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RESEARCH ARTICLE

Study of the antifungal (*Candida albicans*) activity of olive leaves extracted in the intestinal of mice.

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Abstract

Twenty mice were used in study divided into two groups, the first group (control) include mice , while the second 10mice and the group was infected by a fungus Candidaalbicanis then treated extract olive leaf Extracts were prepared from dried and powdered leaves with solvents (ethanol) treated extract olive leaf(0.5mg\ml)for a month (0.5ml) a day for a month to observe effects therapeutic extract in organs (small and large intestine), has been observed histological changes when fungal invasion and after treatment compared with the control group, where it was noted therapeutic response of structures of the gut.

Introduction:-

Olive leaf is the leaf of the olive tree (Oleaeuropaea) have a rich medicinal uses(1) .There are many references citing the medicinal use of the plant (Oleaeuropaea) in ancient times Effects of olive leaves like the antioxidant, hypoglycemic, antihypertensive, antimicrobial, and antiatherosclerotic have been reported in various studies(2).

Oleaeuropaea L. leaves contain number of phenolic compounds that give unique properties to the extracts obtained from it. The most important are natural occurring glycoside oleuropein and its degradation product hydroxytyrosol, which is obtained by chemical hydrolysis. They both have bitter taste and many health or medical benefits(3)Oleuropein have antimicrobial (Bacillus subtilis, B. cereus,taphylococcusaureus Salmonella typhi, Vibrio cholerae,.), anti-protozoal and antiviral activity. Oleuropein acts through elenolic acid, a hydrolysis products. The olive leaf extract is proved to have anti-fungal properties. It is especially useful in cases of candida overgrowth, also known as a yeast infection. This fungal excess may cause a variety of symptoms, including digestive upset, fatigue, and respiratory concerns(4). C . albicans is a harmless commensal yeast-like fungus in healthy humans, which can cause superficial as well as life threatening systemic infections under immune compromised, situations. C .albicans can colonize or infect virtually all body sites because of its high adaptability to different host niches by the activation of appropriate sets of genes in response to complex environmental signals(5)Candida Albicans Possibly the most problematic and widespread fungal infection afflicting the western world, the family of Candida yeasts has developed a high resistance to most antifungal drugs and is commonly contracted in hospitals while patients undergo other surgical procedures.(1)The natural antifungal olive leaf on the other hand, has the potential to destroy many kinds of fungus, or their sub-division of yeasts such as Candida Albicans.(6)

Aims:-

The present study was aimed to evaluate the effects of olive leaves extracts antifungals

Materials and methods:-

Animales:-

Twenty mice weighing between(24–31 g) grams were used in this study. the animals were maintained and acclimatized in the college of veterinary medicine –Tikrit university under laboratory conditions in group cages The experimental animals were housed in standard plastic cages and maintained under controlled laboratory conditions of humidity (65%), temperature (25 ± 1°C), and 12 : 12 h light: dark cycle, with balanced food and water ad libitum. The mice were allocated randomly into two groups 10 each; group(A) was kept as control, group(B) was infected of fungus(candid albicanes)af and then supplemented with (0.2ml\ body weight) olive leaf extract. The extract doses were given individually as an oral daily dose. Treatment was last for thirty days.

Preparation of Olive Leaf Extract:-

Olive leaves used in this study were collected from farmer in Tikrit city . They were collected in winter (January) and properly prepared for drying process in the day they were collected. Leaves were washed to remove impurities such as dust and then dried in an air oven for 3 days at 380C. A standardized solvent extraction protocol was used for the plant material. The air dried plant materials were ground in a blender with a particular size to ensure the plant powders in identical size. 10 g of each plant powder was extracted for 2 hrs with 200 ml of 70% (v/v) aqueous ethanol at 38 ºC by a thermo-shaker which is fixed to 180 rpm. Then the samples were centrifuged at 5000 rpmfor 15 minutes and the supernated parts of the samples were carried to a rotary evaporator to remove ethanol under reduced pressure at 38 ºC, 120 rpm. The remaining aqueous solutions were lyophilized at -50 ºC, 0.028 mbar and the percent (w/w) extraction yields of plant materials were calculated. The crude extracts were kept in refrigerator in glass bottles until the further experiments.High performance liquid hromatography (HPLC) (Shimadzu orporation, Kyoto, Japan) was used to detect theactive compound of the extract.(7)

Samples:-

Isolated from laboratory of veterinary medicine collage of Tikrit university.

Histology:-

The animals were killed at the day after the last dose under intensive dose of chloroform. Large & small intestinal of the animals were rapidly removed and micro dissected to obtain tissue samples for histological examination. Blocks of tissues were immediately fixed in 10% neutral buffered formalin, dehydrated with graded series of ethyl alcohol and embedded in paraffin. Sections of 5 microns were cut and stained with eosin and hemotoxylin according to(8). Photomicrographs of the slides were taken using digital camera attached to light microscope. The whole photomicrographs were compared with those of parts of large & small intestinal of control group (A).

Results and Discussion:-

Control:-

Colon:-

Associated with sloughing of other cell towered the lumen of intestine. The goblet cell were a abundant and are located in between epithelial and intestinal gland ,usually surrounded by congested blood vessel and solitary lymphocyte infiltration (FIG1-A) The colonic folds of the mucosa were very short and mostly have an degenerated epithelial cell its surface C.(FIG1-B).

Jejunum

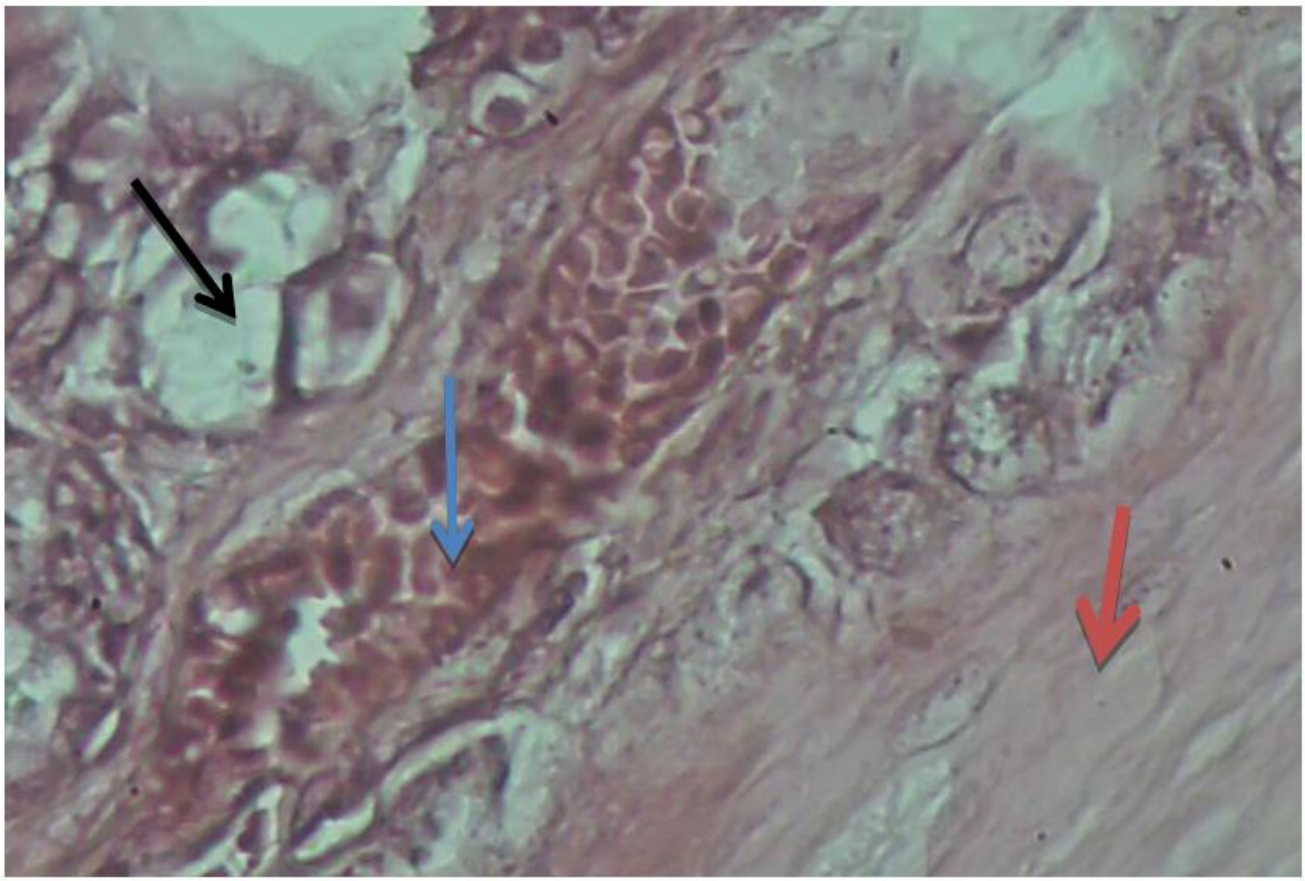
The intestinal villi were present of high level and finger like covered with simple columnar epithelium with a great number of lymphpocytes (FIG2-A) and the cell of c.t. the intestinal gland of mucus type were extended to the bases of the villi and other region of the lamina propria in between there were infiltration of lymphocyte and the blood vessel of the lamina propria and the submucousa were congested with RBC.(FIG-1-B).

