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Experimental study for the effect of Trigonellafoenum-graecum (fenugreek) seeds extract on some biochemical and histo pathological study in induced diarrhea in mice by Klebsiella pneumoniae

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Abstract: This study was designed to evaluate the effect of alcoholic extract of Trigonellafoenum-graecum (fenugreek) seeds in induced diarrhea mice by Klebsiella pneumoniawhich isolated from diarrheal cases in children, besides investigation of its effect on some biochemical parameters and histopathological study. Thirty mice were divided in to three groups: Group I: mice were served as a control negative group. Group II: mice were infected with 1.5×10^8 CFU of *Klebsiella pneumonia* orally for one week, two weeks, three weeks and four weeks. Group III: mice were infected and treated orally with 300 mg/kg .BW of alcoholic extract of fenugreek, the period of treatment were one week, two weeks, three weeks and four weeks, at the end of experiment of period, blood samples were taken from all groups and obtained blood serum to estimate the biochemical measurement such as Serum glucose level, serum cholesterol level, Creatinine, Serum uric acid and serum total proteins level. Mice were sacrificed to examine the histopathological changes .The results illustrated significant decrease ($P < 0.05$) in levels of serum blood glucose, serum cholesterol,creatinine and serum uric acid after treated orally with 300 mg/kg .BW of alcoholic extract of fenugreek .However, no significant changes in serum total protein level in infected and treated groups. Histopathology results of the intestine was showed surface mucosal superficial ulceration and damage with inflammatory cells inside the villi in mice infected with 1.5×10^8 CFU of *Klebsiella pneumonia* orally while, Look-like near or normal structure appearance was reported in the intestine after treated orally with 300 mg/kg .BW of alcoholic extract of fenugreek .

***Key words**:* Klebsiella pneumonia; *histopathological; Trigonellafoenum-graecum (fenugreek) seeds*

I. INTRODUCTION

Diarrhea is the second leading cause of childhood deaths (1),and one of the most common causes of morbidity and mortality among infants and children, especially in developing countries (2) .The etiological agents of diarrhea include a wide range of viruses, bacteria and parasites. In 2009 diarrhea was estimated to have caused 1.5 million deaths in children under the age of 5 years (3).Klebsiella pneumoniae is a Gram-negative which belongs to the family Enterobacteriaceae. It is caused human disease associated with urinary tract infections, pneumonia, and subsequent systemic infections, mortality rates is highly as 60%, (4). In human Klebsiella commonly cause gastrointestinal tract infection,(5) .Klebsiella pneumonia is the Multidrug-resistant (MDR) caused by nosocomial infections in worldwide (6) also it is the most common cause of neonatal sepsis in developing countries (7-8). According to WHO, traditional medicines used by 80% of the world population in developing countries,(9).

One the most medicinal plants widely used in folk Medicine are fenugreek ,this plant is used in diarrhea, diabetes, coughs, congestion, bronchitis, fever, high blood pressure, headache, anemia and other uses (10).The objective of the present work is to assessment antibacterial activity of alcoholic extract of Trigonellafoenum-graecum as well as some biochemical parameters and histopathological study in mice experimental infected with Klebsiella pneumoniae.

II. MATERIALS AND METHODS

Isolates was obtained from diarrheal children in Educational Al-karama hospital in Al-kutcity, cultured on blood agar and MacConkey agar, aerobically at 37 ˚C for 24 hours, microscopic examination, culture characteristics.and API 20E System kit.Antibiotic susceptibility testing (AST) for the *K.pneumoniae* bacteria was done using the Vitek 2 systems AST-GN 69.

Seeds of Trigonellafoenum-graecum (fenugreek) were taken from local market, cleaned, washed, dried and then converted to powder by electrical grinder. 50g of the powder was dissolved in 500 ml of 70% ethyl alcohol and placed on magnetic stirrer for 72 hours .The solution filtered through Whitman filter,the filtrate was put in incubator at temperature of 40c° until dryness then the extract was kept in dark glass container at 4 ºC. Agar well diffusion method was used for inhibitory activity of plant extract against Klebsiella pneumoniae after

preparation of different concentrations of plant extract (100,200,300 mg /ml). Extract was tested for detect the presence of active principles such as alkaloid (11), tannins (12), terpenoid, steroid, carbohydrates, flavonoids (12), (13) and saponnins (13).

