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Influence of the *Chlorophytum Comosum* Leaves Hydroalcoholic Extract on Some Representatives of Intestinal Microflora of Rats

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Abstract

The present study was conducted to evaluate the effect of *Chlorophytum comosum* (*C. comosum*) extract on some of the main representatives of the intestine microflora of rats .

The I Group of test animals received *C. comosum* alcoholic extract for 10 days at the concentration of 5 mg/kg body weight per rat. In the II Group was intestinal dysbiosis induced by gentamicin sulfate, they received the *C. comosum* extract in the same dose. The III Group of animals also with dysbiosis received only a standard diet. After the termination of the oral administration of gentamicin the animals of the III Group was divided on two sub-group, and animals of the second one (experimental) received *C. comosum* extract for 10 days.

Feeding of hydroalcoholic extract of *C. comosum* leaves has no effect on the number of representatives of *Bifidobacterium spp, Lactobacillus spp* during its free reception or with an induced dysbiosis. However, the feeding of the extract promotes moderate stimulation of *Escherichia coli* with normal enzymatic activity and reduction of *Candida spp.*

This allows to recommend *C. comosum* extract as a support means in cases of dysbacteriosis associated with lack of *E. coli*, as well as to reduce the overly high number of *Candida spp*.

Key words: *Chlorophytum comosum*, dysbiosis, extract, microflora, rat.

INTRODUCTION

At the present time still remains urgent searching for low-cost and easily accessible raw materials, which potentially has high biological activity and diverse effects on the organism. One of the promising in this regard is a plant, *Chlorophytum comosum* (*C. comosum*) also known as a Ribbon plant or Spider plant. A wide range of herbs from the genus *Chlorophytum* (*Asparagaceae*) are known for their therapeutic potential with a vast range of pharmacologically important substance, such as alkaloids, vitamins, minerals, proteins, carbohydrates, saponins, polysaccharides, steroids and flavonoids and *C. comosum* is not an exception [7,13,16]. It is a medicinal plant which has got maximum demand and commercial value today as one of the fast growing ever green plants of *Chlorophytum* species traditionally known to be used against bronchitis, cough, fracture, burns, it also shows hepatoprotective, anti-tumor properties and cytotoxicity against cancerous cell line [2,12]. Taking into account the rich biochemical composition of the plant, its extract may also have a potential prebiotic effect in a living system [8]. Considering the above we decided to conduct a study on the effect of feeding the hydroalcoholic extract of *C. comosum* leaves on intestine microbiocenosis of male wistar rats.

MATERIALS AND METHODS

Chlorophytum comosum extract preparation: *C. comosum* was grown in a Scientific research laboratory of immunopathology, immunomorphology and immunobiotechnology of North-Caucasus Federal University (Stavropol, Russia). Extract was prepared using M.V. Leonova, Ju.N. Klimochkin method in our modification [11]. Leaves were collected and washed under water, air dried and placed in a cooling chamber at +4°C for 5 days. Then, shredded into pieces of 1-2 cm size, kept in a low temperature refrigeration chamber (Tefcold se10-45, Denmark) at -40°C for 48 hours. Then the leaf pieces were freeze dried to a moisture level 6% at 70-80 Pa pressure of sublimator and -45°C temperature of the condenser for a total drying cycle of 35 hours. After that 40 gram of dried *C. comosum* leaves was filled with 1000 ml of 70% ethyl alcohol in a ratio of 1:25 and placed in a shaker-incubator (ES-20/60, Latvia) at +60°C and stirring rate of 125 rpm for 1 hour, then removed from the incubator and left at room temperature for 24 hours. The first extract was centrifuged (SL40R cooled centrifuge) at 4700 rpm for 120 minutes at 2-4 °C above zero. After that the supernatant was collected. The processed feedstock was extracted two more times under the same scheme. Three of the resulting extracts were combined and concentrated on a rotary evaporator (IKA RV 10, Germany) at a bath temperature of +40° C and 7 mbar vacuum until alcohol was removed (European Pharmacopoeia). The amount of dry matter in the extract was 0.29%. The extract prepared according to the proposed technology was used for the further experiment.

