



Antibacterial activity of ethanolic extract of Sweet Basil (*Ocimum basilicum* L.) leaves against *Escherichia coli* in experimentally infected Rats

Sarhan R. Sarhan* Orooba M.S. Ibrahim**

*College of Veterinary Medicine, Wasit University

**College of Veterinary Medicine, Baghdad University, Iraq

E-mail: dr_surhan2000@yahoo.com

Abstract

The present study was carried out to investigate the antibacterial activity of *Ocimum basilicum* (OB) ethanolic extract in-vivo by inducing diarrhea in rats which was caused by oral administration of pathogenic *Escherichia coli*. The experiment that performed in this present study included study in-vivo antibacterial activity of the extract after inducing infectious diarrhea with oral pathogenic *E. coli* in five rats groups (eight rats of each). Two doses of O.B extract 100, 200 mg/kg. BW was used to treat this infection for fourteen days orally which compared with group E that treated with Trimethoprim/sulfa at dose 6.85mg/kg BW. The yielding percentage of *Ocimum basilicum* 95% ethyl alcohol leaves extract was 13%. Phytochemicals analysis indicated the presence of alkaloids, phenols, tannins, saponins, flavonoids, steroids and terpenoids, while glycosides were absent, but Alkaloid, phenols, saponins and tannins were seemed to be found in high levels in the crude extract. Biochemical analysis of serum albumin showed different results; Albumin values returned to normal values during treatment with *Ocimum basilicum* leaves extract dose of 200 mg/kg BW and Trimethoprim/sulfa at dose 6.85mg/kg BW also these two kinds of therapy returned intestinal secretory nearly to normal levels after treatment for seven days in comparison with other groups, while a dose of 100 mg/kg BW of OB extract showed a little decrease in albumin concentrations after 7 days of treatment. From the results obtained, it could be concluded that ethanolic extract of O.B leaves at dose 200 mg/kg BW was more effective and safe in comparison with antibacterial agent and other O.B dose, this antidiarrheal activity of O.B ethanolic extract may be due to its constituents of secondary metabolites that are responsible for the antibacterial activity with different mechanisms of action.

Key words: antimicrobial activity _ *Escherichia coli* _ *Ocimum basilicum* _ Diarrhea

التأثير المضاد البكتيري للمستخلص الكحولي لأوراق نبات الريحان ضد الايشيريشيا القولونية في الجرذان المصابة تجريبيا

سرحان راشد سرحان جاسم* عروبة محمد سعيد ابراهيم**

*كلية الطب البيطري، جامعة واسط
كلية الطب البيطري، جامعة بغداد، العراق**

الخلاصة:

أجريت هذه الدراسة لتحديد الفعالية المضادة للبكتيريا للمستخلص الكحولي لأوراق نبات الريحان في الجسم الحي (*in-vivo*) بأحداث الأسهال في الجرذان المختبرية بواسطة عزله محليه من الأيشيريشيا القولونية (*E. Coli*) تجريبيا في خمس مجاميع من الجرذان المختبرية (ثمانية جرذان لكل مجموعة) فمويا، وقد استعملت جرعتين من المستخلص (100، 200) ملغم/كغم لعلاج هذه الإصابة في المجاميع C , D على التوالي ولفترة أربعة عشر يوما - فمويا، لقد قورنت فعالية هذه الجرعة المختلفة مع المجموعة التي عولجت بالمضاد الجرثومي الترايمثوبريم/السفا بجرعة (6,85) ملغم/كغم لفترة أربعة عشر يوما- فمويا، أظهرت نتائج الإستخلاص بواسطة جهاز السكسوليت، أن نسبة المستخلص الكحولي (95%) لأوراق الريحان كانت (13%)، في حين أظهرت نتائج التحليل الكيميائي وجود مركبات الأيض الثانوي التاليه في المستخلص الكحولي : الفينولات، التانينات، الصابونينات، الفلافونيدات والستيرويدات والتيربينويدات. بينما لم تظهر الكلايكوسيدات في التحليل الكيميائي. الفينولات، الفينولات، الصابونينات والتانينات ظهرت بصورة كبيرة في المستخلص الكحولي. إن التحليل الكيميائي الحيوي للألبومين قد اظهر نتائج متباينه، حيث إن جرعة (200) ملغم/كغم من المستخلص أو 6,85 ملغم من الترايمثوبريم/السفا أعادت الألبومين إلى القيم الطبيعية خلال الأسبوع الأول من العلاج، كذلك فإن هاتين الجرعتين قد أعادت الإفرازات المعويه إلى المستوى الطبيعي في الأسبوع الأول من العلاج بينما المجاميع الأخرى تطلب أربعة عشر يوما. في حين اظهر العلاج بجرعة 100 ملغم/كغم من المستخلص الكحولي انخفاض طفيف في القيم خلال فترة العلاج. مما سبق يمكن الاستنتاج بأن العلاج بالمستخلص الكحولي لأوراق الريحان بجرعة 200 ملغم/كغم كان أكثر فعالية وسلامة مقارنة بالمضاد البكتيري أو بالجرعة الأخرى من المستخلص الكحولي، أن هذه الفعالية المضادة للأسهال للمستخلص الكحولي لأوراق الريحان ربما تعود لوجود مركبات الأيض الثانوي في المستخلص والتي تكون مسؤولة عن التأثير المضاد للبكتيريا وبآليات عمل مختلفة

