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Oxidative stress biomarkers in the blood of rainbow trout (*Oncorhynchus mykiss* Walbaum) and equine plasma after incubation with hemp oil "Annabis BIO"

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Industrial hemp is a multi-use crop that has been widely cultivated to produce fibers and nutrients, such as protein, dietary fiber, minerals, and unsaturated fatty acids, which make them a good fortifying component in food production. The antioxidant capability of hemp oils has been reported. In the current study, for evaluating the antioxidant activity of commercial hemp oil "Annabis BIO" derived from certified industrial hemp seeds without the psychoactive substance THC (Olomouc, Czech Republic), biomarkers of oxidative stress [2-thiobarbituric acid reactive substances (TBARS), oxidatively modified proteins (OMP), total antioxidant capacity (TAC)] were used in models of the blood collected from adult healthy rainbow trout (*Oncorhynchus mykiss* Walbaum), the blood of rainbow trout with clinical symptoms of ulcerative dermal necrosis (UDN), and equine plasma. A volume of 0.1 mL of the hemp oil was added to 1.9 mL of fish blood or equine plasma. After incubation of the mixture for 60 min with continuous stirring, biomarkers of oxidative stress were studied in samples. After *in vitro* incubation of hemp oil with the blood of clinically healthy rainbow trout, we noted a statistically significant decrease in biomarkers of lipid peroxidation by 55.6% ($p < 0.05$). The highest increase in TBARS level was observed after *in vitro* incubation of hemp oil with the blood of UDN-affected rainbow trout. *In vitro* incubation of hemp oil with equine plasma resulted in a statistically significant increase in the level of ketonic derivatives (by 29%, $p < 0.05$) and aldehydic derivatives of OMP (by 33.1%, $p < 0.05$). Incubation of hemp oil with the blood of UDN-affected trout resulted in a decrease of the ketonic derivative of OMP (by 43.3%, $p < 0.05$). Incubation of hemp oil with equine plasma, we observed a statistically significant decrease in TAC level by 56.6% ($p < 0.05$). Similarly, after incubation hemp oil with blood samples of UDN-affected trout, a statistically significant decrease in total antioxidant capacity (by 59.3%, $p < 0.05$) was observed. The results suggest that the investigated hemp oil have shown varied antioxidant capacities. Accordingly, this study proposes that the therapeutic benefit of this hemp oil can be, at least in part, attributed to using different biological materials (blood, plasma) used *in vitro* in the current study.

Keywords: hemp oil, oxidative stress biomarkers, rainbow trout, equine plasma, *in vitro*

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Introduction

Oxidative stress (OS), defined as disturbances in the pro- and antioxidant balance, is harmful to cells due to the excessive generation of highly reactive oxygen (ROS) and nitrogen (RNS) species (Filomeni et al., 2015). When the balance is not disturbed, OS has a role in physiological adaptations and signal transduction (Apel and Hirt, 2004; Finkel, 2011). However, an excessive amount of ROS and RNS results in the oxidation of biological molecules such as lipids, proteins, and DNA (Juan et al., 2021). Oxidative stress has been reported in many diseases, due to both antioxidant depletions as well as increased ROS production (Forman and Zhang, 2021). For example, the kidney is a highly metabolic organ, rich in oxidation reactions in mitochondria, which makes it vulnerable to damage caused by OS, and several studies have shown that OS can accelerate kidney disease progression (Sies, 2015). On the other hand, oxidative stress is important in the pathophysiology of altering regulatory factors of mitochondrial activity, modifying the concentration of inflammation mediators associated with a large number and size of adipocytes, promoting lipogenesis, stimulating differentiation of preadipocytes to mature adipocytes, and regulating the energy balance in hypothalamic neurons that control appetite (Jones, 2008).