Dodenum

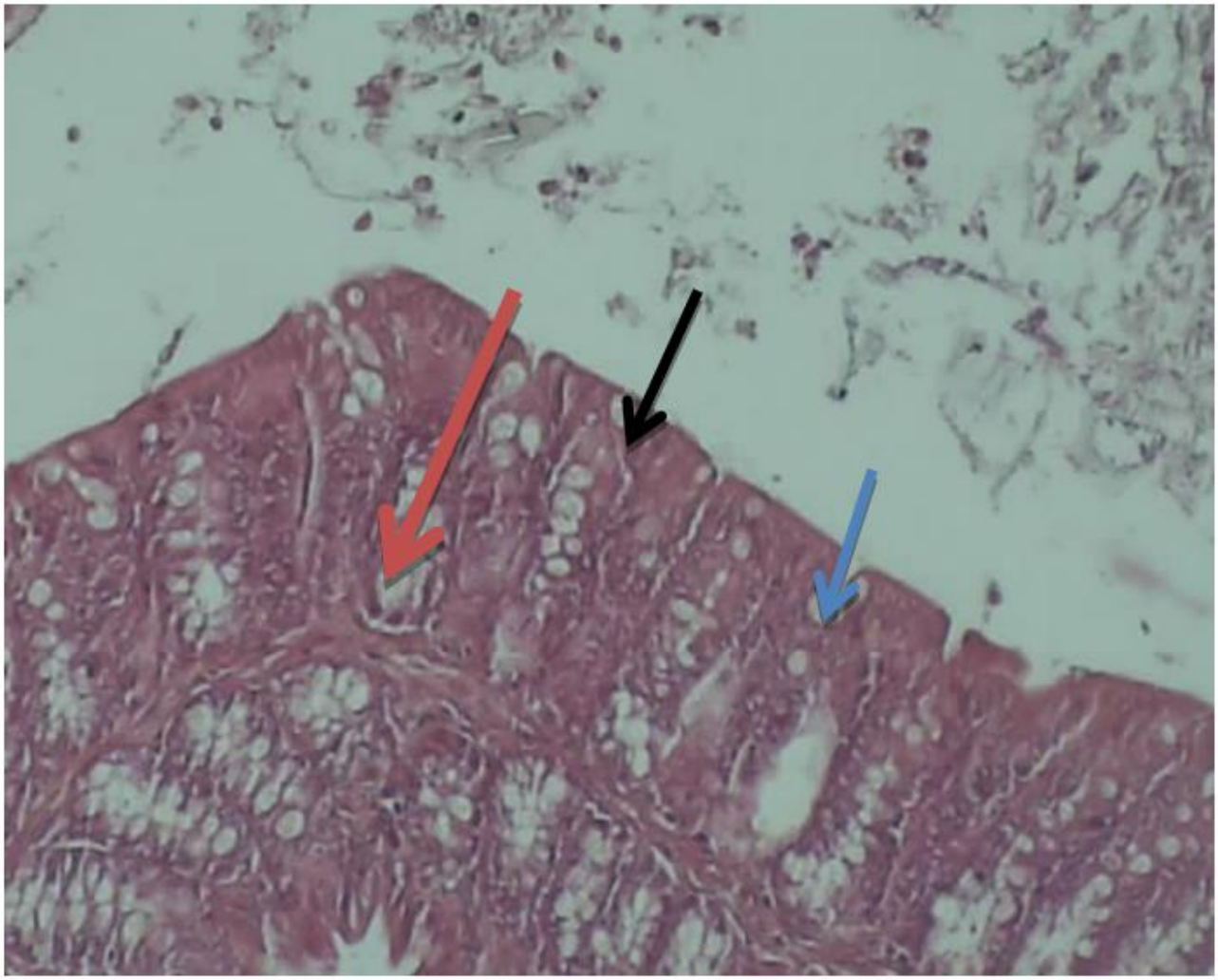
The mucosa of duodenum were formed mainly by the villi which lined by the simple columnar epithelum with goblet cell (FIG3-A).the intestinal gland were extended deeply in the laminapropria and extended even to the submucosa ,these gland were containing the goblet cells and its secretion in the lumen of these gland. There were nodular infiltration of the lymphocytes in between the intestinal gland (FIG3-B) . the blood vessel with RBC were easily demonstrated in laminapropria and mucosa .

Cecum:-

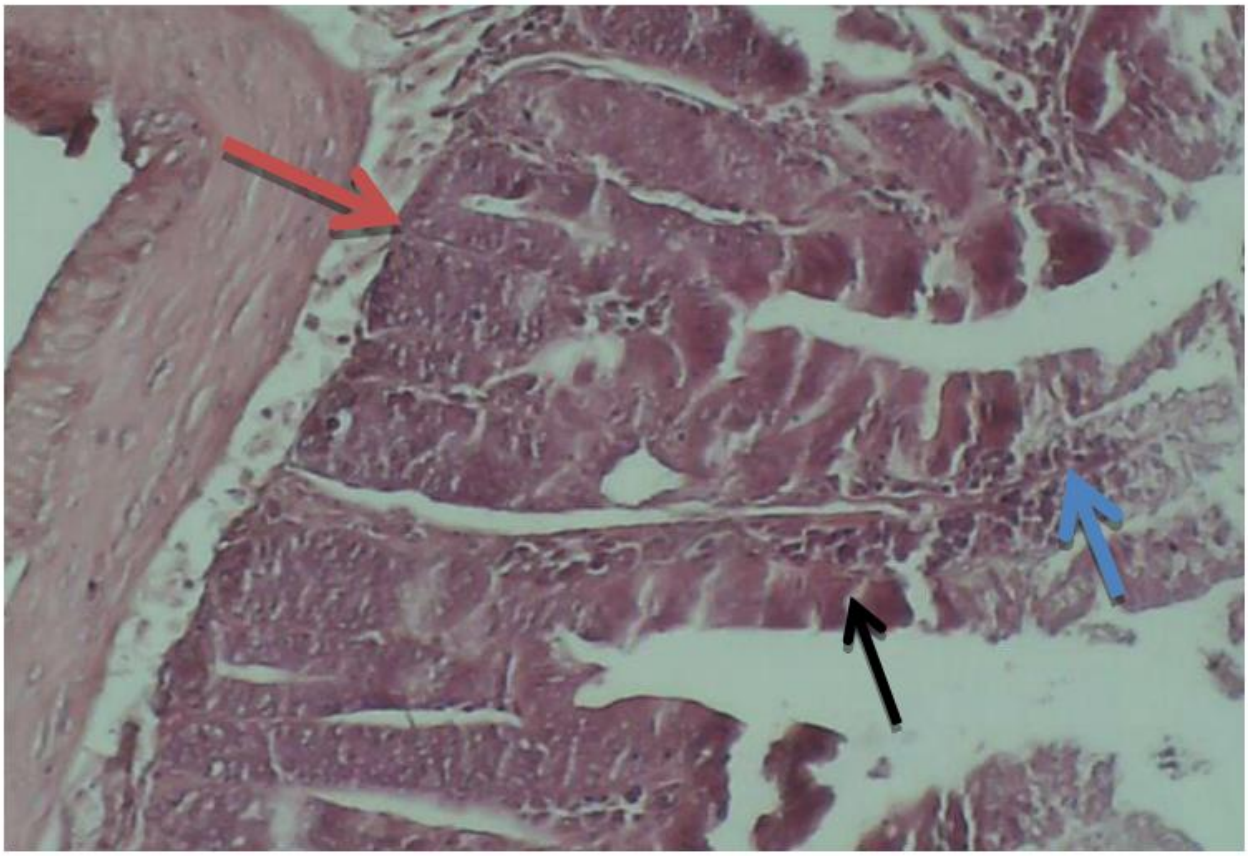
The innermost layer , the mucosa is made of smooth mucous membrane with many goblet cells . surrounding the mucosa is the submucosa layer that contains the blood vessels and nerves that support the surrounding tissue .



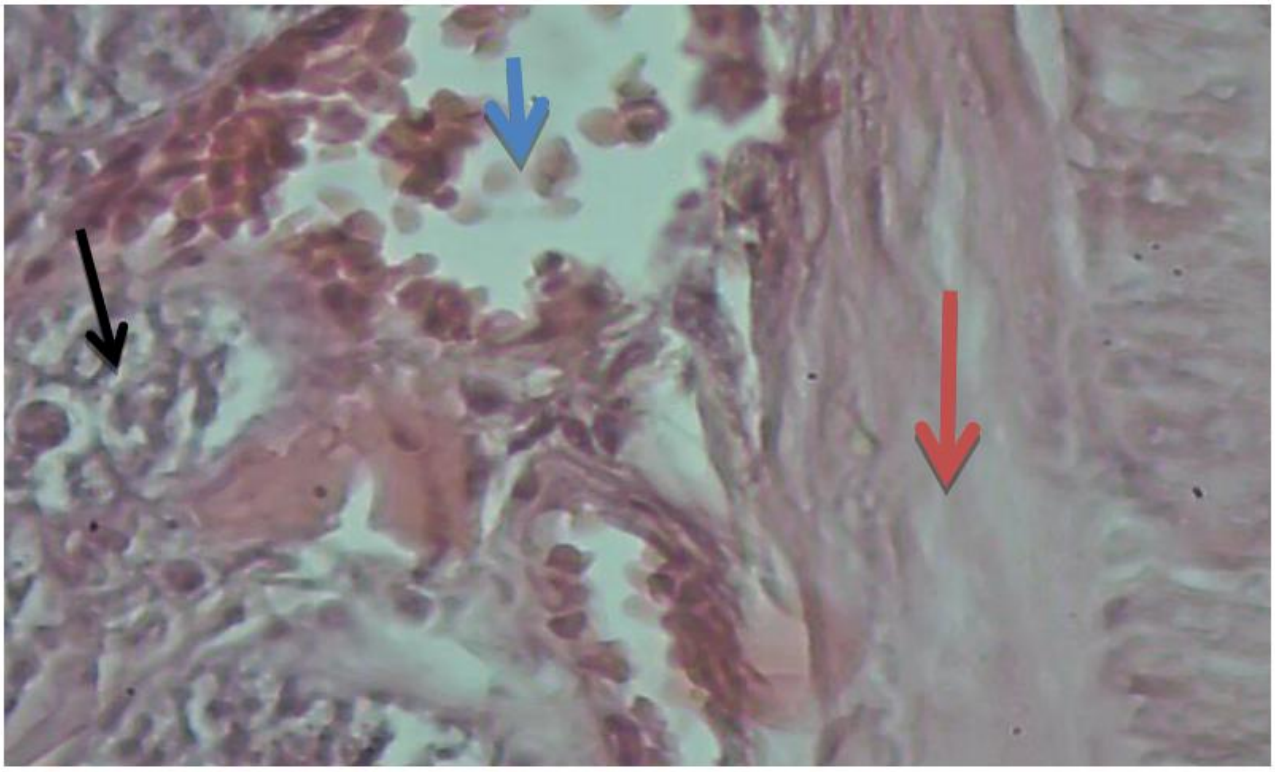
(fig 1) A- COLON CONTROL
Black arrow -intestinal gland of colon
Blue arrow -congested blood vessels of submucosa
Red arrow -muscular layer
(H&E X40)



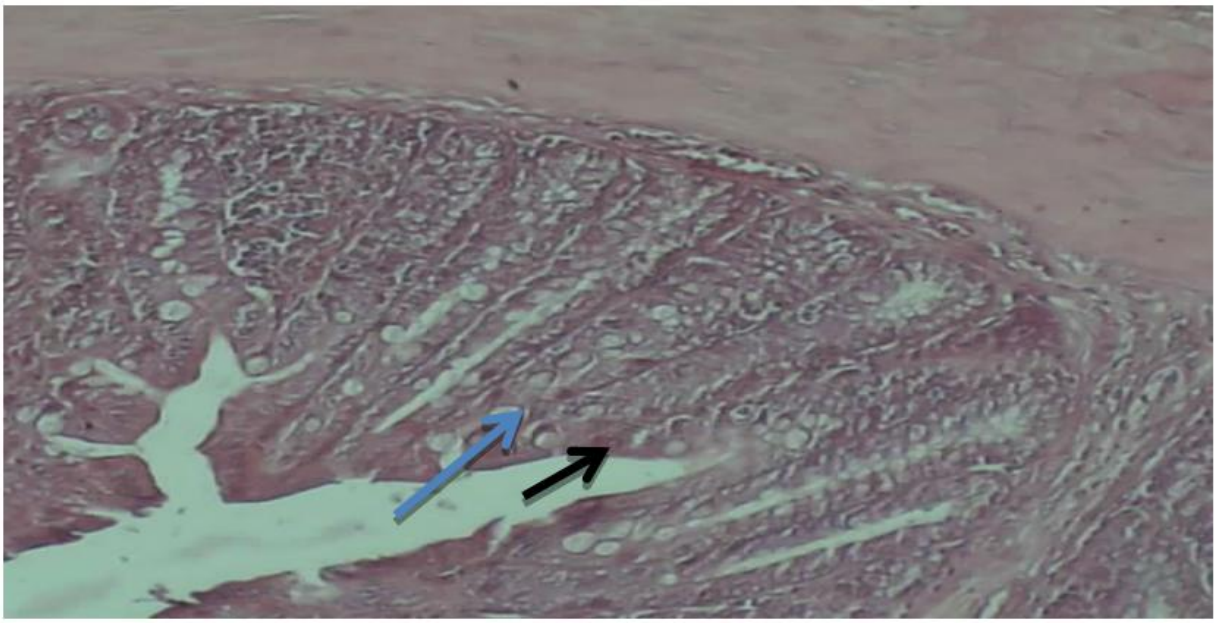
(fig1)B- colon control.
Black arrow - instinal fold colon.
Blue arrow-goblet cell.
-red arrow -intestinal gland.(H&EX40).