Introduction:

Diarrheal diseases are major problem in third world countries which are responsible for death of millions of people and animals each year, diarrhea is an alteration in normal bowel movement and it is characterized by an increase in the water content, volume, or frequency and decrease of dry matter of feces ^(1,2,3). Diarrhea are associated with loss of electrolytes and fecal matter, decreased fluid absorption or increased fluid secretion can lead to dehydration, it can be either acute or chronic ⁽⁴⁾. Diarrhea accounts of 46% of calves and lambs mortality ⁽⁵⁾. The most common causes of acute diarrhea are bacterial and viral infections ^(6,7). Rota virus is responsible for causing severe diarrhea which leads to gastroenteritis ⁽⁸⁾. Bacterial causes like; *Escherichea coli*, *Staphylococcus aureus*, *Sallmonella* and *Vibrio cholera*, also parasitic infection especially protozoa can lead to sever acute diarrhea which is responsible for the high levels of mortality and morbidity in humans and animals ^(6,9). Infections with *Escherichea coli* being one of the major causative agents ⁽¹⁰⁾. *E. coli* infections occur all over the world and affect all farm animals in all ages, it can cause white scour in new born calves and to less extent diarrhea in lambs, kids etc. and septicemic colibacillosis in lamb, foals, calves and kids ⁽¹¹⁾. Calf mortality has been reported to be very high in cow and buffalo neonates ⁽¹²⁾. According to a survey on mortality rate in calves vary from 2% to 20% due to diarrhea in advanced countries. In Iraq ⁽¹³⁾ noticed after study included 100 fecal specimens collected from animals suffering diarrhea from different areas of

Baghdad, *E. coli* was isolated from 68 specimens. The highest infection rate with *E. coli* was recorded by ⁽¹⁴⁾ in Costa Rica, it's was 94% from calves suffering diarrhea. Colibacillosis associated with *E. coli* occurs in all species of new born farms animals and it is a major cause of economic loss in this age. It is a major cause of diarrhea in calves, piglets and lambs and it cause diarrhea particularly in calves less than 30 days of age ⁽³⁾. Resistance to the antimicrobial agents is recognized as a major global public health problem, infectious diseases are for approximately one-half of all cases of death in different beings ⁽¹⁵⁾. For these reasons, international organizations including the ⁽¹⁶⁾ have encouraged studies pertaining to treatment and prevention of diarrheal diseases using traditional medical practice ⁽¹⁷⁾, it is possible that antimicrobial compounds from plants may inhibit bacteria by a different mechanism than the presently used antibiotics and may have a clinical value in treatment of resistant strains ⁽¹⁸⁾. Plants have long been a very important source of new drugs, many plant species have been screened for substances with therapeutic activity and for those medicinal plants is a promising source of antidiarrheal drugs ⁽¹⁹⁾. Therapeutic value of plants used in trade medicine derives from the presence of phytochemicals principles (secondary metabolites) which are found in all parts of plant such as alkaloids, tannins, flavonoid, saponins and phenols ⁽²⁰⁾. One of the most important medicinal plants is *Ocimum basilicum* (Sweet basil), which is a seasonal plant belongs to the family of *Lamiaceae*. *Ocimum* plant is an important medicinal