Induction of infection:Thirty mice were obtained from the national center for drug control and research / Baghdad and divided in to three groups: Group I: Six mice were served as a control negative group. Group II: Twelve mice were infected with 0.25 ml/mice which contain 1.5×10^8 CFU of *Klebsiella pneumonia* orally for one week, two weeks, three weeks and four weeks. Group III:Twelve mice were infected and treated orally with 300 mg/kg .BW of alcoholic extract of fenugreek, the period of treatment were one week, two weeks, three weeks and four weeks. At the end of experiment of period, blood samples were taken from all groups and put in plain centrifuge tube for obtained blood serum to estimate the biochemical measurement such as Serum glucose level (14-15), serum cholesterol level (15),Creatinine (16), Serum uric acid (17) and serum total proteins level (18).Mice were sacrificed to examine the histopathological changes and samples from the lung and intestine were fixed in 10% neutral buffered formalin. (20)**.**Statistical analysis was performed using SPSS-21 (Statistical Packages for Social Sciences- version 21). One Way Analysis of Variance (ANOVA) and Least significant differences (LSD) post hoc test was performed(multiple comparisons) to assess significant difference among means. $P < 0.05$ was considered statistically significant as described by (21).

III. RESULTS AND DISCUSSION

The results in present study indicated that the *Klebsiella pneumoniae*isolates, gram negative, lactose fermentation, mucoids and smooth colonies. Biochemical test were positive Voges-Proskauer test, positive citrate utilization test and positive urease test.

Our results have shown that high level of resistance appeared against different classes of antibioticsas shown in table (1). Antimicrobial resistance is one of the major issues in the world today (21). SikarwarandBatra (22)Showed highly use of broad spectrum antibiotics has led to both increased carriage of *Klebsiella pneumoniae* and the development of multidrug resistant strains like those of Extended Spectrum Beta Lactamases (ESBLs) in hospitalized patients.Over the last two decades, the incidence of infections caused by multidrug resistant *Klebsiella pneumoniae*has increased.(23).

Table 1: Antibiotic susceptibility testing of *Klebsiella pneumoniae* by vitek-2 method

Antimicrobial	MIC	Interpretation
ESBL	POS	+
Ampicillin	≥ 32	R
Amoxicillin/clavulanic acid	8	S
Ampicillin/sulbactam	≥ 32	R
Piperacillin /tazobactam	≤ 4	S
Cefazolin	≥ 64	R
Ceftazidime	16	*R
Ceftriaxone	≥ 64	R
Cefepime	2	*R
Ertapenem	≤ 0.5	S
Imipenem	≤ 0.25	S
Gentamicin	≥ 16	R
Tobramycin	≥ 16	R
Ciprofloxacin	≤ 0.25	S
Levofloxacin	≤ 0.12	S
Nitrofurantoin	128	R
Trimethoprim-sulfamethoxazole	40	S

Results of antibacterial activity reveal the alcoholic extract of plant exhibited antibacterial activity against as shown intable (2). (24) Reported the extract of Trigonellafoenum-Graecum seeds is found to be more active against klebsiella pneumoniae and other gram negative strains.Also (25) has been suggested that the antibacterial activity by fenugreek seed extracts may be refer to its flavonoid content.

Table 2: Antibacterial activity ofalcoholic extract of fenugreek seedsagainst*K. pneumoniae*

Concentration mg /ml	inhibition zone diameter(mm)
100	15.66±0.33 c
200	18.33±0.33 b
300	21.00±0.57 a

Means with different letters differ significantly (P < 0.05)

The phytochemical analysis in the present study has revealed the presence of alkaloid, tannins, terpenoid, steroid, carbohydrates, flavonoids and saponnins as shown in table (3).phytochemical analysis of plants resulted in the discovery of novel effective compounds which would treat the problem of drug resistance.(26). This study was in agreement with (21) which reported Phytochemical screening of hydroalcoholic extract of fenugreek seed revealed the presence of cardiac glycosides, flavonoids, glycosides, phenol, saponins, steroids, tannin, terpenoids and volatile oils.

Table 3: Results of phytochemical analysis of alcoholic extract of fenugreek seeds.

Chemical compounds	Alcoholic extract
Alkaloid (Mayer's)	+
Tannins	+
Terpenoid	+
Steroid	+
Carbohydrates (Benedict’s test)	+
Flavonoids	+
Saponnins	+

Table(4) and Figure (1) illustrated that the blood glucose level was significantly elevated in control (+ve) group after 2, 3 and 4 weeks p<0.05 compared to control negative group .treated groups with 300 mg/kg fenugreek alcoholic extract showed a significantly decreased in blood glucose level .The hypoglycemic effect of fenugreek is retain to its high content of soluble fiber which acts to decrease the rate of gastric emptying by delaying a absorption of glucose from the small intestine (27).

