Original Article

Study of the effect of chitosan and nystatin for Rabbits treated with Candida krusei yeast on blood parameters, electrolytes and intestinal tissue

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Abstract

The study included the effect of chitosan and nystatin on animals treated with C. krusei on blood parameters, electrolytes and intestinal tissue. Domestic male rabbits were used in this study, which indicated a noticeable rise in the concentration of Hb and PCV in the blood of the Chitosan group compare to the control group and a significant decrease in the C. krusei and C group, While the results revealed a noticeable rise in W.B.C in the blood of the treated group Chitosan, C. krusei, C. krusei + Chitosan, and C. krusei + Nystatin compared to the control group. It was found from the results in a table (2) that there was a considerable decrease ($P<0.05$) in the level of K+ concentration in the blood serum in C. krusei group, C. krusei + Chitosan group, and C. krusei + Nystatin group compared to a control group.

While the findings indicated that there was a significant increase in the concentration of Na+ in the blood serum in the treated group C. krusei and group C. krusei + Chitosan and C. krusei + Nystatin group compared with the control group and a significant decrease in the Chitosan group compared to the control group, while it showed a significant decrease in Ca + concentration in group C. krusei+Nystatin compare to the control group.

Through the results, it was discovered that there were no significant differences in the concentration of Mg + in all groups in the blood serum compared to the control group. The results of the microscopic examination in the four groups of intestinal tissue showed the appearance of necrosis and lysis in the cells lining the villi, fluttering and blood congestion with the proliferation of inflammatory cells in abundance in addition to the occurrence of hyperplasia in the mucous layer of the intestine.

Introduction

Chitosan is a complex carbohydrate that varies in its content of acetyl groups. The substance is a homogeneous positively charged repeating unit-based biopolymer of glucose amine. It is derived from chitin and dietary fibers of animal origin. It is a crucial part of the crustacean exoskeleton, insects, and fungi cell walls.[1] Chitosan has many biological and functional functions, including Its activity against microorganisms such as bacteria, fungi, and yeasts, its anti-tumor activity, lowering the level of cholesterol in the blood, its ability to hold water, fats, and dyes, stabilizing emulsions, gel formation, chelating mineral elements, and its antioxidant activity.[2]

Chitin is produced industrially from the exoskeleton of marine invertebrates such as crustaceans such as crabs, shrimp, and lobsters (shrimp), as well as the exoskeleton of insects and the fungi's cell walls and yeasts. In addition to the external structure of insects and the cell walls of fungi and yeasts.[3]

Among fungi, the yeast species Candida is the most prevalent source of fungal infections. There are roughly 20 distinct varieties of Candida; Candida is the most prevalent in human albicans.[4]

This type of fungi (yeasts) lives on the entire body, especially in hot and humid climates. The fungi multiply, in various circumstances and under special conditions, in a very large way and cause inflammation. The places most susceptible to infection are the vagina, mouth, and skin are all affected by Candida and diaper rash in children. Candida penetrates the body, in rare cases, enters the bloodstream, and leads to general pollution, and this is a risky circumstance because roughly 45% of cases result in death.[5]

A series of intestinal illnesses known collectively as "inflammatory bowel disease" (IBD) involves inflammation in the digestive tract, which includes the mouth, esophagus, stomach, small intestine, and large intestine [6]. This disturbs the normal functioning of the digestive system for the process of breaking down food, extracting nutrients from it, and removing waste [7].

Inflammatory bowel disease refers to ulcerative colitis, which causes inflammation and ulcers in the colon and rectum, and Crown's disease, which causes swelling of the lining of the digestive tract. Reducing the inflammation that generates signs and symptoms is the aim of IBD therapy. In the best scenarios, this might result in long-term improvement in addition to symptom alleviation. recovery and a reduced risk of complications. Inflammatory bowel disease treatment includes either medication or surgery.[8]

Anti-inflammatory medications are often the first step in treating IBD. Corticosteroids and amino salicylates, such as mesalamine, are among them (Asacol HD, Delzicol, others), balsalazide (Colaza), and olsalazine (Dipentium). The type of medication the depending on the patient's affected area of the colon.[9]

MATERIALS AND METHODS:-

The study used chitosan obtained from the Chinese company (Xi'an) and it was dissolved in pure water at a rate of 150 mg / 100 ml for the purpose of use .[10]and Candida krusei was obtained from candida cultured dishes. We take a lube campaign and dissolve it with 100 ml of distilled water.

