Annals of R.S.C.B., ISSN: 1583-6258, Vol. 25, Issue 6, 2021, Pages. 1575-1583
Received 25 April 2021; Accepted 08 May 2021.

Investigation the role of *Lactobacillus acidophilus* on *Cryptosporidium parvum*

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Abstract :

Cryptosporidium parvum is a type of parasite with global distributionIt is one of common causes of diarrhea especially in children which is Considered a widespread health problem in the world.This study included the evaluation of the effect of Lactobacillus ssp in cryptosporidium parvum oral inoculation of mice with I $\times 10^8$ Cell/ 0.1 ml Cyst of C. paruum.In the serum of the infected mice was on the level of IL – 10, IFN – y and IL – 4 high compared with uinfected mice.High Level of ggt, Lipase compared with uinfected mice The result of this research show that Lactobacillus acidophilus bacteria and azithromycin Ain have asimillar effect on cryptosporidium parvum infection in vivo and Lactobacillus acidophilus bacteria non toxicity and more safety than azithromycin.

Keyword; Lactobacillus GGT acidophilus, Cryptosporidium parvum,IL-10,IL-4,IFN-Y,Lipase,GGt

Introduction :

Cryptosporidium is an intestinal Coccidian parasite affecting various animals and men. (1) .It causes self limiting acut diarrhea immunocompetent healthy individuals, Where as it is an opportunistic pathogen in immunocompromised patients (HIV) causing chronic presistent life threatening diarrhea. (2) Sporulated Oocyst is the infective form of the parasites Thick walled Oocyst is infectious to other persons where as the thin walled cocyst can cause autoinfection. (3) Who reported that cryptosporidium parvum in 2004 spreading among the poor and may be due to malnution, or un healthy condition especially the tropical to the world. (4) Sponseller et all in 2014 report the infect the uper respiratory system with C. parvum caused infalmentary in mulous of the nose secretory with Chang in the vioco, but When infected the lower respiratory system caused cough, fever and dyspnea. (5). The transmission to human by Oocyst by two method one by ingestion with feces containing thick walled Ootyst and by autoinfection thin walled oocyst can infect the same host. (6) Lactobicilli are normal flora of mouth, intestine and femal genital tract with important role in the control of undesirable microorganisms that can be considered as natural bio preservatives.

Lactobacilli have an important the rise of pathogenic bacteria by producing antimicrobial metàbolites. (7).*L. acidophilus* is Gram positive, rod, in shap short in

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sizef 2-10 **µ** ml. (8).It is produce bacteriacins that can be used as biological preservatives in order to inhibit growth L. monocytogenes in various Kind of food especially fermentative dairy products like Yogurt and cheese. (9)

Materials and method sources of parasite :

Sources of Parasite

The Parasite was obtaine from Baghdad science then isolated by Lumb method. (10).When it put in (PBS) with (200) ml Penicillin (0.2ml) streptomycin (2.5) ml amphotericin and stored in refrigrebor at atemperature (4 e) until they will used. (11) .Diagnosis: The color and strength of the samples, the existence on the absence of odor, either microscopy was examined by force of the great (loox) dyed wet strips and stained by Zieh-Neelsen. (12)

Preparation of free cell and supernatant :

Both cells and supernatant obtained from MRS both culture after incubated for (18 hr) at (37 C^o) in an anaerobic Jar, then the culture was Centrifuged at (10.000 rpm) for 10 **µ**in supermant was removed and filered through (0.22 Mm) pores size filters and concentrated while bacterial cells were taken, then washed and suspended in to contain (1×10^3 cell/0.1 ml) after that stored in refrigerator to use later. (13 , 14) Gamma Glutamyl transferase Measurement depend by Shaw method. (15) Lipase measurement depenet panteghinil method. (16) .

Using (60) mouse white were obtained from national center for research and drug control and average age between (5 – 12) weeks weight (16 – 22 gm).

Divided into six groups each groups include (10) mice (50) mice was given (IXo cyst/0.1 ml) to make infection, and ansure, the more was infected by examined their feces.

