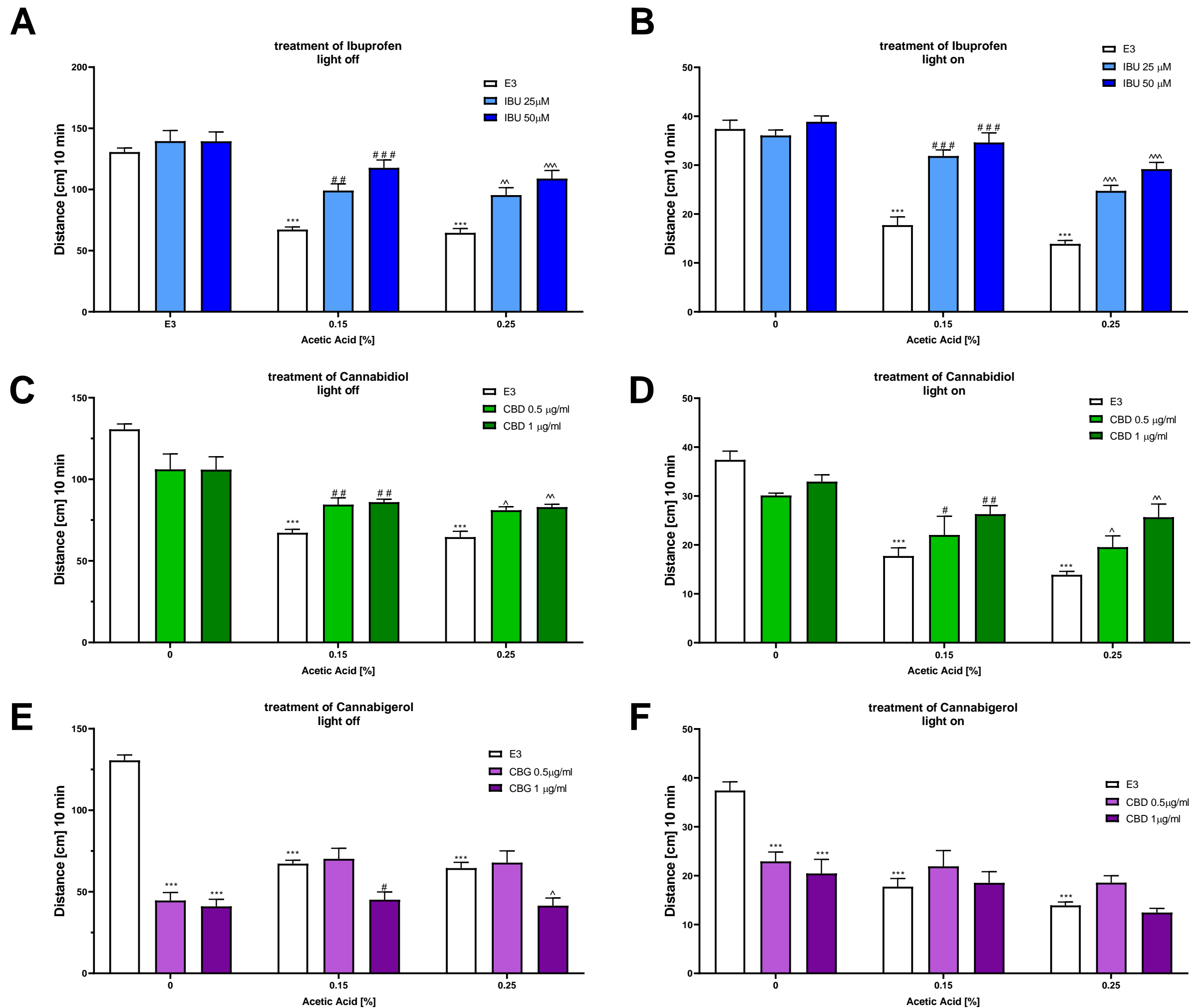

Fig. 5. Comparison the covered distance of zebrafish preincubated in therapeutics (IBU – A, B; CBD – C, D; CBG – E, F) and then incubated in AA. Data collected in trials performed in dark (A, C, E) and in light (B, D, F). Statistical significance respectively: * for comparison to E3 control (*** = p < 0.001); # for comparison to 0.15 AA control (# = p < 0.01, ## = p < 0.05, ### = p <0.001); ^ for comparison to 0.25 AA control (^ = p < 0.01, ^^ = p < 0.05, ^^^ = p <0.001), post-hoc Tukey test.

