Evaluation of Blood Parameters When Infected with *Candida Albicans*and Its Recovery Using Probiotics

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Abstract

Purpose: To study, blood components and some indicators of liver function were measured andCandida albicans was isolated and identified from infected cows' vagina with inflammatory signs then experimentally in rabbits treated with estradiol hormone.

Introduction: Candidiasis is an infection caused by yeast,.Candida can multiply and cause an infection if the environment inside the vagina changes in a way that induces its growth.

Materials and Methods: Isolation and identification *of candida albicans* isolate from cow' vaginitis (40 samples), duration in November 2020 till May 2021 then treated with *saccharomyces cerevisiae. Candida albicans* inoculums at 1.5 $\times 10^8$ cell /ml, in non-pregnant vagainal' Rabbits a weight (2 kg) after treated with estradiol hormone for several days before the infection occurred. The experiment was carried out in the microbiology laboratories in College Veterinary Medicine- the University of Baghdad, the blood, liver parameters, and rabbits' vaginal histology was studied.

Results: The Hb parameters in G2 showed higher significant differences (8.31±0.75107) compared with G1(12.80 ± 2.42782), G3 (13.26 ± 1.465), G4 (12.46 ± 1.987), G5 (12.75 ± 2.128) respectively, the effectiveness of the treatment with *S.cerevisiae* and its ability to return hemoglobin and liver functions to its normal value and the treatment cause inhibitory effect of C.albicans infection, the vagainal histological section in G4 showed narrow folding of mucosa thrown with mild MNCs infiltrate LP and submucosa, no inflammatory cells seen in a muscular layer or in the serosa.

Conclusion: The study proved the effectiveness by using *Saccharomyces cerevisiae* and treating rabbits vaginal infection with pathogenic Candida albicans, that Injected estradiol hormone S\C then, recovery the blood and liver parameters to their normal condition, in addition, the treatment of vaginal tissue and its return to its normal state.

***Key words**: Candida albicans , Blood parameters, probiotics.*

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Introduction

Candidiasis is an infection caused by a yeast (a type of fungus) called Candida, it is normally living inside the body (in places such as the mouth, throat, gut, and vagina) and on the skin without causing any problems, sometimes Candida can multiply

and cause an infection if the environment inside the vagina changes in a way that induces its growth. Candidiasis in the vagina is commonly called a vaginal yeast infection, other names for this infection are vaginal Candidiasis, vulvovaginalCandidiasis, or candidlyVaginitis[1]. Candidiasis is one of the fungal diseases occur saprophytically but only *Candida albicans* is commonly associated with diseases in humans and animals found commensallyof mucocutaneous areas particularly of intestinal and genital tracts[2]. Also, there are many microflora in the vagina like *E.coli, Klebsiella, and Streptococcus faecalis*[3].

Among Candida spp., *Candida albicans* is the most common infectious agent, this dimorphic yeast is a commensal that colonizes skin, the gastrointestinal and the reproductive tracts, and the most frequent manifestations of genitourinary Candidiasis include Vulvovaginal candidiasis (VVC), and it the most prevalent vaginal infection worldwide, and Candida albicans is major agent[4]. VulvovaginalCandidiasis is characterized by disruption of the vaginal microbiota composition, following large spectrum antibiotic use. Recent studies support the effectiveness of oral and local probiotic treatment for prevention of vulvovaginal candidiasis, demonstrate that vaginal administration of probiotic *Saccharomyces cerevisiae* live yeast used as therapeutics, this effect was likely due to multiple interactions of *Saccharomyces cerevisiae* with *Candida albicans*, induced co-aggregation of Candida and inhibited its adherence to epithelial cells, however, only the probiotic yeast was able to suppress some major virulence factors of Candida albicans such as the ability to switch from yeast to mycelia form and the capacity to express several aspartyl proteases[5]*Saccharomyces cerevisiae* is a species of yeast (single-celled fungus microorganisms, yeasts are an important source for obtaining products with probiotic activity, yeast, strains are more well-known in their use as probiotics[6]*Saccharomyces cerevisiae* has developed as a model because it is a single-cell organism, small with a short generation time (doubling time 1.25–2 hours at 30 °C), can be easily cultured, it is a strong economic driver, as a result of its established use in industry[7]Also used as a feed additive in livestock that Contains; proteins, vitamins (vitamin B6, thiamin (B1), biotin, riboflavin (B2), nicotinic acid pantothenic acid (B5) and enzymes that improve weight gain and growth performance [8]. lowers mortality rates, increases hematological parameters decreases serum cholesterol levels, improves the fertility of female[9].