plant as it is a well known source of different phytochemicals (secondary metabolites), it is distributed throughout most of the world and it is abundant in Iraq. *Ocimum basilicum* is used as an antioxidant, antiseptic, preservative, sedative, digestive regulator and diuretic. It is also recommended for treatment of headaches, coughs, infections of upper respiratory tract, kidney malfunction and to eliminate toxins⁽²¹⁾. *O. basilicum* is also used in the treatment of a number of ailments like bronchitis, rheumatism and pyrexia⁽²²⁾. Also *Ocimum* plant rich in secondary metabolites which have antibacterial activity by different mechanisms⁽²³⁾. Therefore this study was presented to evaluate the antibacterial activity of ethanolic extract of *Ocimum basilicum* against *E. coli* as well as treatment of diarrhea and avoiding resistance to antimicrobial agents. These were accomplished by studying the in-vitro antibacterial activity of plant extract and in-vivo through treatment of experimentally infected rats with *E. coli* comparing with different antibiotics. As well as overcome the resistance to antibacterial drugs.

Materials And Methods

Test organism: Pathogenic *E. coli* isolate was obtained from the College of Veterinary Medicine/Department of Internal and Preventive Medicine/University of Baghdad. This isolates spp. was identified by studying morphological and some biochemical characteristics.

Plant Materials: Fresh *Ocimum basilicum* leaves were purchased from a local market in Baghdad during May to August 2010. Later these plant leaves were washed under

tap water, and then dried in room temperature at shade. The dried leaves were crushed to a fine powder by an electrical grinder. The plant classification was done in the Ministry of Agriculture/ State Board for Seeds Testing and Certification S.B.S.T.C in Abu Graib /Baghdad at certificate No. 3670 in 28 / 11 / 2010.

Preparation of Crude Organic Solvent Extract of *Ocimum basilicum* Plant:

Organic solvent extraction of the *Ocimum basilicum* leaves was carried out by using ethanol (95% ethyl alcohol) which is considered as very effective in extracting the active ingredients of the plant according to method described by ⁽²⁴⁾ This was done by using Soxhlet apparatus, 50g of plant leaves powder was put inside the thumble and 500 ml of 95% ethanol was put inside the flask. The extraction was carried out for 24 hours by heating temperature that kept the solvent at 50-60 C° until a clear and colorless solvent appeared in the extracting unit. After that, the extract was dried by using an electric oven at temperature 40-45 C° until dry extract was obtained. The dry extract was placed in an incubator under 38-40 C° for complete dryness of the sample .The final extract was kept frozen at – 20 C° until use.

Experimental Animals: Forty eight male Wister albino rats about three months of age and with body weight ranged between 190-210g were used to perform the experiment of the present study. Rats were housed in plastic cages 20×50×75cm dimension, placed in a special housing room belongs to the Department of Physiology and Pharmacology / College of Veterinary Medicine for two weeks for adaptation. Standard rodent diet (Commercial feed pellets) and tap water were freely available. Housing condition were maintained at 20-25 C° in air-conditioned room, the air of the room

was changed continuously by using ventilation vacuum, while the light/dark cycle was 14/10 in housing place. The litter of the cages was changed weekly.

In-vivo antibacterial activity of ethanolic extract of *Ocimum basilicum* in rats

Challenge Bacteria: Pathogenic isolate of *Escherichia coli* was obtained by Department of Internal and Preventive Medicine in College of Veterinary Medicine/Baghdad university from a calve suffering from diarrhea; it was used as a challenge strain.

Inducing Infection (Diarrhea): After pilot study, the challenge dose which induced infection (acute diarrhea) was 2.5×10^6 cfu/ml of *E. coli* suspension, the inoculums preparation-standardize according to viable counting method-pour plate technique by using serial ten-fold dilutions. 0.5 ml was orally administered into the rats and watched for symptom of diarrhea. The dilution that established infection in the rat which showed by the symptoms was used as infectivity dosage for the rats throughout the infection ^(11,25). Overnight culture (24 h at 37 C°) in brain heart infusion broth dilution of 0.5ml was given with a gavages stomach tube to each rat of infected groups.