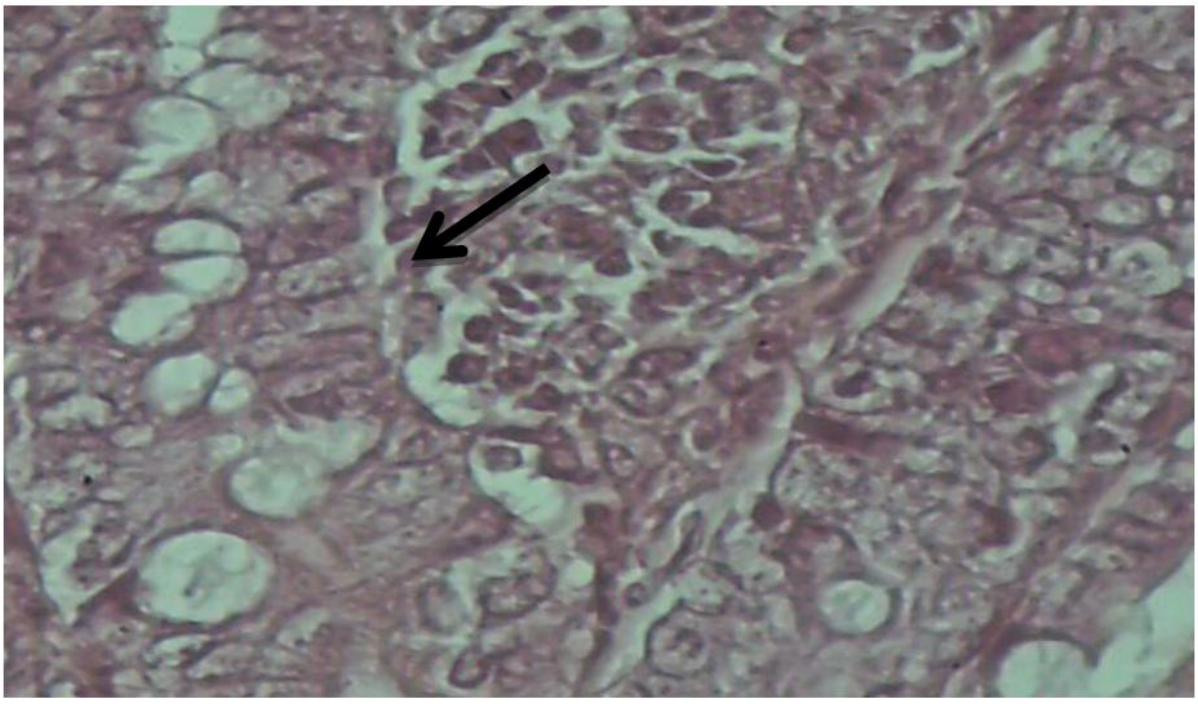
(FIG2)A-jeujenum control .
Black arrow –intestinal villi of jeujenum.
Blue arrow –core of villi with lymphocyte aggregation.
Red arrow-submucosa (H&EX20).



(FIG2)B-
Black arrow-intstinal gland of lamina propria of jeujenum.
Blue arrow-submucus blood vessel.
Red arrow-muscular coat (H&EX40).



(FIG3)A--Duodenum control.
Black arrow-duodenum villi lined with simple columnar epithelium.
Blue arrow-goblet gland (H&EX20) .



(FIG3)B-Duodenum control Nodular lymphocyte aggregation of the lamina propria of the duodenum (H&EX40)

Infected:-

Cecum:-

The mucosal fold of the cecum were contenting simple epithelium of columnar cells certain number of these cells were degeneration and desquamated from the surface (FIG4A)the mucus gland in the lamina propria containing degeneration mucus cells and mucussecretion in lumen of gland (FIG4B).

Jeujenum

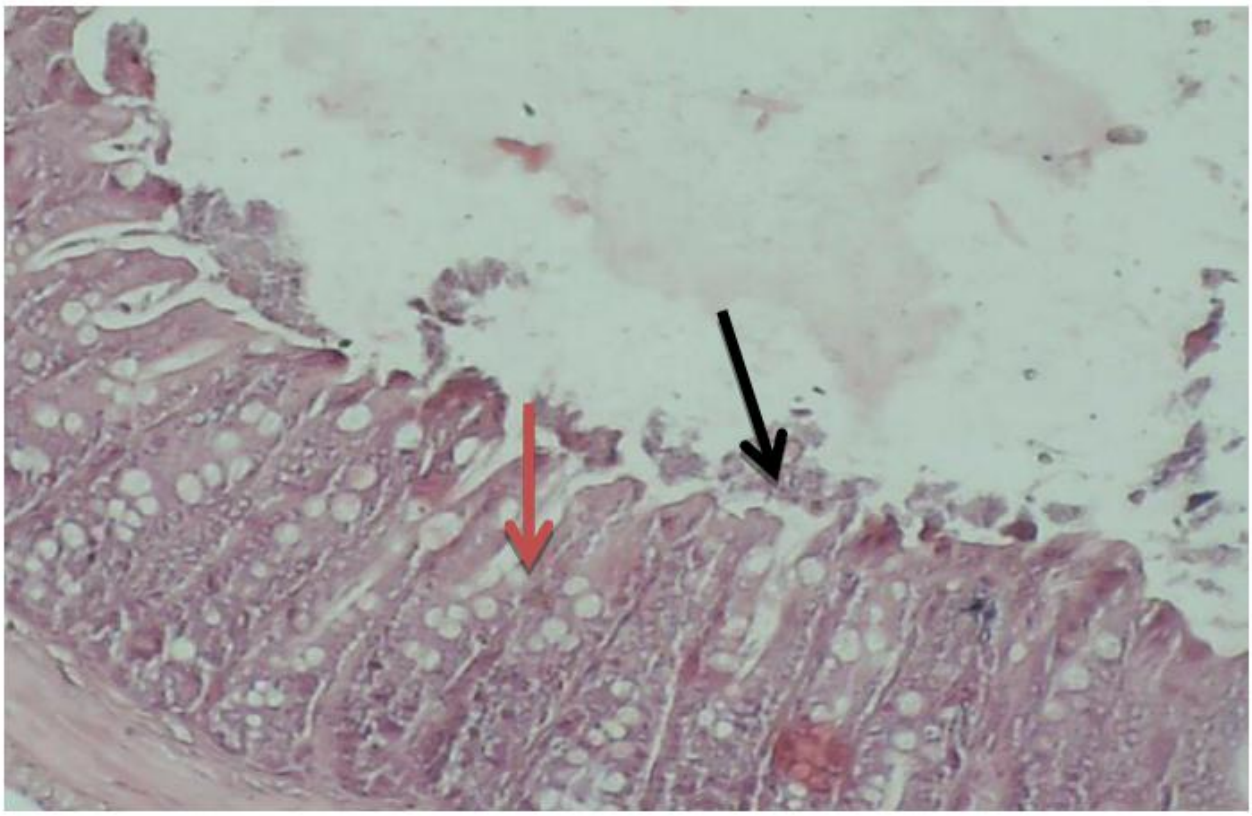
The intestinal villi of jejunum were degeneration its epithelial cells and most the cells and the C.T. of the villi were sloughing in the lumen of intestinal (FIG5A). The mucus cells of the intestinal gland were broken down into a small fragment (FIG5B).the core of certain villi were infiltration with lymphocytes there were congested of the blood vessels. Of the mucosal layer in between intestinal gland.

Deudenum

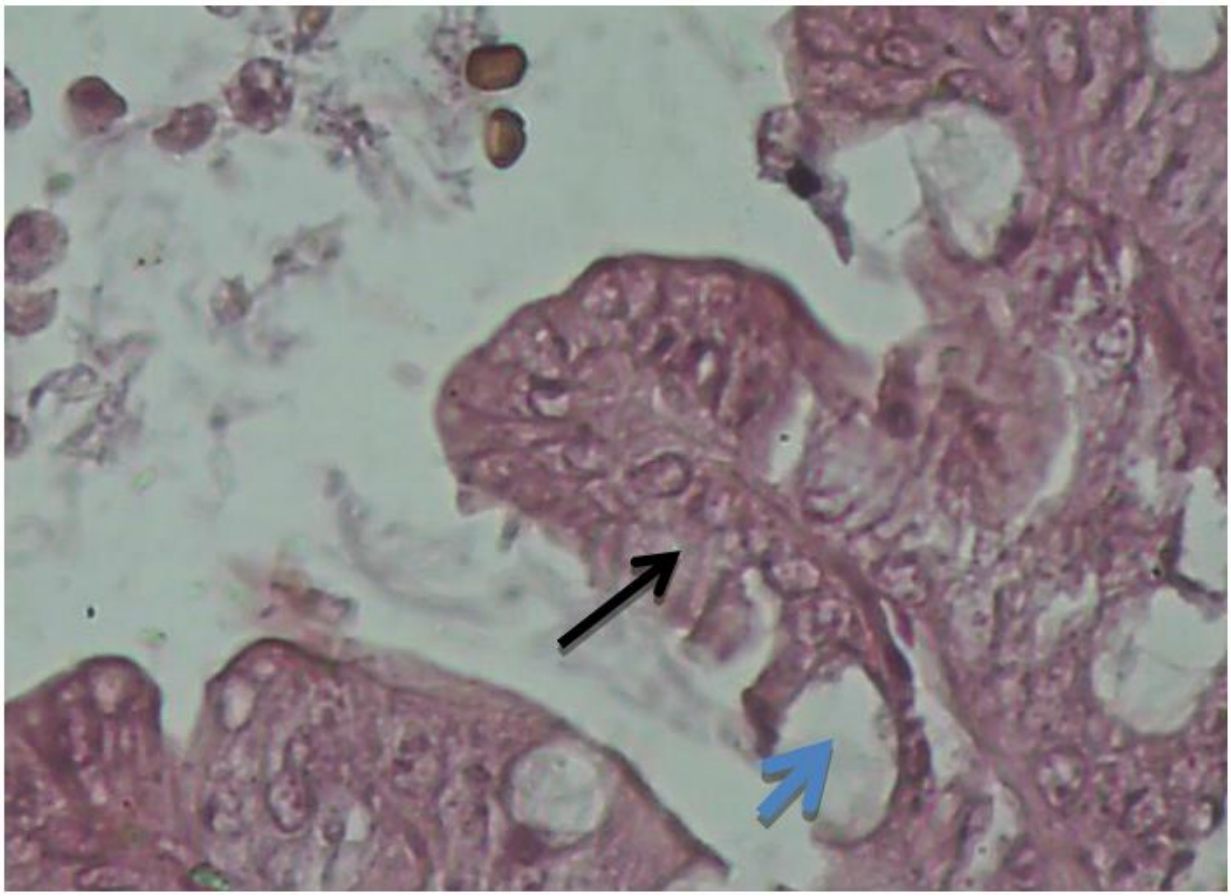
Degenerative changes were demonstrated in the most of the epithelial cells of the surface of the villi,also there were an vacuolation the cytoplasm of these cells and nuclei ,were disappeared (FIG6B).the mucus glands were mostly lost its mucus cells, lymphocyte infiltration in between intestinal gland and the blood vessels in the submucos were enlarged with blood (FIG6A).

