Plant Archives Volume 20 No. 2, 2020 pp. 5053-5059 **e-ISSN:2581-6063 (online), ISSN:0972-5210**

DETECTION OF APOPTOSIS IN MICE INFCTED WITH *SALMONELLA TYPHIMURIUM* AND TREATED WITH PLANT EXTEACTS AND ANTIBIOTICS

Maha Fadhel Mohammad and Muna Sachit Hashim

Department of Pathology, College of Veterinary medicine, University of Baghdad Iraq.

Abstract

Experiments was designed in order to study the pathological changes induced by *Salmonella typhimiurm* in the mice and treated with Olive Leaves Alcoholic Extract and Ciprofloxacin.One-hundred mice both gander were divided randomly into 6 groups and treated as follow: 1st group was injected with 0.1ml /4×10^8 CFU/ ml intraperitoneally bacterial suspension of *S. typhimiurm* as control positive**.** 2nd group were injected intraperitoneally with bacterial suspension of *S. typhimiurm* 0.1 ml 4×10^8 CFU/ ml than injected 30mg/kg /0.3ml of Ciprofloxacin intra muscular injection, 3rdgroup pre-treated with Olive leaf alcoholic extract by gavage-tube for 2 weeks then injected with intraperitoneally 0.1 ml /4×10^8 CFU/ml *S. typhimiurm* after that post-treated with 30mg/kg /0.3ml of Olive leaf alcoholic extract orally by gavage-tube until the end of experimental (30days). 4th group which were given 30mg/kg /0.3ml of Olive leaf alcoholic extract orally only by cavage-tube.5thgroup were treatment with 0.3ml of Ciprofloxacin intramuscular injection only,6th group mice were orally administration of 0.3ml of sterile normal saline as control. Results showed heavy deposition of apoptosis marker in many organs as well as heavy destructive and necrotic changes with sever inflammatory reaction. Conclusion, study reported that salmonella be highly virulence pathogen to induced apoptosis as well as necrosis in infected tissues.

Key words : bacterial infection ; mouse. necrosis ; apoptosis.

Introduction

Invasive non-typhoidal Salmonella infection is mostly caused by Salmonella Typhimurium or Salmonella enteritidis.The incubation period of non-typhoidal salmonellosis is 6-72 hours but illness usually occurs within 12-36 h after exposure (Fournier *et al.,* 2015). *S. typhimurium* was considered as an important pathogen that cause public health problem around the world, this pathogens were usually associated with diarrhea and GIT disorder in human around the world. Some serotypes are usually associated with aself-limited disease or outbreaks of gastroenteritis in humans (Polo *et al.,*1999). *Salmonella typhimurium* form a second cause of food borne disease in European countries in the later two decades (Pereira *et al.,* 2007). The sources of infection by this pathogens were meat and poultry products (Bisbini *et al.,*2010) Food animal and poultry were considered the main source of salmonellosis in the humans,It was recorded 15 cases of salmonellosis per 100,000 persons annually in the USA but this reched 6.8 cases \100.000 individual in the 2010 (Nilsson *et al.,* 2019; Hurtado *et al.,* 2017).

The genus Salmonella *is* closely related to *Escherichia,* however horizontal gene played an instrumental role in its divergence from the *E. coli* lineage transfer (Banesaru, 2015). Disease is commonly manifested by acute diarrhea, abdominal pain, fever, and sometimes vomiting.The illness usually lasts 4-7 days, and most people recover without treatment (Glynn and Palmer,1992). Enteropathogenic bacterial species are common infectious agents in developing countries. Among these bacteria, *Salmonella enterica,* the etiological agent of salmonellosis, which considered to be an important agent of diarrheal and systemic disease (Nakazato *et al.,* 2011). *Salmonella* represents the most common and primary cause of food poisoning in many countries for

over 100 years (Alakomi & Saarela, 2009).

Despite well-established instructions and measures for preventing salmonellosis (*Salmonella* food poisoning), the incidence and severity of human salmonellosis have significantly increased. Most salmonellosis cases are self-limiting (approximately 80%), but large outbreaks caused in schools, hospitals, and restaurants (Guiney *et al.,*1995). At present, over 2500 serotypes of *Salmonella* the most common serotypes associated with human illness in the United States and European countries are *Salmonella enterica* serovar *typhimurium* and *Salmonella enterica* serovar *Enteritidis* (Fierer & Guiney,2001). *Salmonella* was isolated from 2% (5 of 250) of faecal samples, 2% of rumen (5 of 250) and from 7.6% (19 of 250) of carcass samples were collected from cow slaughter house. Three *Salmonella* species were isolated (*S. dublin, S. agona* and *S. typhimurium* DT104) (Mcevoy *et al.,*2003). *S. typhimurium* in a percentage of 5.9% from 320 bovine fecal samples collected from different ages and sexes present in farms and slaughter houses in Diwaniya and Najaf governorates; in addition (AL-Karawiy,2008).Aims of experiment's to diagnosis the pathological changes after and before infection.

Materials and Methods

One hundred Albino mice of gender, aged 7-8 weeks and weight range (20-25) g.They housed at animal house of University of Baghdad College of Veterinary Medicine Department of pathology for 2 months. With controlled conditions of temperature (20 ± 5ºC).The mice were housed in a plastic cage containing hard-wood, and they were fed pellets and given tap water in specials bottles.

Formalin solution 10%

This solution was prepared by adding 10 ml of formalin to 90 ml distilled water, used for fixation of histopathological specimens (Luna and Lee, 1968).