Preparation of Different Concentration of *Ocimum*: Stock solutions were prepared by mixing 1g, 2g from dried extract with 10 ml of 50% DMSO separately, it was filtered

through whatman (No.1).to prepare the concentrations of 100 and 200 mg/ml respectively. These concentrations were used for daily dosing of treated groups.

Experimental Design: Forty rats were divided equally into five groups, eight rats in each group (Treatment begin after 24hrs. after inducing infection). Group(A): positive control (infected and not treated group), Group(B): negative control (not infected group which given only 50% DMSO orally for 14 days, Group(C): infected and treated orally with 100 mg/kg B.W of ethanolic extract of *Ocimum basilicum* for 14 days, Group(D): infected and treated orally with 200 mg/kg B.W of ethanolic extract of *Ocimum basilicum* for 14 days, Group(E): infected and treated orally with 6.85 mg/kg B.W of Trimethoprim /Sulfamethoxazole at concentration 1.35mg/ml for 14 days. The experimental Durations were as follows: One week before inducing infection. Two weeks of treatment after 24hr inducing infection.

Blood Collection: Blood collection was done at zero time in the first week (before inducing infection), after 7 days of treatment and after 14 days of treatment of the experiment. Animals were anesthetized by I.M. injection of ketamine 90 mg/kg B.W and Xylazine 40 mg/kg B.W. Blood samples were obtained via cardiac puncture technique from each anesthetized animals using 1 ml disposable syringe from eight animals in each group. Blood samples were collected in test tubes with no anticoagulant that

allowed standing and coagulating. Serum was separated from coagulated blood samples by centrifugation at 3000 round per minute (rpm) for 5 minutes and then serum samples were stored in a freezer at -8 °C till use for albumin test.

Clinical Signs: Clinical signs were checked continuously for color, consistency of feces and development of diarrhea in infected groups, also any change in activity, behavior, death rate and rectal temperature of the animals were recorded weekly throughout the experiment.

Albumin Test: Albumin liquicolor Kit that made by (Human company/Germany) was used to determine the concentration of serum albumin, the Kit contain one reagent (Bromocresol green 30mmol/l + citrate buffer 260 umol/l, pH 4.2). and Standard (Albumin 4g/dl + Sodium azide 0.095%). Bromocresol green (BCG) forms with albumin in citrate buffer a colored complex. The absorbance of this complex is proportional to the albumin concentration in the sample. The procedure was done according to kit instructions.

Determination of intraluminal fluid accumulation: Intraluminal fluid accumulation was determined by the method of ⁽²⁶⁾. Rats were sacrificed before infection; a day after infection and at the end of 7 and 14 days of treatment, the small intestine was removed after tying the ends with threads and weighed. The intestinal

content was collected by milking into a graduated cylinder and their volume was measured. The intestine was reweighed and the difference between the full and empty was calculated. This was done in order to know the effect of infection and possible effect of extract and antibacterial agent on intestine secretary.

Gross pathological Examination: At the end of the experiment, three animals from each group were sacrificed by injection of a high dose of ketamin hydrochlorid and post mortem examination was done for animals, the macroscopic appearance was recorded to detect any abnormal gross change in the small intestine.

Statistical Analysis: Data were analyzed statistically using the Microsoft Program (SPSS). Statistical analysis of data was performed on the basis of Two-Way Analysis of Variance (ANOVA) using a significant level of ($P < 0.05$). Specific group differences were determined using least significant differences (LSD) as described by ⁽²⁷⁾.

Results And Discussions

Extraction of *Ocimum basilicum*: Extraction of *Ocimum* leaves with 95% ethanol gave a deep green color extract with plant powder yield percentage of 13 % , this was determined by using the following equation: Percentage yield of the extract = weight of extract (gm) / weight of *Ocimum* powder (gm) \times 100.

=13 (gm) / 100 (gm) \times 100 =13% ⁽²⁸⁾. This result is almost similar to the results of ⁽²⁹⁾ who found that the percentage recovery of ethanolic extract was 10.6 %w/w from fine *ocimum* leaves powder which was extracted by using a soxhlet apparatus. The near similarity in yield percentage may be attributed to the same solvent which was used in the present extraction.