Materials and Methods

Isolation of *candida albicans* from cow' vaginitis (40 samples), and Preparation of *saccharomyces cerevisiae*according to [10].and

Candidaalbicans inoculums' concentration 1.5 $\times 10^8$ cell /ml according to[11], Experimental animals (Rabbits eight for each group).

Group1: untreated (control).

Group2: Inject female rabbits S/C with 5mg estradiol in 50µl sesame oil for 5 days prior to injecting vaginal inoculation (2 ml/ rabbit Candida albicans) for 7 days (daily intake) / Intravaginal.

Group3 : Same group2 + 2ml /kg B.W (orally) of *Saccharomyces cerevisiae* for 7 days (daily intake) at the same time.

Group4: Same group 2 + 2ml /kg B.W (orally) of *Saccharomyces cerevisiae* for 10 days, daily intake treat after final doses of *Candida albicans*.

Group5 : Treated with *Saccharomyces cerevisiae* 2ml /kg B.W (orally) for 7 days only (daily intake).

Methods:

Preparation of culture media:

Sabouruad dextrose agar (SDA), Cornmeal agar

(CMA), Glucose Peptone Yeast Broth Medium (GPYB) The medium was prepared according to the manufacturer's directions.

Candida albicans were described based on morphological characteristics of the culture medium, the development of germ tubes, and the production of Chlamydospores[12,13]

Histopathological Examination

VagainalRabbits tissueshave been prepared for histopathological examination according to[14]

Statistical Analysis

The results data were analyzed statistically by using the Microsoft Program (SPSS). Statistical analysis of data was performed on the basis of Analysis of Variance (ANOVA) and specific group differences were determined using least significant differences (L.S.D), as described by[15]

Results and Discussion

Candida albicans identification by typical and rapid methods: -

Candida albicans were described based on morphological characteristics of the culture medium, the development of germ tubes, and the production of Chlamydospores.

Cultural Characteristics on Sabouraud's dextrose agar:

Candida albicans was cultivated on Sabouruad dextrose agar for 48-72 hours, and colonies appeared as thin, smooth white-cream, glistening round and curved colonies at 37 °C for 2-3 days after inoculation; these results are consistent with those obtained using Sabouruad dextrose agar for 48-72 hours [16].Candida albicans colonies on sabouruad dextrose agar revealed white creamy opaque pasty colonies under the microscope. as in figure (1).

Figure (1): *Candida albicans***colonies on sabouruad dextrose agar showed white creamy opaque pasty .**

Microscopical Characteristics:

Using Lactophenol Cotton Blue stain, microscopically analysis revealed the presence of pseudohyphae with clusters of budding cells. These morphological features of isolates were *Candida albicans*.fig(2)

