ISSN 2330-6440 Vol 3, No 3, July 2016

Study of the antifungal (*Trichophyton rubrum*)activity of olive leaves extracted in the intestinal of mice

Buthina abdulhameed

Department of pharmacology,

buthinaabad67@yahoo.com

Hiba Younes

Department of microbiology ,

Reem suheel

Deportment of chemistry

Veterinary medicine collage \Tikrit university

.

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 ***Trichophyton rubrum*** 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 (o.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 (Olea europaea) have a rich medicinal uses(Soni MG et.al.. 2006)..There are many references citing the medicinal use of the plant (Olea europaea) in ancient times Effects of olive leaves like the antioxidant, hypoglycemic, antihypertensive, antimicrobial, and antiatherosclerotic have been reported in various studies(Somova LI,et.al.. 2003)

Olea europaea 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(Aziz NH,et.al. 1998) .Oleuropein have antimicrobial (Bacillus subtilis, B. cereus,taphylococcus aureus 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, Skin, hair, nail, and subcutaneous tissues in human are subjected to infection by many organisms, mainly fungi named dermatophytes and cause dermatophytoses (Valeria et al., 1996;). dermatophytoses is widely distributed all over the world with various degrees and more common in men than in women. There are three genera of mould that cause dermatophytosis. These are Epidermophyton, Trichophyton and Microsporum. ,high cost of treatment, difficulty of control and the public health consequences explain their great importance (Chermette et al., 2008) However, their clinical is differentiation is difficult The clinical care is required care by a physician or other healthcare professional in the treatment of these diseases (Beentje, 1994). Plant extract has been used to treat a number of infectious diseases including those caused by bacteria, fungi, protozoa and viruses (Soylu et al., 2005; Yoshida et al.,) A many reports are available in vitro and in vivo efficacy of plant extract against human pathogens causing fungal infections (Natarajan et al., 2003). **Aims**:- The present study was aimed to evaluate the effects of olive leaves extracts antifungals

Key words :- olive leaves extracts, antifungals, ***Trichophyton rubrum***

Materials and methods

1-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.

2. 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.(Lafka, T.Iet.al., 2013)

3-Organisms:-

Isolated from laboratory of veterinary medicine collage of Tikrit university **.** *s*

4-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(-Jon,D. and Alan ,S. 1989). 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

Organs Control

1-colon

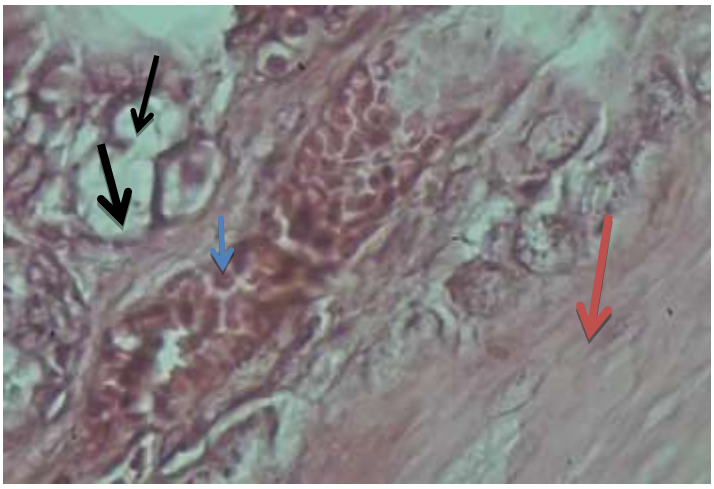
associated with sloughing of other cell towered the lumen of intestine. The goblet cell were a abundant and are located in between epithelial and intestinal gland ,usually surrounded by congested blood vessel and solitary lymphocyte infiltration (FIG1-A) The colonic folds of the mucosa were very short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

2-jejunum

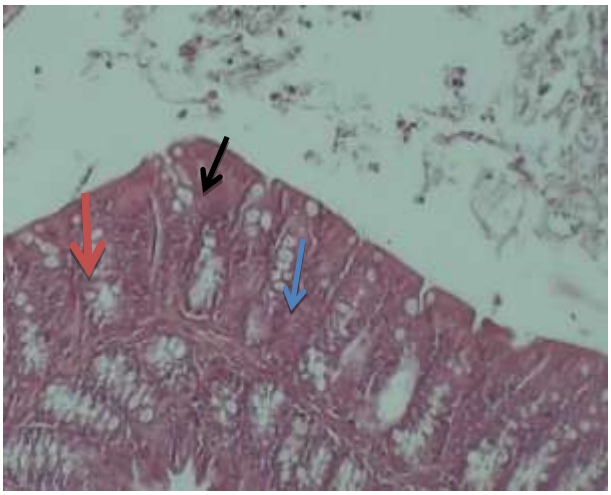
The intestinal villi were present of high level and finger like covered with simple columnar epithelium with a great number of lymphpocytes (FIG2-A) and the cell of c.t. the intestinal gland of mucus type were extended to the bases of the villi and other region of the lamina propria in between there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