Detection of phytochemical components in *Ocimum basilicum* leaves powder and its extracts: The plant leaves powder screening showed the presence of the following phytochemicals; alkaloids, phenol, saponins, flavonoids, tannins and glycosides, while steroids, terpenoids did not exist in plant powder. The detection of phytochemicals in ethanolic extract gave an evidence of existence of the following components; alkaloid, phenols, steroids, saponins, flavonoids, tannins and terpenoids, while glycosides were absent. Alkaloid, phenols, saponins and tannins seemed to be found in a high level in crude extract. ⁽³⁰⁾ referred that the initial screenings of plants for possible antimicrobial activities typically begin by using crude aqueous or alcoholic extractions and it can be followed by various organic extraction methods. Since nearly all of the identified components of the plant which are known by their activity against microorganisms are aromatic or saturated organic compounds, they are most often obtained through ethanol or methanol extraction. The presence of

alkaloids, phenols, steroids, tannins and terpeoids in 95% ethanolic extract was confirmed by a previous finding of ⁽²⁹⁾. ⁽³¹⁾Supported phytochemical screening of the current study when he found the presence of alkaloids, saponins, tannins and flavonoids in *Ocimum basilicum*.

Identification of *E. coli*: The strain had a smooth circular pink colony on MacConkey agar and displayed wide hemolytic zones around the colonies on Blood agar and a metallic green sheen on Eosin Methylene Blue agar. The challenge bacteria were the same strain which was used in in-vitro antibacterial activity assay.

Physiological Changes:

Clinical Signs: Before induction of infection, healthy animals presented normal feces; solid molded, rough with dark brown color, while after inducing infection animals, were suffering from anorexia, dehydration, little fever or no fever and the feces was unmolded-wet loose or liquid appearing and light brown in color table (1); the frequency of diarrheal feces increased gradually from the first day after infection. All the animals exhibited clinical signs of abnormal behavior like; calmness, less mobile and curled up. The animals of group C which received 100 mg/kg B.W of ethanolic extract of *Ocimum basilicum* for 7 days did not show complete recovery at the end of 7 days of treatment and clinical signs were relatively mild until 14 days of treatment the signs began subside, while the animals of group D which

dosed with 200 mg/kg B.W of ethanolic extract of O.B and group E which dosed with 6.85mg/kg B.W of Trimetoprim /Sulfamethoxazole for 7 days exhibited faster recovery. Group A (+ve control) untreated group showed; severe diarrhea, dyspnea, emaciation, rough body coat, poor body weight gain and reduced elasticity of skin indicating dehydration. Morbidity (infection) rate was 100% in infected groups, the highest recorded mortality rate was 75% in group A (+ve control) along the period of experiment while groups C showed fluctuating rates of mortality, groups D and E did not lose any animals during the period of experiment, as showed in table (2). ⁽³²⁾ reported in rat that infectious diarrhea with *E. coli* gave clinical signs developed in steps began with production of thin and watery feces, fever, loss of appetite, signs of dehydration appear (sunken eyes, dry mucus membranes, rough hair), unable to rise body, loss of consciousness, dehydration and death can be less than 24 hours. During diarrhea, large amounts of water and electrolytes are lost from the body. Water moves from the extracellular fluid (the blood and the interstitial space between cells) into the intestinal lumen. *E. coli* infections are mostly tend to cause hypersecretory diarrhea with rapid and severe water loss. Speed with which dehydration occurs during these infections may not provide enough time for the lungs to compensate for the rapid onset of acidosis ⁽³³⁾.

Table (1): Fecal characteristics of Rats In different groups infected and treated with different doses of ethanolic extract of *O.B* and Trimethoprim/Sulfamethoxazole or kept without treatment. Group rat no. = 8. +ve control: infected not treated group, –ve control: not infected, not treated group

Period and rates Group	Week before infection		24 hrs after inducing infection		After 7 days of treatment		After 14 days of treatment		Total Mor. Rate
	Infection rate	Mort* rate	Infection rate	Mort. Rate	Infection rate	Mort. rate	Infection rate	Mort. rate	
G A +ve control	–	–	100%	–	100%	50%	100%	25%	75%
G B –ve control	–	–	–	–	–	–	–	–	–
G C: O.B extract 100mg/kg	–	–	100%	–	100%	25%	100%	–	25%
G D: O.B extract 200mg/kg	–	–	100%	–	100%	–	100%	–	–
G E: Trim/Sulfa. 6.85 mg/kg	–	–	100%	–	100%	–	100%	–	–

Table(2) : Infection rate and mortality rate of rats in different groups infected and treated with different doses of ethanolic extract of *O.B* and Trimethoprim/Sulfamethoxazole or kept without treatment during the course of experiment.