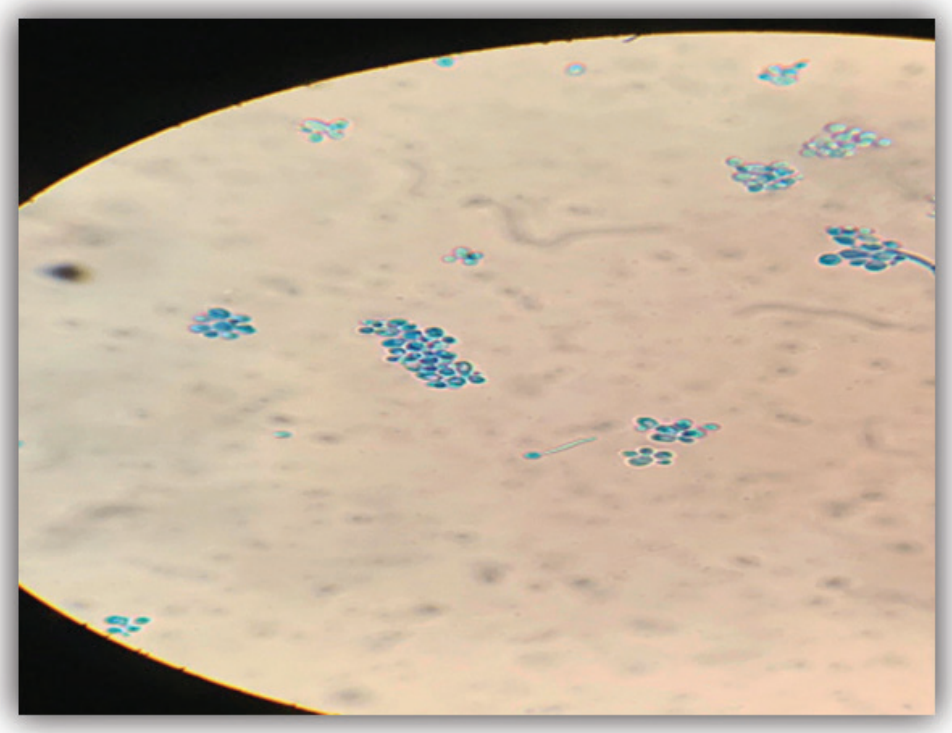


Figure (2): Microscopically appearanceChlamydospores of *Candida albicans*.

Germ tubes formation:

Forming long tube-like projections from yeast cells,these tubular extensions are an early stage in the formation of hyphae in the hyphae formation process. The germ tubes developed within 2-3 hours of incubation at 37 °C when *Candida albicans* was inoculated in the human serum , which is a distinct diagnostic feature of *Candida albicans* that distinguishes it from other Candida species this study is agree with [17]Fig(3).

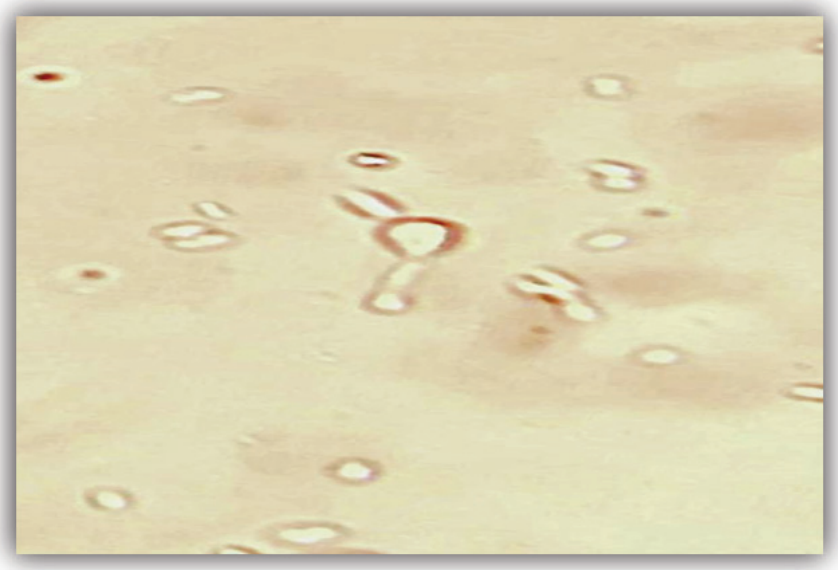


Figure (3): Germ tube production after 3 hours of growth in fresh human's serum at 37°C. (40X).

Chlamydospores production

Another measure for the detection of *Candida albicans*, the ability to produceChlamydospores was tested using corn meal agar, and the results were consistent [18] .. When the isolates are cultured on corn meal with 1% of tween-80 agar and incubation at 30 °C for 48 hours (Dalmau plate technique), The growth was then stained with lacto phenol cotton blue stain and analyzed under a microscope with a 40X objective, which agree with [19]. The hematologicparameters showed theHB is higher significant differences at level ($P \leq 0.05$) in G2 (8.31±0.75107) when the vagaina's rabbit infected by *C.albicans* compared with G1(12.80 ± 2.42782), G3 (13.26 ± 1.465), G4 (12.46 ± 1.987),G5 (12.75 ± 2.128) respectively. the results showed the effectiveness of the treatment with *S.cerevisiae* and its ability to return hemoglobin to its normal value and its inhibitory effect on the growth of *C.albicans* isolates, this result is similar to the researcher's study an agreement with[20],. they found in their research that the Hemolysin are involved in *Candida albicans* virulence because they are responsible for the destruction of red blood cells and the acquisition of iron. Iron and other inorganic elements are required for the development of Candida cells and the establishment of the infection process,the synthesis of haemolysin by *Candida albicans* could beassociated with reductions in red blood cell and hemoglobin levels throughout the infection period [21].