Colon

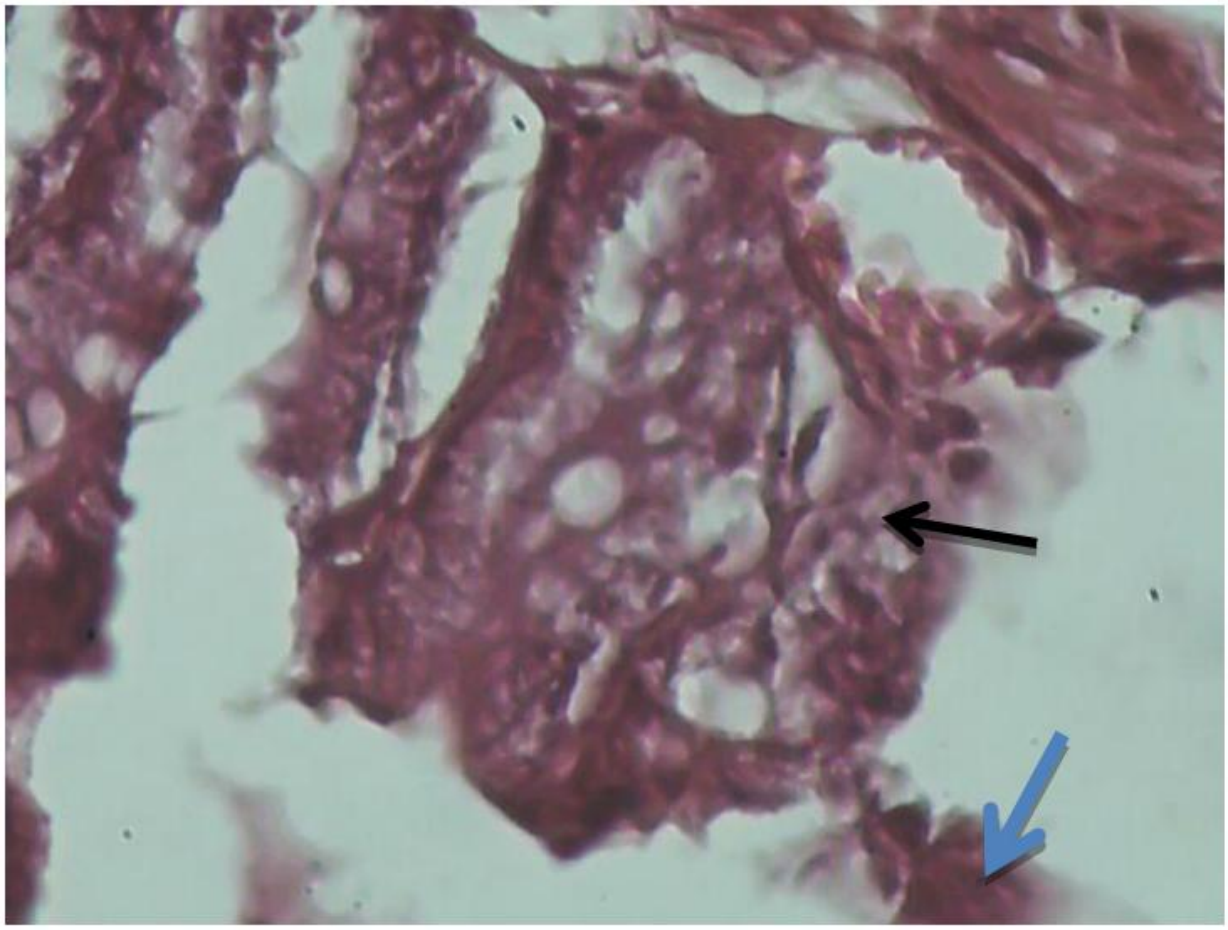
The colonic fold of the mucosa were very short and mostly have an degenerated epithelial cells on its surface (FIG 7-A).associated with sloughing of other toward the lumen of intestinal .the goblet cells were a abundant and are located in between epithelium cells and intestinal gland ,usually surrounded by congested blood vessels and solitary lymphocytes infiltration (FIG7B).



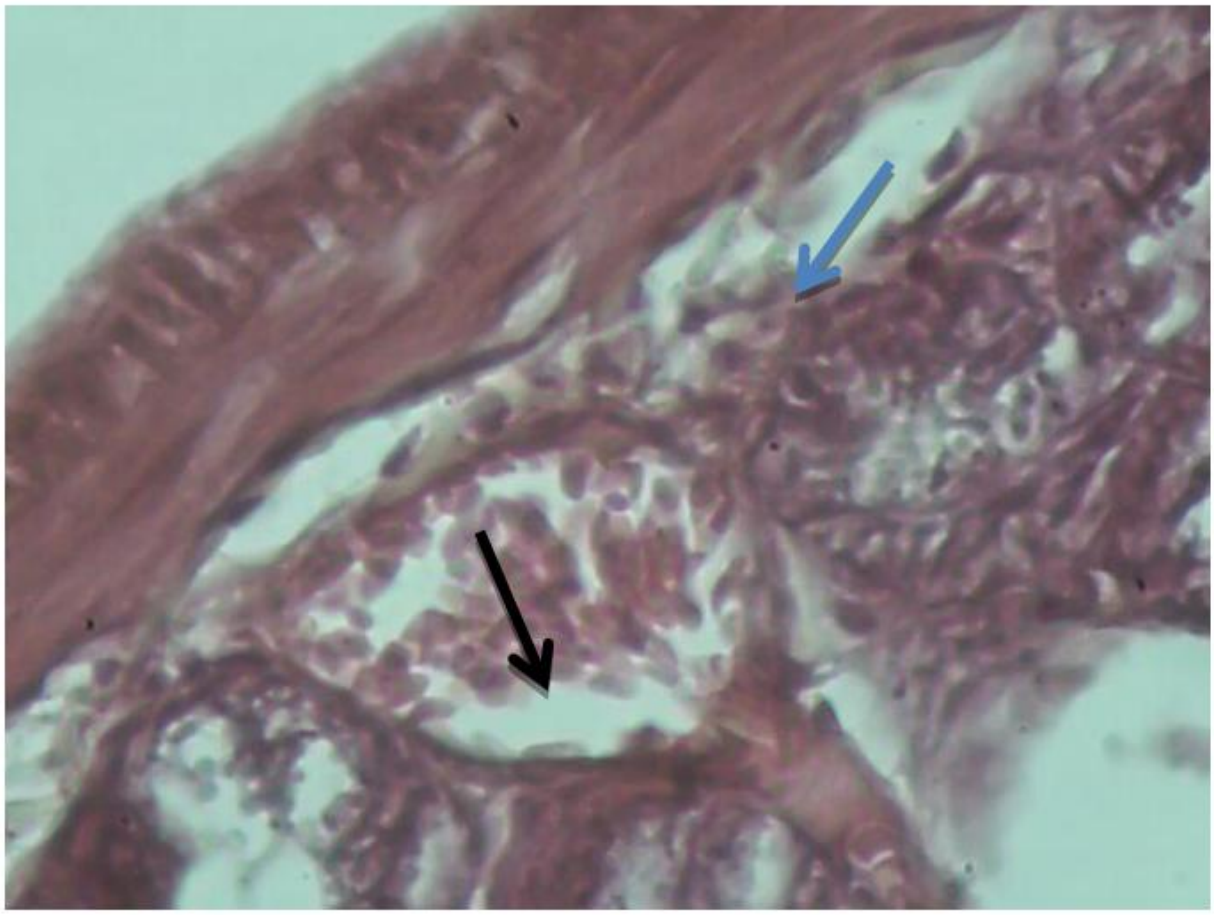
(FIG4)A-cecum infected by fungus:-black arraw-desquamated epithelium cells of the cecalfold&degeneration epithelium cells.
Red arrow-mucus cells of cecum intestinal gland.(H&EX20) .



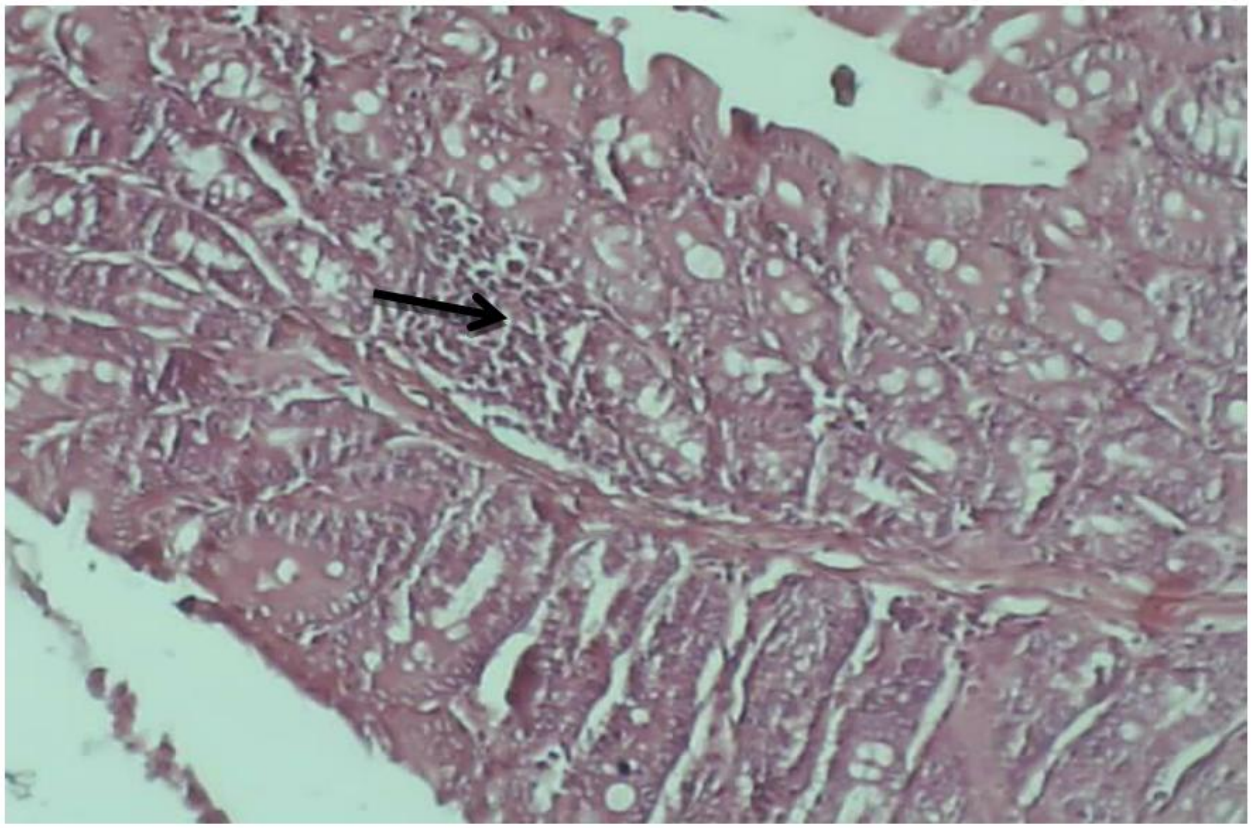
(FIG 4)B-Cecum infected by fungus:-Black arrow -degeneration epithelium cells of cecal.
Blue arrow-degeneration ofgoblet cells(H&E X40).