3-dodenum

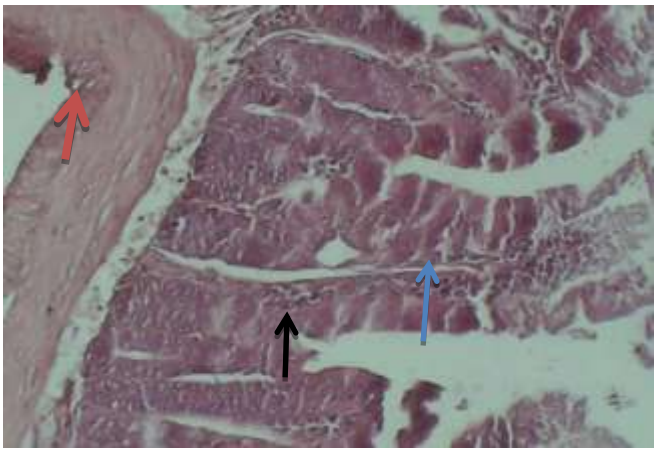
The mucosa of duodenum were formed mainly by the villi which lined by the simple columnar epithelum with goblet cell (FIG3-A) .the intestinal gland were extended deeply in the laminapropria and extended even to the submucosa ,these gland were containing the goblet cells and its secretion in the lumen of these gland. There were nodular infiltration of the lymphocytes in between the intestinal gland (FIG4-B) . the blood vessel with RBC were easily demonstrated in laminapropria and mucosa .



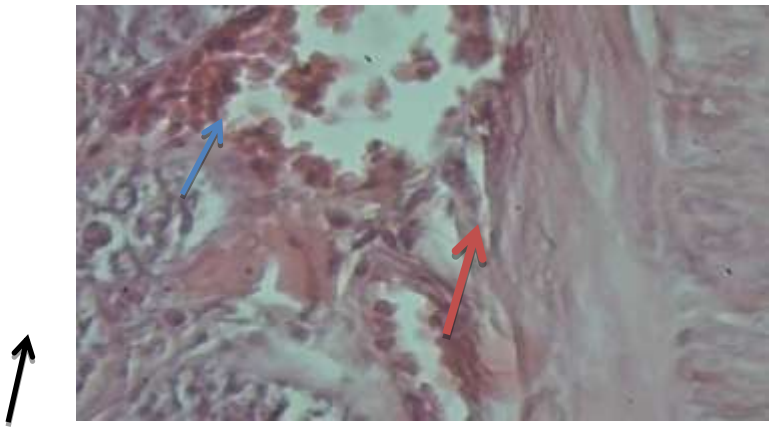
(fig 1) A- COLON CONTROL **Black arrow** -intestinal gland of colon
Blue arrow -congested blood vessels of submucosa
Red arrow -muscular layer
(H&E X40)



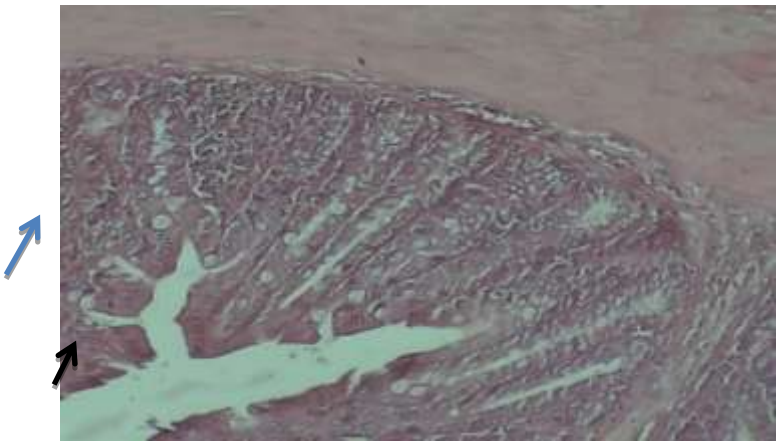
(fig1)B- colon control
Black arrow - intestinal fold colon
Blue arrow-goblet cell