In the last decades, a lot of attention has been paid to the compounds present in medicinal *Cannabis sativa* L., such as Δ^9-tetrahydrocannabinol (Δ^9THC) and cannabidiol (CBD), and their effects on inflammation and other disorders (Pellati et al., 2018). *Cannabis sativa* L. is a dioicous plant of the Cannabaceae family and it is widely distributed all over the world (Pellati et al., 2018). It has been used as a psychoactive drug, as a folk medicine ingredient, and as a source of textile fibre since ancient times. *Cannabis* is thought to have originated from central Asia and has been domesticated for over 5,000 years (Irakli et al., 2019; Farinon et al., 2020). *Cannabis* varieties that are low in psychoactive cannabinoids are used to produce fiber and oilseed. However, the most valuable cannabis product today is the terpene- and cannabinoid-rich resin with its various psychoactive and medicinal properties. The resin is produced and accumulates in glandular trichomes that densely cover the surfaces of female (pistillate) inflorescences and, to a lesser degree, the foliage of male and female plants. In total, more than 150 different terpenes and approximately 100 different cannabinoids (House et al., 2010; VanDolah et al., 2019). *C. sativa* is characterized by a complex chemical composition, including terpenes, carbohydrates, fatty acids and their esters, amides, amines, phytosterols, phenolic compounds, and the specific compounds of this plant, namely, the cannabinoids. In the ambit of nonpsychoactive compounds, cannabichromene (CBD) represents the most valuable one from the pharmaceutical point of view, since it has been found to possess a high antioxidant and anti-inflammatory activity, together with antibiotical, neuroprotective, anxiolytic, and anticonvulsant properties. Cannabidiolic acid (CBDA) has antimicrobial and antinausea properties, while cannabigerol (CBG) has anti-inflammatory, antimicrobial, and analgesic activities. Thanks to its lack of psychoactivity, CBD is one of the most interesting compounds, with many reported pharmacological effects in various models of pathologies, from inflammatory and neurodegenerative diseases to epilepsy, autoimmune disorders like multiple sclerosis, arthritis, schizophrenia, and cancer (Sommano et al., 2020). Concerning other phenolics present in *C. sativa*, several flavonoids have been identified, belonging mainly to flavones and flavonols, together with cannflavins A and B, which are *C. sativa* typical methylated isoprenoid flavones. *Cannabis* flavonoids exert several biological effects, including properties possessed also by cannabinoids and terpenes. Anti-inflammatory, neuroprotective, and anti-cancer activities have been described for these compounds (Rupasinghe et al., 2020).

Hemp essential oil can inhibit or reduce bacterial growth, also exerting antioxidant activity, and therefore it can find an advantageous application in the food processing field (Pellegrini et al., 2021). Hemp essential oil can inhibit or reduce bacterial proliferation and it can be a valid support to reduce microorganism contamination, especially in the food processing field (Iseppi et al., 2019). Hemp inflorescences can be used as a source of natural antioxidants in vegetable oils and lipid products to retard their oxidation, especially those characterized by a high degree of unsaturation (Cantele et al., 2020). Hempseed and hempseed oil can safely be utilized as feed ingredients for laying hens to produce table eggs that are enriched in essential fatty acids. Additionally, the eggs procured from these hens had a similar aroma and flavor compared to eggs from hens that did not feed any hemp. The greater the dietary hemp inclusion, the more pigmented the resulting yolks became in terms of darkness, redness, and yellowness (Goldberg et al., 2012). Also, hemp seed products could be recommended as a feed ingredient for enhancing the essential fatty acid contents of fish which in turn can have a good impact on consumer health (Afridi et al., 2019). Protein content and amino

acids profile of hempseed and hempseed derivatives (cake, meal, and oil) make these products suitable for inclusion in ruminant diets. In addition, the fatty acid composition of hemp oil allows to transfer of the PUFA and in particular, n-3 fatty acid to the milk of dairy ruminants (Bailoni et al., 2021).

The oil extracted from hemp seeds has significant biological properties through the unique composition of polyunsaturated fatty acids, chemical elements, and various antioxidant compounds (Vitorović et al., 2021). The potential of this oil for the prevention of oxidative stress and for the treatment of oxidative-stress-induced ailments is of increasing interest (Vitorović et al., 2021).

In the current study, for evaluating the antioxidant activity of commercial hemp oil "Annabis BIO" derived from certified industrial hemp seeds without the psychoactive substance THC (Olomouc, Czech Republic), oxidative stress biomarkers [2-thiobarbituric acid reactive substances (TBARS), oxidatively modified proteins (OMP), total antioxidant capacity (TAC)] were used in models of the blood collected from adult healthy rainbow trout (*Oncorhynchus mykiss* Walbaum), the blood of rainbow trout with clinical symptoms of ulcerative dermal necrosis (UDN), and equine plasma.

Material and methodology

Hemp oil

Cold-pressed commercial hemp oil "Annabis BIO" 250 ml (Olomouc, Czech Republic) from certified industrial hemp seeds, without the psychoactive substance THC, was used for the current study. The greatest treasure of hemp oil is the unsaturated fatty acids Omega-3 and Omega-6, which are about 75%, including linoleic, alpha-linolenic, gamma-linolenic, and oleic acids. The ratio of these acids is 3 : 1 (Omega-6 to Omega-3). Hemp oil is a source of valuable vitamins (i.e. A, B_1, B_2, B_3, B_6, C, D, E) and minerals (calcium, magnesium, sulfur, potassium, iron, zinc, and phosphorus). In addition, "Annabis BIO" hemp oil is rich in vitamin K, essential for the synthesis of important proteins and enzymes, which has anti-hemorrhagic, antifungal, antibacterial, anti-inflammatory, and analgesic properties. Vitamin K also plays an important role in calcium metabolism. "Annabis BIO" hemp oil also contains health-promoting phytosterols and phospholipids, contained in cell membranes in all living organisms. It contains 20 essential amino acids, including 9 essential amino acids that cannot be synthesized by the body itself, so must be provided through the diet. The green color of the oil is caused by the presence of a large amount of chlorophyll, which acts as a strong antioxidant, possessing antiseptic, astringent, and regenerating properties. It also improves the blood supply to the skin, therefore it contributes to its oxygenation and nourishment. Hemp oil is credited with improving the functioning of the cardiovascular system, i.e. supporting the heart and blood vessels, regulating blood pressure and cholesterol levels. In addition, it supports the immune system and thus contributes to the building of the body's immunity (https://dobrekonopie.pl/product/olej-konopny-bio-250ml/).