Period Group	Week before infection			24 hrs after inducing infection			After 7 days of treatment			After 14 days of treatment		
	Color	Texture	Remark	color	Texture	Remark	color	Texture	Remark	color	Texture	Remark
G A +ve control	Dark brown	Sold molded	–	Light brown-yellow	Wet lose feces	+++	Light brown-yellow	Wet lose feces	++	Light brown	Soft and moist	+
G B –ve control	Dark brown	Sold molded	–	Dark brown	Sold molded	–	Dark brown	Sold molded	–	Dark brown	Sold molded	–
G C: O.B extract 100mg/kg	Dark brown	Sold molded	–	Light brown-yellow	Wet lose feces	+++	Light brown	Soft and moist	++	Dark brown	Sold molded	–
G D: O.B extract 200mg/kg	Dark brown	Sold molded	–	Light brown-yellow	Wet lose feces	+++	Light brown	Sold molded	+	Dark brown	Sold molded	–
G E: Trim/Sulfa. 6.85 mg/kg	Dark brown	Sold molded	–	Light brown-yellow	Wet lose feces	+++	Light brown	Sold molded	+	Dark brown	Sold molded	–

Group rat no. = 8 +ve control: infected not treated group,
–ve control: not infected, not treated group.* = Mortality

Serum albumin: The values of albumin for the five groups were gm/dl for groups A-E respectively, as showed in table (3). There were no significant differences ($P<0.05$) between the five groups before infection. The second step of measuring of albumin levels was done after 24 hrs of inducing infection. Results showed a significant decrease ($P<0.05$) in albumin concentration in all infected groups, the concentration decreased significantly ($P<0.05$) in five challenged groups in the same time when they were compared with group B (-ve control), also there was a significant decrease in albumin concentration of infected groups when they were compared with albumin concentration before inducing infection, just group B did not show a significant change in its albumin concentration through the experiment period. There were no significant differences ($P<0.05$) between infected groups after 24 hrs of inducing infection. These results are in agreement with ⁽³⁴⁾ which discriminated between the effects of infection and of anorexia associated with infection by *E. coli*. ⁽³⁵⁾ Referred that hypoalbuminaemia is one of the most common and dramatic events that characterizes the metabolic response to infection in humans and animals. Moreover, injury is associated with malnutrition, essentially due to a reduction in the intake of nutrients and

calories. One of the most important factors in the regulation of albumin synthesis is the nutritional state. Protein-free diets are associated with depleted plasma albumin levels and reduced albumin synthesis in rats ⁽³⁶⁾. Studies in humans were conducted at only one stage of the inflammatory process, mostly during the acute phase, and it is possible that albumin synthesis varies with time following injury, as shown in a rat model of peritonitis ^(37,38). In the present study, animals were treated for up to 14 days after infection. During this period, infected animals presented a rapid decrease in albumin concentration and persistent hypo-albuminaemia in group A (+ve control) compared with group B (-ve control). Albumin synthesis was always significantly lower in infected rats than in control animals. After treatment, group D and E showed significant increases ($P<0.05$) in albumin concentration, and returned to normal values at the first week of treatment, while the other groups showed a little change in albumin concentration after seven days of treatment. These results gave good evidence about the suitable therapeutic dose of plant extract that can be used as an antibacterial agent against *E. coli*. These findings are supported by the results of other in-vivo and in-vitro studies which referred to a high potency of different ethanolic extract concentrations of *Ocimum basilicum* plant against *E. coli*.

table (3): Albumin concentration (gm/dl) values of rat in different groups infected and treated with different doses of ethanolic extract of *O.B* and Trimethoprim/Sulfamethoxazole or kept without treatment during the course

Group \ Period	Week before infection	After 24 hrs. inducing infection	After 7 days of treatment	After 14 days of treatment
G A +ve control	3.80±0.36 A a	2.80±0.59 B b	1.40±0.74 E c	2.42±0.63 E d
G B -ve control	3.80±0.73 A c	3.85±0.51 A c	3.83±0.51 A b	3.82±0.41 A a
Gc: O.B extract 100mg/kg	3.80±0.43 A a	2.86±1.10 B d	2.94±0.20 D c	3.60±0.96 C b
G D: O.B extract 200mg/kg	3.80±0.23 A a	2.82±0.33 B c	3.78±0.078 C b	3.82±0.81 B a
G F: Trim/Sulfa. 6.85 mg/kg	3.80±0.31 A a	2.85±0.71 B b	3.82±1.02 C a	3.81±1.04 B a

of experiment.