Preparing the animals:

In this study, local male rabbits whose weight varied between (1300-1800) grams were used, and they were placed in metal cages with metal covers and dimensions
(120) x 60 x 60 cm, with a floor furnished with sawdust, and the care aspect was taken into account. Cages are cleaned and sterilized, with sawdust replaced every two days. The animals were kept in situations where there was a 12-hour cycle of light and a 12-hour cycle of darkness. The animals were fed with a mixture of feed made up of (35% wheat, 34% yellow maize, 20% soybeans, 10% animal protein, and 1% milk powder, to which 50 grams of preservatives and anti-fungal materials are added), and food and water were provided continually and in ample amounts during the raising period. rearing and treating animals for a month.

Preparation of the yeast suspension:

The yeast suspension was prepared by transferring part of the growing colony with lube and placing it in a sterile tube containing 5 ml of saline solution at a concentration of 4.50
- 5.00%. (cells/ml) .[11] Experiment design:

(25) local male rabbits were utilised in the study, divided randomly into (5) groups, each group included (5) animals, and the weights of each group were taken into account as much as possible. before starting the study.

Groups-:

The first group: The rabbits were fed this group on ration and normal water.

The second group: the group of animals given chitosan at a concentration of 150 mg/kg animal on a day.

The third group: was injected with a suspension of C. krusi yeast at a concentration of 1.5 x 108 cells/ml, once daily until symptoms appear.

Fourth group: Animals given chitosan at a concentration of 150 mg/kg animal/day, and suspension of C.krusi yeast at a concentration of 1.5 x 108 cells/ml, once daily.

Fifth group: They were injected with a suspension of C. krusi yeast at a concentration of 1.5 x 108 cells/ml, once daily. With an antagonist nystatin at a concentration of 1.42 mg / kg / 2 days.

Draw of blood :-

After the expiry of the specified period of the experiment
(30) days. Animals were spent starving for 24 hours, and then anesthetized by chloroform. After that, blood samples were drawn directly from the heart using the cardiac heart puncture method. Approximately (8-10) ml of blood was withdrawn, and 2 ml of it was placed in tubes containing an anticoagulant substance K-EDTA to perform the examination of the total number of Hb, PCV, the rest of the WBC was placed in test tubes free of anti-throat material, which was kept at room temperature for around 30 minutes, after which the serum was separated by centrifugation at 3000 rpm for 15 minutes, and the serum was kept at (-20) degrees Celsius in fresh tubes of plastic. Clean prior to biochemical tests are performed. Na , K , Mg , Ca .[12]

Preparation of tissue samples:

After dissecting and extracting the animals the small intestines necessary for the current study, the organs were

weighed and using a physiological solution for washing then they were placed in a buffer neutralized formalin at a concentration of 10% for 24 hours, and the surplus fixative solution was then removed from the samples by washing them with tap water for 30 minutes, and then a series was conducted on them , of the operations based on the method
[13] which are 1- Indentation and dredging 2- Impregnation 3- Burying 4- Slicing 5- Staining and loading [6 - Microscopic examination of tissue sections.

Results and Discussion :-

It was found through the results that indicated a noticeable rise at the level ($P < 0.05$) in the effect of chitosan on PCV Hb ratio in rabbits, as the effect ratio was 12.7 and 41.0, respectively, compared to the control group, while the effect of C. krusei yeast and C. krusei + Nystatin was shown in comparison. The control demonstrated a significant decrease at the level ($P < 0.05$), where the effect ratio was
12.7 and with a total of 41.0, respectively.

It showed, through the results, a significant increase at the level ($P < 0.05$) in W.B.C in all groups compared to the control group, where the highest percentage of increase was in group C. Krusei + Nystatin, followed by C. krusei + chitiosan group, which reached 9.0 and 7.40 (×103/L) respectively. As shown in Table and figure No. (1)

Table (1): Effects of chitosan treatment on Hb, PCV and W.B.C of Rabbits.