Previous research has shown that incubating zebrafish larvae in AA solution leads to damage of caudal fin external tissue [8]. Our experiment proved that this effect can be reduced by preincubation of animals in the solution of analgesic drugs (Fig. 3**.**). Acute inflammation is an inherent part of the healing process, but incubation in AA may lead to excessive inflammation, affect surrounding cells, and increase tissue damage. IBU is a well-known, widely used non-steroidal anti-inflammatory drug (NSAID). Its properties are connected with the inhibition of cyclooxygenase enzymes (COX), which induce expression of inflammatory mediators [14]. It is known that COX occurs not only in humans, but also in other species like zebrafish [15], thus protective mechanisms in aquatic animals may be

connected with anti-inflammatory properties of IBU. Our studies shown, that damage made by AA can be also reduced by incubating larvae in CBD or CBG solution. Since the zebrafish has an endocannabinoid system similar to humans [16], cannabinoids can help to avoid excessive inflammation, but their mechanism of action is different than IBU and does not include COX inhibition. Endocannabinoid receptors 2 (CB_2) are expressed in T cells, B cells, and macrophages. Anti-inflammatory properties of cannabinoids are based on inhibition of activation and proliferation of immune response cells involved in inflammation. Despite different mechanisms, the overall effect is similar to IBU [17]. However, while analyzing the results of the experiment with CBD and CBG, their physical properties should also be considered. Cannabinoids hardly dissolve in water, thus they could partly cover larvae epidermis making a physical barrier to AA. Finally, we should also consider analgesic properties of IBU and cannabinoids, and hypotheses where preincubated larvae experience reduced pain, decreased movement, and were less exposed to AA compared to fish without preincubation. Nonetheless, to define which mechanism is responsible for protective properties of researched drugs, further research should be performed.

Studies confirmed that incubation of zebrafish larvae in AA lead to significant changes in their behaviour, which is believed to be connected with experiencing pain (Fig. 4. A, B). Additionally, preincubation in IBU and CBD can lower reduction of animal activity affected by noxious stimuli. Analgesic properties of IBU are already well known and described. The mechanism is based on reduction of prostaglandins level via blocking COX-1 and COX-2, which lead to desensitisation of free nerve endings, considered as nociceptive receptors [18]. Previous studies showed that some cannabinoids may also have analgesic properties [19]. However, the way those compounds influence the human body is still not fully understood. There are different hypotheses of pain-relieving ability of CBD. Since CBD can function as an inhibitor of humoral and cell-mediated immunity, it may reduce noxious stimuli connected with inflammation [20]. CBD can also influence brain neurotransmitter pathways. This cannabinoid can elevate serotonin level and reduce reuptake of anandamide – endocannabinoid involved in pain modulation. Finally, CBD can desensitise nociceptive receptors via affecting transient receptor potential (TRP) channels, and directly inhibit experiencing of noxious stimuli [21]. CBG is a one of the less-known cannabis derived cannabinoids. It is believed that this compound may have similar properties to CBD, but with lower toxicity [22]. Our studies showed that preincubation in CBG, unlike other used therapeutics, lead to reduction of zebrafish larvae locomotor activity. Due to lack of proper research, only hypothesis could be drawn why zebrafish larvae were less mobile than animals incubated in AA. Previous studies showed that CBG is a partial agonist of CB

receptors [23]. Agonizing CB_1 in the brain leads to inhibition of gamma-aminobutyric acid (GABA) release [24]. GABA is a primary inhibitory neurotransmitter in the brain, thus blocking its release to synapse will lead to constant stimulation of nerve and its overexcitability [25]. Reduced capability to conduct nerve stimuli may lead to decreased locomotor activity.