The PCV parameter showed the higher significant differences at level ($P \leq 0.05$) in G2 (26.78 ± 3.98) when the vagaina's rabbit infected by *C.albicans* compared with G1(38.33 ± 8.09), G3 (38.58 ± 7.54), G4 (38.86 ± 8.12),G5 (43.73 ± 8.96) respectively. the results showed decrease in PCV level this results were an agreement with [22]when he found a PCV less than 30 percent indicating anemia with infected specially particularly if hemoglobin levels are low.

The WBCs count parameters showed the higher significant differences at level ($P \leq 0.05$) in G2 (29.35 ± 3.46) when the vagaina's rabbit infected by *C.albicans* compared with G1 (8.24 ± 2.20), G3 (10.91 ± 1.77), G4 (7.37 ± 1.27),G5 (8.67 ± 2.61) respectively. Laboratory investigations have shown increase in the total number of circulating leukocytes in rabbits injected with bacteria or yeast. An agreementwith [23].

The cholesterol counts parameter showed the higher significant differences at level ($P \leq 0.05$) in G2 (90.22 ± 4.73) when the vagaina's rabbit infected by *C.albicans* compared with G1 (58.98 ± 15.75) G3 (71.86 ± 10.19), G4 (47.41 ± 19.46),G5 (45.78 ± 16.99) respectively. Metabolic effects of*S. cerevisiae*lower the cholesterol levels, this results were an agreement with [24] .

The ALT test counts parameter showed the higher significant differences at level ($P \leq 0.05$) in G2 (361.21 ± 65.21) when the vagaina's rabbit infected by *C.albicans* compared with G1 (96.30 ± 24.83), G3 (95.34 ± 28.17), G4 (129.60 ± 52.15),G5 (85.34 ± 25.23) respectively, indicated high significant differences in group 2 the resultsan agreement withKretschmar[25]who found that Alanine Aminotransferase (ALT) is abundant in many tissues, it is of limited use in rabbit liverdisease, however, if ALT levels are abnormally high, it could suggest liver inflammation and necrosis.

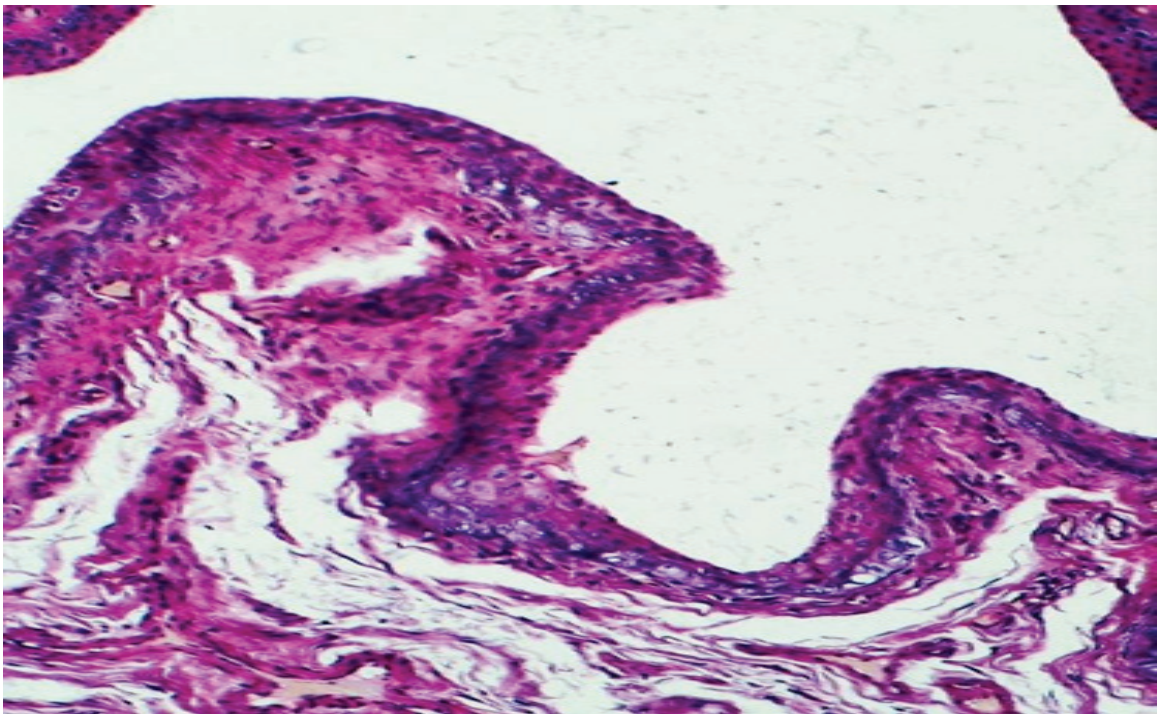
The AST test counts parameter showed the higher significant differences at level ($P \leq 0.05$) in G2 (180.85 ± 61.62) when, the vagaina's rabbit infected by *C.albicans* compared with G1 (55.67 ± 20.39), G3 (61.61 ± 13.23), G4 (83.60 ± 13.79),G5 (44.46 ± 17.92) respectively. The enzyme aspartate aminotransferase (AST) is present in a number of tissues in the rabbit, including the liver, heart, pancreas, and muscle. Elevations in this enzyme can be caused by liver cell necrosis.[26](Table 1).