Parameters / Groups	Hb (g/dl)	PCV (%)	W.B.C (× 10^3/µL)
Chitosan	12.70±0.16 a	41.0±1.00 a	5.00±1.77 c
***C. krusei*+Nystatin**	10.90±0.18 c	36.0±1.02 c	9.00±1.14 a
C. krusei	10.90±0.14 c	36.0±1.11 c	5.40±1.27 c
***C. krusei*+Chitosan**	11.50±0.11 b	38.0±0.90 b	7.40±1.51 b
Control	11.51±0.12 b	38.0±1.21 b	3.90±1.30 d

The values represent mean ±S.E

- Different of letters vertically mean significant difference at the level of significance ($P<0.05$).

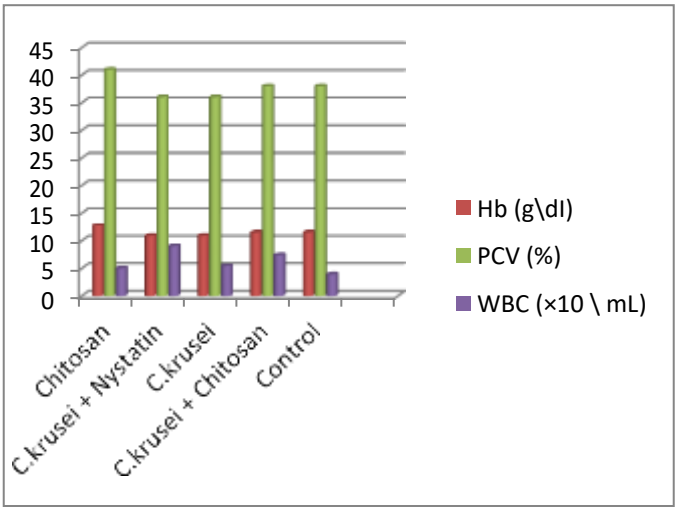


Figure (1): Effects of chitosan treatment on Hb, PCV and W.B.C of Rabbits.

White blood cells are a defensive barrier and a dam against the attacks that attack the animal by microorganisms such as

bacteria, fungi and viruses. White blood cells are classified according to the shapes of the nucleus and pigment, and each type has a specific function in the immunological system of the body. The number of white blood cells in rabbits ranges from 5-12.5 x 103 / mm the proportion of lymphocytes is 30-85 %, mononuclear cells 4-1, neutrophils 20-75 %, eosinophil's 5 %, and basophils 0 - 8 %. [14], Chitosan is a stimulant substance for the work of the immune system attached to the gastrointestinal tract of mammals through its binding to the receptor (Phagocytosed

(S) and affects the host defense systems by several mechanisms, including the accumulation and activation of macrophages and the activation of natural killer cells in mice lymphocytes and phagocytes [15] .The macrophage has special receptors for the side branches of a number of complex sugars, and it has special receptors for the side branches of a number of complex sugars, especially for beta-glucans and chitin. Stimulates the formation of T- lymphocytes, B-cells, NK-cells, and cytokines, in addition increases the formation of immunoglobulins (lgs), especially IgA. In some studies, chitosan can be used as an alternative to antibiotics in farm animals .[16]

(T) Table (2) Effects of chitosan treatment on electrolyte tests of Rabbits

Parameters / Groups	K^+ (mmol/L)	Na^+ (mmol/L)	Ca^+ (mg/dl)	Mg^+ (mg/dl)
Chitosan	4.80±1.26 a	155.0± 3.11 e	9.50±0.18 a	2.00±0.17 a
***C. krusei*+Nystatin**	3.70±1.16 b	217± 1.15 b	7.00±0.19 c	2.10±0.89 a
C. krusei	3.40± 1.00 c	240.0± 1.21 a	8.10±0.67 b	1.90±0.84 a
***C. krusei*+Chitosan**	4.00± 1.08 b	200.0± 2.13 c	9.20±0.82 a	2.03±0.91 a
Control	4.60±0.90 a	190.0± 2.10 d	9.00±0.70 ab	1.80±0.81 a

-The values represent mean ±S.E

Different of letters vertically mean significant difference at the level of significance (P<0.05).