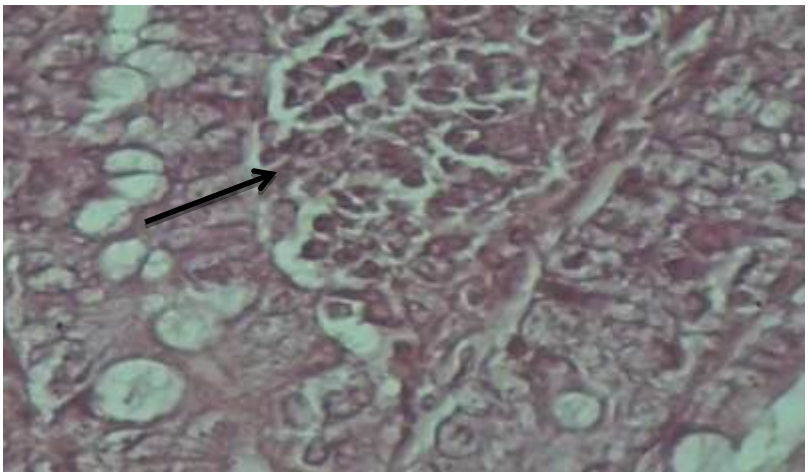
(FIG2A)-jeujenum control
Black arrow –intestinal villi of jeujenum
Blue arrow –core of villi with lymphocyte aggregation
Red arrow-submucosa (H&EX20)



(FIG2B)-
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-goblt gland (H&EX20)



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

Treatment organs by Olive leaf extract

1-duodenum treatment

The intestinal mucosa was containing an villi some of the villi had degeneration epithelial cells on its surface fig (4A).also there were sloughed cells in the lumen of duodenum the intestinal gland contains basophilic granules in the cytoplasm of cell fig (4B).indicating necrosis of these cell surrounded by lymphocytic infiltration .the submucosa blood vessel were noted engorged with RBC.

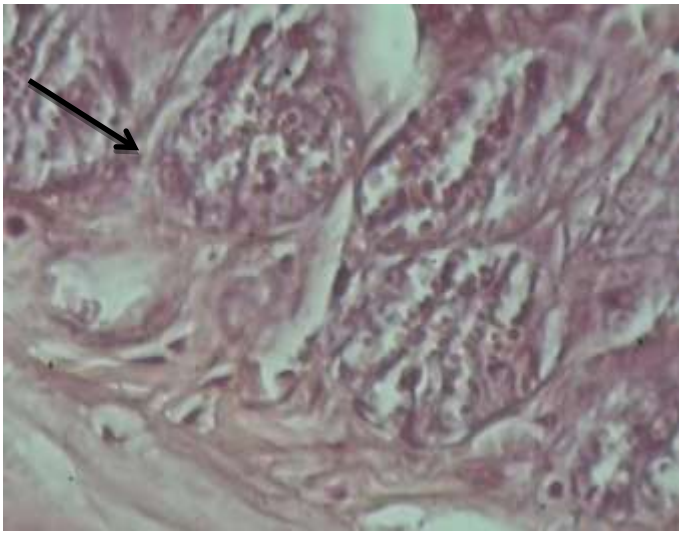
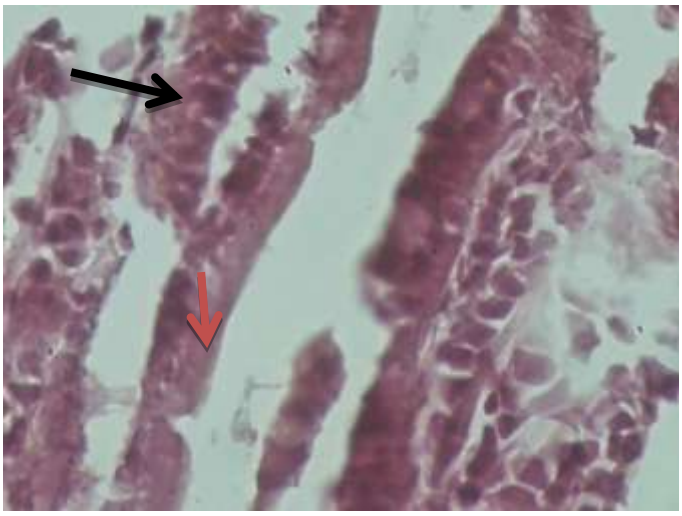