Values represent mean ±S.E, Group rat no.= 8.

Different small letters means significant ($P < 0.05$) results between periods.

Different capital letters means significant ($P < 0.05$) results between groups.

+ve control: infected not treated group, -ve

control: not infected, not treated group

Interluminal Fluid Accumulation:

Inducing infection in different groups lead to increase in intestinal secretory in rats. The effect of infection and treatment on fluid accumulation (ml) and weight of intestinal contents (gm) are listed in table (4). The results showed a significant increase ($P < 0.05$) in fluid accumulation during after inducing infection in all five infected groups in comparison with group B (-ve control) at the same period of the experiment. After

seven days of treatment, groups D and E showed a significant decrease ($P < 0.05$) in fluid accumulation, While groups C did not show important or little intestinal secretory decrease in comparison with group B (-ve control), while treated groups after 14 days of treatment had less significant in intraluminal fluid accumulation in comparison with seven days of treatment and they began to return to normal content. The pathogenic *E. coli* species comprise a very versatile group with numerous virulence determinants (virulence factors) including adhesins, invasins, toxins and secretion systems that allow them act as causative agents in both human and veterinary medicine^(39,40,41).

ETEC strains cause cholera-like watery diarrhea through the elaboration and action of LT (heat-labile) and/or ST (heat-stable) enterotoxins or both. The ability of ETEC strains to produce diarrheal illness by either or both of these enterotoxins is what defines an ETEC. There are two types of LTs, LT-I and LT-II that are commonly found in human and animal isolates. However, the term LT refers to LT-I, which is associated with disease in both humans and animals, while LT-II is expressed only in animals, but it is rarely associated to disease^(42,39). LT-I is constituted by ~80% amino acid identity with cholera toxin that consists of a single A subunit and five identical B subunits. The A subunit is responsible for the enzymatic activity, and the B subunits are responsible for the toxin binding to the cell surface. After endocytosis the A subunit stimulates a series of intracellular processes leading to an increased level of cyclic adenosine monophosphate (cAMP), resulting in an increased phosphorylation of chloride channels, and hence a reduced absorption of NaCl. This increased extracellular ions content results in osmotic diarrhea. Besides, there is an increased secretion of ions in the villi crypts⁽³⁹⁾. STs are small peptides including two unrelated classes, STa and STb, which differ in structure and mechanism of action. Only toxin of the STa class has been associated with human and animal diseases. It was established that the STa receptor is located on the apical surface of enterocytes and that binding to the receptors leads to an increased intracellular cyclic guanine monophosphate (cGMP) levels, which affects the electrolytic balance in a similar manner as LT^(39,41). Series of studies on the resistance of *E. coli* which were isolated from animals and humans have strongly suggested that those bacteria which are resistant to antimicrobials used in

animals would also be resistant to antimicrobials used in humans^(43,44,45). Therefore, the search for safe, effective chemotherapeutic drugs is one of our interesting. So that why we chose the *Ocimum basilicum*, is one of the medicinal plants that have antimicrobial effect. Probably extract increased the reabsorption of sodium chloride and water by decreasing intestinal motility as observed by a decrease in intestinal transit by charcoal meal suggesting its sympathomimetic activity of *Ocimum basilicum*, these results were confirmed by⁽⁴⁶⁾ who confirmed that the leaf extract of *Ocimum gratissimum* also inhibited the intestinal motility caused by contact of the tissue with acetylcholine, a substance that was known to contract smooth muscles of ileum. Recent findings by⁽⁴⁷⁾ showed that the essential oil of *Ocimum gratissimum* reversibly and concentration-dependently reversed the tonic contractions induced by acetylcholine. Therefore, the relaxant property of *Ocimum gratissimum* extract portrayed in this study may be ascribable to these essential oils. On the other hand, this relaxant activity was similar to that observed with adrenaline and noradrenaline. The antidiarrheal activity of the extract may also be due to the presence of denature proteins forming protein tannates, which make the intestinal mucosa more resistant and reduce secretion⁽⁴⁸⁾. The medicinal value of these plants depends on bioactive phytochemical constituents that produce definite physiological action. Some of the most important bioactive phytochemical constituents include alkaloids, flavonoids, phenolics, essential oils, tannins and saponins steroids and terpenoids⁽⁴⁹⁾. These constituents mediated the antidiarrheal activity of the leaves extract of *Ocimum basilicum*. The overall possible mechanisms may be due to, inhibition of release of autacoids and