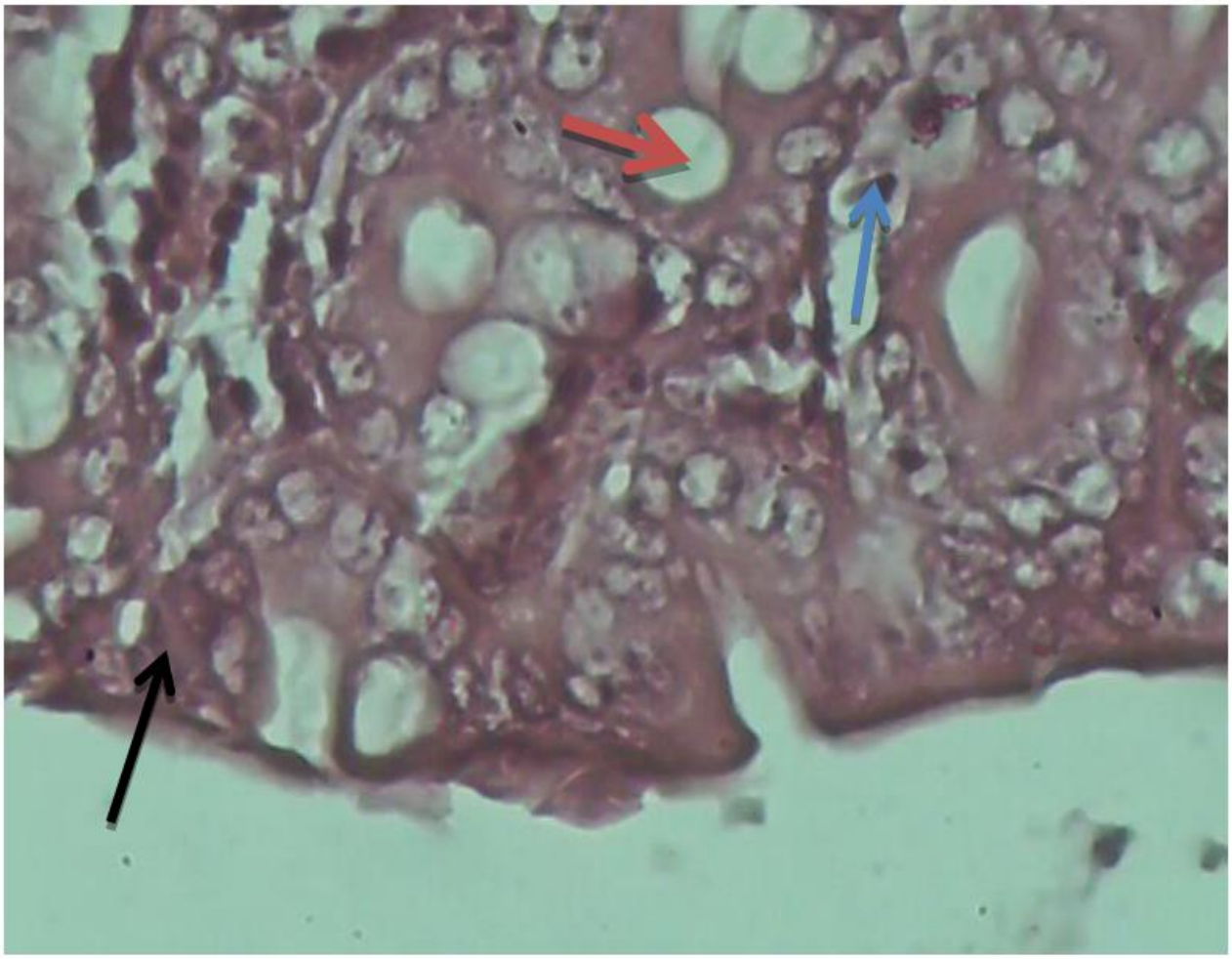
(FIG5)A:-Jeujenuminfected by fungus:black arrow-degeneration epithelium cells jejunum villi
Blue arrow-sloughed epithelium cells .



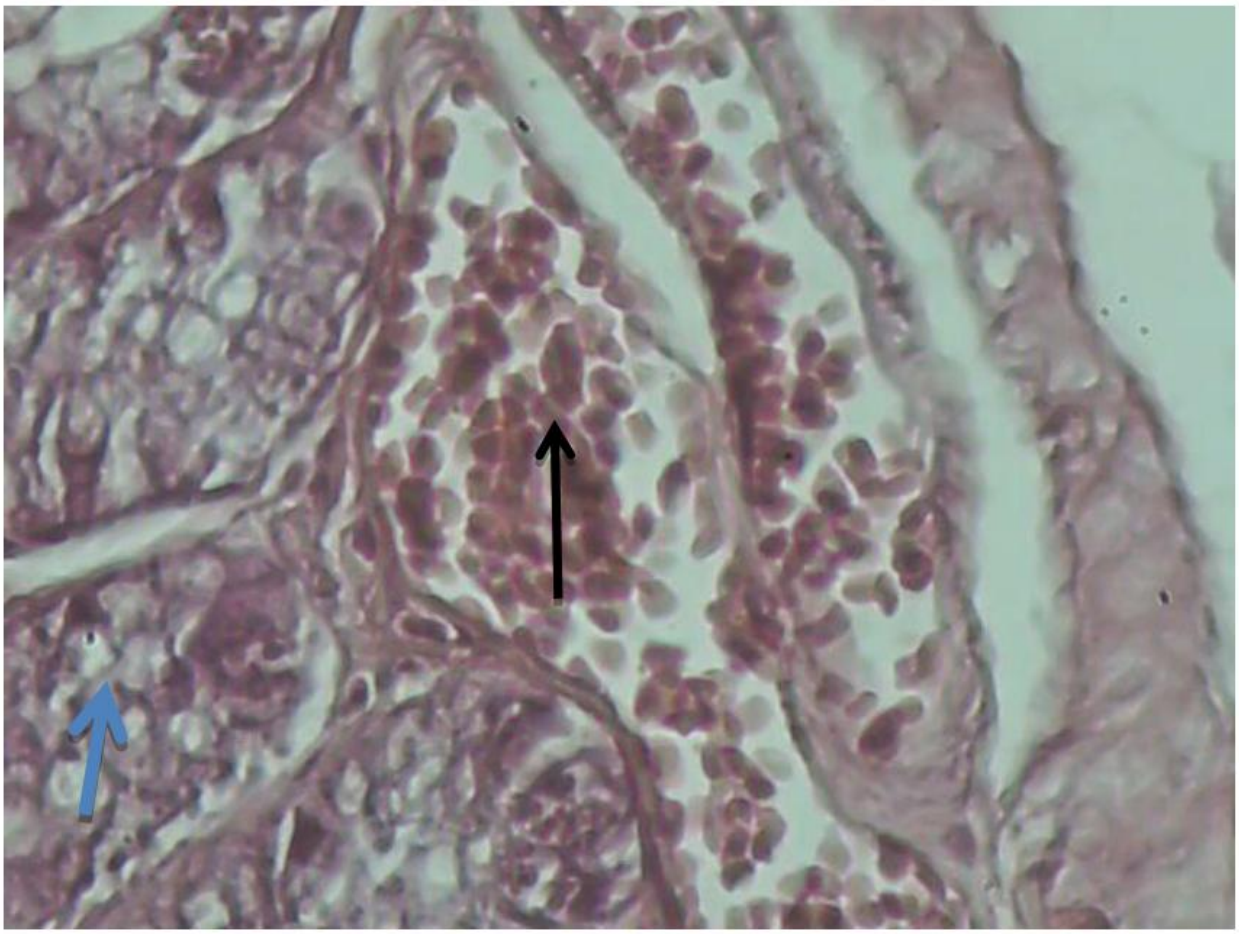
(FIG5)B-jejunum infected by fungus:-black arrow-congested blood vessel of the submucus of the jejunum
Blue arrow:-lymphocyte aggregation .



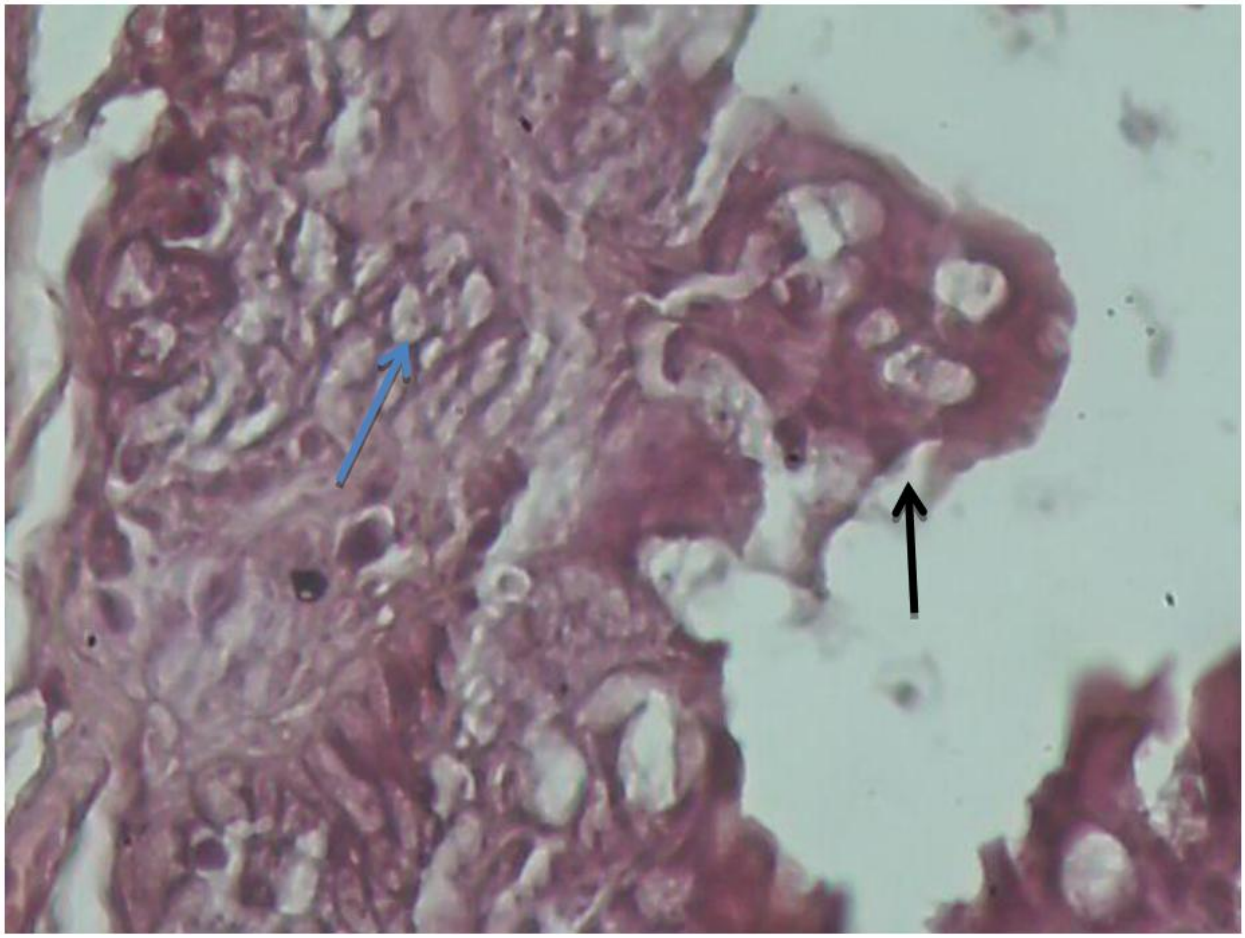
(FIG6)A-duodenum infected by fungus :-black arrow:-lymphocyticaggregation of the lamina propria of duodenum (H&EX20).



