

STUDY THE HISTOLOGICAL LESIONS THAT INDUCED BY SHIGELLA FLEXNERI FOR AND THE ROLE OF ANTHEUM GRAVEOLENS AND TO TREATMENT IN COMPARATIVE WITH AN ANTIBIOTIC

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ABSTRACT

The present study was carried out to investigate the histological lesions and morphological changes caused by the experimental *Shigella flexneri* bacteria in both the small intestine and the large intestine in albino mice *Mus musculus* and attempt to treat them using the *Antheum graveolens* and antibiotic amikacin. And study the inhibitory activity of the extract of the plant in vitro. *Shigella flexneri* was tested for a variety of antibiotics and showed that it was sensitive to (Amikacin, Erythromycin Tobramycin, Gentamycin, Ceftriaxone, Cefotaxime and Nalidixic acid) and resistant to (Ampicillin, Trimethoprim and Aztreonam). The inhibitory activity test was performed of *Antheum graveolens* extract toward *Shigella flexneri* and was found to be sensitive and high. Both ID-50 and LD-50 LD dose were determined. In the present study, the laboratory animals were divided into 36 mice and randomly assigned to 6 groups. Each group consisted of six mice. These groups are control group, ID-50 group, LD-50 group, group that treated with water *Antheum graveolens*, group that treated with alcoholic *Antheum graveolens* and antibiotic group. The bacterial infection of the mice was induced by mouth, the animals left dosage of the doses pathogenic from bacteria for a four-day and the appearance of the disease. On the fourth day, The results of the histological examination of the small and large intestines showed satisfactory changes in the different tissue layers of these structures. microscopic examination showed less enhancement in groups that treatment with alcoholic *Antheum graveolens* and antibiotic compared with groups that administrated water *Antheum graveolens*

INTRODUCTION

Despite the remarkable development of science, medical plants have proved to be a major source of many medicinal properties. Their various extracts contain effective substances widely used in the development of various properties that have been successfully replaced by chemical properties[1],to the use of natural alternatives due to the side effects of various chemicals and their effectiveness in the treatment of some of the most difficult diseases and newly known, and many of the antibiotics have lost their effectiveness in treatment for the emergence of new bacterial strains resistant to it and more[2]. Among these natural alternatives are the plant *Anthem graveolens*.

The plant is an annual or annual plant, native to the Arab Maghreb, Spain, Portugal, Cyprus and some parts of Europe and is today in many parts of the world. Southwest Asia and southwestern Europe are cultivated, and in the rest of the world they are now

temperate. It is an existing plant with a height of 70-120 cm and has a root and tendon resembling carrots. Its leaves are composed of feathers, which are divided into striped floral and full yellow floral parts within the tentacles of a 15 cm diameter site. The leaves are five leaves green Color The leaves are also five leaves yellow color, while the stems are smooth and smooth green color[3]. The flowers develop into dark-brown pressed seeds that appear to be cut, and in ideal conditions the root of the plant can reach deep into the soil[4]The plant belongs to the Umbrella or tent family, which contains more than 250 species of crops[5]. The herbaceous plants that belong to this family include parsley, celery, coriander and fennel, which is similar to the plant of the net. Where the appearance, except for the lack of hair [6],The mature leaf plant is characterized by a pungent, pungent smell that grows from seed and usually takes two and a half months to produce new seeds [7]In Iraq, it is traditionally used as a taste enhancer for children.

The active substances have been categorized in *Anthem graveolens* The phytochemical study showed the leaves, stems and roots are rich in tannins, free quinones, , flavonoids, alkaloids, steroids, saponins and glycosides[8], In the last few decades, some bacteria have acquired resistance to known antibiotics Because most cases of diarrhea are

produced by different types of microorganisms that directly affect the channel, especially the small intestine and large intestines and cause inflammation of the intestine.

MATERIALS AND METHODS

Antibiotic Sensitivity Test:-

This test was performed using the modified Bauer-Kirby method .by [9], and depended by the World Health Organization(WHO).

Bacterial isolated:-

The standard *Shigella flexneri* isolation of ATCC12022 was obtained from the Media / Erbil / Microbiology Laboratory. Some laboratory tests were carried out to

confirm the validity of the diagnosis, and the type of biochemical tests was determined using VITEK® 2 Compact.