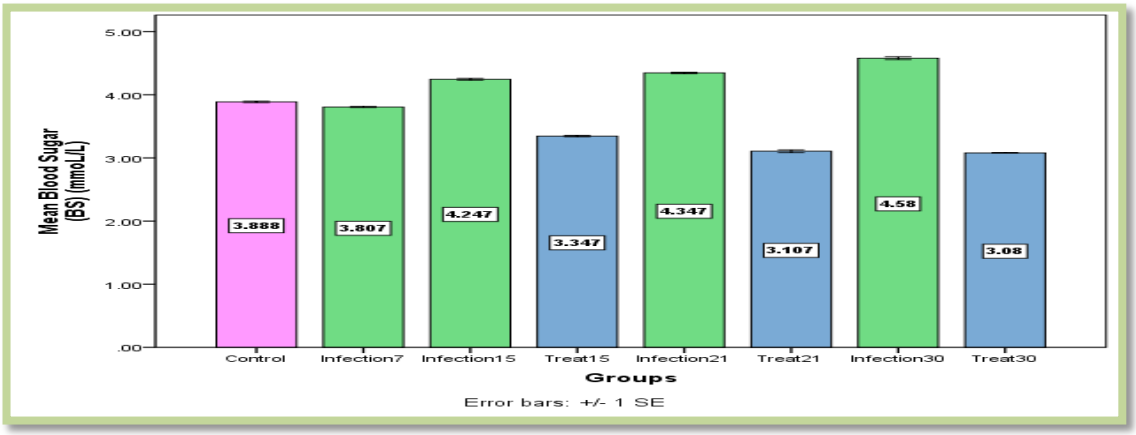


Figure (1): comparison of serum glucose levels (mmoL/L) of the experimental mice groups

Table (4): Mean values ± SE of serum glucose levels (mmoL/L) of the experimental mice groups

Groups	**Mean±SE**
Control	3.88±0.01 d
Infection after 7 days	3.80±0.008e
Infection after 15 days	4.24±0.01 c
Treated after 15 days	3.34±0.01e
Infection after 21 days	4.34±0.01f
Treated after 21 days	3.10±0.02 g
Infection after 30 days	4.58±0.02 a
Treated after 30 days	3.08±0.05g

Means with different letters differ significantly (P< 0.05)

Table (5) and Figure(2)illustrated that the control (+ve) mice group showed a significant increase in serum cholesterolserum cholesterol level at ($p < 0.05$) compared to control (-ve) group. Alcoholic extract of fenugreek with 300 mg/kg mice group exhibited significant reduction in the elevated levels of serum cholesterol level. (28) Reveals a significant reduction in the total cholesterol concentration in the diabetic animals treated with fenugreek extract. The administration of fenugreek decreased the serum cholesterol in diabetic rats (29).

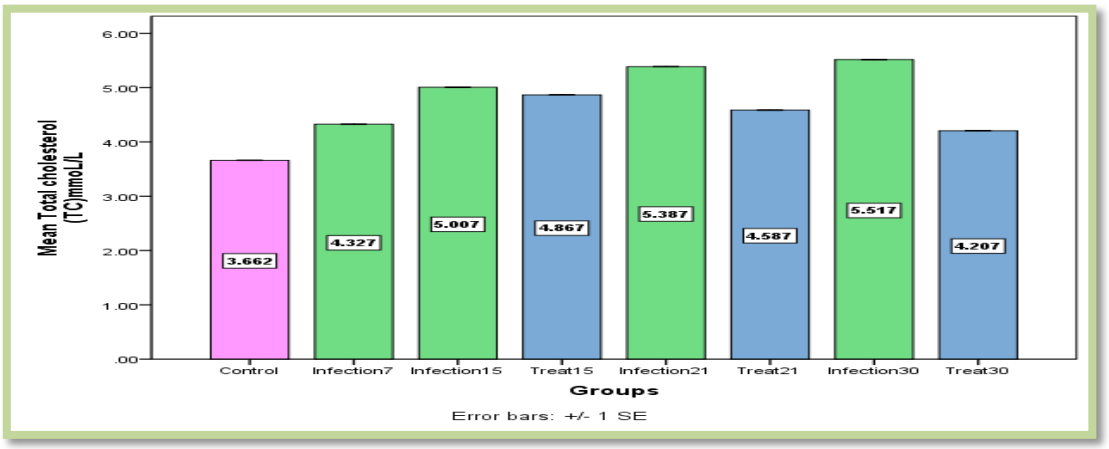


Figure (2): comparison of serum cholesterol levels (mmoL/L) of the experimental mice groups

Table (5): Mean values ± SE of serum cholesterol levels (mmoL/L) of the experimental mice groups