prostaglandins thus inhibiting the motility and secretion induced by *E. coli*. The decrease in the intestinal secretory in group (ve control) is in agreement with ⁽⁵⁰⁾ +A (who reported that illness is typically abrupt, but can vary from mild, brief, and self-limiting to a severe disease similar to that seen in *Vibrio cholerae* infection.

Pathological Gross Examination: The gross examination performed to the scarified animals of infected group, intestine lumen showed; edema, congestion, gas production, pitechial hemorrhage, with thickening its wall and hemorrhagic ulceration of intestinal lumen. After treatment, groups D and F, gross pathological findings were returned to normal, While groups C and E did not show important or little intestinal morphological changes in comparison with group B (–ve control), as illustrated in figure (1) The intestinal lumen lesions were on one line with ⁽⁵¹⁾ who pointed that a variety of cytokines possess catabolic activity like tumor necrosis factor (TNF) which thought to be the agent responsible for wasting in

infected animals. *E. coli* toxin can destroy cells in the intestinal tract and, if they enter the bloodstream, can impair or destroy the kidney, heart and the liver. The intestinal damage causes a lot of bleeding which can be lethal. In other cases damage to the kidney, heart and liver can be permanent or even lethal ⁽⁵²⁾. Decreased cell proliferation and increased apoptosis may be the main mechanisms responsible for intestinal mucosal injury ⁽⁵³⁾. In case of absence or incomplete treatment with *Ocimum basilicum* extract, *E. coli* pathologically developed because it possesses colonization factors, which bind bacteria to specific receptors on the intestinal cell membrane, where the organism produces powerful enterotoxins, caused hemorrhagic of intestinal lumen which followed by ulcerative then translocation to different vital body organs.

Table (4): Intestinal contents volum(ml) and Intestinal content weight (gm) in different groups infected and treated with different doses of ethanolic extract of *O.B* and Trimethoprim/Sulfamethoxazole or kept without treatment during the course of experiment.

Period Group	Week before infection		After 24 hrs. inducing infection		After 7 days of treatment		After 14 days of treatment	
	Volume	Weight	Volume	Weight	Volume	Weight	Volume	Weight
G A +ve control	1.37±0.73 A d	2.30±0.39 A c	3.55±0.83 A a	3.90±0.36 B a	3.00±0.18 A b	3.20±0.13 A b	2.00±0.91 A c	2.75±0.18 A d
-ve control	1.37±0.36 A a	2.30±0.73 A b	1.36±0.55 C a	2.31±0.56 C b	1.38±0.35 D a	2.34±0.28 D b	1.36±0.33 C a	2.30±0.13 C b
G C: O.B extract 100mg/kg	1.37±0.22 A c	2.30±1.12 A c	3.51±1.07 B a	3.91±0.22 A a	1.90±0.43 C b	2.60±0.32 C b	1.38±0.43 C c	2.32±0.42 C c
G D: O.B extract 200mg/kg	1.38±0.39 A b	2.33±0.52 A b	3.56±0.22 A a	3.90±0.44 B a	1.40±0.26 D b	2.35±0.91 D b	1.34±0.19 C b	2.23±0.92 D c
G F: Trim/Sulf a. 6.85 mg/kg	1.36±0.45 A c	2.35±0.45 A b	3.56±0.72 A a	3.60±0.80 A a	1.38±0.60 D b	2.30±0.40 D b	1.36±0.59 C c	2.27±0.80 D c

Values represent mean ±S.E, Group rat no.= 8.

Different small letters means significant ($P < 0.05$) results between periods.

Different capital letters means significant ($P < 0.05$) results between groups .

+ve control: infected not treated group, -ve control: not infected ,not treated group



Figure (1): A- Infected Rat Intestine B- Normal Rat intestine



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