Antimicrobial activity test extracts of *Antheum graveolens*. against *Shigella flexneri*

Method was adopted by [9]

Methods of detection of active compounds in *Antheum graveolens*:-

1 - **Detection of glycosides:** (Benedict reagent) This detection is done by adding (1) ml of raw extract to (2) ml of the Benedict reagent and then placed in the water bath for several minutes the appearance of blue indicates the presence of the glycosides.

2.**Detection of tannins:** The water solution (1%) of the ferric chloride and add a quantity of it to an equal amount of the plant extract, the appearance of green sediment blue indicates the presence of tannins.

3- **Detection of flavonides:** Add (4) ml of ethyl alcohol 95% to (1) ml plant extract in a glass test tube and then put the test tube in boiling water bath for a period of (25-30) minutes and after the removal of the bath Watery added drops of potassium hydroxide solution (0.5). The appearance of dark color indicates a positive assay (presence of flavonoids).

4-Detection of Phenols: This examination is done by adding (0.5) of the plant extract to a few drops of ferric chloride solution (0.5) The criteria for the appearance of dark green color indicates the presence of phenols.

5- Trepenoids: This detection is added by adding (5) ml of plant extract to a mixture of (2) ml of chloroform and (3) ml of concentrated sulfuric acid. The emergence of a medium layer of reddish brown indicates the presence of turbines.

6. Detection of alkaloids: 5 ml of plant extract was added to 1 mL of Mayer reagent prepared from the use of solvents A and B as follows: Solution A was prepared with 1.58 g of HgCl₂ in (60 ml of distilled water. B solution Prepare 5 ml of KI in 10 ml of distilled water. Immediately before detection, mix the solids and complete the mixture to 100 mL with distilled water. The appearance of the white precipitate indicates a positive test.

7. Detection of Saponins: This is done by preparing a water solution of dry sample powder and placing it in a test tube and well rolled. The appearance of dense foam remains for a long time indicative of the positive examination [11].

Preparation of histological sections:-

attended histological sections based on the method mentioned in [12], in the Laboratory of Life Sciences - college of Science / Tikrit University The organs to be studied immediately after the dissection of mice for the studied groups were fixed with formalin stabilizers for 24 hours the samples were then washed with a running water for half an hour to remove the excess adhesive from the fabric. then passed with a series of concentrations of 70% -100% ethyl alcohol for the purpose of drawing water from samples for 30 minutes per concentration. The sample mixture from xylene and paraphein wax 1:1 was placed in a oven temperature for 15 minutes, then transferred to a half-fused wax. The samples were then immersed in the same type of wax used by pouring the molten wax and quietly into an iron mold. L shape, the mold-shaped wax molds were cut by a sharp knife after placing the mold on the rotary microtome holder. The sections were cut with a thickness of 7.5 cm. Using the hematoxyline type of Haris Hematoxylin & Eosin, Light microscope (Olympus / Japan) After recording the footage was filmed using a Sony type camera and pulled the images to a 2400.Hp color printer.

RESULTS AND DISCUSSION

Confirmation diagnosis of *Shigella flexneri* :-

The diagnosis of the bacteria was confirmed by appearance, size, texture, the bacteria grown on the MacConkey agar medium were small in size and pale yellow and sometimes colorless. While the bacteria grown on the S. S agar appeared as smooth, colorless circular colonies.

Biochemical diagnosis:-

Biochemical diagnosis was performed using the VITEK® 2 compact device. The initial diagnosis and biochemical tests of the bacteria were identical to those reported by [13] and as shown in Table (1)

Table (1) Results of biochemical diagnosis using VITEK® 2 compact

Identification Information		Analysis Time:	6.00 hours	Status:	Final
Selected Organism		99% Probability	<i>Shigella flexneri</i>		
ID Analysis Messages		Bionumber:	0005411140100210		