Animals used in study: Experiments were performed on 60 male Wistar rats, about 9 months old, the average weight 250g. All animals were maintained in conventional conditions in an environmentally controlled room (20–22°C, 12 h light:dark cycle), with food and water *ad libitum*. All the animal experiments were performed according to the compliance with the EC

Directive 86/609/EEC and with the Russian law regulating experiments on animals.

Treatment Design: At the first stage of experimental influence rats were randomly divided into 3 groups (n=20/group). All animals received a standard diet recommended by the Institute of Nutrition (Moscow, Russia). Animals of the I Group received *C. comosum* leaves hydroalcoholic extract at the concentration of 5 mg/kg bw per rat (Areshidze *et.al.* 2013) during 10 days in addition to the usual diet. For the purpose of suppression of intestinal microflora and subsequent monitoring of its restoration the intestinal dysbiosis of animals Group II and III was induced by gentamicin sulfate in dose of 10 mg per rat twice a day through a gastric tube for 10 days for all the rats. The dose of antibiotic was selected according to the method of dysbiosis simulations in laboratory animals [6], considering the conversion factor per unit body surface for rats [9]. Animals of the Group III received a standard diet, and the animals of the Group II received *C. comosum* extract at the concentration of 5 mg/kg bw per rat every day during the antibiotic influence, in addition to the usual diet.

After the termination of the oral administration of gentamicin, animals of Group III was divided randomly into two sub-groups (n=10/group). During the next 10 days rats of the Group IIIC received a standard diet recommended by the Institute of Nutrition, and the animals of the Group IIIE received *C. comosum* extract at the concentration of 5 mg/kg bw per rat.

Bacteriological examination: At the initial stage of the experiment, before extract and antibiotic influence, the background bacteriological examinations of the large intestinal microflora of all animals were conducted. Then, fecal examinations on 3, 6, 10 day was performed by selection of faeces individually from each animal in sterile sealable glass containers with the aim of counting the number of *Bifidobacterium spp, Lactobacillus spp, Escherichia coli* (with normal enzymatic activity) and *Candida spp* after the completion of the dysbiosis protocol. The number of viable bacteria was determined by serial dilutions method. Serial tenfold dilution of cecal contents was prepared with sterile phosphate buffer saline (pH 7.2) solution. Planting on selective solid culture media (Lactobacillus Selection Agar Base (HiMedia, India), Agar for bifidobacteria (HiMedia, India), Hottinger agar (Medgamal, Russia), Endo (HiMedia, India), Candida Medium (HiMedia, India) in petri plates and counting colonies of bacteria after the incubation time of 48 hours at 37 °C was carried out. Cultivation of *Bifidobacteria* and *Lactobacilli* was performed on solid nutrient media under microaerophilic conditions using the anaerobic system for the cultivation (INFORS MT Multitron, Switzerland). The identification was performed by the characteristic cultural properties on the corresponding nutrient media, as well as by the presence of specific cells in the smears stained by Grams stain. The confirmation of normal enzymatic activity of *E. coli* was performed using test system for biochemical identification and differentiation of *Enterobacteria* (DS-DIF-entero-24, Cat P-1441, Russia). Microscopy was carried out using a Carl Zeiss Axio Imager 2 (A2) (Jena, Germany) with Zeiss AxioCam MRm for obtaining digital images (Zeiss AxioVision Release 4.8.1). Morphologically distinct organisms were counted in appropriate aliquots and an estimate of the abundance of distinct organisms was obtained using microorganisms counter Scan 100 (Interscience, France).