Groups	**Mean±SE**
Control	3.66±0.004h
Infection after 7 days	4.32±0.006 f
Infection after 15 days	5.00±0.006 c
Treated after 15 days	4.86±0.006 d
Infection after 21 days	5.38±0.006 b
Treated after 21 days	4.58±0.006 e
Infection after 30 days	5.51±0.006 a
Treated after 30 days	4.20±0.006 g

Means with different letters differ significantly (P< 0.05)

As seen in (table ,6) (Figure,3) and (table , 7) (Figure, 4) respectively , the mean value of Serum creatinine and uric acid ,it showed a significant elevation in creatinine and uric acid (P < 0.05) in mice that infected with Klebsiella pneumoniae as compared with control group . Results have also shown that treated group with alcoholic extract of fenugreek caused a significant decrease in creatinine and uric acid (P<0.05) as compared with infected and control groups.(30)Revealed that the fenugreek extract at concentration 200 mg/kg was reduced the levels of creatinine potassium was the highest component of mineral. The uric acid in serum was increased in kidney diseases. (31) Revealed the group which consumed fenugreek aqueous (IV) and ehanolic (VI) extract exhibited decreased level of uric acid when compared to the diabetic group.

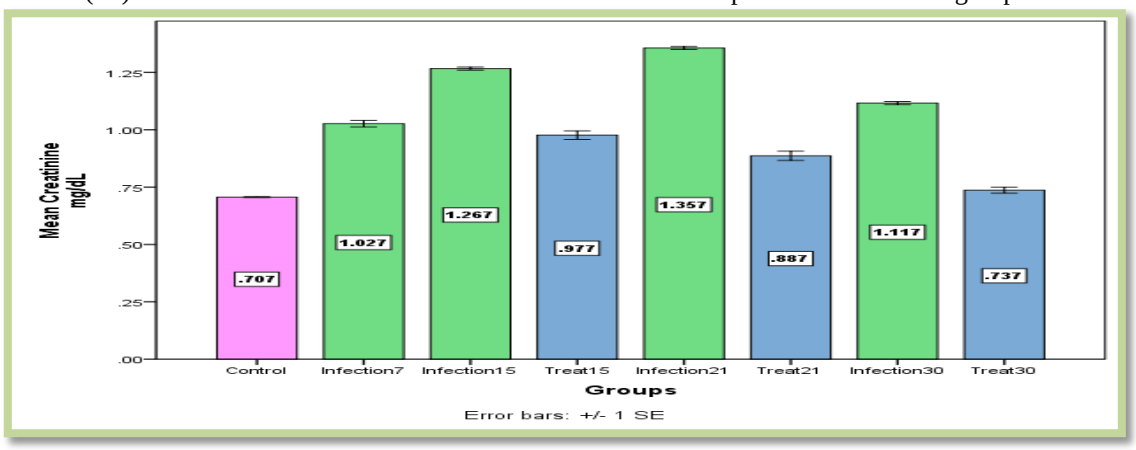


Figure (3): comparison of serum creatinine levels (mg/dl) of the experimental mice groups

Table (6): Mean values ± SE of serum cholesterol levels (mg/dl) of the experimental mice groups

Groups	Mean ± SE
Control	0.70±0.002 g
Infection after 7 days	1.02±0.01 d
Infection after 15 days	1.26±0.006 b
Treated after 15 days	0.97±0.01e
Infection after 21 days	1.35±0.006 a
Treated after 21 days	0.88±0.02 f
Infection after 30 days	1.11±0.006 c
Treated after 30 days	0.73±0.01 g

Means with different letters differ significantly (P< 0.05)

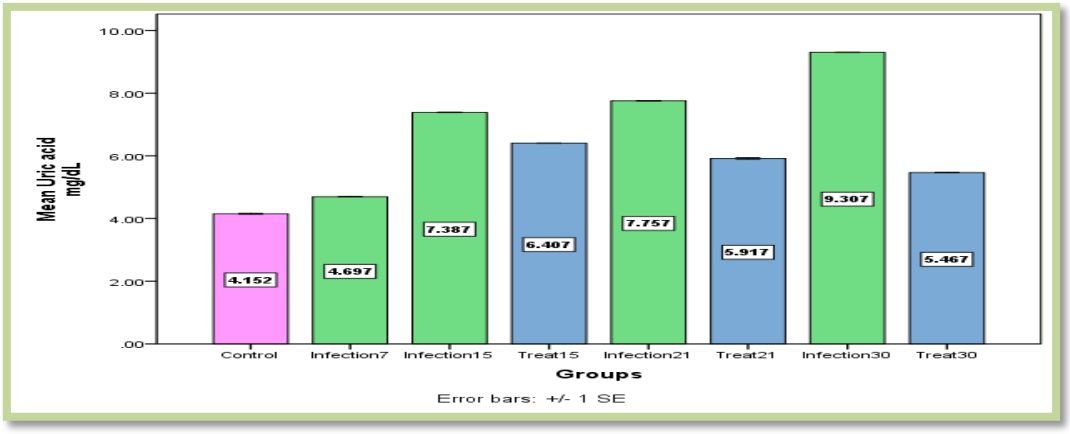


Figure (4): comparison of serum uric acid levels (mg/dl) of the experimental mice groups

Table (7): Mean values ± SE of serum uric acid levels (mg/dl) of the experimental mice groups.