Table (1): Blood parameters in rabbits infected with *Candida albicans*and treatment with probiotics.

Group Parameter	GROUP 1 Control negative	GROUP 2 Control positive infected with c.albicans 7 days	GROUP 3 Infection with C.albicans 7 days & treatment with S.cerevisiae 7 days in the same times	GROUP 4 Infection with C.albicans7 days Then treated with S.cerevisiae 10 days	GROUP 5 Only Treated with S.cervesiae 7 days	NO.	LSD
Hb	12.80 ± 2.42782A	8.31±0.75107 B	13.26 ± 1.46574 A	12.46 ± 1.98778 A	12.75 ± 2.12804 A	8	1.863
PCV	38.33 ± 8.09 A	26.78 ± 3.98 B	38.58 ± 7.54 A	38.86 ± 8.12 A	43.73 ± 8.96 A	8	7.62
WBC	8.24 ± 2.20 C	29.35 ± 3.46 A	10.91 ± 1.77 B	7.37 ± 1.27 C	8.67 ± 2.61 BC	8	2.40
Cholesterol	58.98 ± 15.75 BC	90.22 ± 4.73 A	71.86 ± 10.19 B	47.41 ± 19.46 C	45.78 ± 16.99 C	8	14.58
ALT	96.30 ± 24.83 BC	361.21 ± 65.21 A	95.34 ± 28.17 BC	129.60 ± 52.15 B	85.34 ± 25.23 C	8	42.87
AST	55.67 ± 20.39 BC	180.85 ± 61.62 A	61.61 ± 13.23 BC	83.60 ± 13.79 B	44.46 ± 17.92 C	8	31.56
Different Capital letters mean significant differences horizontally ($P \leq 0.05$) between groups							

