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ANTI-BLASTOCYSTIS ACTIVITY OF HOP EXTRACTS IN VITRO

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Key words: *anti-Blastocystis activity, hop extracts, metronidazole*
Ключові слова: *антибластоцистна активність, екстракти хмелю, метронідазол*

Abstract. Anti-blastocystis activity of hop extracts in vitro. Pokhil S.I., Kazmirchuk V.V., Tymchenko O.M., Yevsiukova V.Y., Melnyk A.L. *Blastocystis sp. are the most common, unicellular, anaerobic parasites of the intestinal tract of many animal and human species that can cause various digestive diseases. Metronidazole has long been used as a first-line treatment for blastocystosis, but recent clinical and in vitro studies have demonstrated its low efficacy against Blastocystis sp. The aim of this study was to determine the in vitro sensitivity of Blastocystis sp. clinical isolates to carbonic acid hop extracts and alcohol hop extracts in comparison with metronidazole. Five cultures of Blastocystis sp. were isolated from faecal samples from patients with irritable bowel syndrome with predominant diarrhoea (IBS-D, Rome IV). The parasites were identified by microscopy of faecal smears permanently stained with trichrome, Wheatley's modification and Heidenhain's iron-haematoxylin. Blastocystis sp. was cultivated at 37°C under anaerobic conditions on*

RPMI-1640 with antibiotics and horse serum, taking into account their growth characteristics. To detect the anti-blastocystic activity hop extracts and metronidazole were tested in the range from 1000 μg/ml to 1 μg/ml.The presence and number of viable Blastocystis sp. cells were determined after 24, 48, 72 and 96 hours. Blastocystis sp. cells were counted in a hemocytometer using the trypan blue dye exclusion test. All experiments were performed in triplicate. According to the results of in vitro sensitivity of 5 clinical isolates of Blastocystis sp. to the action of carbonic acid hop extract, alcohoicl hop extract and metronidazole, a direct positive pattern in the dose-response and contact time-response effects was established. Alcoholic hop extract showed the highest level of antiblastocystic activity with indicators (for 72-hour parasite cultures) of the minimum inhibitory concentration (which inhibits the parasite cultures growth by 50%) (2.8±0,8) μg/ml and the minimum lethal concentration (which destroys parasite cells by 100%) – 8 μg/ml, being 2.4 and 4.5 times lower than the minimum inhibitory concentration and 8 and 62.5 times lower, than the minimum lethal concentration for hop extract and metronidazole, respectively ($p<0.05$). It has been shown that alcoholic hop extract (≥16 μg/ml) causes gradual morphological changes in Blastocystis sp. cells, leading to their complete destruction.In contrast to metronidazole, subinhibitory concentrations of hop extract (<2 μg/ml) do not stimulate the proliferation of Blastocystis sp. cells in vitro.