Groups	Mean±SE
Control	4.15±0.01 h
Infection after 7 days	4.69±0.006 g
Infection after 15 days	7.38±0.006 c
Treated after 15 days	6.40±0.006 d
Infection after 21 days	7.75±0.003b
Treated after 21 days	5.91±0.02e
Infection after 30 days	9.30±0.006 a
Treated after 30 days	5.46±0.006 f

Means with different letters differ significantly (P< 0.05)

Depending on the results in (Table, 8) and (Figure,5) the serum total protein level in infected and treated groups did not cause significant changes. Each change had similar to that control. The current findings agree with previous reported (32) which founded that the Supplementation of Fenugreek in high cholesterol diets shows no significant differences in serum total proteins, albumin and globulins of all experimental groups, also shows no literature demonstrated the effect of Fenugreek seed powder or extract on serum proteins total, albumin and globulin. Addition of fenugreek has not resulted in any change in total proteins of turkey Poults.(33).

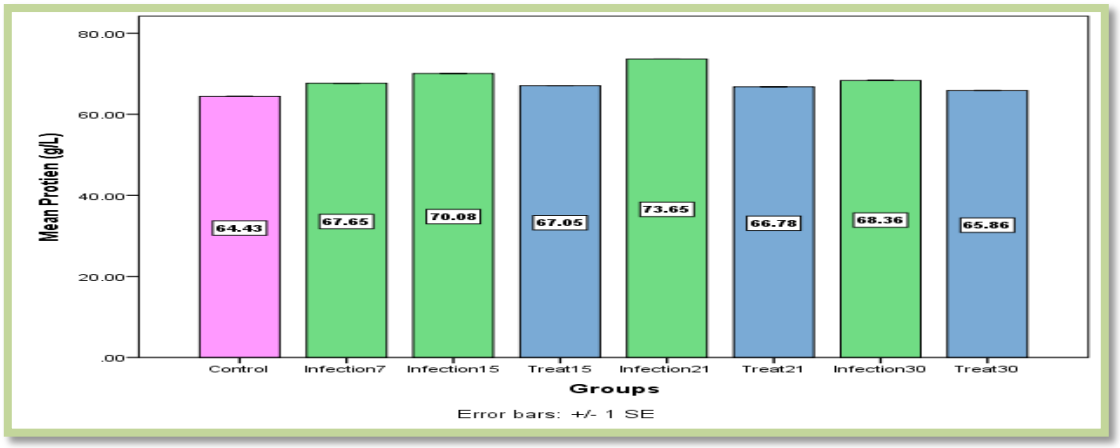


Figure (5): comparison of serum total protein levels (g/L) of the experimental mice groups

Table (8): Mean values ± SE of serum total protein levels (g/L)) of the experimental mice groups

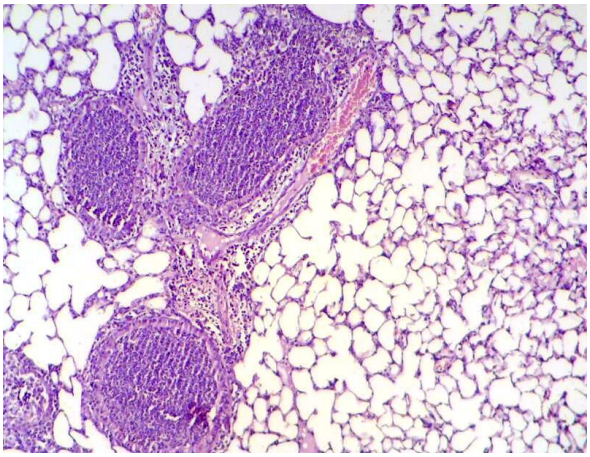
Groups	Mean±SE
Control	64.43±0.008h
Infection after 7 days	67.65±0.006 d
Infection after 15 days	70.08±0.006 b
Treated after 15 days	67.05±0.006 e
Infection after 21 days	73.65±0.006a
Treated after 21 days	66.78±0.006 f
Infection after 30 days	68.36±0.006 c
Treated after 30 days	65.86±0.03g

Histopathology results of the second group, the intestine was showed slightly shortening of intestinal villi (malabsorption syndrome) after 1st week of infection (Figure,6). Also showed surface mucosal damage with mild inflammatory cell infiltration after 2nd weeks (Figure ,8) ,while intestine showed surface mucosal superficial ulceration and damage with inflammatory cells inside the villi after 3rd weeks (Figure 10,11) in addition to present lymphoid follicular hyperplasia with increase of immunity (Peyer's Patches) (Figure ,12) , these evidence was agreement with (34) who reported that , the diffuse nodular lymphoid hyperplasia of the intestine is a rare lymphoproliferative disorder, which associated with gastrointestinal symptoms such as abdominal pain, chronic diarrhea, or rarely intestinal obstruction.