Fish and collection of blood samples

Clinically healthy rainbow trout (*Oncorhynchus mykiss* Walbaum) with a mean body mass of 300–350 g were used in the experiments. The study was carried out in the Department of Salmonid Research, Stanisław Sakowicz Inland Fisheries Institute (Rutki, Poland). The experiments were performed in water at 14.5 ±0.5 °C and pH 7.2–7.4. The fish were fed a commercial pelleted diet. Adult fish, 3–5 years of age, were collected from the site on the Słupia River, Słupsk, the central part of northern Poland. The blood sampling for analysis from males and females of trout affected by ulcerative dermal necrosis (UDN) syndrome was collected directly after the catch. After catching, microbiological tests were also performed. These tests revealed that *Aeromonas* spp. complex caused the UDN syndrome. Blood was drawn from the efferent branchial arteries of the rainbow trout. Blood was stored in tubes with sodium citrate as the anticoagulant and held on the ice. A volume of 0.1 mL of the hemp oil was added to 1.9 mL of blood samples. For positive control, 4 mM phosphate buffer (pH 7.4) was used. After incubation of the mixture at 25 °C for 60 min with continuous stirring, biomarkers of oxidative stress were studied in samples.

Horses and collection of blood samples

Eighteen healthy adult horses from the central Pomeranian region in Poland (Strzelinko, N 54º 30' 48.0" E 16º 57' 44.9"), aged 8.9 ±1.3 years old, including 6 Hucul pony, 5 Thoroughbred horses, 2 Anglo-Arabian horses, and 5 horses of unknown breed, were used in this study. All horses participated in recreational horseback riding. Horses were housed in individual boxes, with feeding (hay and oat) provided twice a day, at 08.00 and 18.00 h, and water available *ad libitum*. All horses were thoroughly examined clinically and screened for hematological, biochemical, and vital parameters that were in the reference ranges. The females were non-

pregnant. Blood was drawn from the jugular vein of the animals in the morning, 90 minutes after feeding, while the horses were in the stables (between 8:30 and 10:00 AM). Blood samples were stored in tubes with 3.8% sodium citrate as the anticoagulant and held on the ice until centrifugation at 3000 rpm for 5 min to remove plasma. The pellet of blood was re-suspended in 4 mM phosphate buffer (pH 7.4). A volume of 0.1 mL of the hemp oil was added to 1.9 mL of plasma. For positive control, 4 mM phosphate buffer (pH 7.4) was used. After incubation the mixture at 37 °C for 60 min with continuous stirring, biomarkers of oxidative stress in samples were studied. Plasma aliquots were used in the study.

The 2-Thiobarbituric acid reactive substances (TBARS) assay

The level of lipid peroxidation was determined by quantifying the concentration of 2-thiobarbituric acid reacting substances (TBARS) with the Kamyshnikov (2004) method for determining the malonic dialdehyde (MDA) concentration. This method is based on the reaction of the degradation of the lipid peroxidation product, MDA, with TBA under high temperature and acidity to generate a colored adduct that is measured spectrophotometrically. The nmol of MDA per mL was calculated using $1.56.10^5$ $mM^{-1}.cm^{-1}$ as the extinction coefficient.

The carbonyl derivatives content of protein oxidative modification (OMP) assay

To evaluate the protective effects of hemp oil against free radical-induced protein damage in equine plasma and fish blood, a carbonyl derivatives content of protein oxidative modification (OMP) assay based on the spectrophotometric measurement of aldehydic and ketonic derivatives in the erythrocyte suspension and plasma was performed. The rate of protein oxidative destruction was estimated from the reaction of the resultant carbonyl derivatives of amino acid reaction with 2,4-dinitrophenylhydrazine (DNFH) as described by Levine et al. (1990) and as modified by Dubinina et al. (1995). DNFH was used for determining carbonyl content in soluble and insoluble proteins. Carbonyl groups were determined spectrophotometrically from the difference in absorbance at 370 nm (aldehydic derivatives, OMP_{370}) and 430 nm (ketonic derivatives, OMP_{430}).

Measurement of total antioxidant capacity (TAC)

The TAC level in the samples was estimated by measuring the 2-thiobarbituric acid reactive substances (TBARS) level after Tween 80 oxidation. This level was determined spectrophotometrically at 532 nm (Galaktionova et al., 1998). Sample inhibits the Fe^{2+}/ascorbate-induced oxidation of Tween 80, resulting in a decrease in the TBARS level. The level of TAC in the sample (%) was calculated with respect to the absorbance of the blank sample.