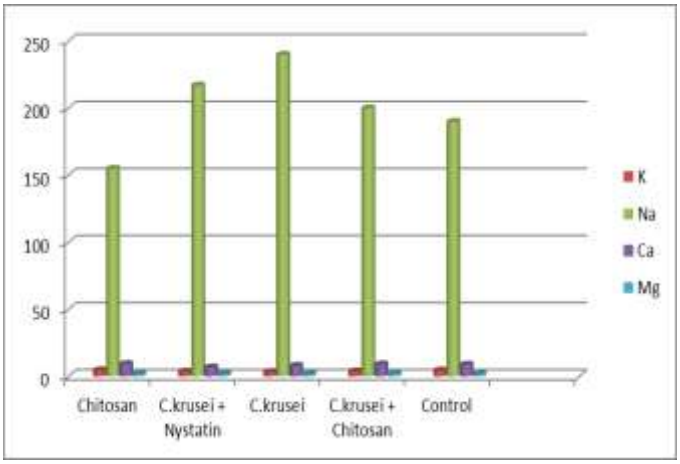


Figure (2) Effects of chitosan treatment on electrolyte tests of Rabbits

It was found through the results, as shown in Table and Figure No. (2), that it was a significant decrease in the level (P < 0.05) in the concentration of K+ in serum in both C. krusei group, C. krusei + Chitosan group, and C. krusei + Nystatin group. Compared to the control group, the effect ratio was 3.4, 4.00, and 3.7 mmol/L, respectively.

The findings revealed a substantial rise in concentration at the level (P 0.05). of Na+ in C. knusei group, C. kusei + Chitosan group, and C. krusei + Nystatin group compared with the control group, and a significant decrease in the Chitosan group compared to the control group. While the results showed a significant decrease at the level (P < 0.05) in the Ca+ concentration in the C. kusei + Nystatin group compared to the control group, while the Mg+ concentration did not show any significant differences in the blood serum compared to the control group.

Chitosan has the ability to dissolve in water, which facilitates its absorption through the intestines and its entry into the bloodstream. It has multiple vital activities. It is an anti-fungal that has the effect of improving immunity by increasing the weight of organs, immune cells and cytokines. .[17] [18 .]As for nystatin, it is also considered an antifungal, as it belongs to the polyene group. Numerous fungi and yeasts, including candida, can be cured by it. This compound shows toxic effects when administered when it is effectively absorbed by intact skin or through mucous membranes.[19] It is also considered one of the safe drugs when treating oral and capacitive fungal infections, as is the case in the rest of the antifungals belonging to the polyene group.[20]

Nystatin binds to ergosterol, cytoplasmic membrane's primary constituent in the fungus. It also leads, when available in appropriate concentrations, to creating a membrane with perforations, which leads to potassium leakage out of the cell and thus cell death.[21]

Fungi release toxins in the body (acetaldehyde compounds). It is one of the carcinogenic compounds and is responsible for the following symptoms: nausea, flatulence, gas, headache, fatigue and liver damage .[22]

Candida also withdraw magnesium from the body, which leads to constipation. When the percentage of magnesium in the body decreases, the nerves of the colon and muscles become tense and complicated. Colon tissue begins to swell and swell, making constipation worse. Gases accumulate and may lead to colon blockage .[23]

The most common cause of a significant loss of potassium is often associated with a massive loss of fluid from the body. This is usually the result of diarrhea, excessive sweating, or losses related to muscle crush injuries.[21] Vomiting may also cause hypokalemia, although a person does not lose a large amount of potassium in the vomiting itself, but loses large amounts of potassium through the urinary tract due to excessive bicarbonate. Urinary diabetic ketoacidosis is a unique instance of potassium depletion brought on by vomiting.[24] And low potassium levels in both the overall body and the cells are indicative of hypokalemia in this situation. In addition to the urinary loss due to polyuria and volume contraction, potassium is also lost through the renal tubules as the positively charged's companion writer, beta- hydroxybutyrate.[25]

Hypokalemia may result from low blood magnesium levels. Magnesium is important for potassium processing. This may become apparent when hypokalemia persists despite taking potassium supplements. Various electrolytes disturbances may also be present.[26]