Statistical method: To facilitate the statistical presentation of data on the number of colony forming units of bacteria logarithms were used (base 10). All analyses were performed using the Statistical Package for the Social Sciences (SPSS) for Windows, version 11.0 packed program. Data was presented as mean ± standard deviation. The difference between the control and experimental groups was analyzed using Mann-Whitney U test. $P \leq 0.05$ was considered statistically significant. Statistical processing of the results of the research was carried out using the program Primer of Biostatistics (Version 4.03).

Table 1. The dynamics of the intestinal microflora of rats in applying them an extract from *Chlorophytum comosum*

Bacterial group	Numbers of the monitored bacterial groups in 1 gram of faecal microflora of experimental animals Log_{10} CFU/g											
	Before the influence			Day 3			Day 6			Day 10		
	I	II	III	I	II	III	I	II	III	I	II	III
Bifidobacterium spp	8.15 ±0.5	8.24 ±0,5	8.12 ±0,4	8.12± 0.6	6,9 ±0.4*	7.1 ±0.2*	8.11 ±0.5	5.11 ±0.2*	5.8 ±0.4*	8.14 ±0.4	4.4 ±0.4*	4.2 ±0.5*
Lactobacillus spp	8.25 ±1,2	8.22 ±0,3	8,3 ±0,2	8.23± 1.2	6.4 ±0.2*	6.7 ±0.3*	8.21 ±1.1	5.6 ±0.3*	5.2 ±0.4*	8.28 ±1.1	4.1 ±0.4*	4.5 ±0,5*
Escherichia coli with normal enzymatic activity	5.12 ±0.3	5,55 ±0.74	5,2 ±0.2	5.13± 0.5	4.5 ±0.4*	4.12 ±0.2*	5.55 ±0.4	4.1 ±0.2*	3.4 ±0.2*	6.25 ±0.2*	3.5 ±0.4*▲	2.2 ±0.4*▲
Candida spp	3.2 ±0.2	3.1± 0.25	3.2 ±0.4	3.55± 0.43	3.1 ±0.5	3.5 ±0.8	2.9 ±0.5	3.2 ±0.5	3.67 ±0.5	2.25 ±0.2*	3.8 ±0.15*▲	4.5 ±0.2*▲

I –group, n=20, animals received *C. comosum* extract at the concentration of 5 mg/kg.bw per rat in addition to the usual diet

II –group, n=20, animals with experimental antibiotic-associated dysbiosis received *C. comosum* extract at the concentration of 5 mg/kg.bw per rat

III – group, n=20, with experimental antibiotic-associated dysbiosis

* Indicates difference from beginning of treatment (P≤0.05)

▲Indicates difference between groups (P≤0.05)

Table 2. The dynamics of the recovery of the intestinal microflora of rats after antibiotic-associated dysbiosis in applying them an extract from *Chlorophytum comosum*

Bacterial group	Numbers of the monitored bacterial groups in 1 gram of faecal microflora of experimental animals Log_{10} CFU/g						
	The end of the gentamicin influence	Day 3		Day 6		Day 10	
	Unified group	IIIC	IIIE	IIIC	IIIE	IIIC	IIIE
Bifidobacterium spp	4.2±0.4	3.8±0.2	3.6±0.5	4.5±0.4	4.6±0.5	5.5±0.4*	5.7±0.2*
Lactobacillus spp	4.5±0.5	4.1±0.2	3.9±0.4	4.7±0.5	4.7±0.5	6.1±0.4*	6.6±0.4*
Escherichia coli with normal enzymatic activity	2.2±0.4	2.2±0.5	2.8±0.4	2.8±0.4	3.2±0.2*	3.5±0.2*▲	4.5±0.2*▲
Candida spp	4.5±0.2	4.5±0.4	3.5±0.3	4.2±0.3	3.4±0.2*	3.7±0.3*▲	2.8±0.2*▲

I – control sub-group, n=10, animals received a usual diet
II – experimental sub-group, n=10, animals received *C. comosum* extract at the concentration of 5 mg/kg.bw per rat in addition to the usual diet.
* Indicates difference from beginning of treatment (P≤0.05)
▲ Indicates difference between groups (P≤0.05)