Biochemical Details																	
2	APPA	-	3	ADO	-	4	PyrA	-	5	IARL	-	7	dCEL	-	9	BGAL	-
10	H2S	-	11	BNAG	-	12	AGLTp	-	13	dGLU	+	14	GGT	-	15	OFF	+
17	BGLU	-	18	dMAL	-	19	dMAN	+	20	dMNE	+	21	BXYL	-	22	BAIap	-
23	ProA	+	26	LIP	-	27	PLE	-	29	TyrA	+	31	URE	-	32	dSOR	-
33	SAC	-	34	dTAG	-	35	dTRE	+	36	CIT	-	37	MNT	-	39	5KG	-
40	ILATk	+	41	AGLU	-	42	SUCT	-	43	NAGA	-	44	AGAL	-	45	PHOS	-
46	GlyA	-	47	ODC	-	48	LDC	-	53	IHISa	-	56	CMT	+	57	BGUR	-
58	O129R	+	59	GGAA	-	61	IMLTa	-	62	ELLM	-	64	ILATa	-			

Bacterial susceptibility to antibiotic:-

The sensitivity test was carried out against (10) different types of antibiotics, The results showed that *Shigella flexneri* was sensitive to antibiotics (Amikacin, Tobramycin, Cefotaxime, Ceftriaxone, Gentamicin, Erytheromycin, Nalidixicacid) and antimicrobial resistance (Ampicillin, Aztreonam, Trimethoprim). Our findings agreed with [14-15] *Shigella flexneri* resistant and high-grade antibiotic Ampicillin. Our findings did not agree with the findings of

[16].It was found that all isolates of the bacteria *Shigella flexneri* were sensitive to Ampicillin but agreed with him that the bacteria were sensitive to Erythromycin.

Evaluation of the antimicrobial activity of extracts *Antheum graveolens* against *Shigella flexneri*

The results of the inhibitory effect of the plant extract against the *Shigella flexneri* bacteria showed the sensitivity of the bacteria to both the water and alcohol extracts, showing a high sensitivity to the concentration of 200 mg / ml, the least sensitive to the concentration of 1.5 mg / ml of the concentrations used. The inhibitory action of the aquatic extract of the plant is attributed to the presence of many chemical compounds acting as antimicrobials, including essential oils, terpenoids, oxypenedanin and falcarindiol [17]Our results have been agreed upon by [18-20]) in their study on the inhibitory effect of *Staphylococcus aureus*. The results showed a clear inhibitory effect.

Active substance in *Antheum graveolens* extracts:-

The results of the general chemical tests conducted on the plant showed the presence of a number of vital compounds. Our results were agreed with the [7]which gives the plant the importance of being one of the important medicinal plants developing in Iraq. As shown in the table(2) .

Table (2) Results of the qualitative reagents of *Antheum graveolens*

Alkaloids	Saponins	trephines'	Phenols	Flavonoids	glycosides	reagents
+	+	+	+	+	+	results

Results of histological examination of the large intestine of the groups of mice with *Shigella flexneri* and treatment with plant extract and antibiotic:

1- control group:-

The general histology of the large intestine consists of four layers: mucus, submucosa, muscularis, serosa Serosa, as shown in Figure1.

2. Infected dose 50 (ID-50):-

Large intestine:

The results of the histological examination showed significant damage to the large intestine in mice which the dose with infected dose. There is a variation in the length of the villa and the loss of some of the surface epithelial cells. The original plate is not distinct in terms of content. Most epithelial cells are separated or decomposed and their residues are present in the intestinal cavity. The cavernous cells appear large and there is a thin layer of mucus on the villa surface The outer muscle layer appears separated from the sub-mucous layer by a secrete separation. The circular and longitudinal muscle layers are separated from each other and secondary, appearing irregularly as shown in picture (2).

3-Lethal -dose LD-50

Large intestine:

The results of the histological examination of the large intestine showed the damage caused by the bacteria. The villa appear irregular, sometimes appear short, and the epithelial cells are not distinct, The cytoplasm appears as a continuous piece without a dividing line between the cells, and the senescence passes through the various stages of decomposition, the large and large number of coliform cells, the mucus is more rigid, the pigment appears darker than it is in the natural state. Bleeding is in the original plate and the subcutaneous layer is necrotic. Bleeding is in the original plate and the subcutaneous layer is inflated. The mucosal layer is clearly visible and the intestinal glands. The outer muscle layer is thin, separated from the subcutaneous layer. The longitudinal muscle layer is not distinguishable from the circular muscle layer and the serosa layer. It is also separated from the external muscular layer as in the picture. The histological changes infective and lethal dose causes the fact that the bacteria *Shigella* have the ability to cross the defenses of the host body, including acidity of the stomach, mucous cells and the movement of the intestinal tract [21], We also agree with [22] that the bacteria *Shigella flexneri* have the ability to pass the acidity of the stomach and the invasion of epithelial cells and multiplication within these cells producing the bacterial toxins affecting the mucous layer of the intestines. Diarrhea may be due to the proliferation of bacteria inside the epithelial cells and their secretion of poisons Which requires that the diarrhea of the host body as a defensive means of defense to get rid of the agent causing the damage as shown in picture(3).