Statistical analysis

The mean ± S.E.M. values were calculated for each group to determine the significance of the intergroup difference. All variables were tested for normal distribution using the Kolmogorov-Smirnov and Lilliefors test ($p > 0.05$). The significance of differences between the levels of oxidative stress biomarkers (significance level, $p < 0.05$) was examined using the Mann-Whitney *U* test (Zar, 1999). All statistical calculation was performed on separate data from each individual with Statistica 13.3 software (StatSoft, Krakow, Poland).

Results and discussion

The batch spectrophotometric and spectrofluorometric 2-thiobarbituric acid (TBA)-based methods are the most commonly used assays to measure lipid peroxidation (Tsikas, 2017). Level of lipid peroxidation determined by the concentration of TBARS in the blood of clinically healthy rainbow trout, the blood of rainbow trout with clinical symptoms of UDN, and equine plasma after incubation with commercial hemp oil "Annabis BIO" (Olomouc, Czech Republic) was presented in Figure 1.

Analyzing data presented in Figure 1, we obtained statistically significant changes in TBARS content in the blood and plasma samples after *in vitro* incubation with hemp oil. After *in vitro* incubation of hemp oil with the blood of clinically healthy rainbow trout, we noted a statistically significant decrease in biomarkers of lipid peroxidation by 55.6% ($p < 0.05$), i.e. TBARS content was (6.15 ±0.46 nmol. mL^{-1}) compared to the control samples (13.85 ±0.85 $nmol.mL^{-1}$). The contrary tendency was observed after incubation of hemp oil with blood samples of rainbow trout with clinical symptoms of UDN, where we recorded a statistically significant increase in TBARS content by 594.2% ($p < 0.05$) compared to the values in control untreated samples (79.49 ±5.1 nmol. mL^{-1} *vs.* 11.45 ±1.13 $nmol.mL^{-1}$). Also, when hemp oil was exposed to the equine plasma, we observed a statistically significant increase in the concentration of lipid peroxidation end products by 64.5% ($p < 0.05$),

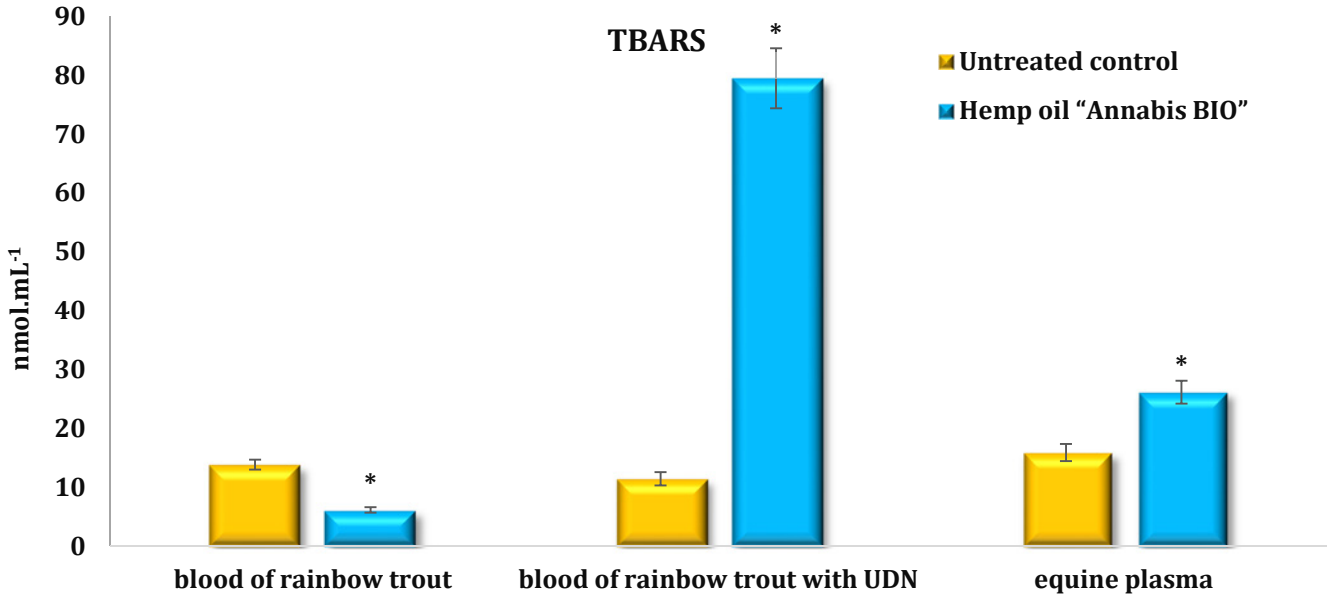

Figure 1 Level of lipid peroxidation determined by the concentration of 2-thiobarbituric acid reactive substances in the blood of clinically healthy rainbow trout, the blood of rainbow trout with clinical symptoms of ulcerative dermal necrosis (UDN), and equine plasma after incubation with commercial hemp oil "Annabis BIO"
Results are presented as the mean (M) ± the standard error of the mean (S.E.M.)
* changes were statistically significant ($p < 0.05$) compared to the untreated controls

i.e. (26.15 ±1.94 nmol.mL^{-1}) compared to untreated controls (15.9 ±1.45 nmol.mL^{-1}) (Figure 1).