RESULTS

It should be noted that pathogens and microorganisms of the genus *Proteus*, *Klebsiella*, *Sitrobacter*, hemolytic flora were not detected in any of the animals before and after the experiment. The results obtained at the first stage of the experiment are shown in the Table 1. Found that the use of *C. comosum* leaves hydroalcoholic extract in the selected dose during 10 days on rats Group I did not have any depressing or stimulating effect on the target microorganisms of intestinal microbiocenosis, in particular representatives of *Bifidobacterium spp, Lactobacillus spp*: to the 10th day of study their number was not significantly increased or decreased. The number of *E. coli* with normal enzymatic activity by the end of the experiment increased by one order of magnitude (P≤0.05), which indicates a moderate stimulating effect of *C. comosum* extract. In relation to *Candida spp* a moderate inhibitory effect (P≤0.05) is observed.

The use of gentamicin in Group II and III fairly quickly (already on the 3d day) led to the suppression of the studied microorganisms that logically amplified to the 10th day of exposure. In this case the use of the *C. comosum* extract did not allowed to reduce the rate of fall of the intestinal microflora, but nevertheless, can be noted that the reduction in the number of *E. coli* in the Group II going slowly than in Group III, and eventually led to the fall not so strong. The general weakening of the body significantly increases the amount of *Candida spp* in both groups, but in the Group II receiving *C. comosum* extract increase was not as strong as a Group III (P≤0.05).

The results obtained after the termination of gentamicin exposure are shown in the Table 2. It can be noted that the animals of the both sub-groups show a low rate of recovery of own microflora. On the 3rd day after the termination of the influence of gentamicin, a insignificant continuing decline in the number of *Bifidobacterium spp* and *Lactobacillus spp* on the background of the increased number of *Candida spp* was observed. By the 10th day the microflora level could not be restored to their original values (Table 1), but the growth of *E. coli* in a sub-group with *C. comosum* came faster and its final number was higher (P≤0.05) than the control sub-group. Reducing the amount of *Candida spp* in the experimental group was also faster than control sub-group.

DISCUSSION AND CONCLUSION

Plant extracts have diversified action in relation to various microorganisms. There are extracts from plants, having a prebiotic effect [4], and also some that on a contrary have an antimicrobial properties [3, 8]. In this case it was interesting to determine the effect of the hydroalcoholic extract of *C. comosum* leaves in a living system of warm blooded animals, taking into account the fact that it is a well known medicinal plant. Many authors [5,10,14] reported a pronounced antimicrobial activity of plant *Chlorophytum*, which allowed to suggests a similar effect of the test extract in relation to the main representatives of intestinal microflora during its free reception or the general weakening of the body with antibiotic. Our research was the first to reveal that *C. comosum* extract prepared as described above in the selected dose has no influence on the amount of *Bifidobacterium spp, Lactobacillus spp,* but moderately stimulates growth of *E. coli* with normal enzymatic activity, which is in contrast with the findings of the Sundaram *et.al*. [14], who reported about the antibacterial activity of the acetic acid, acetone and ethanol *Chlorophytum* extracts in relation to *E. coli* which was found during *in vitro* tests. Valya *et.al*. (2009) [15] and Ahmad *et.al*. (2014) [1] in their research also reported about the antibacterial activity of *Chlorophytum borivilianum* to *E. coli* but the effect was less pronounced than in relation to *Staphylococcus.* Our results can be explained with special relationships inside the microbiocenosis system of the intestine. The results of the present study also revealed weak antimycotic action of the *C. comosum* extract in relation to *Candida spp* that in general is confirmed by Valya *et.al*. [15] and Chakraborthy *et.al.* [5].

This allows to recommend *C. comosum* extract as a support means in cases of dysbacteriosis associated with lack of *E. coli*, as well as to reduce the overly high number of *Candida spp*.

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CONFLICT OF INTEREST

The authors declare that there is no conflict of interests regarding the publication of this paper.

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