Therapeutic dose

4 - group of mice dose with Infected dose and treated with water extract

Large intestine:

The results of the histological examination of the large intestine treated with the water extract of the denture showed improvement in the structural system of the layers of the large intestinal wall, Where the villa appear more stable and the original plate is regular in terms of contents and nucleus is clear, , As for the outer layer of circular and longitudinal muscle is regular and closer to the natural state and surrounded by the serosa layer consisting of one row of squamous cells and as shown in the image (4). The reason for this improvement to the nature of the **Antheum graveolens** components of the and its unique therapeutic properties and ownership of many materials that Act as antibacterial and viral antibodies that have the potential to inhibit and kill pathogenic microorganisms [23]

5 - group of mice dose with Infected dose and treated with alcohol extract

Large intestine:

The results of the histological examination of the large intestine in the group of mice treated with the alcoholic plant extract showed A pretty good improvement ,Where the outer muscle layer is distinct and consists of two layers of longitudinal and circular. The nuclei are clearly shown with very little cellular decomposition features, surrounded by the serous layer and the subcutaneous layer are clear and characterized by the large number of epithelial cells and villa damage is shown in the(5). The reason to the fact that the alcohol dissolves all the active substances in the plant faster than in the water extract, leading to the concentration of the active substances and more , And may be due to the duration of the treatment led to the emergence of the side effects of this extract rather than the emergence of the improvement of the tissues of tissue layers forming the wall of the intestine.

6-group of mice dose with Infected dose and treated with antibiotic treatment Amikacin

Larg intestine:

The results of the histopathological examination of the large intestine of the antibiotic-treated mice group showed a good improvement, showing the improvement in epithelial cells The superficial and sides borders are arranged on the surface of the broad villa, Crypts of Librokhn be few, and the mucous membranes are numerous and full of mucus due to the animal's need for mucus to stand out, The external longitudinal muscle layer is less

diametrically than the external circular muscular layer and the nuclei are regular We did not notice any separation of this layer from the subcutaneous layer as shown in Figure 7. This indicates the role of the antibiotic in killing the bacteria or inhibition of the enzymes necessary for the multiplication of the bacteria. The appearance of the tissue lesions may be due to the fact that the treatment period is short and the number of toxins secretion [24].