(FIG6-B)duodenum infected by fungus :-black arrow:-degeneration epithelium cells of the villi of the duodenum
Blue arrow:-irregular nuclei of epithelial cells.
Red arrow-hyperatrophy of goblet cells.



(FIG7–A)colon infected by fungus –black arrow-congested blood vessel of the submucus of colon.
Blue arrow:-intestinal gland (H&EX20).



"(FIG7 -B)colon infected by fungus –black arrow:-degeneration epithelium cells of the mucus of the colon
Blue arrow:-intestinal gland (H&EX40).

Treatment

Cecum

Degenerative process of the epithelial cells of the colonic fold of mucosa were demonstrated (FIG8-A) but still there were caration in the cytoplasm,the lamina propria and submucosa were containing longest blood vessel and the muscular coat of the colon appeared (FIG8-B).

Dodenum

Intestinal villi were prominent and cover by simple columnar epithelium with goblet cells (FIG 9A). The lamina propria was engorged with a great number of intestinal gland which lined by the goblet cells of large size and globe like in between the intestinal mucus gland there was infiltration of the lymphocytes. Some of these lymphocytes were arranged in pattern (FIG 9-B) .the intestinal gland were extended to the submucosa which had congested blood vessels.

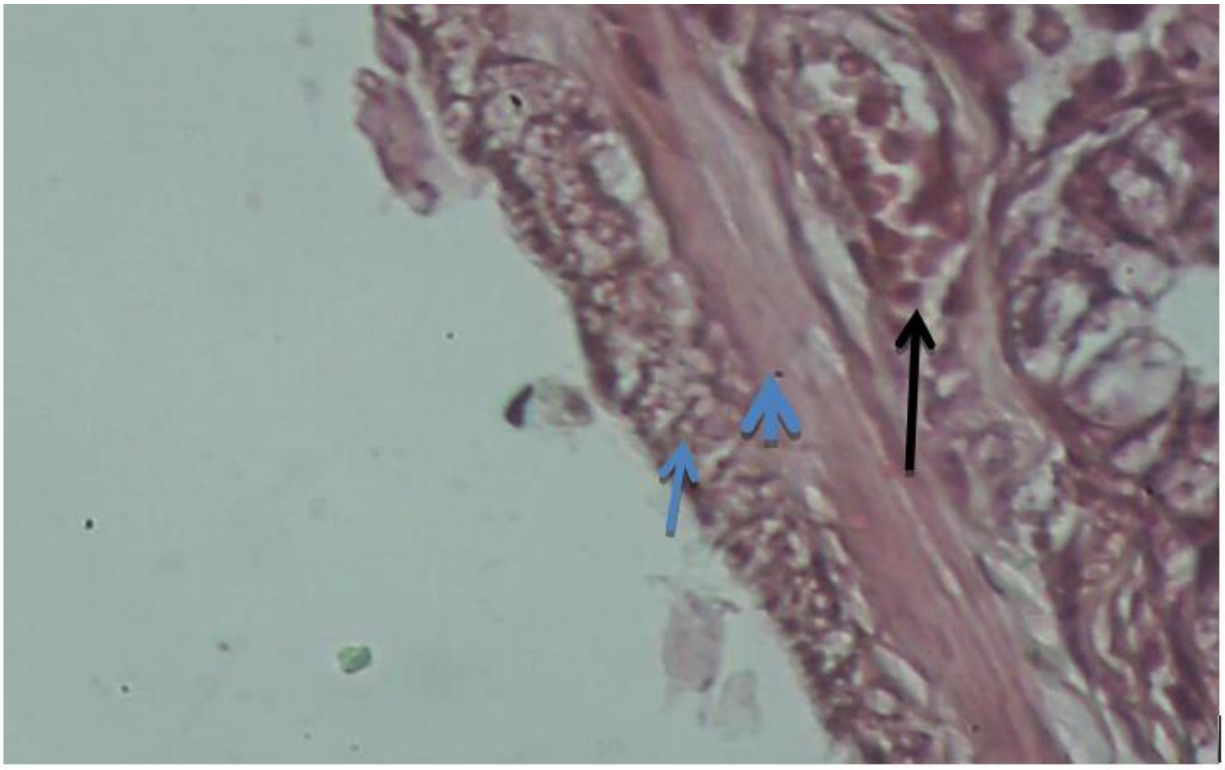
Colon

The intestinal were very tall lined by simple columnar epithelium and covered the surface of these cell by the microvilli the intestinal mucus gland were containing a great size of goblet cells with secretion (FIG10-A)the lateral side and the base of certain villi were surrounded by a lymphocytic aggregation of the lymphocytes c(Fig 10-B) .

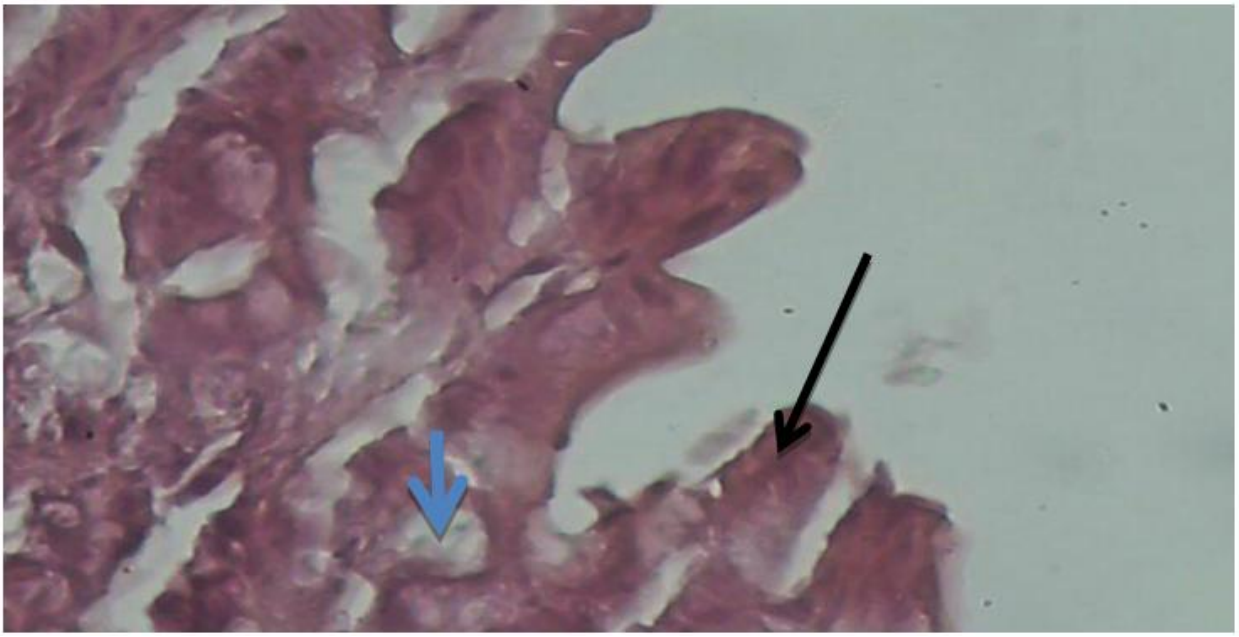
Jejunum

The villi of intestine were finger –like covered with tall columnar epithelium cells (FIG11-A).There was a certain number of epithelium cells on the surface of villi still in the form of degeneration (FIG11-B).The of each villi was

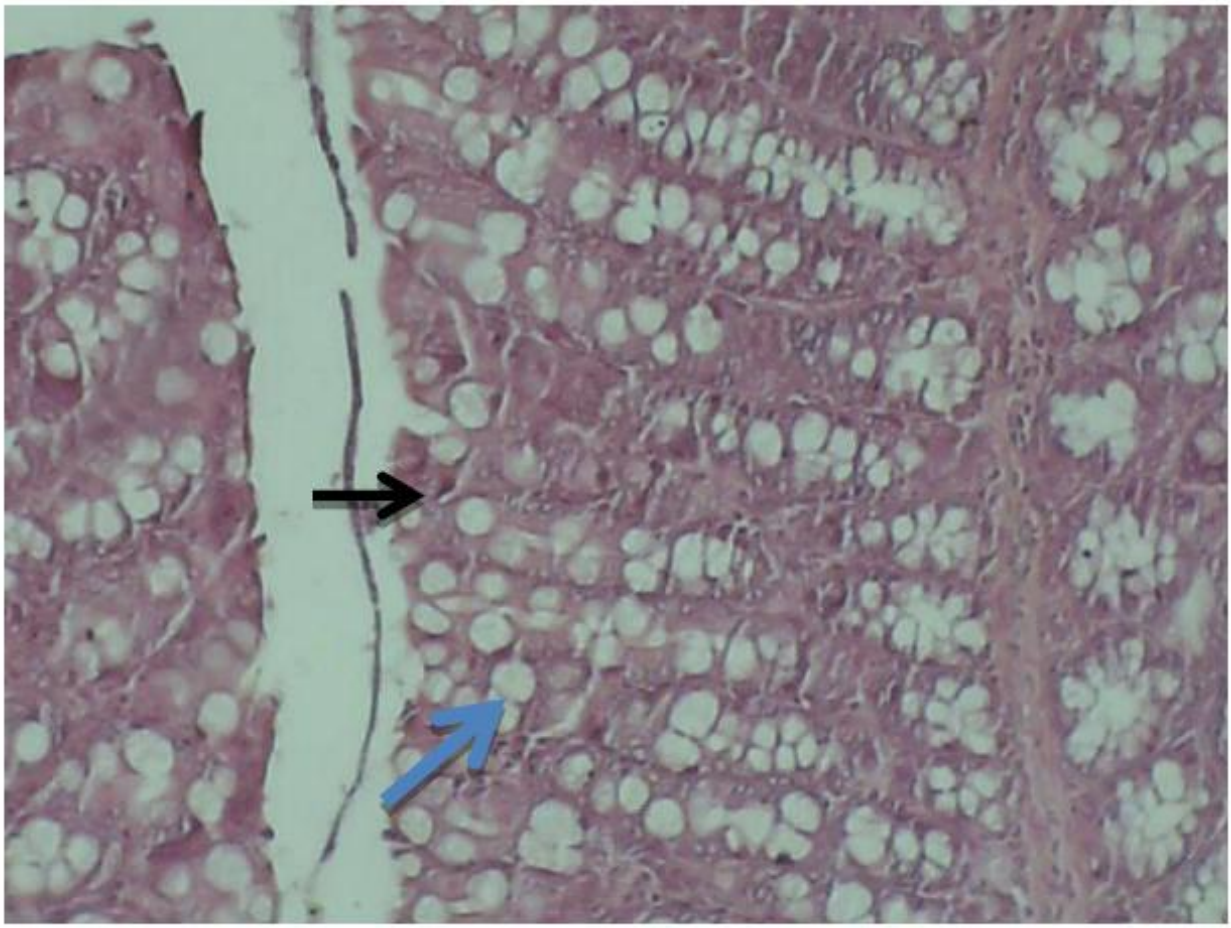
Infiltration by lymphocytes, the goblet cells in between the epithelial cells were variety seen the intestine mucus gland were atrophied in the lamina propria.