1- Groups, one (uninfected): inoculated with normal saline Consider is Control negative.
2- Group two (infected): inoculated orally with (0.1ml/day) with normal saline consider it as control positive.
3- Group three (infected): inoculated orally (0.1ml/day) Lactobacillus acidophilus.
4- Group four (infected): inoculated orally with (0.01ml/day) of free cetts which contains I $\times 10^3$ cell/ml day.
5- Group five (infected): inoculated orally with (0.1 ml/day) of supernatant.

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6- Group six (infected): inoculated orally with (0.1 ml/day) of azithromycin.

During the experiment, Stools of mice were collected and cheeked by light microscope and numerate the number of paraste after the end of experiment all mice were killed and the blood Collected from ocular vein of mice L_{10} , L_4 , IFN – y measurement depend k/t.

Result and Discussion :

Table (1): The level of IFN-Y in serum in treated group and control.

Treatment group Time (day) pg/ml			
	3	7	12
Control negative	30.6 ± 0.56	32.14 ± 3.42	33.44 ± 2.74
control positive	40.60 ± 1.60	40.90 ± 0.80	41.64 ± 2.55
Azithromycin	43.10 ± 2.70	49.00 ± 0.97	46.10 ± 70.0
bacterial cells 1×10^8 cell/ml/day	74.20 ± 16.22	60.00 ± 1.80	52.16 ± 1.32
Sediment	50.35 ± 12.90	50.00 ± 0.77	41.50 ± 5.55
supernatant	52.22 ± 10.22	54.16 ± 0.24	50.16 ± 1.26

The level ofI IfN-y appears significantly ($P<0.05$) increased in azithromycin and treatment groups Compared with control positive groups Level of IFN – y as show in table (1) in all treated group after (12) days of treatment after infected the mice by C. parvum the result show in treated group with bacterial cells ($IX10^8$ Cell/ml/day) after 3, 7, 12 days (74.20 ± 16.22), (60.00 ± 1.80), (52.16 ± 1.33) respectively while in infected group treated with sediment after 3, 7, 12 days (50.35 ± 12.9), (50.00 ± 0.77), (41.50 ± 5.55) respectively, but in treatent with supernatant the level of INF – y was (52.22 ± 10.22), (54.16 ± 0.21), (50.16 ± 1.26) respectively but in azithromycin treatment group INF – y Livet (43.10 ± 2.70), (48.00 ± 0.97), (46.10 ± 7.00) respectively compared with control group.

In the control positive group noticed that the level of IFN – y after 3, 7, 12 days is (40.60 ± 1.60), (40.90 ± 0.80), (41.64 ± 2.55) pg/ml irrespectively compared with control group.

Table (2) : The level of IL-10 in serum in treated proups and control.

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Treatment group Time (day) pg/ml			
	3	7	12
Control negative	80.6 ± 4.6	78.0 ± 10.5	87.5 ± 6.6
control positive	117 ± 14.0	114.7 ± 14.0	111.0 ± 5.5
Azithromycin	88.5 ± 8.7	120.0 ± 11.5	98.3 ± 5.5
bacterial cells 1×10^8 cell/ml/day	123.0 ± 11.5	121.6 ± 9.0	120.0 ± 14.0
Sediment	96.5 ± 6.00	96.3 ± 2.5	87.7 ± 3.38
supernatant	115.7 ± 18.77	108.8 ± 16.5	105.0 ± 13.0

In table (2) shows the level of IL – 10 in all treated groups in bacterial cells treatment group the results were shown after 3, 7, 12 days (123.0 ± 11.5), (121.6 ± 9.0), (120.0 ± 14.0) respectively compared with control group the level of Il – 10 in sediment treatment group was (96.5 ± 6.00), (96.3 ± 2.3), (87.7 ± 3.38) respectively compared with control group was (115.2 ± 18.77), (108.8 ± 16.5), (105.0 ± 13.0) respectively.

In azithromycin areatment group was the level of IL – 10 (88.5 ± 8.7), (120.0 ± 11.0), (98.3 ± 5.5) respectively Compared with Control group while in Control positive group the level of this was (117.0 ± 14.0), 114.1 ± 14.0), (111.0 ± 5.5) respectively compared with Control group.