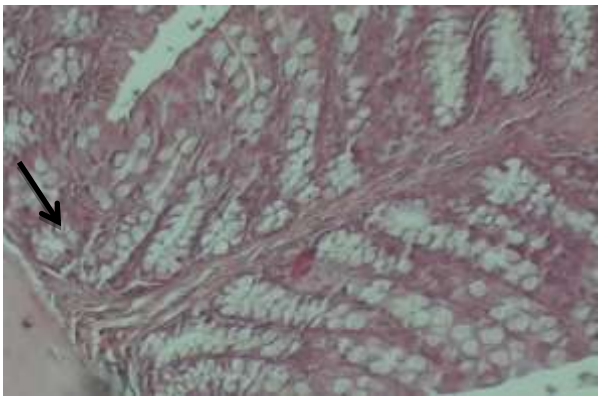
Fig (4A):-basophilic granules of the intestinal mucus gland of the duodenum **(H&EX40)**



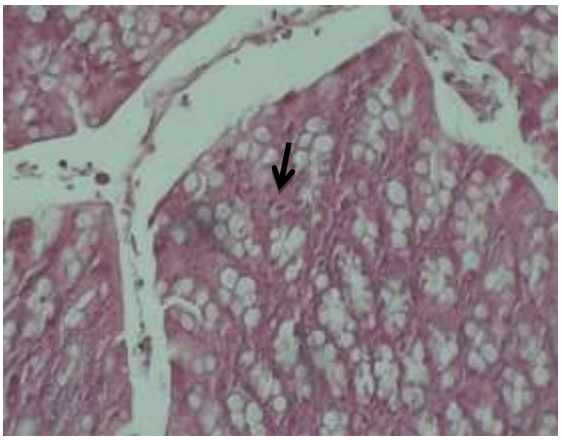
Fig(4B):-degeneration changes of the epithelial cells of the duodenum **(arrow black)**
-sloughed epithelial degeneration cells **(arrow red) (H&EX40)**

2-colon treatment

The mucosa of the colon was formed by fold projected toward the lumen fig (5A).covered by simple columnar epithelium and the whole laminapropria of the colon was greatly engorged with mucus cells of globe like extended in the form of lobular gland to the end of the lamina propria fig (5B).The base of the fold had individual lymphocytes distributed in between the mucus gland.



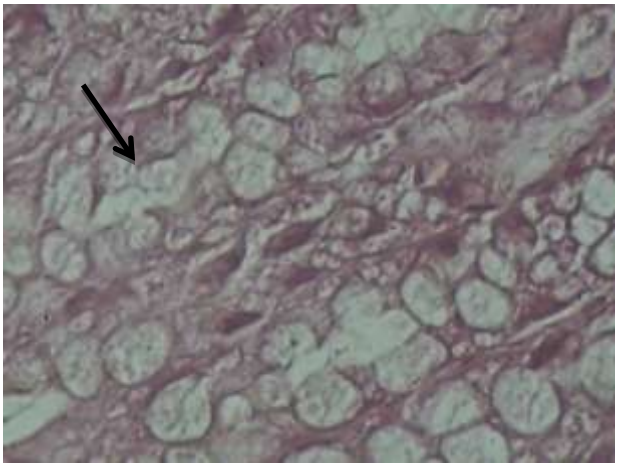
Fig(5A):-tubular mucus intestinal gland of the colon (normal) **(H&EX20)**



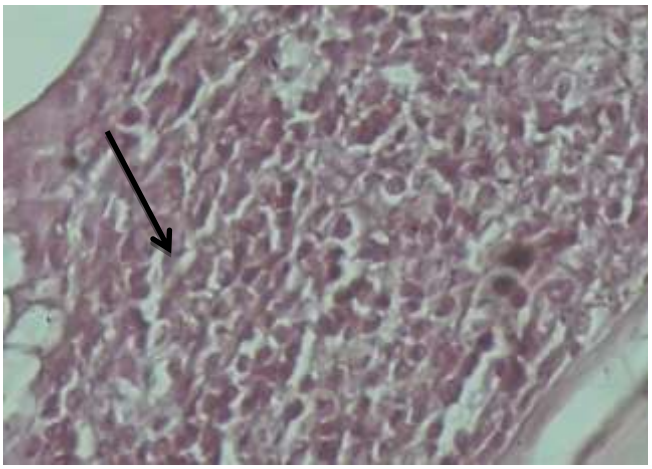
Fig(5B):-intestinal aggregation of globet cells of the colon(normal)(H&EX40)