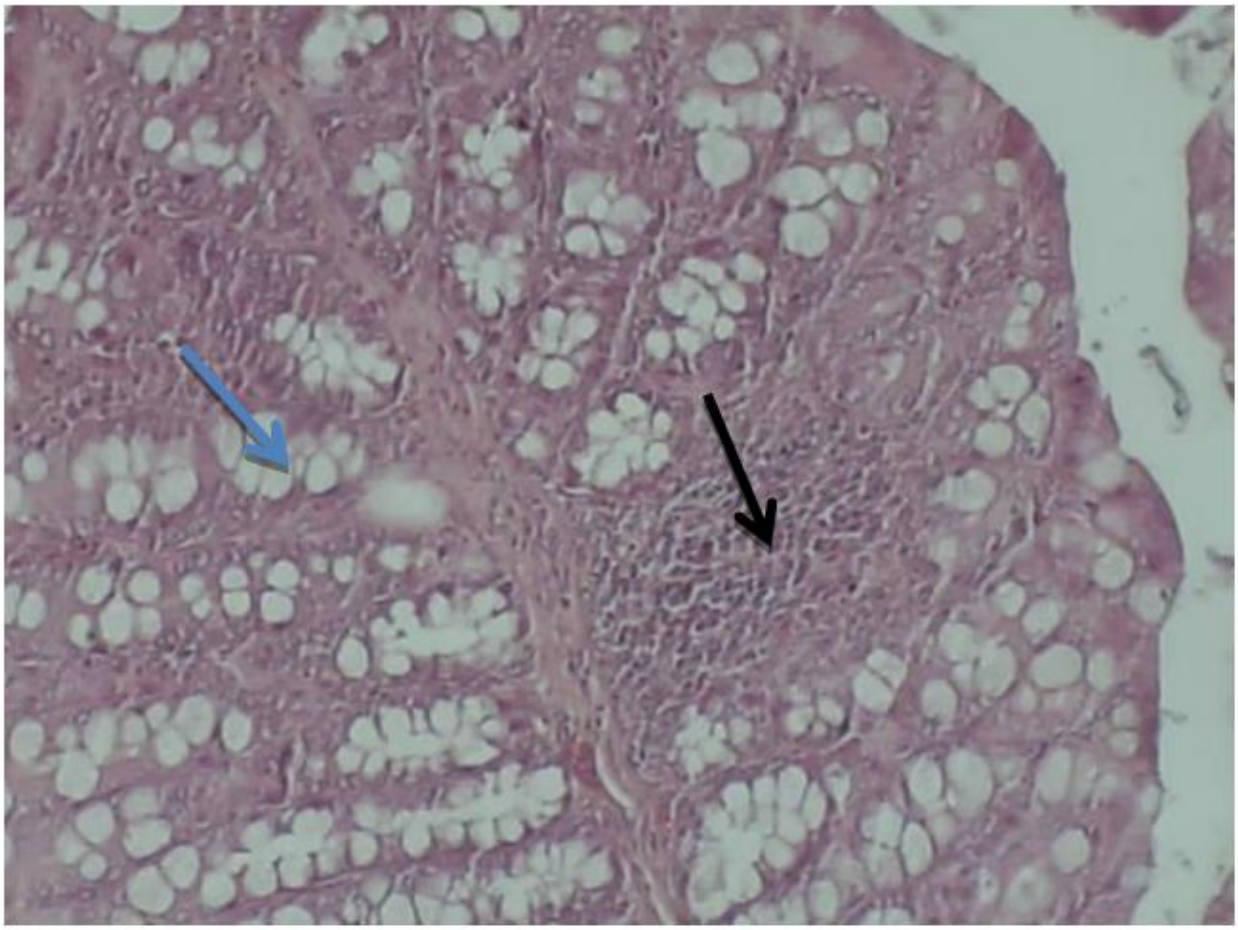
(FIG8-A-_Cecum treatment)black arrow-submucus congestion of blood vessels
Blue arrow-muscular ,coat inner circular ,outer longitudinal (H&EX40)



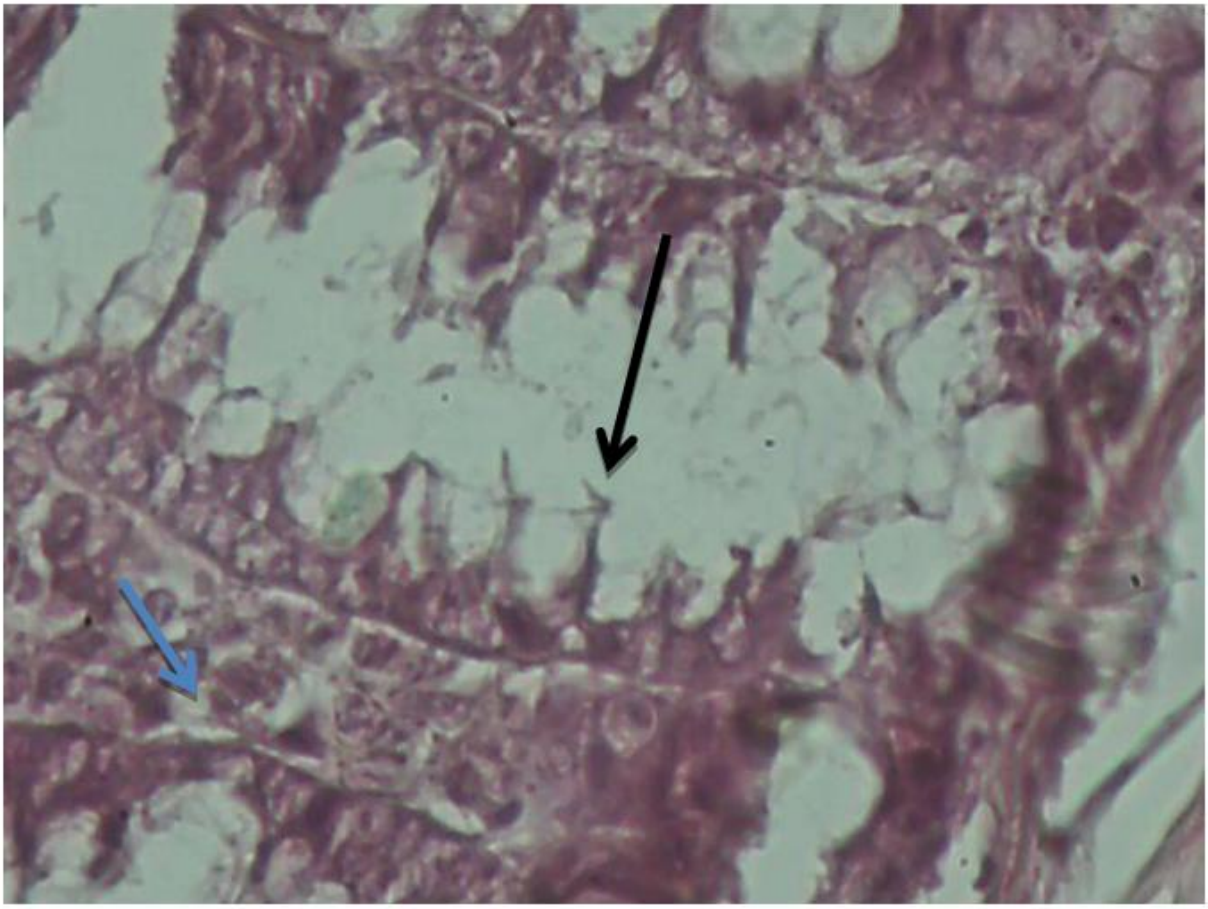
(FIG8-B)-cecum treatment :black arrow :- epithelium cells of cecum
Blue arrow:-goblet cells (H&EX40).