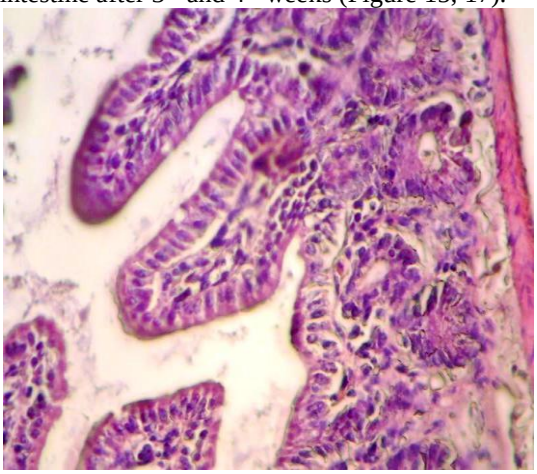
Also the result revels shortening of the intestine villi after 4th weeks (Figure ,13),this finding agreed with (35) who described intestine of suckling mice infected with K. pneumoniae was villous atrophy , degeneration and vascular congestionas well as tissue damage of the mucosa necrosis and entire sheets of epithelial cells had sloughed offin the in some parts.Furthermore, it has been reported that overgrowth of Bacteria in Small intestinal lead to wide spectrum of symptoms that mimic irritable bowel syndrome which caused severe malnutrition and diarrhoea (36).

In lung histopathological revealed severs destruction (necrosis) of parenchymal tissue with infiltrate of inflammatory cells after 2nd weeks of infection (Figure, 7). In addition to present severs destruction (necrosis) of lung parenchymal tissue with abundant infiltration ofinflammatorycells after 3rd weeks (Figure, 9). (37)Revealed necrosis in lung infections with K.pneumoniae. Furthermore, Klebsiella pneumoniae has been caused pneumonia and invasive bacterial disease in healthy and immunocompromised patients. (38-39). Klebsiella pneumoniae is gram negative opportunistic pathogens, saprophyte of the gastrointestinal and respiratory mucosa in humans (40-41)

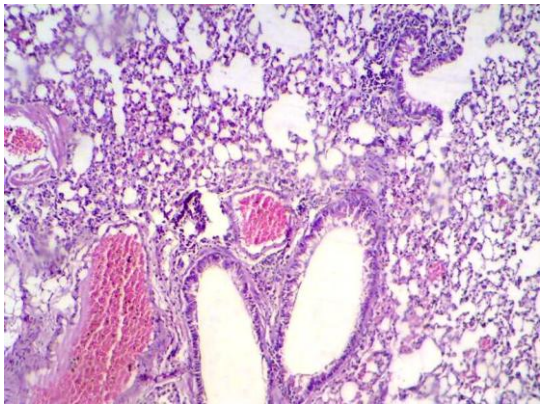
Histopathology results of the third group, the lung was showed still mild inflammatory cells infiltration and presence of emphysema after 2nd weeks (Figure, 14) .In other section lung showed look-like near or normal with mild destruction of alveolar septae with mild inflammatory cell infiltration after 3rd weeks (Figure, 16). Look-like near or normal structure appearance was reported in the intestine after 3rd and 4th weeks (Figure 15, 17).



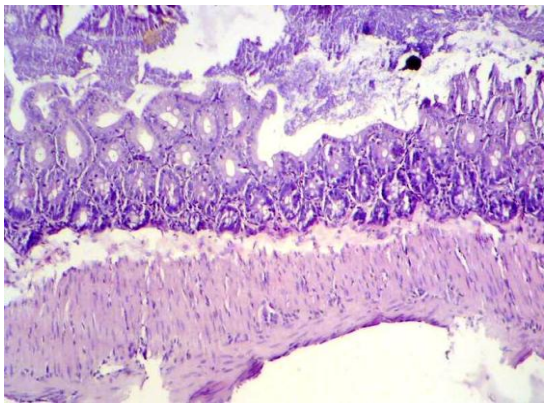
Figure, (6): section in intestine shows *slightly* shortening of intestinal villi (malabsorption syndrome) of lung (H&E 400 X).