Level of aldehydic derivatives of OMP in the blood of clinically healthy rainbow trout, the blood of rainbow trout with clinical symptoms of UDN, and equine plasma after incubation with commercial hemp oil "Annabis BIO" was presented in Figure 2. After *in vitro* incubation of hemp oil with equine plasma, we observed a statistically significant increase in the level of aldehydic derivatives of oxidatively modified proteins by 33.1% ($p < 0.05$), i.e. (19.45 ±0.89 nmol.mL^{-1})

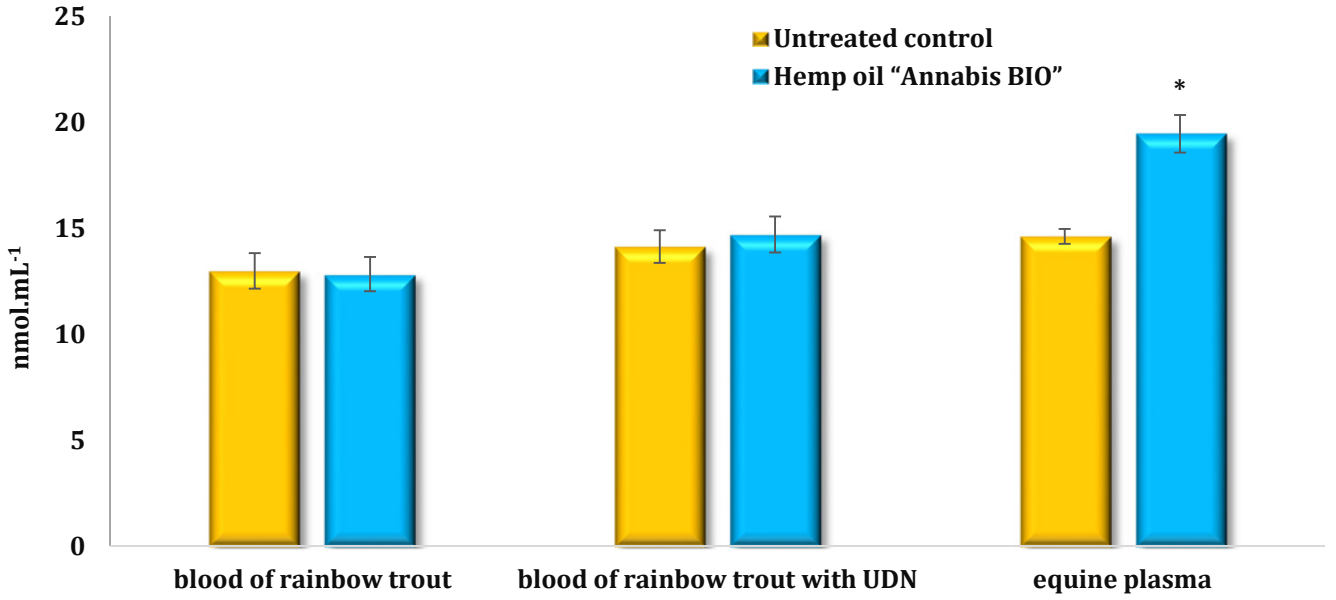

Figure 2 Level of aldehydic derivatives of oxidatively modified proteins in the blood of clinically healthy rainbow trout, the blood of rainbow trout with clinical symptoms of ulcerative dermal necrosis (UDN), and equine plasma after incubation with commercial hemp oil "Annabis BIO"
Results are presented as the mean (M) ± the standard error of the mean (S.E.M.)
* changes were statistically significant ($p < 0.05$) compared to the untreated controls