Реферат. Антибластоцистна активність екстрактів хмелю in vitro. Похил С.І., Казмірчук В.В., Тимченко О.М., Євсюкова В.Ю., Мельник А.Л. *Blastocystis sp. – найбільш поширені одноклітинні анаеробні паразити кишкового тракту багатьох видів тварин і людини, які здатні спричинювати різні захворювання органів травлення. Для лікування бластоцистозу тривалий час в якості препарату першої лінії застосовується метронідазол, проте результати нещодавніх клінічних досліджень та експериментів in vitro продемонстрували його низьку ефективність щодо Blastocystis sp. Метою роботи було визначення чутливості in vitro клінічних ізолятів Blastocystis sp. до дії вуглекислого та спиртового екстрактів хмелю порівняно з метронідозолом. П'ять культур Blastocystis sp. було виділено зі зразків фекалій від хворих із синдромом подразненого кишківника з переважанням діареї (СПК-Д, Рим IV). Ідентифікацію паразитів проводили за результатом мікроскопії мазків фекалій, перманентно зафарбованих трихромом у модифікації Вітлі та залізним гематоксиліном за Гендейгайном. Культивування Blastocystis sp. проводили при 37°С в анаеробних умовах на RPMI-1640 з антибіотиками та сироваткою коня, ураховуючи їхні особливості росту. Для виявлення антибластоцистної активності екстракти хмелю й метронідазол тестовано в діапазоні від 1000 мкг/мл до 1 мкг/мл. Наявність і кількість життєздатних клітин Blastocystis sp. визначали через 24, 48, 72 і 96 годин. Підрахунок клітин Blastocystis sp. проводили в гемоцитометрі із застосуванням тесту на виключення барвника трипанового синього. Усі експерименти виконано в трьох повторах. За результатами визначення чутливості in vitro 5 клінічних ізолятів Blastocystis sp. до дії вуглекислого екстракту хмелю, спиртового екстракту хмелю та метронідазолу встановлено пряму позитивну закономірність в ефектах "доза-відповідь" та "час контакту-відповідь". Спиртовий екстракт хмелю виявив найбільш високий рівень антибластоцистної активності з показниками (для 72-годинних культур паразитів) мінімальної інгібуючої концентрації (яка пригнічує ріст культур паразитів на 50%) 2,8±0,8 мкг/мл та мінімальну летальну концентрацію (яка на 100% знищує клітини паразитів) – 8 мкг/мл, які були нижчими у 2,4 та 4,5 рази за мінімальну інгібуючу концентрацію та у 8 та 62,5 рази за мінімальну летальну концентрацію для вуглекислого екстракту хмелю та метронідазолу відповідно ($p<0,05$). Показано, що спиртовий екстракт хмелю (≥16 мкг/мл) викликає поступові морфологічні зміни в клітинах Blastocystis sp., призводячи до повного їх руйнування. На відміну від метронідазолу, субінгібуючі концентрації спиртового екстракту хмелю (<2 мкг/мл) не стимулюють розмноження клітин Blastocystis sp. in vitro.*

Blastocystis sp. (formerly *Blastocystis hominis*) belongs to the family of Blastocystidae, class Blastocystea, superclass Opalinata, clade Opalozoa, type Bigyra, kingdom Heterokonta/Chromista, supergroup SAR, domain Eukaryota and is the most common anaerobic unicellular parasite of the intestinal tract of many animal species, colonizing more than a billion people worldwide [1, 2]. Although, it is well known that blastocystosis is asymptomatic, successes in investigating the virulent potential of these parasites have revealed their pathogenic activity on both the human body and the intestinal microbiota [3, 4]. This contributed to the conclusion of a long scientific debate on whether it is appropriate to formally recognise a separate nosological disease caused by *Blastocystis* sp., which is now included under the code "1A35 Blastocystosis" in the International Statistical Classification of Diseases and Related Health Problems of the Eleventh Revision [5].

The pathogenesis of blastocystosis is mainly attributed to the occurrence and development of various ("non-specific") inflammatory bowel diseases (IBD) including traveller's diarrhoea and irritable bowel syndrome (IBS), which are sometimes accompanied by allergic reactions – eosinophilia and manifestations of skin lesions, especially urticaria [6, 7, 8].

The density of parasite colonisation in the intestinal tract determines the degree of manifestation of disease symptoms and justifies the appropriateness and tactics of etiotropic therapy [8, 9, 10]. In cases where the latter is justified, metronidazole (MTZ) has long been used as the first-line treatment for blastocystosis [8, 9, 10, 11]. However, the results of recent clinical studies and *in vitro* experiments have

demonstrated low sensitivity of *Blastocystis* sp. strains to both MTZ and a number of other traditional antiparasitic drugs [12, 13, 14, 15].

Therefore, an urgent scientific task today is the search for new compounds with pronounced anti-*Blastocystis* activity and the development of drugs based on them, among which special attention is paid to preparations of plant origin due to the many potential advantages of phytotherapy (greater safety, simultaneous achievement of several beneficial effects, relatively low cost, etc.) [16-18].