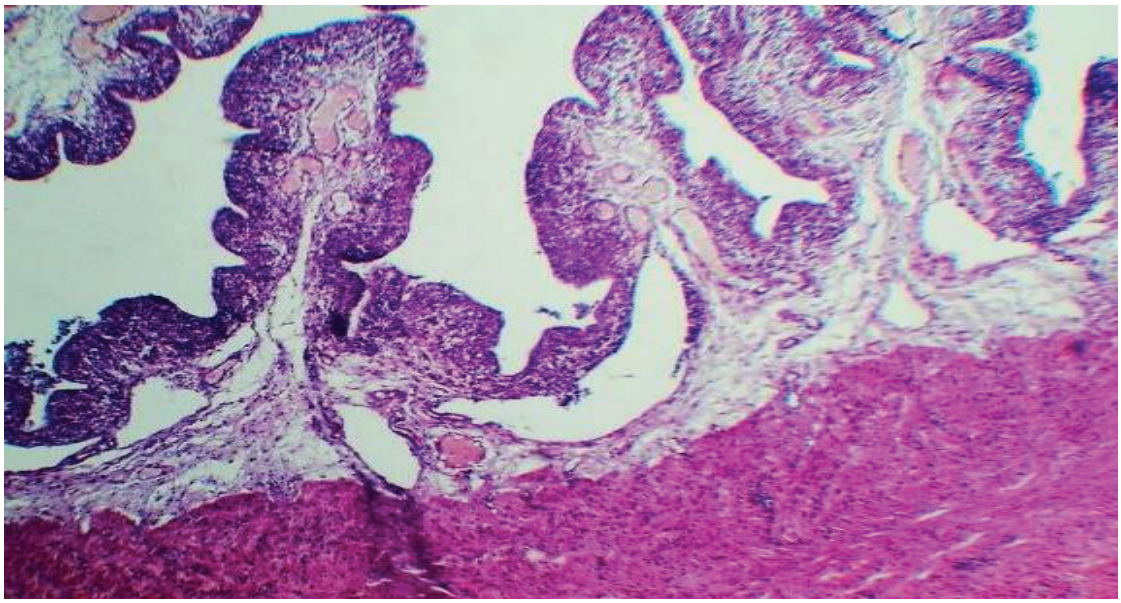
Histological Examination

In G1 the microscopic examination of control animals revealed the vaginal wall by light microscope, in lower 1/3 of vaginal duct the mucosa consist from 2 to 3 layers of stratified squamous epithelium and numerous vascular sinusoids lined by endothelial cells in muscularispropria the muscular layer consist from compact arrangement of muscle strands (inner longitudinal and cross external layer) without sinusoidal vascular in muscularispropria.



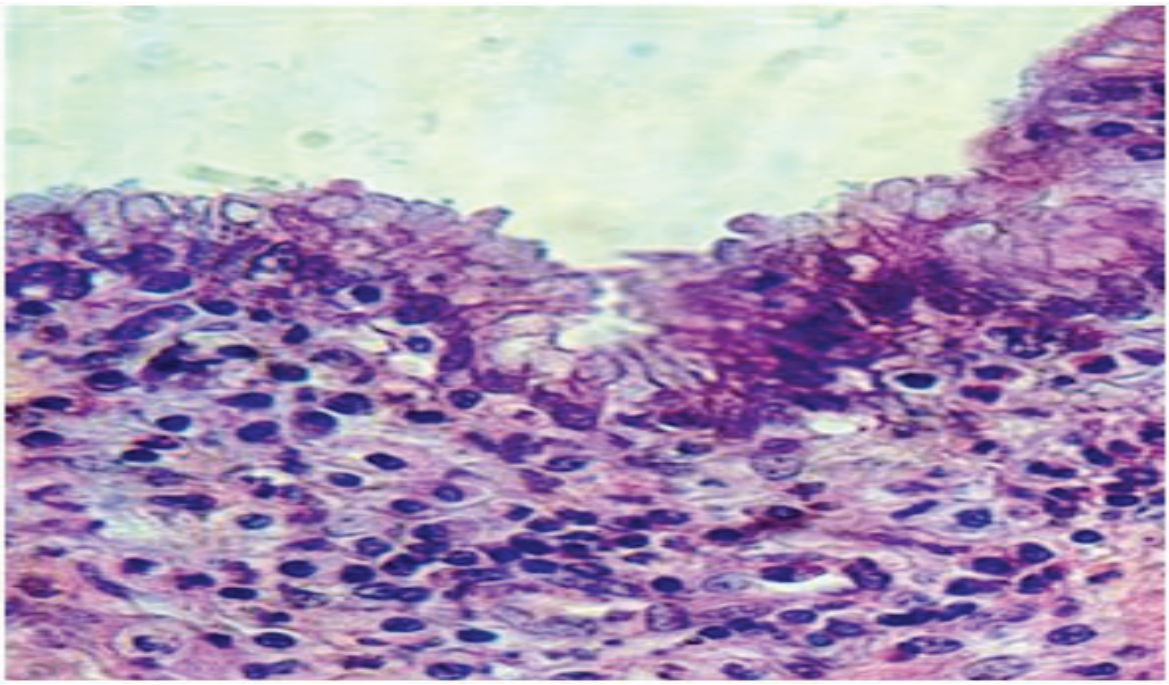
Figure(4) : Histopathologic section in vaginae of rabbit in G1 showed normal architecture of vaginae wall lined by stratified columinar epitheliumvascular sinusoids in muscularispropria. (H&E stain, 100X).