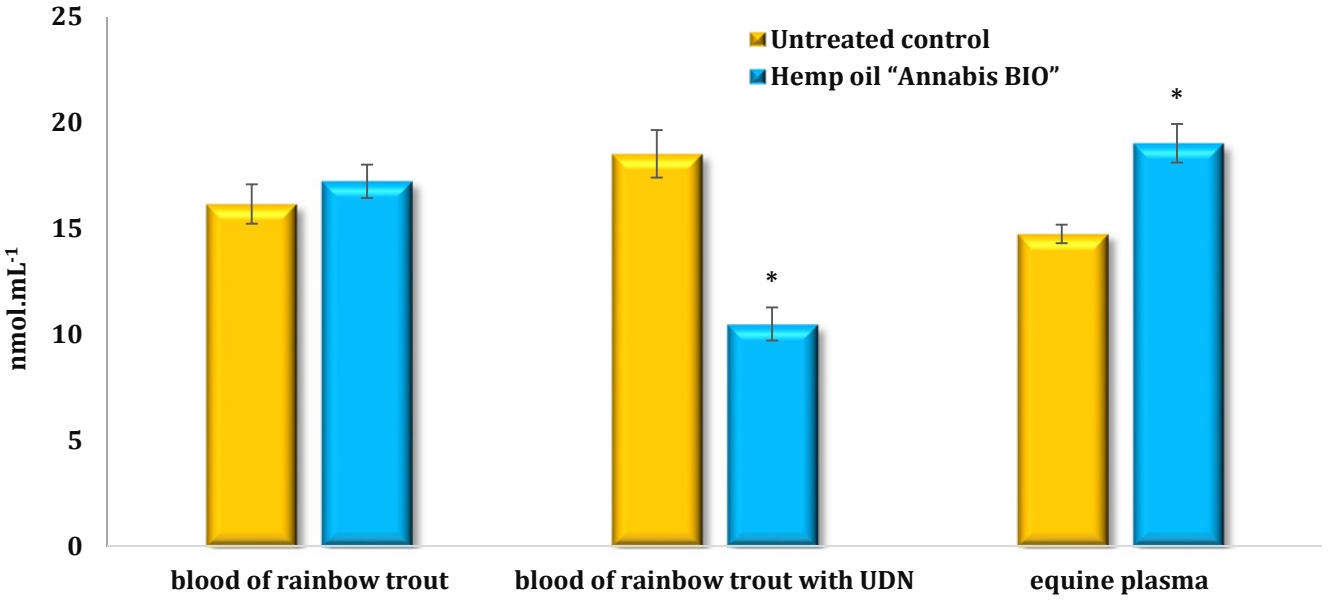

Figure 3 Level of ketonic derivatives of oxidatively modified proteins in the blood of clinically healthy rainbow trout, the blood of rainbow trout with clinical symptoms of ulcerative dermal necrosis (UDN), and equine plasma after incubation with commercial hemp oil "Annabis BIO"
Results are presented as the mean (M) ± the standard error of the mean (S.E.M.)
* changes were statistically significant ($p < 0.05$) compared to the untreated controls

compared to untreated controls (14.61 ±0.35 nmol.mL^{-1}). On the other hand, after incubation of hemp oil with the blood of UDN-affected rainbow trout, we also observed an increase in the level of aldehydic derivatives of OMP (14.7 ±0.85 nmol.mL^{-1}), but this increase was statistically not significantly (by 4%, $p > 0.05$) compared to the untreated samples (14.13 ±0.77 nmol.mL^{-1}). The contrary tendency was observed after incubation of hemp oil with the blood of clinically healthy rainbow trout, where the level of aldehydic derivatives of OMP was statistically non-significant decreased (by 1.2%, $p > 0.05$) compared to untreated controls (12.83 ±0.81 nmol.mL^{-1} *vs.* 12.98 ±0.84 nmol.mL^{-1}) (Figure 2.).

Level of ketonic derivatives of OMP in the blood of clinically healthy rainbow trout, the blood of rainbow trout with clinical symptoms of ulcerative dermal necrosis, and equine plasma after incubation with commercial hemp oil "Annabis BIO" was presented in Figure 3. Different trends were observed when we examined the concentration of ketonic derivatives of oxidatively modified proteins in the blood of rainbow trout and equine plasma samples. *In vitro* incubation of hemp oil with equine plasma resulted in a statistically significant increase (by 29%, $p < 0.05$) in level of ketonic derivatives of OMP, i.e. (19.03 ±0.91 nmol.mL^{-1}) compared to untreated controls (14.75 ±0.44 nmol.mL^{-1}). On the contrary, after incubation commercial hemp oil "Annabis BIO" with blood sampled from UDN-affected rainbow trout, we recorded a statistically significant decrease in the level of ketonic derivatives of oxidatively modified proteins (by 43.3%, $p < 0.05$) compared to untreated controls, i.e. (10.5 ±0.48 nmol.mL^{-1}) *vs.* (18.53 ±1.12 nmol.mL^{-1}). When commercial hemp oil "Annabis BIO" was incubated with the blood of clinically healthy rainbow trout, a non-statistically significant increase (by 6.6%, $p > 0.05$) in the concentration of ketonic derivatives of OMP was observed, i.e. (17.23 ±0.79 nmol.mL^{-1}) compared to untreated controls (16.16 ±0.93 nmol.mL^{-1}) (Figure 3).