An increase in blood pH may lead to temporary hypokalemia by one of the following two mechanisms. First, Potassium is first moved into cells through alkalosis from the plasma and interstitial fluid, possibly by stimulating the Na+ -H+ exchanger that activates the sodium-potassium pump.[27]

Histological sections prepared from the intestines of white mice dosed with saline showed the normal shape of all layers of the intestinal tissue (mucosal layer, submucosal layer, muscle layer and serous layer) in figure No. (3)

The results of microscopic examination in the four groups also showed the appearance of necrosis and lysis in the cells lining the villi, fluttering and blood congestion with the proliferation of inflammatory cells in abundance in addition to the occurrence of hyperplasia in the mucous layer of the intestine as shown in figures (4,5,6,7) compared to the control group.

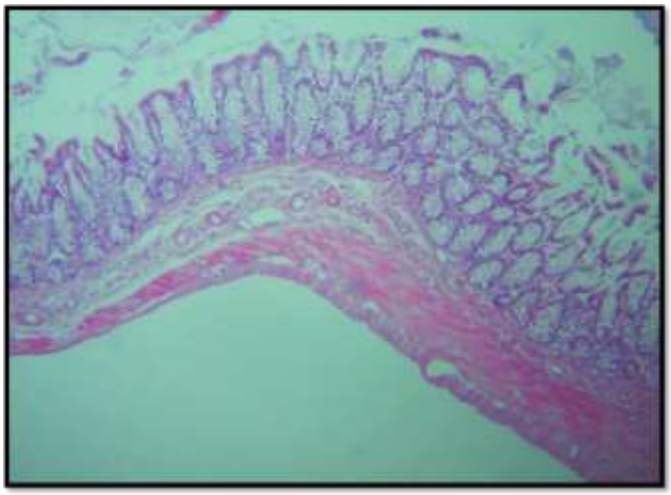


Fig (3): Histological section in the intestine of control group showed four layers: Mus) Mucosa, SMus) submucosa, Mu) Muscularis and S) Serosa (H&E; 10x).

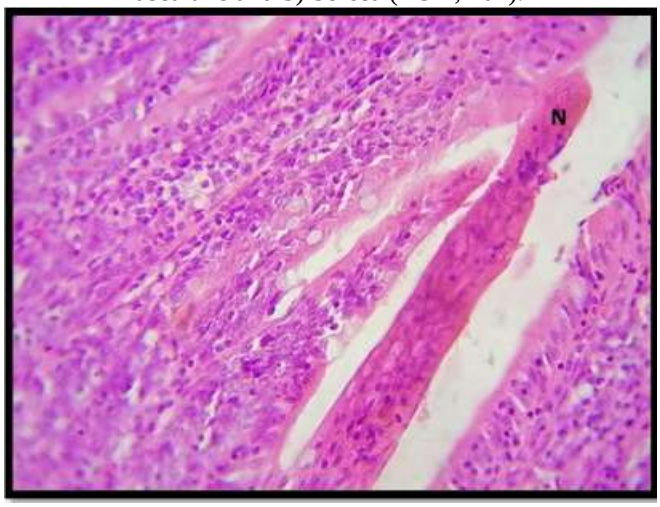
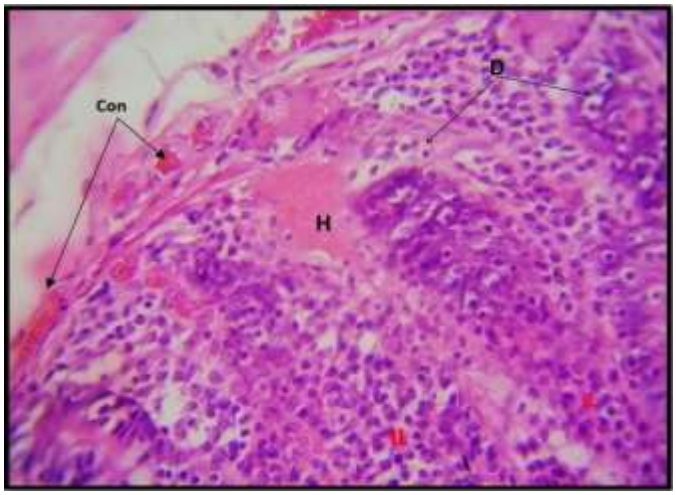
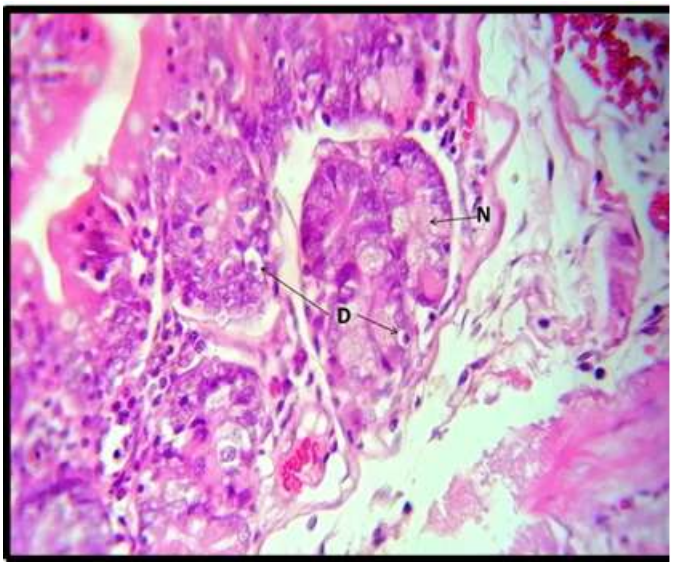