The common hop (*Humulus lupulus L.*) belongs to the family of Cannabaceae in the order Urticacales and is a widespread (cultivated) perennial plant throughout the world whose female inflorescences (cones) contain a huge number of bioactive compounds, which leads to the versatile use of their total extracts and individual purified components, including in the field of medicine as agents with antioxidant, anti-inflammatory, metabolic, neuroprotective, cardioprotective, antiviral, antibacterial, antifungal and anticarcinogenic effects [19, 20, 21]. However, data on the activity of hop extracts and compounds against pathogens of human parasitic diseases remain extremely limited [22, 23, 24]. This is particularly true for the blastocystis pathogen (*Blastocystis sp.*), the sensitivity of which to the action of hop extracts has so far remained unclear.

The purpose of the study was to determine the *in vitro* sensitivity of clinical strains of *Blastocystis* sp. to the action of carbon acid and alcoholic hop extracts as compared with metronidozole.

MATERIALS AND METHODS OF RESEARCH

The study was conducted *in vitro*. No experiments were conducted on humans or animals, as evidenced by the extract from the minutes of the meeting of the Biomedical Ethics Committee of the State Institution "I. Mechnikov Institute of Microbiology and Immunology of the National Academy of Medical Sciences of Ukraine" No. 3 of 17.02.2022.

Five cultures of *Blastocystis sp.* were isolated from faecal samples from patients with irritable bowel syndrome with predominant diarrhoea (IBS-D, Rome IV). All fecal specimens contained ≥5 parasite cells per field of view in wet smear preparations, stained with 1% Lugol solution, under light microscopy at a total magnification of ×400. *Blastocystis sp.* identification was carried out by microscopic examination of the fecal smears permanently stained with trichrome, Wheatley's modification and Heidenhain's iron-haematoxylin [25].

Blastocystis sp. was cultured at 37°C under anaerobic conditions (anaerostat ANS1) in tubes (16×100 mm) with a screw cap (not fully closed to ensure proper gas exchange) containing 5 ml of RPMI-1640 liquid nutrient medium with L-glutamine (Biovest International, Inc.) and enclosed antibiotics (ampicillin 12 mg/ml, streptomycin 4 mg/ml) and 10% heat-inactivated horse serum. The *in vitro* cultivation of *Blastocystis* sp. took into account their growth characteristics in RPMI medium [26]. Stabilized (long-term) xenic parasite subcultures were obtained from primary cultures after ten successive cultures in a new portion of medium. The sensitivity of *Blastocystis sp*. to extracts of hop, metronidazole and different concentrations of ethanol was determined at the initial concentration of parasite cells in suspensions 2×10^5/ml. Samples of (Hercules variety) carbon dioxide hop extracts (CO_2HE) and alcoholic (AHE) were provided by Polysya Institute of Agriculture of the National Academy of Agrarian Sciences of Ukraine (NAAS Ukraine). The Polysya Institute of Agriculture, NAAS Ukraine determined quality indicators for hop extract samples and determined their component composition: CO_2HE – mass fraction of α-acids – 53.3%, mass fraction of β-acids – 13.8%, β/α-acids ratio 0.26, kugomulon as α-acid – 31.6%, kolupulon as β-acid – 52.3%, xanthohumol – missing (test report No. 7 from 15.04.2021); AHE – mass fraction of α-acids 7.8%, mass fraction of β-acids 8.9%, β/α-acid ratio 1.14, congumulon in α-acids 29.5%, colupulon in β-acids 47.5%, xanthohumol – 0.63% (test report No. 8 of 19.05.2022). From the above hop extracts 1% (mass/volume) base solutions in 96% ethanol were made.

"Metronidazole-Darnitsa" infusion solution with MTZ concentration of 5 mg/ml (PJSC "Pharmaceutical firm "Darnitsa", Ukraine) was used as a reference drug.

For the detection of anti-*Blastocystis* activity, extracts of hop and metronidazole were tested in the range from 1000 μg/ml to 1 μg/ml (by halving their successive concentrations). Each series of experiments comprised five control tubes for the parallel evaluation of the growth intensity of *Blastocystis* sp. in RPMI medium without any antiparasitic agents added and in suspensions with final contents of 96 % ethanol 10.0%, 5.0%, 2.5% and 1.0% (volume/volume) to evaluate the role of ethanol in the effects of the corresponding hop extracts concentrations.