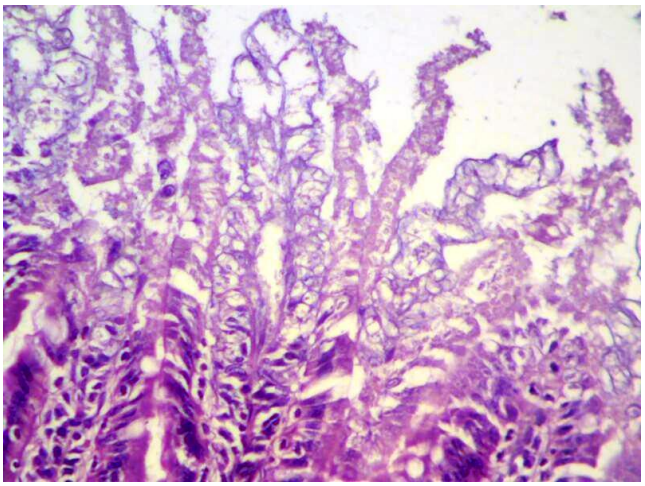
Figure, (7): section in lung shows severs destruction (necrosis) parenchymal tissue with infiltrate of inflammatory cells (H&E 200 X).



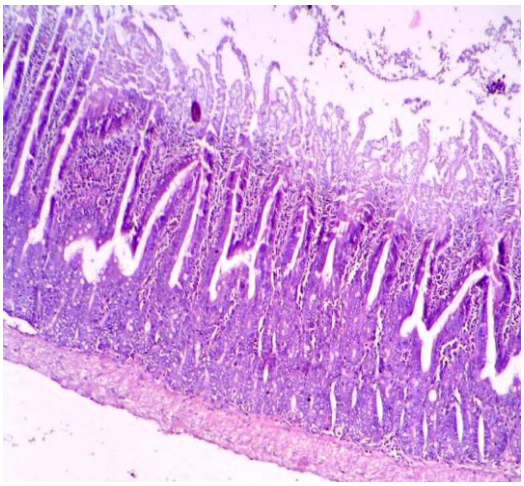
Figure, (8): section in intestine shows surface mucosal damage with mild inflammatory cell infiltration (H&E 200 X).



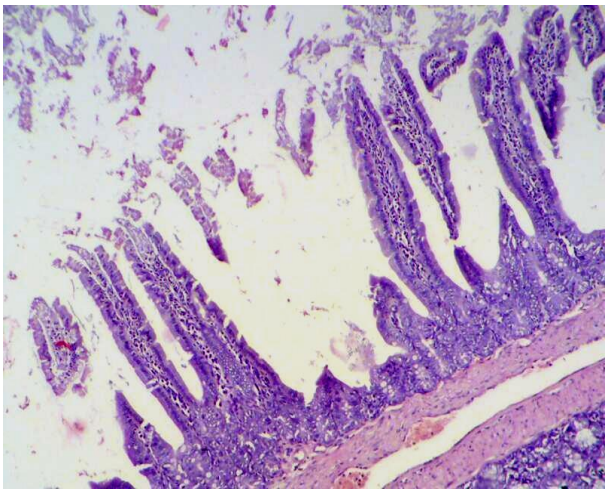
Figure, (9): section in lung shows severs destruction (necrosis) of lung parenchymal tissue with abundant infiltration ofinflammatory cells (H&E 200 X).



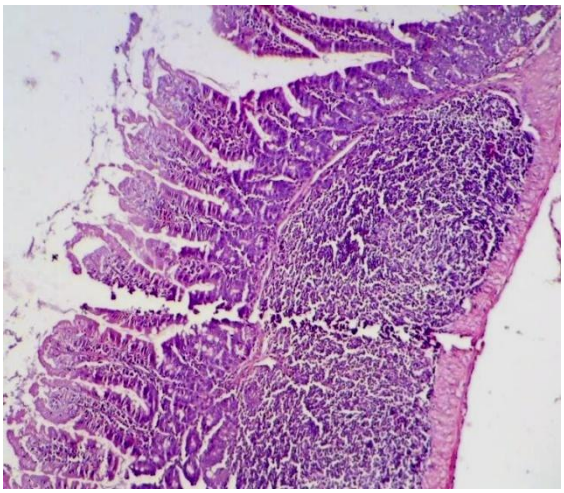
Figure, (10): section in intestine shows surface mucosal superficial ulceration and damage with inflammatory cells inside the villi (H&E 200 X).