3-jejunum treatment:-

The intestinal villi were easily recognized associated with simple columnar epithelium covering its surface with microvilli and containing goblet cells in between . the intestinal gland were mostly forward by goblet cells mucus (fig 6A) which extended to the end of the villi .there were nodular aggregation of lymphocytes in the lumina propria in between the intestinal gland (fig6B)



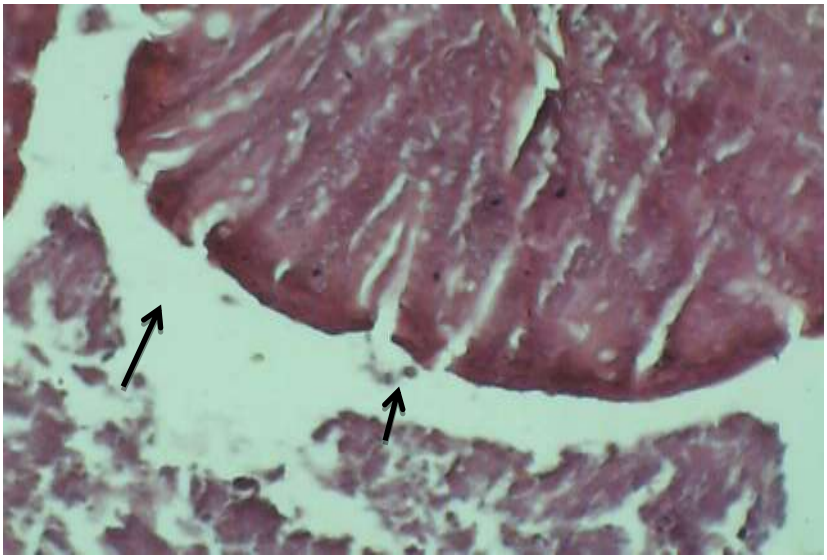
Fig(6A):-intestinal hypertrophy mucus gland (**H&EX40)**



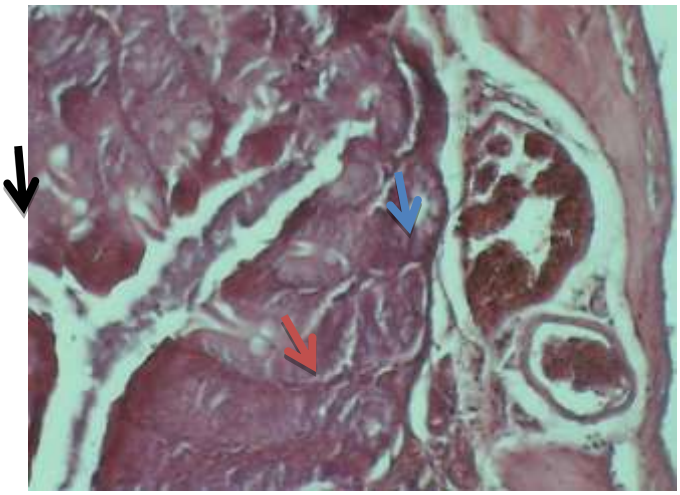
Fig(6B):-nodular lymphocytic infiltration in the lamina propria **(H&EX20)**

INFECTED ORGANS BY FUNGUS

1-duodenum :-The surface of the simple columnar epithelium of the duodenum villi were thickened fig (7A),there was necrotic foci in the epithelium and lymphocytes infiltration in the bass of villi in the lamina propria was demonstrated .the blood vessels of the sub mucosa were engorged with blood fig (7B).