The presence and number of viable cells of *Blastocystis sp.* in all test tubes was determined daily for four days (24, 48, 72 and 96 hours), which is due to the beginning of a natural decrease in the concentration of parasites when they are grown in the RPMI environment [26]. Cell count of *Blastocystis sp.* was performed in a hemocytometer using the trypan blue dye exclusion test, which was reproduced according to the basic protocol [27] with the difference that cells were washed from the

medium serum by centrifugation at 500 g for 5 min. Cell counting techniques of *Blastocystis sp.* and criteria for assessing their viability as outlined in [18, 28]. Indicator of inhibition of growth (reproduction) of *Blastocystis sp.* cells as a result of the effect on parasite cultures, different concentrations of CO_2HE, AHE, MTZ and ethanol were calculated daily according to the formula: $GI_{\%}$=(A–B)/A×100, where $GI_{\%}$ – level of growth inhibition of *Blastocystis sp.* in percentages (%), A – the average number of viable cells of parasites in a test tube of an adequate control, B – the average number of viable cells in an experimental test tube with a culture of parasites to which CO_2HE, AHE, MTZ or ethanol was added. To calculate $GI_{\%}$ MTZ and ethanol, tubes containing cultures of *Blastocystis sp.* in RPMI medium without added antiparasitic agents (hereafter untreated control) served as an adequate control, for – $GI_{\%}$ CO_2HE and AHE control tubes with parasite cultures grown in the presence of appropriate concentrations of ethanol were used as control tubes to account for its role in the anti-*Blastocystis* action extracts of hop.

The anti-*Blastocystis* activity of both types of hop extracts and metronidazole has been characterised by a minimum (lowest) inhibitory drug concentration, which inhibits the growth of all parasite cultures by 50% (MIC_{50}); a minimum lethal concentration (MLC) that completely (100%) destroys the cells of all parasite strains The effect of complete death of *Blastocystis sp.* cells was assessed by the presence/absence of growth from four-day suspensions in which parasite cells with dubious signs of viability were microscopically detected. For this purpose, 1.0 ml of the investigated suspension of *Blastocystis sp.* was taken, parasite cells were washed twice from drug residues by centrifugation in RPMI medium (volume ratio 1:9; centrifugation mode 500 g, 5 min), the obtained sediment was resuspended in 0.3 ml of fresh RPMI and incubated for four days as previously indicated.

Morphological changes in *Blastocystis* sp. cells induced by hop extracts were described according to the results of their phase-contrast microscopy at total magnification ×600.

Statistical processing of the obtained data was performed using the LibreOffice 7.6 Calc software package. The difference in mean values (M±m) was considered statistically significant at $p<0.05$ [12].

Studies were not conducted on humans or animals.

RESULTS AND DISCUSSION

In recent decades, the scope of use of hop and its compounds in medicine has been steadily expanding [19, 20, 21]. One of the promising areas of their use concerns the field of medical parasitology. Articles [22, 23] show the anti-parasitic effect of hop extract chalcones against strains of *Plasmodium falciparum* (the causative agent of tropical malaria), *Trypanosoma brucei* (one of the causative agents of African trypanosomiasis), and *Leishmania mexicana* (the causative agent of cutaneous leishmaniasis in Mexico and Central America). The authors of another work [24] established a high sensitivity of representatives of free-living Ciliates (*Paramecium caudatum, Tetrahymena pyriformis*), Flagellates (*Euglena* sp., *Polytomella papillata*) and Amoebae (*Amoeba proteus, Chaos sp.*) to the action of xanthohumol and β-acids and, to a lesser extent, to α-acids of hops.

As far as we know, this article is the first to present the results of an *in vitro* study of the anti-*Blastocystis* activity of CO_2HE and AHE, which is compared with the effect of MTZ. The level and features of the anti-*Blastocystis* effect of CO_2HE, AHE and MTZ were assessed by MIC_{50} and MLC indicators, which were determined on 5 clinical strains of *Blastocystis* sp., detected by special methods of light microscopy in the feces of patients with IBS-D (Fig. 1, A-C) and grown in anaerobic conditions on RPMI medium.