Level of total antioxidant capacity in the blood of clinically healthy rainbow trout, the blood of rainbow trout with clinical symptoms of ulcerative dermal necrosis, and equine plasma after incubation with commercial hemp oil "Annabis BIO" was presented in Figure 4. When evaluating the total antioxidant capacity after *in vitro* incubation of hemp oil with equine plasma, we observed a statistically significant decrease in TAC level by 56.6% ($p < 0.05$), i.e. (33.34 ±5.19%) compared to the untreated controls (76.77 ±6.54%). Similarly, after incubation hemp oil with blood samples of UDN-affected rainbow trout, a statistically significant decrease in total antioxidant capacity (by 59.3%, $p < 0.05$) was observed (30.66 ±8.54% *vs.* 75.39 ±10.13%). In contrast, after *in vitro* incubation of hemp oil with blood samples of clinically healthy rainbow trout, we recorded a non-statistically

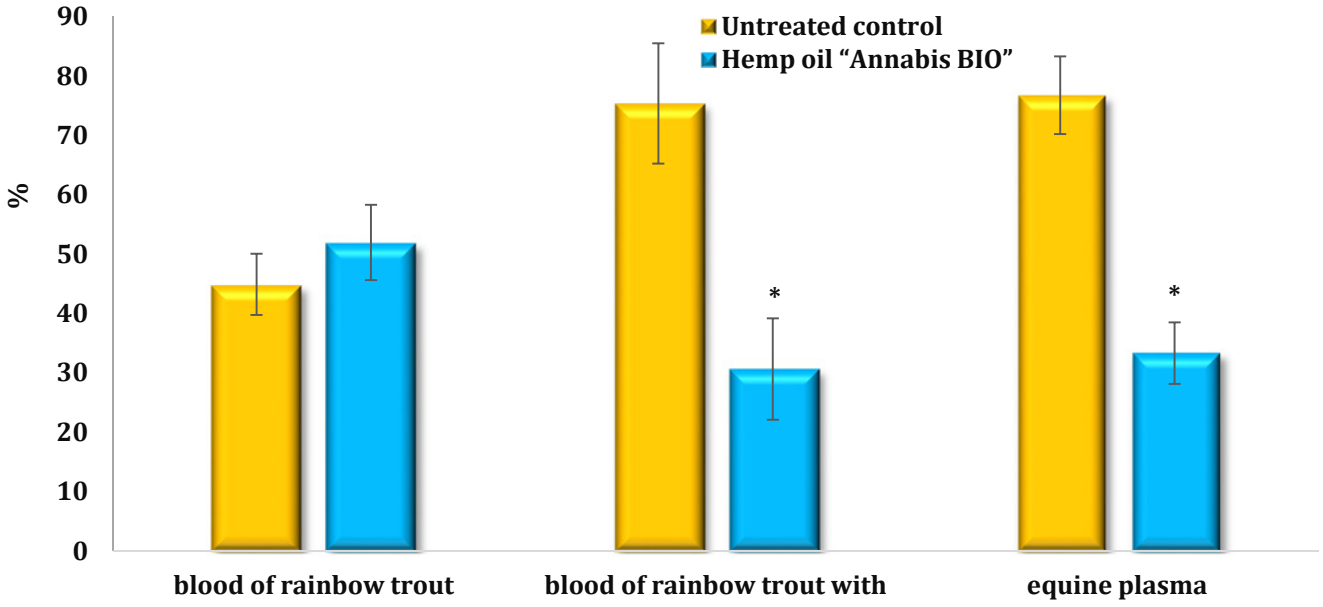

Figure 4 Level of total antioxidant capacity in the blood of clinically healthy rainbow trout, the blood of rainbow trout with clinical symptoms of ulcerative dermal necrosis (UDN), and equine plasma after incubation with commercial hemp oil "Annabis BIO"
Results are presented as the mean (M) ± the standard error of the mean (S.E.M.)
* changes were statistically significant ($p < 0.05$) compared to the untreated controls

significant increase in TAC levels (by 15.7%, $p > 0.05$), i.e. (51.98 ±6.34%) compared to the untreated controls (44.92 ±5.15%) (Figure 4).

Thus, the incubation of hemp oil "Annabis BIO" resulted in different changes in the concentration of TBARS as biomarkers of lipid peroxidation, aldehydic and ketonic derivatives of oxidatively modified proteins, and total antioxidant capacity in the blood of healthy rainbow trout, the blood of rainbow trout with clinical symptoms of ulcerative dermal necrosis, and equine plasma. Incubation of hemp oil with the blood of healthy rainbow trout caused a decrease in TBARS level without statistically significant changes in the level of aldehydic and ketonic derivatives of OMP and TAC. When the blood of UDN-affected rainbow trout was incubated with hemp oil, the content of ketonic derivatives of OMP was significantly decreased, while TBARS as biomarkers of lipid peroxidation was increased with simultaneously decreased TAC level. When equine plasma was incubated with hemp oil, a statistically significant increase of biomarkers of oxidative stress with simultaneously decreased TAC level (Figure 1–4).

Recent investigations have associated plants belonging to the *Cannabis* genus with antioxidant, anticonvulsant, anti-inflammatory, and neuroprotective properties that may impact human health (Ford et al., 2017). For example, *Cannabis* whole extracts acted on both phases of lipid oxidation in copper-challenged LDL. Those effects were just partially related to the content of cannabinoids and partially recapitulated by isolated pure cannabinoids (Musetti et al., 2020). In the current study, the antioxidant activity of hemp oil *in vitro* was detected using the blood of healthy rainbow trout, and this finding is in line with literature data suggesting that this species possesses antioxidant properties (Hacke et al., 2019).