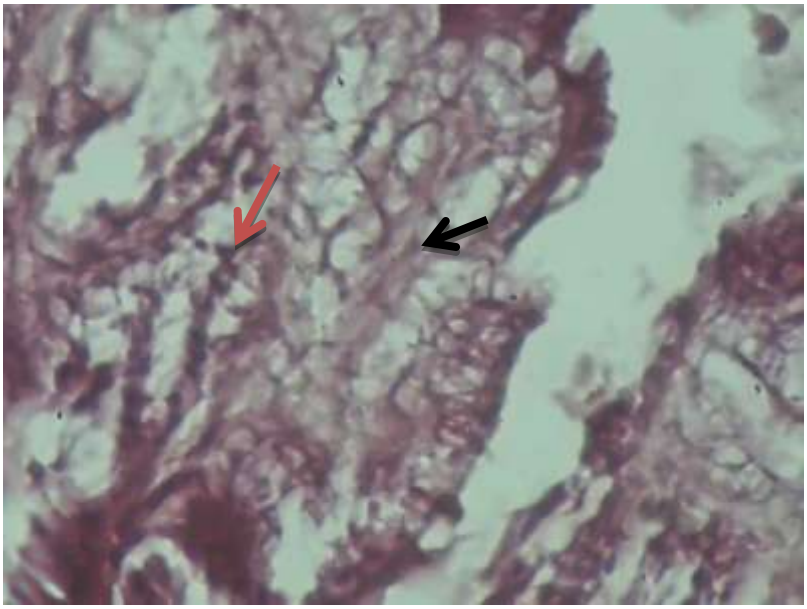
Fig(7A):-thickening of the surface epithelium of the duodenum villi**(H&EX20)**



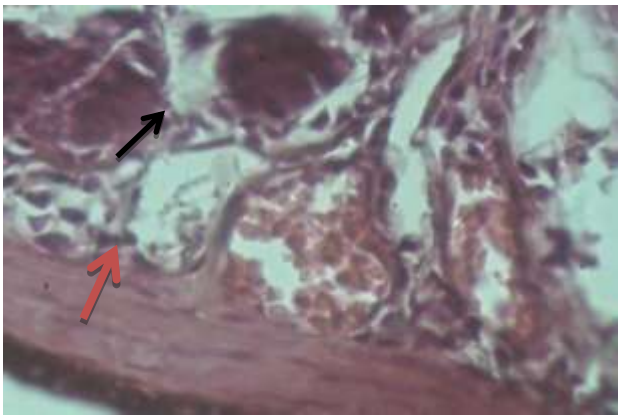
Fig(7B):-necrotic foci of the epithelium **(arrow black)**
-lymphocytic aggregation **(arrow red)**
-Submucous blood vessels congestion **(arrow blue) (H&EX40)**

2-jeujenum infected

The intestinal villi were completely degenerated with its simple columman epithelium fig(8A).the intestine gland in the lamina propria were a atrophied with its mucus cells and mostly surrounded by the lymphocytic infiltration and the adjacent blood vessel were congested fig (8B).Also was nodular lymphocytic aggregation in the lamina propria.



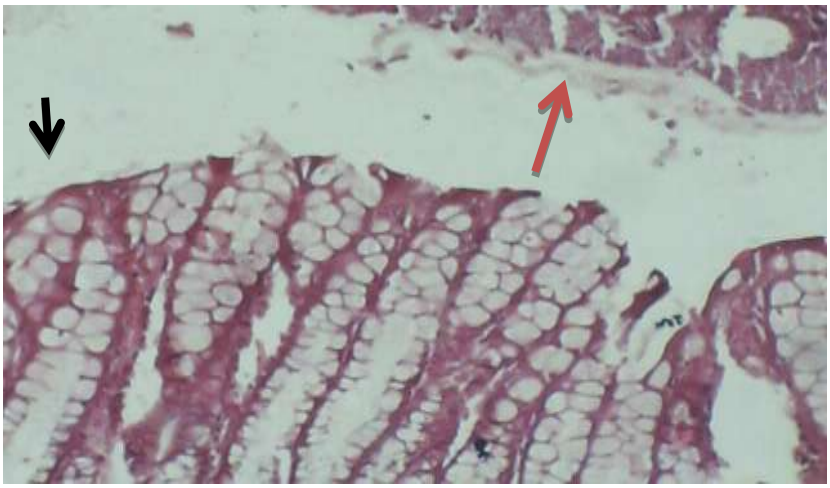
Fig(8A):-epithelial degeneration of the intestinal villi **(arrow black)**
- degeneration intestinal gland (mucus cells)(**arrow red) (H&EX4O)**