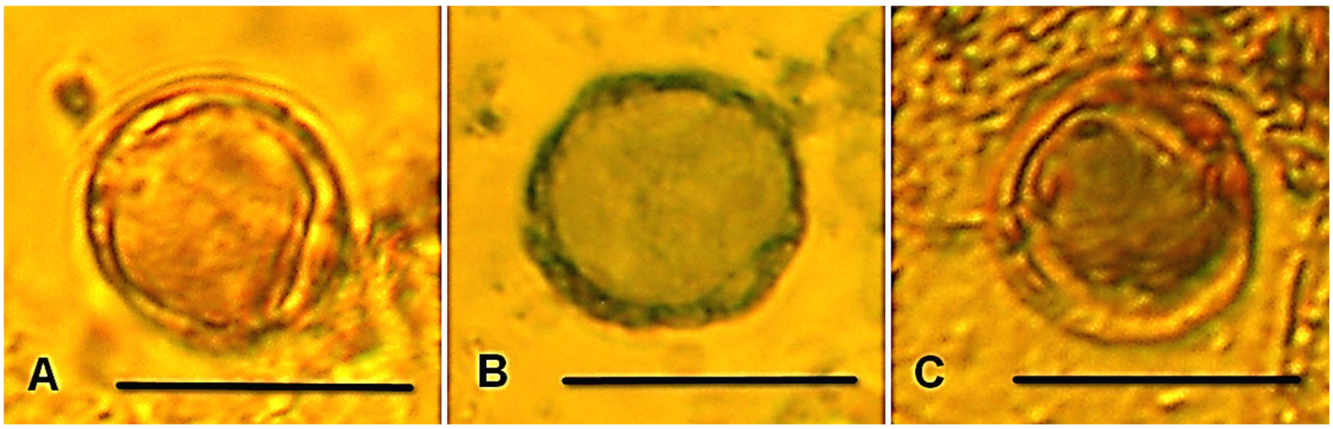

Fig. 1. Vacuolar forms of *Blastocystis sp.* in the preparations of faecal smears of patients with IBS-D, stained with 1% Lugol's solution (A), Heidenhain's iron-hematoxylin (B) and trichrome stain, Wheatley's modification (C) (light microscopy, bar=10 µm)

The results of the experiments showed that in the untreated control after the first day of cultivation of *Blastocystis sp.* the concentration of viable parasite cells was $(2.8\pm0.5)\times10^5$/ ml, in the future, an exponential increase in their number was observed with the achievement of the maximum concentration $(56.6\pm9.0)\times10^5$ cell/ml in three-day parasite cultures. In suspensions of one-day cultures of *Blastocystis sp.* the relative number of viable cells was (98±2)%, and on the fourth day of incubation it decreased to (81±7)%.

The inhibitory effect of ethanol directly depended on its concentration in the medium and was most pronounced after the first day of incubation of cultures of *Blastocystis sp.* and gradually decreased until the end of the observation period, which is probably a consequence of the regressive decrease of the initial amount of ethanol. In one-day and four-day cultures of *Blastocystis sp.* GI% of ethanol was, respectively: (32±5)% i (19±4)% – at the initial concentration of ethanol 10 %; (18±4) % i (5±1) % – at the initial concentration of ethanol 5%; (4±1)% i 0%– at the initial concentration of ethanol 2.5%; at 1% of ethanol in the environment, the effect of inhibiting the growth of *Blastocystis* sp. was not observed.

In general, the manifestations of anti-*Blastocystis* activity of CO_2HE, AHE and MTZ that we have established can be described by a direct positive regularity in the "dose-response" and "contact time-response" effects. That is, the higher the doses of the drugs were used and the longer their duration of action, the more pronounced were the manifestations of anti-*Blastocystis* activity. However, as the presence of different strains of *Blastocystis sp.* of a certain variation in the level of sensitivity to the action of CO_2HE, AHE and MTZ, as well as determined for the entire sample of strains of these protozoa values MIC_{50} and MLC differed significantly (Table). At the same time, in the studied cultures of *Blastocystis sp.* a relatively more pronounced variation of sensitivity to CO_2HE and MTZ than to AHE was observed. In different strains of parasites, the values of the sensitivity indicators are the closest to the MIC_{50} values and MLC varied within two consecutive concentrations of CO_2HE and MTZ, and for AHE – were limited to one concentration for all strains of *Blastocystis sp.*