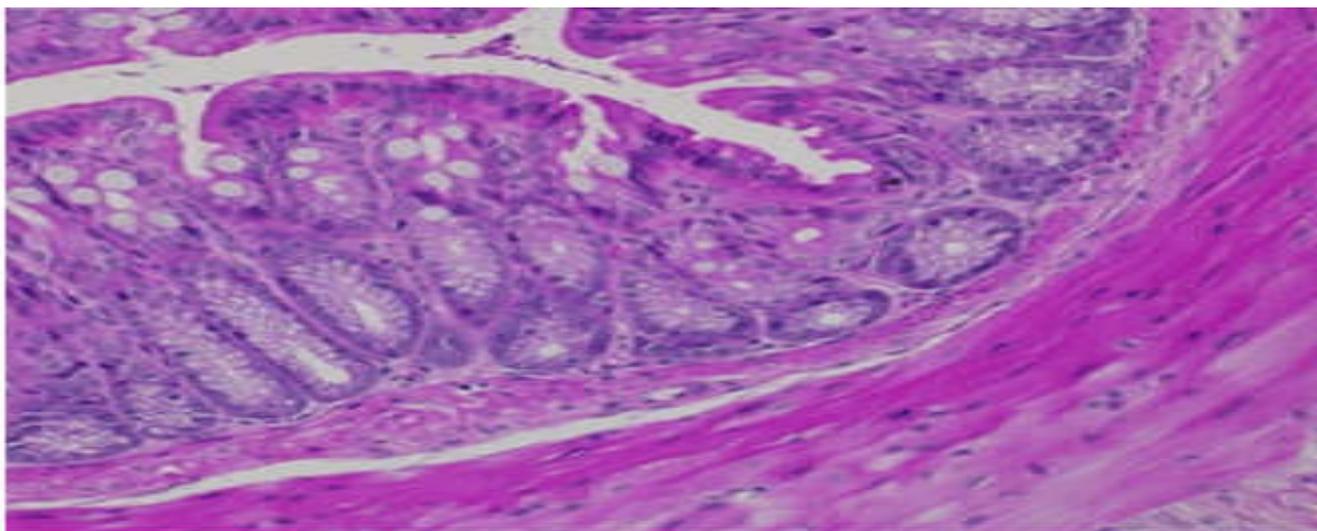


Fig (1) Large intestine for the control group with 40x strong and dye H and E

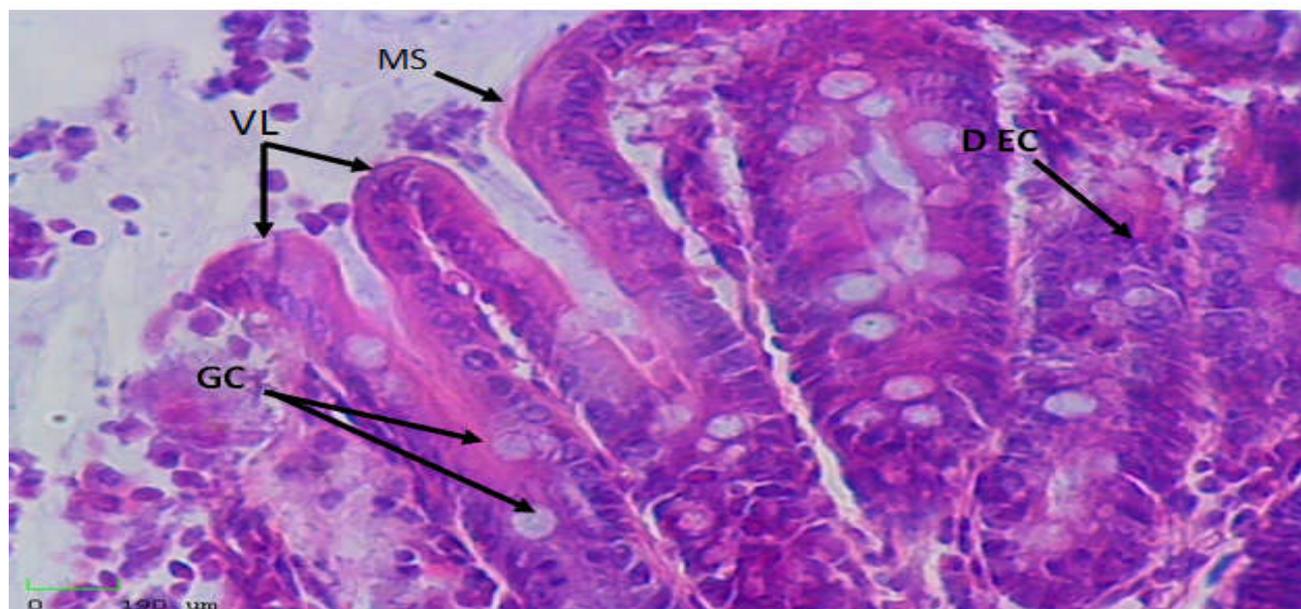


Fig (2) Decomposing epithelial cells DEC and increasing the cells of the Goblet cell GC and mucus cells MS on the surfaces of short villi in the large intestine of the pathogenic group