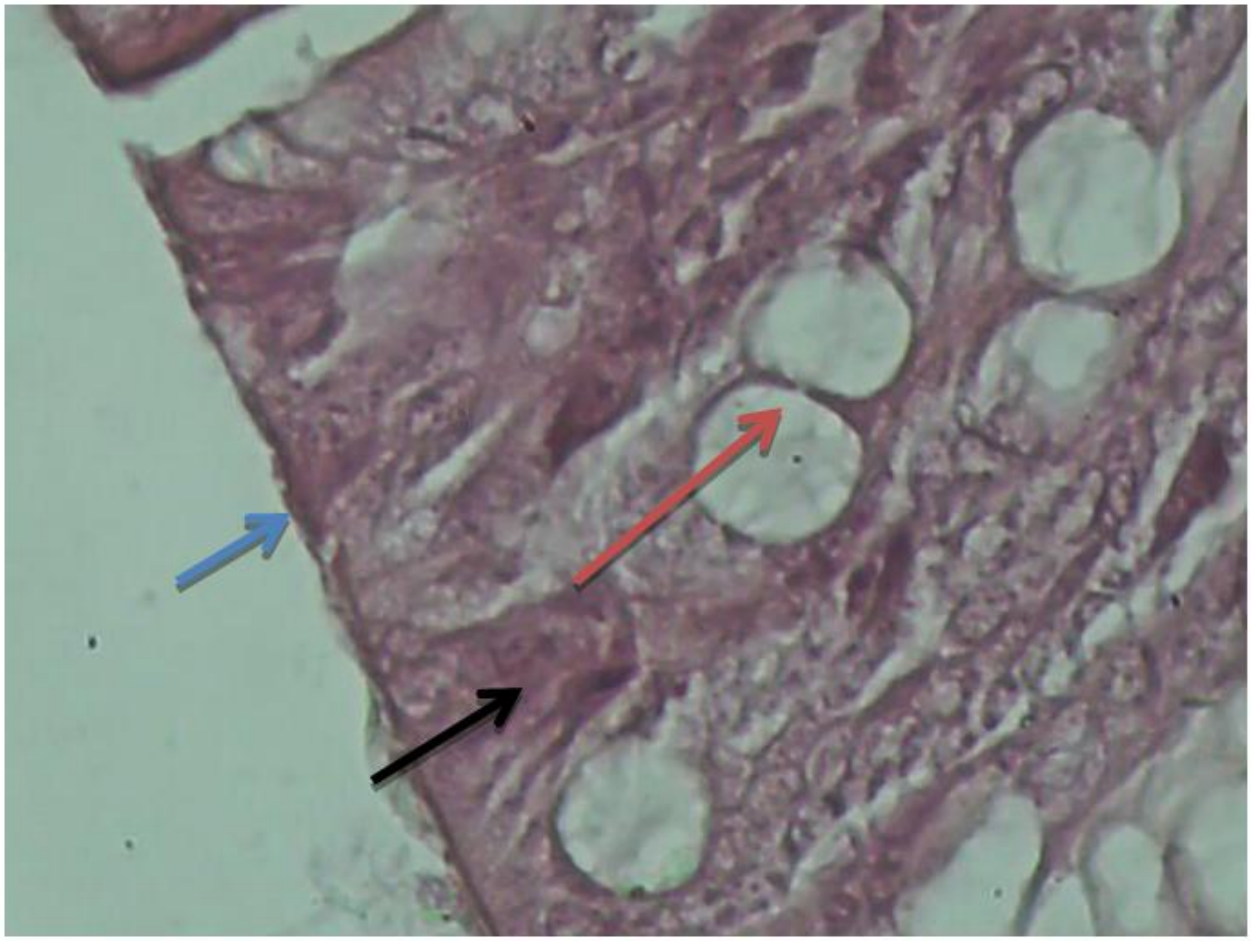
(FIG 9-A-)duodenum treatment _black arrow-intestinal villi of duodenum Blue arrow:-engorged with goblet cell(H&EX20).



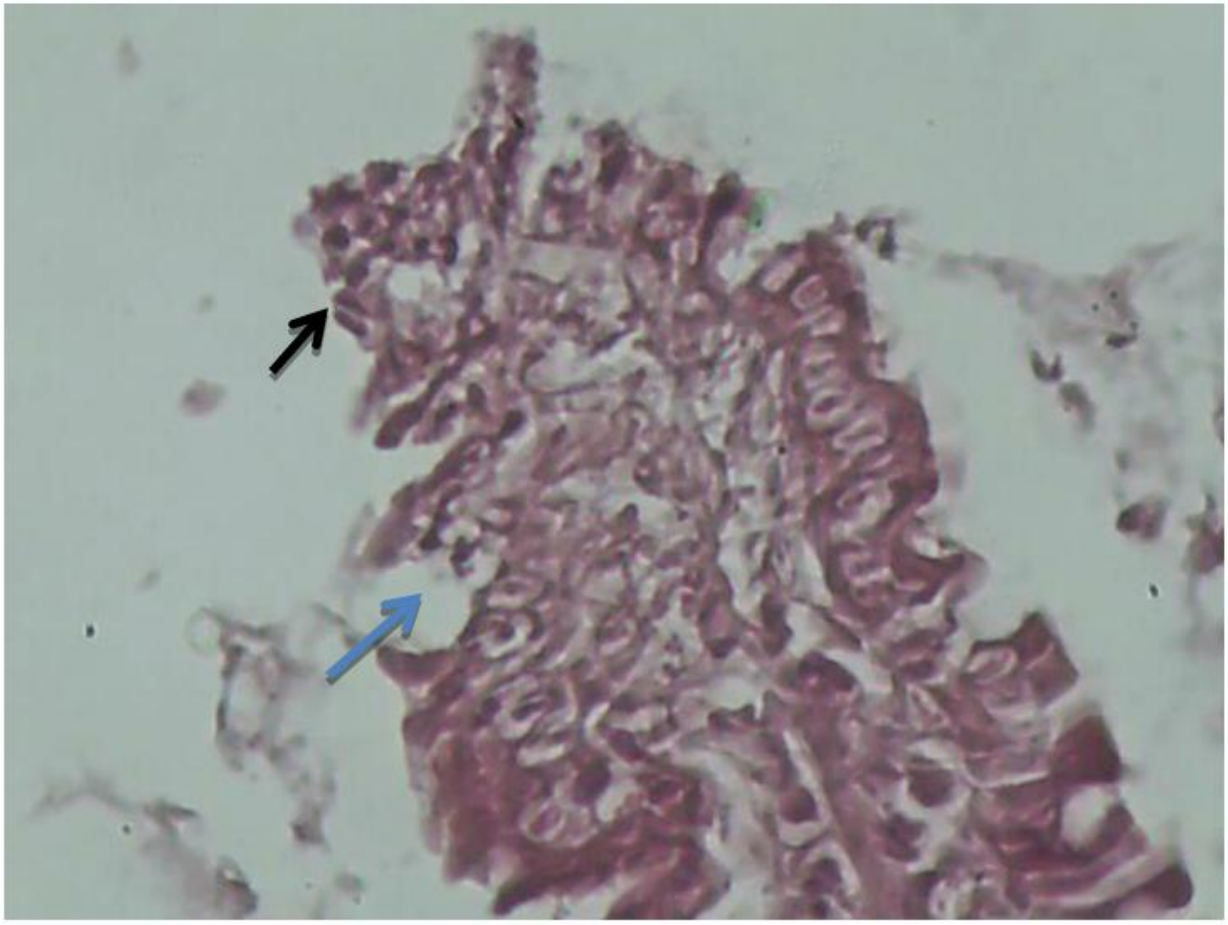
(FIG 9-B-)duodenum treatment _black arrow-lymphocyte aggregation of lamina propria Blue arrow:=-submucosa gland (H&EX20).



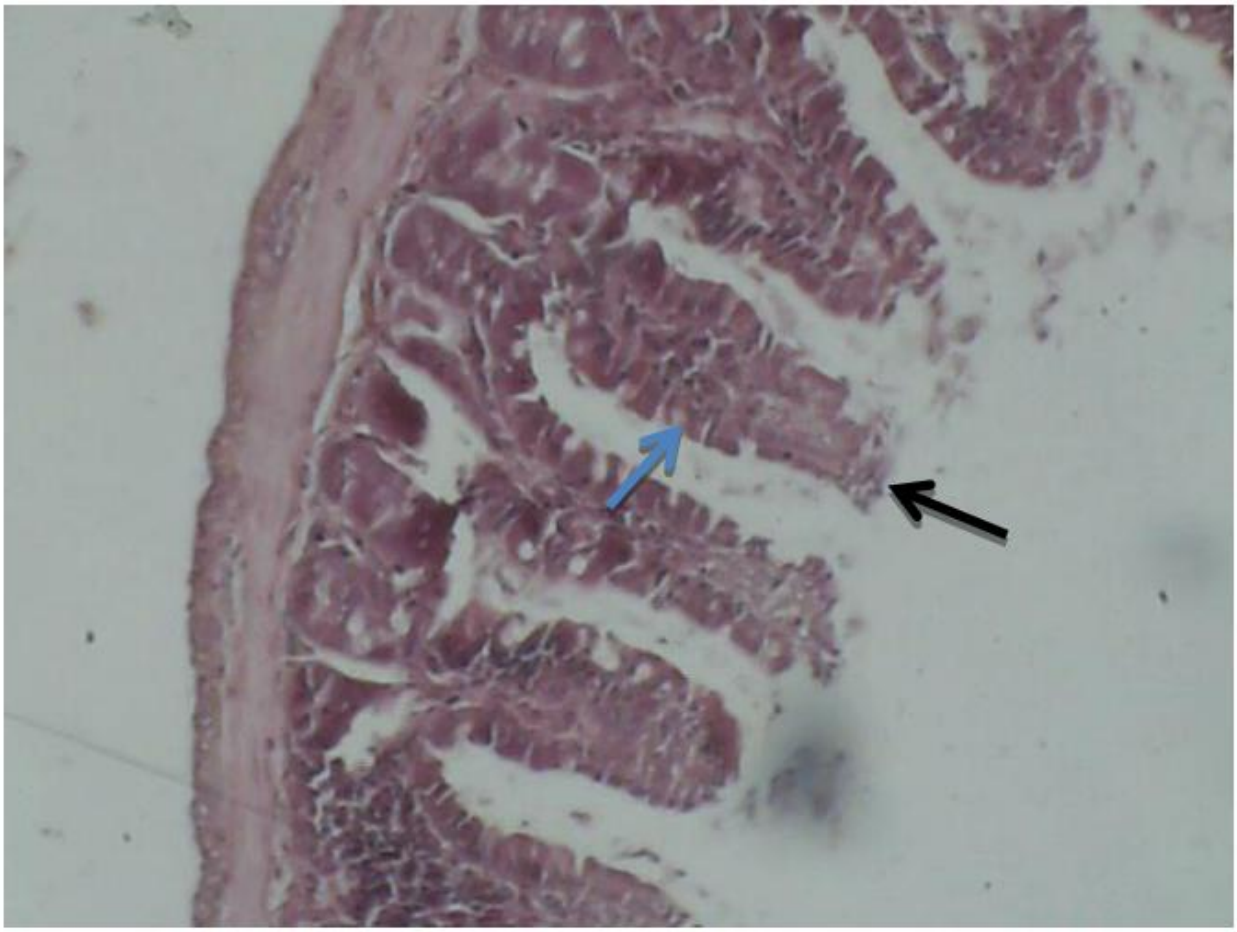
(FIG10-A)Colon treatment Black arrow-degeneration mucus cells of the colon Blue arrow-lymphocyte infiltration (H&EX40).



(FIG10-B) Black arrow-Simple columnar epithelium -blue arrow –microvilli normal Red arrow –enlargement mucus cell(H&EX40).



(FIG11-A-)Jejunum treatment:-black arrow-normal epithelium cells of jejunum Blue arrow-degeneration goblet cells(H&EX20).



(FIG11-B -)Jejunum treatment black arrow-jejunum is demonstration the finger like villi Blue arrow-simple columnar epithelium (H&EX20).

Discussion:-

The cell structure of fungi is similar to human cells, hence difficult to destroy without damaging human cells as well. Synthetic antifungal drugs thus tend to have serious side-effects &most antifungal drugs only inhibit the growth of fungi (fungistatic), which gives them opportunity to mutate and become drug-resistant. Natural antifungal treatments are becoming increasingly important as yeasts and fungi become progressively resistant to conventional antifungal drugs.Olive leaf extract can be a valuable preventative natural antifungal supplement to be taken during some of these treatments. Most doctors are not familiar with olive leaf extract but should nevertheless be consulted if you wish to take it under these circumstances. Olive leaf extract contains compounds with potent antimicrobial activities against bacteria, fungi, and mycoplasma [7]. The reports describing antimicrobial properties of phenolic compounds in olive products refer to compounds obtained from olive fruit particularly hydroxytyrosol and oleuropein(8)

The results clearly demonstrate that the olive leaves extracts can act as a potent antifungal agent against C. albicans

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