Table (3): The level of IL – 4 in serum intreated groups and control.

Treatment group Time (day) pg/ml			
	3	7	12
Control negative	29.5 ± 4.3	29.6 ± 4.3	29.5 ± 4.4
control positive	103.5 ± 7.5	104.2 ± 7.0	105.7 ± 8.0
bacterial cells 1×10^8 cell/ml/day	127.4 ± 4.6	127.0 ± 4.5	127.5 ± 4.5
Sediment	30.5 ± 1.7	30.6 ± 1.6	30.7 ± 1.7
supernatant	119.0 ± 4.5	115.0 ± 18.4	119.0 ± 4.3
Azithromycin	90.0 ± 6.7	90.2 ± 6.8	90.3 ± 6.8

In table (3) shows the level of the of IL-4 in all treated groups in bacterial cells treatment group the results were shown after 3, 7, 12 days (127.4 ± 4.6), (127.0 ± 4.5),

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(127.5 ± 4.5) respectively compared with Control group in sediment treatment group level of IL-4 was (30.5 ± 1.7), (30.6 ± 1.6), (30.7 ± 1.7) respectively While in supernatant treatment group was (119.0 ± 4.5), (115.0 ± 18.4), (119.0 ± 4.3) respectively, compare control group.

In azithromycin treatment group the level of IL-4 was (90.0 ± 6.7), (90.2 ± 6.8), (90.3 ± 6.8) respectively but in Control positive group was (103.5 ± 7.5), (104.2 ± 7.0), (105.7 ± 8.0) respectively.

Table (4): The level of that enzymes in serum interned group and control.

Treated group	GGtu/L Mean ± S. D	Lipase Mean ± S.D
Control negative	18.50 ± 4.77	303.50 ± 90.5
control positive	23.8 ± 9.10	325.40 ± 9.55
bacterial cells 1×10^8 cell/ml/day	21.5 ± 9.11	320.4 ± 90.5
Sediment	19.7 ± 9.10	314.4 ± 90.5
supernatant	20.7 ± 9.5	310.61 ± 90.5
Azithromycin	19.8 ± 9.10	307.9 ± 90.2

In table (4) Shows Level of GGt in serum the result were show (21.5 ± 9.11) in bacterial cell treatment group but was (19.7 ± 9.10) in sealiment treated group while was (20.7 ± 9.5) in supernatant treated group. Compare with Control negative (18.50 ± 4.77). In azithromycin treated group was (19.8 ± 9.10) but in control positive way (23.8 ± 9.10).

The lipase was (32.4 ± 9.5) in bacterial Call treated group and (314.4 ± 90.5) in sedment treated group and (310 ± 9.11) in supernatant treated group compared with control negative (303.5 ± 90.5) in azithromycin way (307.9 ± 90.2) and Control positive was (325.4 ± 90.55).

Lactobacillus acidophilus that orally adimistered to the mice stimulated specific systemic immune functions such as macrophages increases in Lymphocyte proliferative responses and enhanced stimulation of IL10 and IFN – y cytokines. (17)

Mice noculated with *L. acidophilus* ., had significantly enhanced IL10 and

IFN – y Levels in the serum compared to control mice.In azithromycin group The result showed decreased IFN – y and IL10 due to the important role of drug as antimicrobial agent that shown to be effective against other protozal infection and causes damage to DNA and proteins in the cell B.(19)

In table (3) the result indicated that most of positive control group have high concentration of the in their sera in comparison with the other control group in this study and these result were also indicated by (20,21).

IL-4 represented as one of the cytokines which produced by Th2 cells and act as a cofactor inactivation of humoral immunity by activation of B. cells and T cells proliferation and differentiation. (22. 23).

In table (4) is the results indicates GGT serum levels were singnifican increased.The GGt is present in hepatocyte and biliary epithelial cells. An elevated serum or in the bile duct. (24).The Lipase occurs in many tissues and organs include the heart brains muscles veins kidneys, spleen, Lungs, Livers Fatty tissue and plasmn. (25).Lipase level is correlate with inflammation markers, the increased concentration of extracellular Lipase Oocurs as areaction of the of initiation of aninflammatory response. (26).

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