In G2 there was marked acute Vaginitis described as severe vacuolar degeneration and necrosis of squamous epithelium in mucosa with heavy infiltration of inflammatory cells hetrophiles and few mononuclear cells mainly macrophages and few lymphocytes , also infiltrated in submucosa few perivascular in the outer muscular layer extend to serosa with dilation of serosal blood vessels, fibrinousexudates as fibrils network precipitate in submucosa with congestion of blood vessels dilated and contained few inflammatory cells.



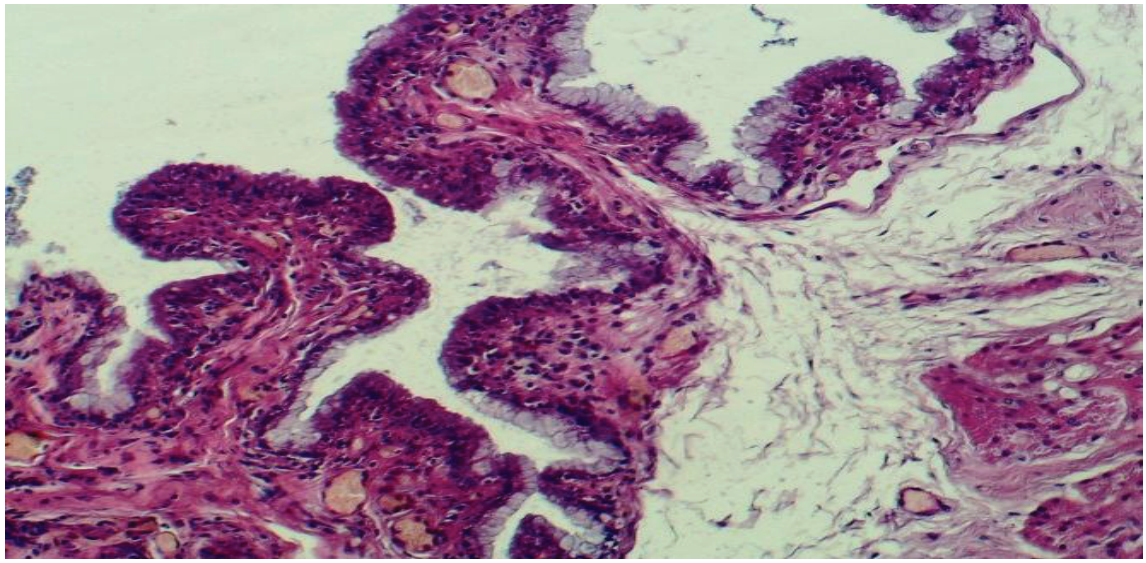
Figure(5) : Histopathologic section in vagina of rabbit in G2 shows severe vaginitis as thickening of mucosa protrude likepapillae in lumen. (H&E stain, 100X) .

In G3 moderate to mild degeneration of squamous epithelial cells (vacuolar degeneration) and moderate mononuclear cells infiltrate lamina propria in mucosa. None inflammatory cells seen in muscular layer and serosa.