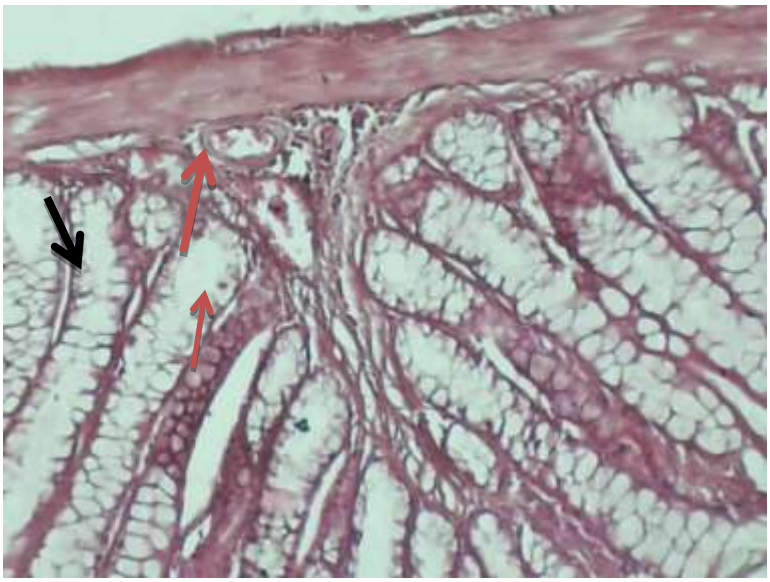
Fig(8B):-lymphocytic infiltration around the mucus gland of the jejunum **(arrow black)**
-congestion blood vessel of submucus **(H&E X20)**

3-colon infected:-

The surface epithelium of intestinal fold were degenerated fig (9A). The intestinal mucus gland containing a great amount of mucus secretion inside the lumen of gland .these gland were surrounded by infiltration lymphocytes congested blood vessels around the gland and in the base of these gland fig (9B)



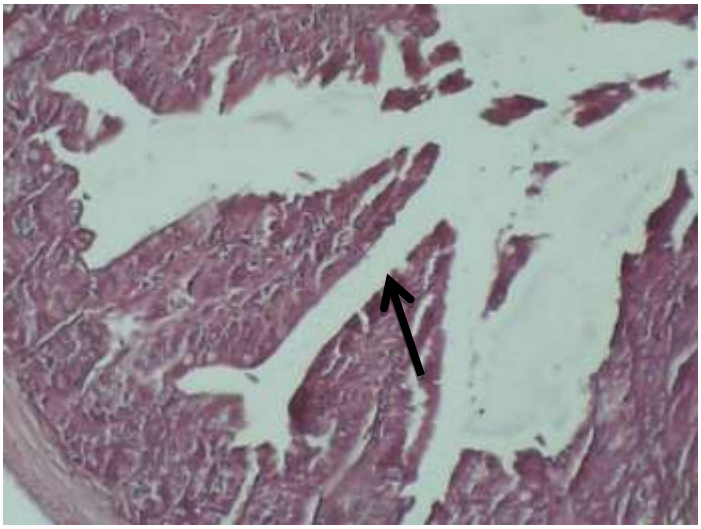
Fig(9A):-colon fold indication the presence of degeneration epithelial cells**(arrow black)**
-intestinal lumen with Deloris of desquamated cells **(arrow red)**



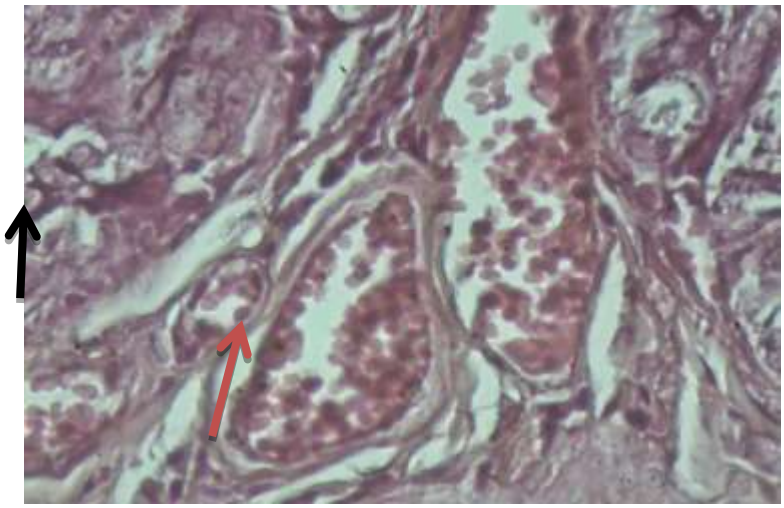
Fig(9B):-extensive amount of the mucus gland **(arrow black)**
-lymphocyte infiltration in the lamina propria and submucus **(arrow red) (H&EX40)**