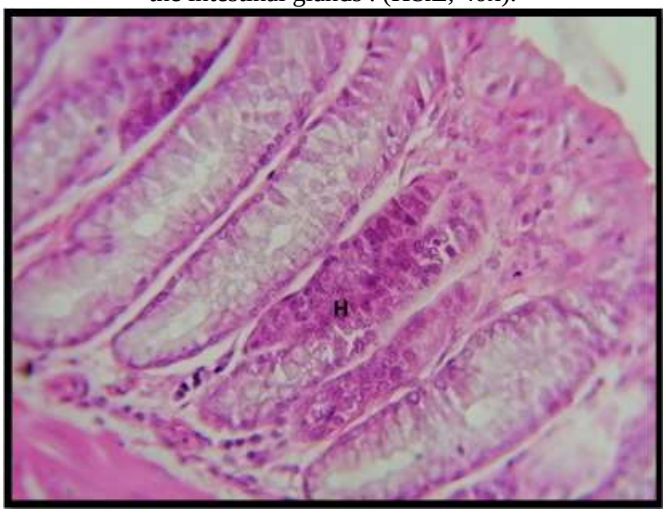
Fig (4): Histological section in the intestine treated with chitosan showed : N)necrosis of the villi top in several area. (H&E; 40x).



Fig(5): Histological section in the intestine treated with C.krusei+ Nystatin showed : H)hemolysis between the crypts with D)presence of degenerated cells, IL) Infiltration of lymphocytes and Con) Congestion .(H&E; 40x).



Fig(6): Histological section in the intestine treated with c.krusei showed : N)necrotic, He) Hemorrhage and D)degenerated cells in the intestinal glands . (H&E; 40x).



Fig(7): Histological section in the intestine treated with c.krusei + chitosan) showed : H)hyperplasia of the intestinal cells .(H&E; 40x).

Intestinal fungi help digest sugars and starches when they are within normal rates, so digestive problems arise when their balance is disturbed or abnormally increased, and these problems include: constipation, bloating, diarrhea, nausea or colic, and can also develop to cause Crown's disease or Ulcers.[28]

Fatigue is the result of the multiplication of these fungi associated with a deficiency of some important necessary likes fatty acids, vitamin B6, magnesium, and other vitamins and minerals [29] [30] .

Conclusions :-

- We conclude through the study that there is a significant effect of C. krusei on blood parameters that reduce the proportion of Hb, PCV, and Wbc, and that chitosan improves the effect of C. krusei, which leads to an increase in Hb, PCV
- C. krusei affects blood electrolytes by reducing K, Ca, and chitosan improves Ca, k concentration.
- C. krusei negatively affects the small intestine and treatment with chitosan improves the effect of C. krusei.

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