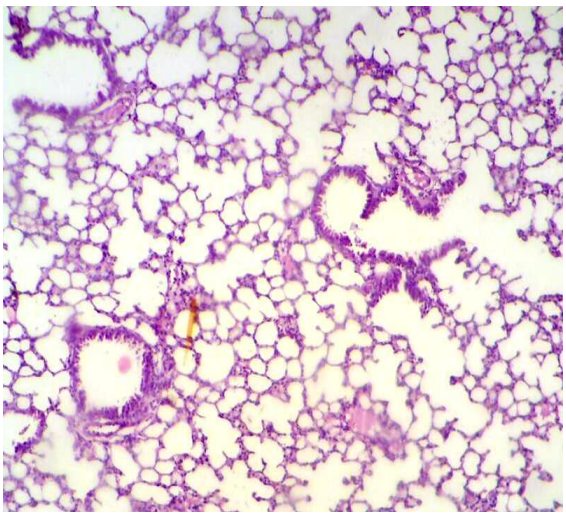
Figure,(11): section in intestine shows surface mucosal superficial ulceration and damage with inflammatory cells inside the villi (H&E 400 X).



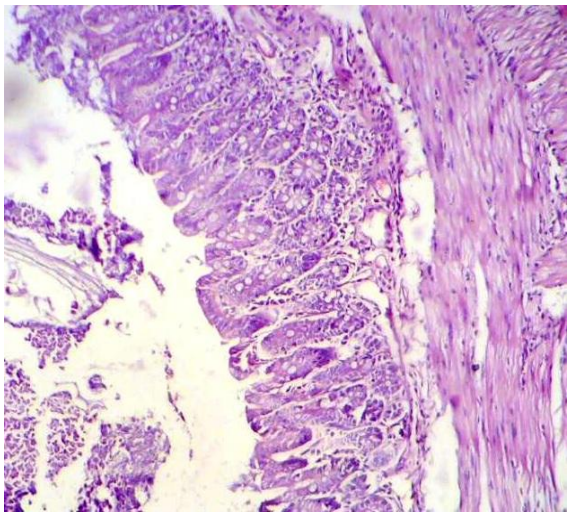
Figure, (12): section in intestine shows lymphoid follicular hyperplasia due to increase of immunity Peyer's Patches . (H&E 400 X).



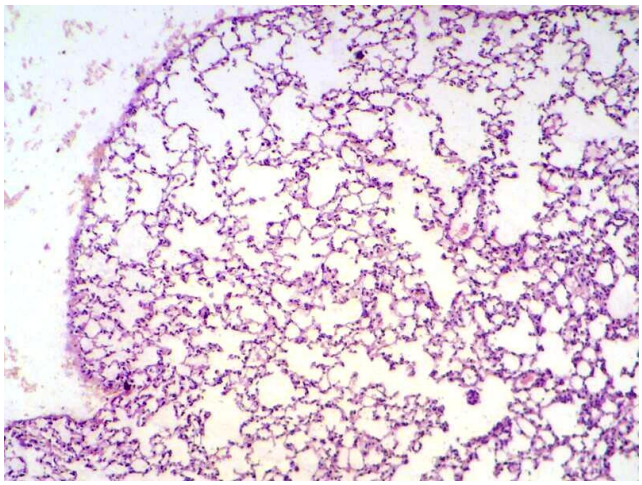
Figure, (13): section in intestine shows shortening of the intestine villi . (H&E 200 X).



Figure, (14): section in lung shows still mild inflammatory cells infiltration and presence of emphysema. (H&E 200 X).



Figure, (15): section in intestine showslook-like near or normal structure appearance (H&E 200 X).



Figure, (16): section in lung look-like near or normal with mild destruction of alveolar septae with mild inflammatory cell infiltration (H&E 200 X).

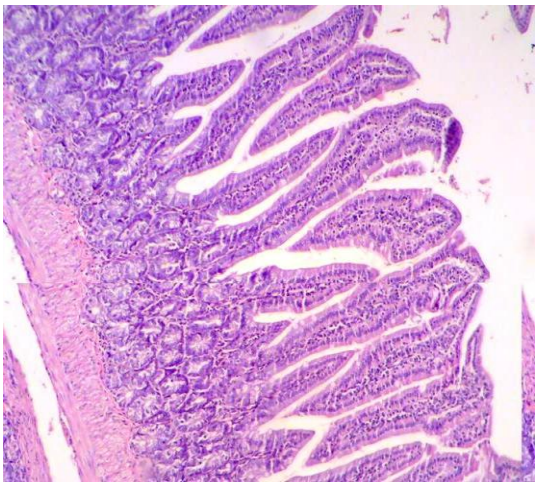


Figure (17): section in intestine, normal appearance of intestine villi (H&E 200 X).

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