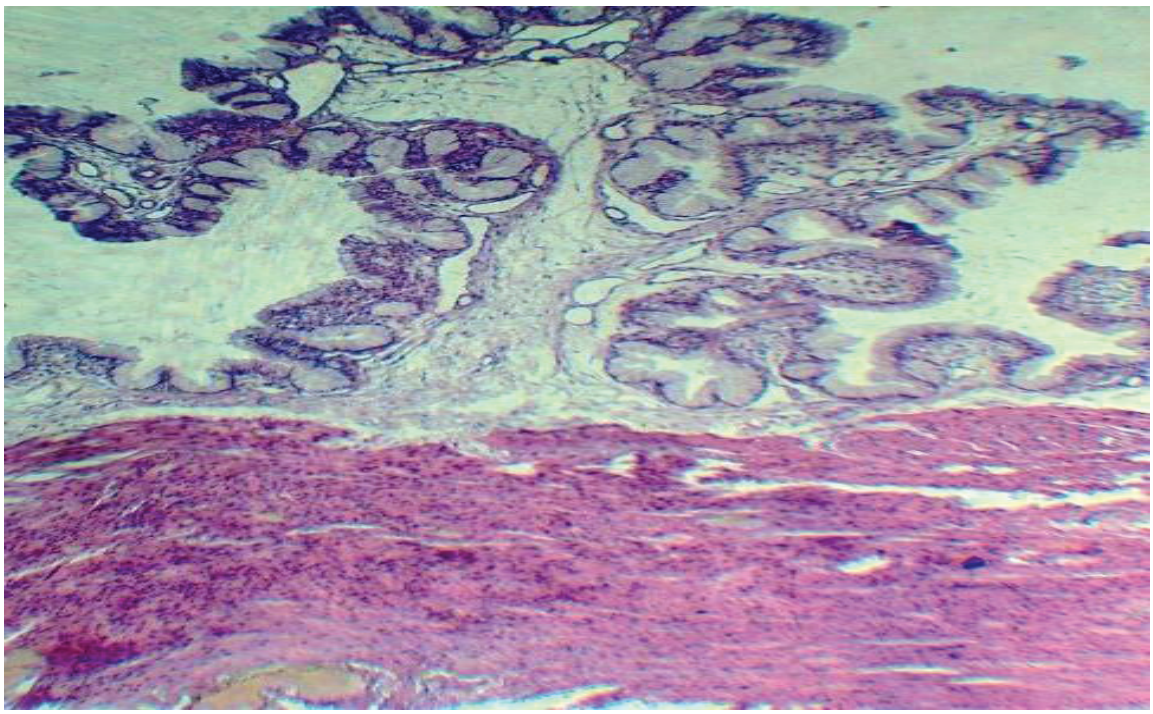
Figure(6) : Histopathologic section in vagina of rabbit in G3 showed: mild to moderate vacuolardegeneration of squamous epithelium, andmononuclear cells infiltration in lamina properia and in muscular layer perivascular, B shows normal villi and mucosal epithelium. (H&E stain, 400X).

In G4 narrow foldings of mucosa thrown into with mild MNCs infiltrate LP and submucosa, no inflammatory cells seen in muscular layer or in serosa.



Figure(7) : Histopathological section in vagainal of rabbit in G4 shows mucosal folding ,few mononuclearcell infiltrate lamina propria. (H&E stain, 100X).

In G5:the architecture of mucosa appeared as normal histology, in lamina propria very mild mononuclear cells seen, no congestion of vascular sinusoids in muscular layer. (Figure 8)



Figure(8): Histopathological section in vagina of rabbit in G5 shows: numerousinfoldings in mucosa, infiltrate lamina propria with lymphocytic cells. (H&E stain, 400X).

The overall summary the probiotic treatments are useful for managing common vaginal infections .[27.] The present study confirms the results of other reports in a quantitative manner, namely that probiotics as a supplement to conventional pharmacological treatments are effective in the short term for the treatment of common vaginal infections in non-pregnant adult females. However, high-quality evidence for the effectiveness of probiotics alone in recurrent or curative vaginal infections is limited. Further high-quality clinical trials are necessary to identify the most effective probiotic strains, the most effective treatment regimens (with or without antibiotics, and females (e.g. pre-menopausal vs. post-menopausal) that may benefit the most from probiotics.

The beneficial effect of both live and inactivated *S. cerevisiae* was due to a co-aggregation of *Candida* and consequently to its inability to adhere to the mucosal surface, protecting the vaginal epithelium from the fungus induced damage [28]. However, only the live and not the attenuated yeast strongly suppressed some of the crucial virulence factors of *C. albicans*, such as its capacity to switch from the yeast to the hyphae form and the ability to express aspartyl proteases These effects were related to the ability of the live yeast to significantly inhibit the expression of two important hyphae growth-associated genes, in particular the hyphae wall protein and extent of cell elongation ,as well as the two secretory aspartyl proteinases ,which play a key role in the immunopathogenesis of vaginal Candidiasis[29].

Conflict of Interest: None declared.

Ethical Clearance: Taken from institutional ethical committee.

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