In addition, based on the results of testing 5 strains of *Blastocystis sp.* AHE also showed the highest level of activity in terms of actual MIC_{50} and MLC values (Table). In terms of the maximum number of viable parasite cells (in 72-hour cultures) values of MIC_{50} and MLC for AHE made accordingly (2.8±0.8) μg/ml and 8 μg/ml and were 2.4 and 4.5 times lower MIC_{50} i – 8 and 62.5 times MLC for CO_2HE and MTZ, respectively ($p<0.05$). AHE is characterized by a stronger inhibitory and lethal effect on strains of *Blastocystis sp.* than a number of other extracts of plant origin: *Artemisia judaica, Allium sativum, Zingiber officinale, Cuminum cyminum* and *Piper nigrum* [16, 17, 18]. In turn, CO_2HE showed a moderate ability to inhibit the growth of *Blastocystis* sp. cultures, especially inferior to AHE in concentrations causing a lethal effect. The different level of anti-*Blastocystis* activity of CO_2HE and AHE can be explained by the difference in the composition of these extracts. First of all, in contrast to CO_2HE, the composition of AHE contains xanthohumol (0.63 мас.%), which according to the authors of the study [24] shows a high protozoicidal effect against *P. caudatum* and *Chaos sp.* in concentrations of 0.05-5.0 μg/ml.

Anti-*Blastocystis* activity of CO_2HE, AHE and MTZ according to MIC_{50} and MLC indicators

Active substance	MIC_{50} (M±m, μg/ml)				MLC (μg/ml)			
	24 h	48 h	72 h	96 h	24 h	48 h	72 h	96 h
CO_2HE	15.9±8.5	8.9±1.8	6.8±1.9*	3.8±1.2	500	125	64	64
AHE	4.9±0.9	4.0±0.6	2.8±0.8*	1.9±0.6	16	16	8	8
MTZ	20.1±8.1	14.3±2.6	12.5±3.2*	6.4±1.8	-**	1000	500	250

Notes: * – the difference in the average MIC50 values of CO2HE, AHE and MTZ is significant ($p\leq0.05$); ** – the concentrations of MTZ used did not provide the effect of complete cell death *Blastocystis sp.*

Currently, the specifics of the mechanism of inhibitory/lethal effect of AHE on cells of *Blastocystis sp.* is unknown. AHE-induced stepwise morphological changes in *Blastocystis sp.* cells, which were observed by phase-contrast microscopy in one- to four-day cultures of parasites with the added lethal concentration of AHE (16 μg/ml), are presented in Fig. 2 (A-F). It was established that with the lethal effect of AHE in typical vacuolar forms of cells of *Blastocystis sp.* (A) the central body decreases and the

process of granule formation starts (B), the further increase in the intensity of granule formation is combined with the appearance of numerous vacuoles (C), the stage of complete granulation of cells is accompanied by the thinning of their outer shell and its loss of clear smooth contours (D), with the subsequent significant degradation of the shell, granulated cells acquire irregular shapes (E), finally, the shell is completely destroyed, and in the place of the cells the detritus of their internal contents appears (F).