Previous studies showed that zebrafish larvae prefer light over dark environments,thus there are mostly diurnal animals [26]. However, our research showed that 96 to 120 hpf zebrafish larvae are typically more active in dark than in light (Fig. 4**.** A, C compared to B, D; Fig. 5. A, C, E compared to B, D, F). This phenomenon may be connected with the presence of melanopsin-expressing cells in the preoptic region of zebrafish. These photoreceptors are activated by the loss of illumination and can trigger light-seeking behaviour. Despite being diurnal, zebrafish larvae in dark are looking for a source of light, thus animals exhibit higher locomotor activity in dark, compared to light [27], [28]. Nonetheless, the possibility that reduction of larvae mobility was triggered by stress caused via sudden turning on the light should also be considered.

Material and methods

In vitro study:

Cytotoxicity assays: cell proliferation - MTT assay

PG-4 (S+L) a glial, astrocyte cell line that was isolated in 1980 from the brain of a normal embryo was used to determine cytotoxicity versus brain cells - neurotoxicity. This cell line was deposited by KJ Dunn and can be used in neuroscience research. Normal brain cat (Feline catus) astrocyte cell line (ATCC-CRL-2032™:) was cultured using McCoy's 5A medium supplemented with 10% heat-inactivated FBS (v/v), 100 U/ml penicillin, and 100 µg streptomycin (complete medium). Cells were maintained at 37°C in a humidified atmosphere of 95% air/5 % CO_2. Cultures were subcultivated every 3-4 days by trypsinization (0.25 % trypsin/EDTA). For toxicity evaluation cells were plated in complete medium (10% FBS) at density $2*10^5$ cells/ml in 96 well plates 24 hours before treatment, and then exposed to IBU, CBD and CBG in serum free medium in sextuplicate. The cells were incubated for 24, 48 and 72 hours and then 10 µL of a 0.5 mg/mL MTT (3-(4,5-Dimethyl-2-thiazolyl)-2,5-diphenyl-2H-tetrazolium bromide by Sigma Aldrich cat no. M5655-1G) solution was added to each well, followed by incubation for 3 h at 37°C. The supernatants were removed and 100 µl of DMSO (Sigma Aldrich) per well was added to dissolve precipitated formazan. The plate was agitated for 10 min and absorbance was measured at 560 and 620 nm using an BioTek Epoch plate

reader (BioTek Instruments, United States). From cells viability values IC_{50} was calculated for each substance (Figure 1).

In vivo study:

Danio Rerio of AB OMD strain were maintained at 28.5°C, with a 14/10h light/dark cycle. Parameters of breeding were consistent with standard aquaculture conditions. Eggs were sourced via natural spawning. Unfertilized eggs were removed. Embryos were incubated in standard light/dark cycle in embryo medium: pH 7.1–7.3, 17.4 µM NaCl, 0.21 µM KCl, 0.12 µM $MgSO_4$ and 0.18 µM $Ca(NO_3)_2$ in an incubator at 28.5°C up to 96 hpf, but no longer than 120 hpf, when the local ethic committee approval is needed. In the experiment the 96 hpf larvae were proceeded into studies. Due to EU Directive, 2010/63/EU, all procedures have been terminated before larvae reached 120 hpf.

Fish Embryo Toxicity Test

The toxicity of the studied chemical compounds was evaluated to define the highest, non-toxic to fish larvae concentration. Procedure has been performed according to OECD *Guidelines for the Testing of Chemicals, Test No. 236*. Researched chemicals include: Ibuprofen (IBU), Cannabidiol (CBD) and Cannabigerol (CBG).