3-ilum infected :-

The ileal villi were lined by degenerated epithelium cells and most of the cell were sloughed from the surface of villi to be corn inside the lumen of intestinal fig (10A). The intestinal gland had degeneration cell. The base of phase gland were unsheathed by zone of lymphocyte and other leukocyte also small blood vessels were filled with blood fig (10B)



Fig(10A):-sloughed epithelial cells of the ileal villi **(H&EX20)**



Fig(10B):-lymphocytic infiltration around intestinal gland of ileum **(arrow black**)
-congestion blood vessels **(arrow red)(H&EX40)**

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),.therefor use Herbal treatments are against yeasts and fungi instead of antifungal drugs .like Olive leaf extract can be a valuable preventative natural antifungal supplement to be taken during some of these treatments. In the present study Olive leaf extract was evaluated for its potential against fungus trichophyton. pharmalogical properties of olive oil, the olive fruit and its leaves have been recognized as important components of medicine and a healthy diet because of their phenolic content.(.Visioli F,et.al.. 2002). Oleuropein has been shown to have strong antimicrobial activity, In 2001, Saija and Uccella . proposed that the glycoside group modifies the ability to penetrate the cell membrane and get to the target site. Effective interference with the production procedures of certain amino acids necessary for the growth of specific microorganisms has also been suggested. Another mechanism proposed is the direct stimulation of phagocytosis as a response of the immune system to microbes of all types.

Conclusion

.. The results clearly demonstrate that the olive leaves extracts can act as a potent antifungal agent against ***Trichophyton rubrum***

Reference

Aziz NH, Farag SE, Mousa LA, Abo-Zaid MA. 1998. Comparative antibacterial and antifungal effects of some phenolic compounds. Microbios.;93:43–54.

Beentje, H.J., 1994. Moringaceae. In Kenya Trees Shrub and Lianas. Majestic Printing Works Ltd., Nairobi, Kenya. Chapter 37.

Chermette R, Ferreiro L, Guillot J, 2008. Dermatophytoses in animals. Mycopathologia, 166: 385-405

-Jon,D. and Alan ,S. 1989. Theory and Practice of histological Techniques.second edition.Chichill Livinstone,;(606-608).

. Lafka, T.I.; Lazou, A.E.; Sinanoglou, V.J.; Lazos,E.S., 2013. Phenolic extracts from wild olive leaves andtheir potential as edible oils antioxidants, Foods, 2, 18-31.

Natarajan V, Venugopal PV, Menon T, 2003. Effect of azadirachta indica (neem) on the growth pattern of dermatophytes. Indian J. Med. Microbiol., 21: 98-10.

Saija A, Uccella N. 2001; Olive biophenols: functional effects on human well-being. Trends Food Sci Technol. 11: 357–363. doi:10.1016/S0924-2244(00)00068-6..

-Somova LI, Shode FO, Ramnanan P, Nadar A. 2003. Antihypertensive, antiatherosclerotic and antioxidant activity of triterpenoids isolated from Olea europaea, subspecies Africana leaves. J Ethnopharmacol

84:299-305-

-Soni MG., Burdock GA., Christian MS, Bitler CM, Crea R. 2006. Safety assessment of aqueous olive pulp extract as an antioxidant or antimicrobial agent in foods. Food Chem Toxicol 44:903–915

Soylu EM, Tok FM, Soylu S, Kaya AD, Evrendilek GA, 2005. Antifungal activities of essential oils on post harvest disease agent Penicillium digitatum. Pak. J. Biol. Sci., 8: 25-29.

Valeria FM, Preve L, Tullio V, 1996. Fungi responsible for skin mycoses in Turin (Italy). Mycoses, 39: 141-150.

Visioli F, Poli A, Galli C. 2002.Antioxidant and other biological activities of phenols from olives and olive oil. Med Res Rev.; 22: 65–75. doi:10.1002/med.1028

Yoshida M, Fuchigami M, Nagao T, Okabe H, Matsunaga K, Takata J, Karube Y, Tsuchihashi R,Kinjo J, Mihashi K, Fujioka T, 2005.Antiproliferative constituents from Umbelliferae plants VII. Active triterpenes and rosmarinic acid from Centella asiatica. Biol. Pharmacol. Bull., 28: 173-175..