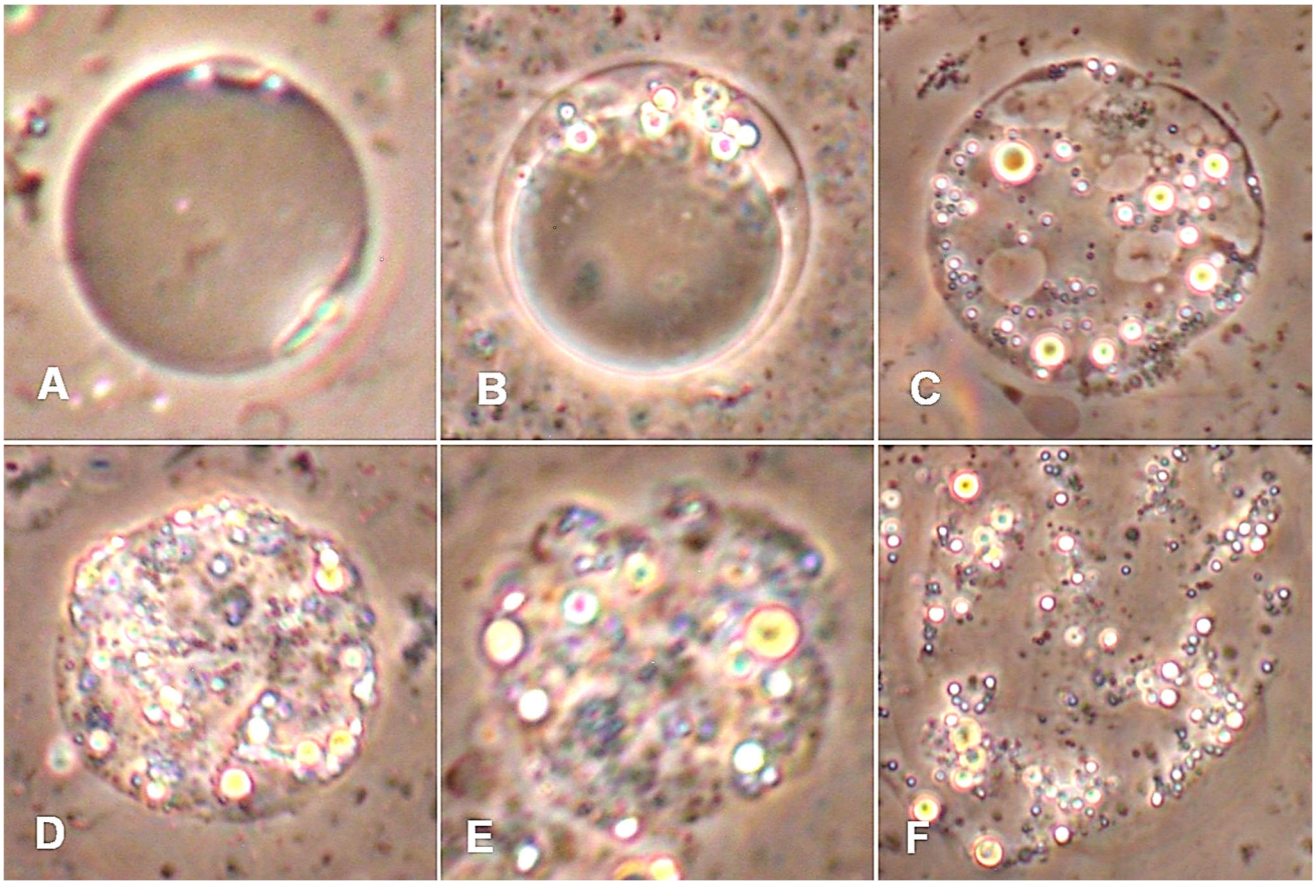

Fig. 2. Morphological changes in the cells of *Blastocystis* sp., which gradually occur as a result of the lethal effect of AHE (16 μg/ml): typical intact vacuolar form (A), reduction of the central body and the formation of peripheral granules (B), intensive granulation and the appearance of numerous vacuoles (C), complete granulation, thinning of the outer shell and its loss of clear smooth contours (D), significant degradation of the shell and transformation of granulated cells into irregular shapes (E), complete destruction of the shell and formation of detritus in the place of cells of their internal contents (F) (phase-contrast microscopy with a total magnification of ×600)