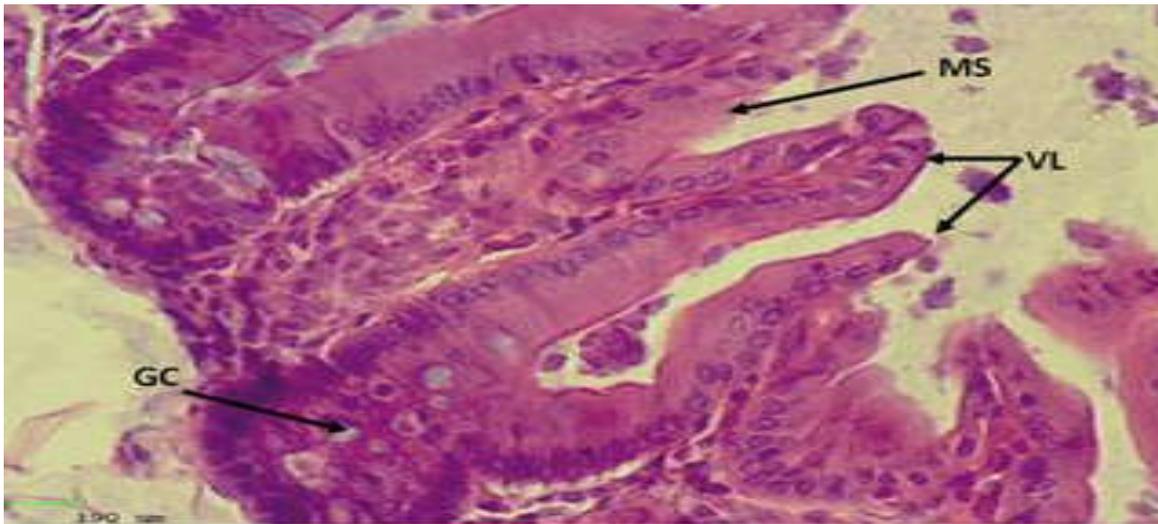
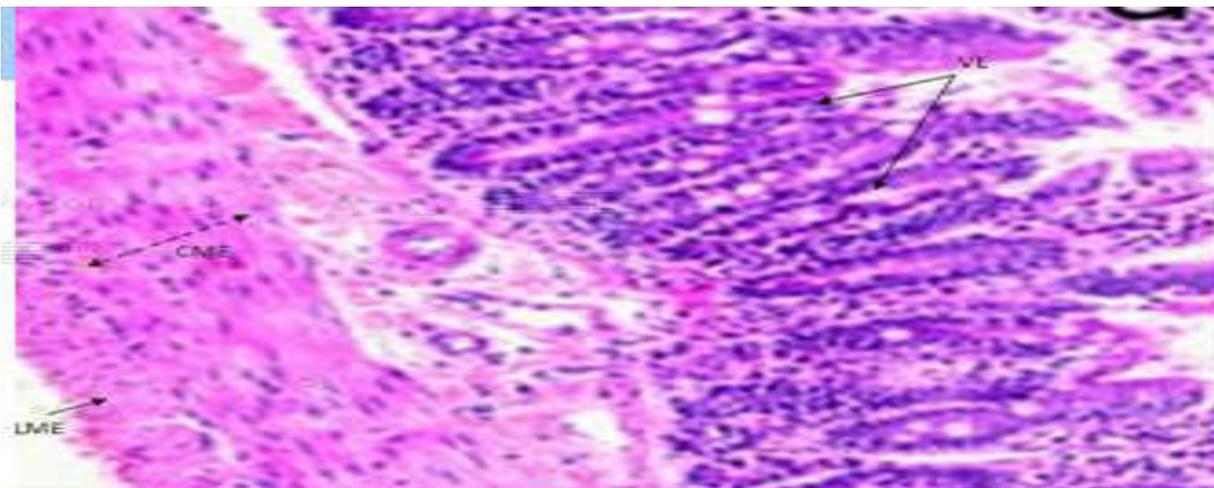
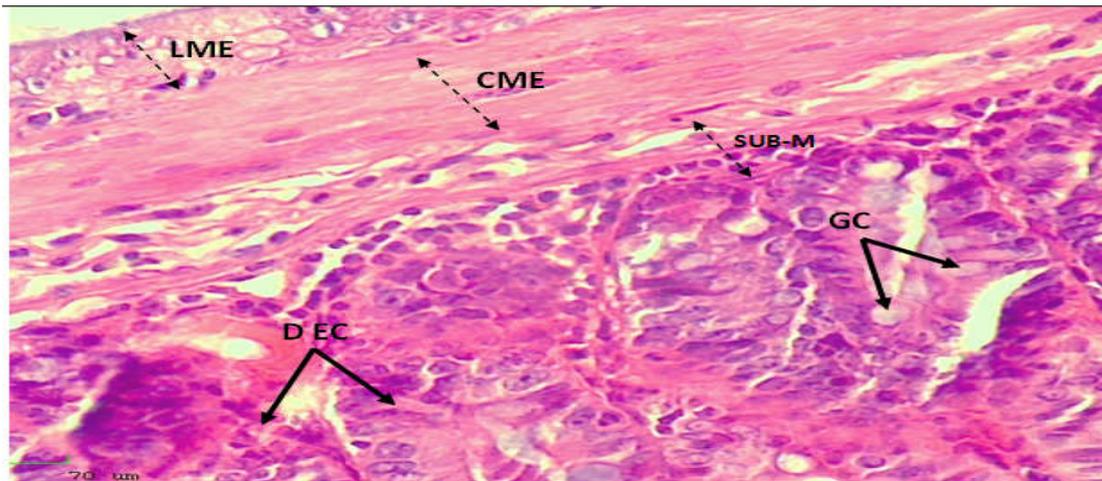