Hemp seed oil is effective for reducing oxidative stress at the cellular level. The results obtained by Vitorović et al. (2021) point to the potential of hemp seed oil for the prevention and treatment of conditions caused by the action of reactive oxygen species. These authors have evaluated the hypothesis that hemp seed oil at different concentrations improves the oxidative state of *Drosophila melanogaster* under non-stress as well as hydrogen-peroxide-induced stress. These authors have analyzed the effects of hemp seed oil on oxidative stress markers and on the life cycle of *D. melanogaster* under non-stress and hydrogen-peroxide-induced stress conditions. The results revealed that under non-stress conditions, oil concentrations up to 62.5 $\mu L.mL^{-1}$ did not induce negative effects on the life cycle of *D. melanogaster* and maintained the redox status of the larval cells at similar levels to the control level. Under oxidative stress conditions, biochemical parameters were significantly affected and only two oil concentrations, 18.7 and 31.2 $\mu L.mL^{-1}$

provided protection against hydrogen peroxide stress effects. A higher oil concentration (125 µL.mL^{-1}) exerted negative effects on the oxidative status and increased larval mortality. The tested oil was shown to contain polyunsaturated fatty acid triglycerides and low levels of tocopherols. The high levels of linoleic and linolenic acids in the oil are suggested to be responsible for the observed *in vivo* antioxidant effects (Vitorović et al., 2021).

The significant antioxidant properties shown by hemp seed oil might generally depend on the phenolic compounds, especially flavonoids, such as flavanones, flavonols, and isoflavones. Smeriglio et al. (2016) have characterized the polyphenolic compounds and antioxidant activity of cold-pressed seed oil from Finola cultivar of industrial hemp. Several methodologies have been employed to evaluate the *in vitro* antioxidant activity of Finola hempseed oil (FHSO) and both lipophilic (LF) and hydrophilic fractions (HF). From the results is evident that FHSO has high antioxidative activity, as measured by DPPH radical (146.76 mmol of TE.100 g^{-1} oil), inhibited β-carotene bleaching, quenched chemically generated peroxyl radicals *in vitro*, and showed high ferrous ion chelating activity. Reactivity towards 2,2'-azino-bis (3-ethylbenzothiazoline-6-sulfonic acid) radical cation and ferric-reducing antioxidant power values were 695.2 µmol of TE.100 g^{-1} oil and 3690.6 µmol of TE.100 g^{-1} oil respectively. FHSO contains a significant amount of phenolic compounds of which 2780.4 mg of QE.100 g^{-1} of total flavonoids. The whole oil showed higher antioxidant activity compared with LF and HF (Smeriglio et al., 2016).

Afridi et al. (2019) examined if the inclusion of dietary hempseed (HS) and hempseed oil (HO) in the diet of the fish could revert the copper-induced toxic effects on the muscle fatty acid profile of rohu (*Labeo rohita*) and mrigal (*Cirrhinus mrigala*). Fingerlings of both species were exposed to a sub-lethal concentration of copper i.e., 20% of LC_{50} (1.34 ppm for rohu and 1.52 ppm for mrigal) for 96 h for 30 days. Following exposure, fish were maintained on graded levels of HO (1, 2, and 3%) or on HS (5, 10, and 15%) for 50 days. Copper exposure showed a significant effect on the fatty acid composition of both species; increased their saturated (SFA) to unsaturated (USFA) and altered their omega-3/omega-6 (ω-3/ω-6) ratios. However, feeding graded levels of hempseed products reverted the toxic effects of copper on the fatty acid profile of both the species, significantly increased muscle total fatty acid contents, improved ω-3/ω-6 ratios, and decreased SFA/USFA ratio in % inclusion dependent manner. Furthermore, hemp seed products showed a species-specific effect on USFA. The ω-3/ω-6 ratios decreased in the muscle of *C. mrigala* whereas an increasing trend with an increase in hempseed product % inclusion was observed in *L. rohita* (Afridi et al., 2019).

Conclusions

Our studies have shown that commercial hemp oil has an antioxidant effect only after incubation *in vitro* with blood samples of clinically healthy rainbow trout, as the values of the oxidative stress biomarkers (TBARS and OMP) have decreased with simultaneously increasing the total antioxidant capacity. When the blood of UDN-affected rainbow trout was incubated with hemp oil, the content of ketonic derivatives of OMP was significantly decreased, while TBARS as biomarkers of lipid peroxidation was increased with simultaneously decreased TAC level. When equine plasma was incubated with hemp oil, a statistically significant increase of biomarkers of oxidative stress with simultaneously decreased TAC level. These results may prompt veterinarians and biologists to carry out further studies to elucidate the dose-dependent antioxidative effects of hemp oil.

Conflicts of interest

The authors declare no conflict of interest.

Ethical statement

This article does not contain any studies that would require an ethical statement.

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