Necrosis cells staining

Five days post fertilisation (dpf) zebrafish larvae were placed in a FALCON 60 x 15 mm Tissue Culture Dish, 50 larvae per dish. Fish were incubated in E3 media, or E3 with therapeutics treatment for 40 minutes. After incubation larvae were placed in the NEST 6 well Cell Culture Plates fitted with Falcon 70 µm Cell Strainer, 10 larvae per well. Fish were exposed to E3 media, or AA solutions (0.15, 0.25, 0.5%) made from a 99.9% AA stock solution in E3 media for 90 s. Optimal concentration of AA was not evaluated in this experiment, but was defined based on L. D. Ellis at al., 2018 [8]. Larvae were washed in HE3 media (E3 + HEPEs 10 mM, pH 7.2), and incubated for 40 s in 10 µl propidium iodide (PI, 1.5 mM) solution in 8 ml HE3 media. To minimise light degradation, PI has been added to HE3 media directly before placing larvae. Liquid which remained in the Cell Strainer during moving it to another solution, was removed with a paper towel before next incubation to decrease dilution. After last incubation fish were removed from the Cell Strainer and moved to the 2 ml of tricaine solution in HE3 (50 µl of 4 mg/ml tricaine per 2 ml HE3). Immobilize larvae were placed in a FALCON 60 x 15 mm Tissue Culture Dish in a drop of the last solution (tricaine in HE3 media), and imaged using a Carl Zeiss Microscopy GmbH SteREO Discovery V8 fluorescent microscope at 4 x zoom, dark: 200 – 400 ms / light: auto. Images

were processed with Zeiss ZEN Microscopy Software. Used therapeutic and concentrations: IBU 5.156 – 10.314 µg/ml (~25-50 µM), CBD 0.5-1.0 µg/ml, CBG 0.5-1.0 µg/ml.

Activity tracking

96 to 120 hpf zebrafish larvae were placed in a FALCON 60 x 15 mm Tissue Culture Dish, 15 larvae per dish. Fish were incubated in E3 media, or E3 with therapeutics for at least 40 minutes. After incubation larvae were placed in a NEST 6 well Cell Culture Plates fitted with Falcon 70 µm Cell Strainer, 15 larvae per well. Fish were steeped in E3 media, 0.15% AA solution, or 0.25 % AA solution made from a 5 % AA stock solution in E3 media for 90 s. Larvae were washed in HE3 and moved to a NEST 96 well Cell Culture Plates, one larva per well. Solution in which larvae were placed in wells has been replaced with 100 µl of HE3 media. The plates were placed into the Noldus DanioVision plate holder. The room temperature of 28°C has been maintained with an electric heater. Larva activity was tracked with Noldus EthoVision XT 17 software. Protocol included 10 min in dark and 10 min in light behavioural analysis, during which images have been taken every 1 s. Lack of movement during 20 min of trial was considered as a death of larva and those data were excluded from further analysis. Used therapeutic and concentrations: IBU 5.156 – 10.314 µg/ml, CBD 0.5-1.0 µg/ml, CBG 0.5-1.0 µg/ml. Number of used larvae: E3 control – 240, AA controls – 140 for each concentration, test groups – 90 for each concentration of IBU and CBD, 70 for each concentration of CBG.

Conclusions

Our studies confirmed that incubation of zebrafish larvae in acetic acid lead to the damage of caudal fin. It was also demonstrated that this effect could be mitigated by preincubation of the animals in therapeutic drugs. Decreased animal locomotor activity due to potentially painful stimuli was observed in behavioral tests. However, it was shown that this reduction could be alleviated by preincubation in ibuprofen or cannabidiol. Furthermore, it was demonstrated that preincubation in cannabigerol not only failed to improve fish locomotor activity but also exacerbated the decrease.

Additionally, it was confirmed that zebrafish larvae exhibited higher activity in dark conditions compared to light conditions, despite being diurnal. In summary, an easy and accessible zebrafish larvae-based model of nociception was developed through our research. Nonetheless, to fully all processes and mechanisms which took place in this model, further studies should be performed.

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