Fig (3) The lack of boundary between epithelial cells DEC in the villa , Goblet cell GC and mucous membranes MS in the large intestines of the group LD-50



Fig(4) the Villa and the muscle layer of the large intestine of the mice treated with the water extract of Anthemum graveolens



Fig(5) Muscular cell, subcutaneous cells, Goblet cell, degeneration of epithelial cells of the large intestine, treatment with alcohol extract of *Antheum graveolens* 400x

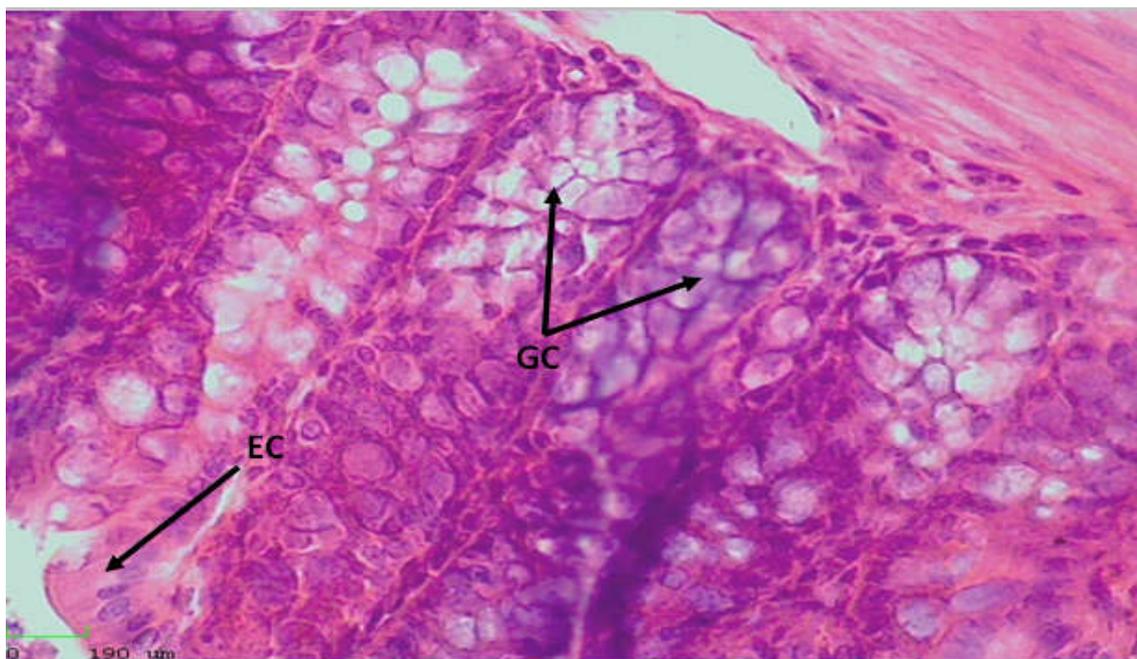


Fig (6) Goblet cell and epithelial cells in the large intestines of the group of mice treated with antibiotics Amikacin 400X /

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