Among the substances we studied, MTZ demonstrated the lowest anti-*Blastocystis* effect (Table), however, the level of antiparasitic activity of MTZ determined in this study (MIC_{50} from (20.1±8.1) μg/ml to (6.4±1.8) μg/ml) was significantly higher than that given in the works [15, 16] and comparable to the data of the publication [18]. The authors of the article [15] indicated that the MIC of MTZ for different strains of *Blastocystis sp.* ranged from 250 μg/ml to 64 μg/ml, but even when using its maximum concentration of 1000 μg/ml, they did not manage to achieve complete liberation of cultures from parasite cells throughout the entire cultivation period. We also observed the presence of (1.0-4.0)×10^3 cells /ml of *Blastocystis sp.* in all crops treated with high doses of MTZ (≥250 μg/ml). At the concentration of MTZ ≤16 μg/ml in the phases of the beginning and exponential growth (24-72 h) in cultures of *Blastocystis sp.* the vacuolar form of cells was significantly predominant (78-94%), and at higher concentrations of MTZ, the specific proportion of granular cells increased (35-82%). In a number of two- to four-day cultures with a high concentration of MTZ (1000-250 μg/ml), protozoan cells, although they retained the granular morphology characteristic of apoptosis [14, 15], and in the test for the exclusion of trypan blue, they had doubtful signs of viability, however, after washing from the residues MTZ did not grow in fresh RPMI

medium. Therefore, in our opinion, MTZ in high concentrations is able to ensure the effect of complete death of *Blastocystis sp.* cells *in vitro* (Table).

On the contrary, as it has already been shown by many scientists, low concentrations of MTZ stimulate the reproduction of *Blastocystis sp.* cells and increase their level of virulence due to more intense formation of amoeboid forms, production of proteases and solubilized antigens, which intensify the proliferation of colon cancer cells [14, 15, 29, 30]. In our conditions, an increase in the number of parasite cells compared to the untreated control was observed in cultures of *Blastocystis sp.* with a concentration of MTZ ≤4 μg/ml, while the greatest (1.4-fold) increase in the number of protozoan cells was observed in cultures with a subinhibitory concentration of the drug of 2 μg/ml ($p<0.05$). Unlike MTZ, subinhibitory concentrations of both CO_2HE (<4 μg/ml) and AHE (<2 μg/ml) did not stimulate the proliferation of *Blastocystis sp.* cells.

Thus, according to the level of anti-*Blastocystis* activity, assessed by MIC_{50} and MLC indicators, AHE significantly outperforms MTZ and CO_2HE. Although subinhibitory concentrations of AHE do not stimulate the proliferation of *Blastocystis sp. in vitro*, further studies should clarify the effect of such concentrations of AHE on the virulence potential of parasites (intensity of formation of amoeboid forms, production of proteases and antigens with pathogenic properties), which will contribute to the creation of a more effective and safe composition for the treatment of blastocystosis.

CONCLUSIONS

1. Based on the results of determining the in vitro sensitivity of 5 clinical strains of *Blastocystis sp.* to the action of carbonic acid hop extract, alcoholic hop extract and metronidazole, a direct positive regularity was established in the "dose-response" and "contact time-response" effects. Alcoholic hop extract showed the highest level of antiblastocystic activity with values of MIC_{50} (2.8±0.8) μg/ml and minimum inhibitory concentration of 8 μg/ml (for 72-hour parasite cultures), which were lower by 2.4 and 4.5 times than MIC_{50} and – 8 and 62.5 times lower than minimum inhibitory concentration for carbonic acid hop extract and metronidazole, respectively ($p<0.05$).

2. The lethal effect of alcoholic hop extract (≥16 μg/ml) causes pronounced gradual morphological changes in the cells of *Blastocystis sp.*: reduction of the central body and the formation of the first groups of peripheral granules, an increase in the intensity of granulation and the appearance of numerous vacuoles, the onset of complete granulation of cells with thinning of the outer membrane and loss of clear smooth contours, subsequent significant degradation of the shell and transformation of granular cells into irregular shapes, finally complete destruction of the shell and the formation of detritus of their internal contents in the place of cells.

3. Unlike metronidazole, subinhibitory concentrations of alcoholic hop extract (<2 μg/ml) do not stimulate the reproduction of *Blastocystis sp.* cells *in vitro.*

Prospects for further research are establishing the effect of subinhibitory concentrations of alcohol hop extract on the level of virulence of *Blastocystis sp.* (intensity of formation of amoeboid forms, production of proteases and antigens with pathogenic properties).

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