Apoptotic marker deposition by Tunel assay

Tunel staining has been adopted as the method of choice for detecting apoptosis it should be recognized that Tunel staining is not limited to the detection of apoptotic cells. Tunel staining may also be used to detect DNA damage associated with non- apoptotic events such as necrotic cell death induced by exposure to toxic compounds other insults (Ansari,1993).Therefore Tunel staining may be considered generally as a method for the detection of DNA damage (DNA fragmentation), and under the appropriate circumstances, more specifically as a method for identifying apoptotic cells.

Preparation of Olive Leaf Alcoholic Extract

Olive leaves were Collected AL-Mahmoodia City in Baghdad, Iraq. Then, they were weighted, washed and air-dried at room temperature (24±2°C) for 4 weeks to remove moisture. The dried leaves were weighted, ground into fine powder.(50g) was taken and macerated in 95% ethanol at room temperature for 48hr, forming green-brown solution then the mixture was filtered, putting in rotary evaporator at 200RPM,50°C for 30 minute to suppurated the ethanol from the Olive leaf extract**.** (Shen *et al.,*2014).

Determination of the infected dose of Salmonella typhimurium

The counting was made by using (Miles& Misra.,1938) The animal was infected with challenge with 0.1 ml / 4×10^{8} CFU/ ml intraperitoneally According to(Al-saadi, 2013).

Results and Discussion

Apoptotic marker deposition

First group: This group was injected with bacterial suspension at 0.1 ml / $4\times10^{8.}$ CFU / ml I/P dose of *S. typhimurium* as control positive. Tissues specimens were processing according to manufacture of company of Tunel kit production.And examined under light microscope at Oil emersion lenses 100X. Brain sections examination's showed heavy deposition of apoptosis marker as dark brown discoloration around degenerated neurons as appears in (Fig. 1). Spleen's tissue appear increase hyper cellularity with heavy deposition of apoptotic marker around lymphocytes.As shows in (Fig. 2). Sections' of liver shows**,** hepatocytes nuclei absents,others cells appear surrounded with dark brown pigmentation due to apoptosis marker deposition (Fig. 3, 4). Lung's tissues also seen very condensing with marker depositions, (Fig. 5).

Second group: Injected with bacterial suspension at 0.1 ml / $4\times10^{8.}$ CFU / ml I/P dose of *S. typhimurium* and treated with Ciprofloxacin I/M injection 30mg/kg / 0.3ml. Lung and spleen and liver showed heavy color, others sections showed moderated deposition of apoptosis marker referring to reduction of infection after treatment, (Fig. 6), (Fig. 7), (Fig. 8) and (Fig. 9).

Third group: Injected with *S. typhimurium* 0.1 ml / 4×10^{8} CFU/ ml I/ P and treated with 30mg/kg / 0.3ml of Olive leaf alcoholic extract orally. Lung, spleen, brain and liver showed lower apoptosis marker deposition after treatment with Olive leaf alcoholic extract, as shows in (Fig. 10), (Fig. 11), (Fig. 12) and (Fig. 13).

Fourth group: This group was orally treated with with 30mg/kg / 0.3ml Olive leave alcoholic extract only. After application of immunohistochemistry staining, all

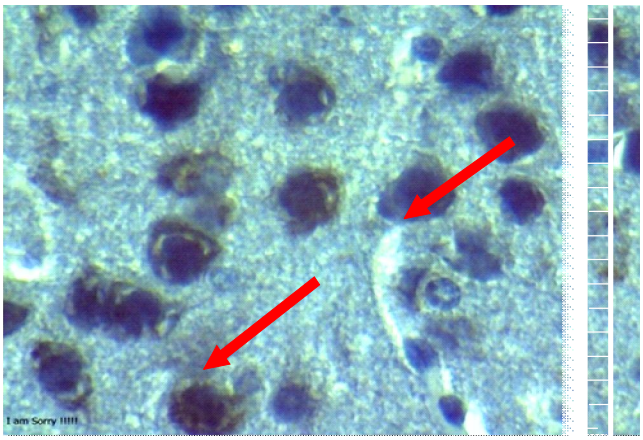

Fig. 1: Brain of mouse injected with bacterial suspension at 0.1 ml / $4\times10^{8.}$ CFU / ml I/P dose of *S. typhimurium* shows heavy deposition of apoptosis marker over neuron 100X.Dap immunohistochemistry staining.

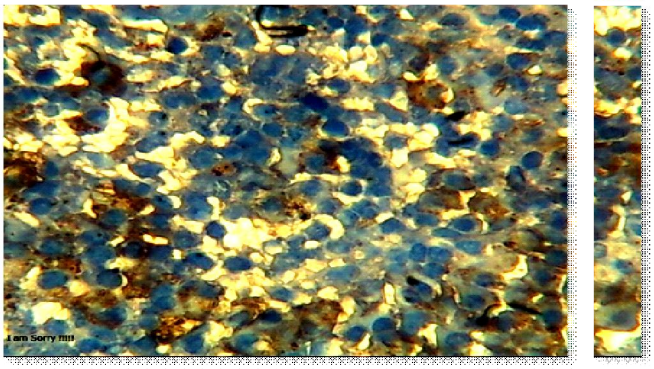

Fig. 2: Spleen of mouse injected with bacterial suspension at 0.1 ml / $4\times10^{8.}$ CFU / ml I/P dose of *S. typhimurium* shows heavy deposition of apoptosis marker around lymphocytes. Dap Stain immunohistochemistry staining, 100X.

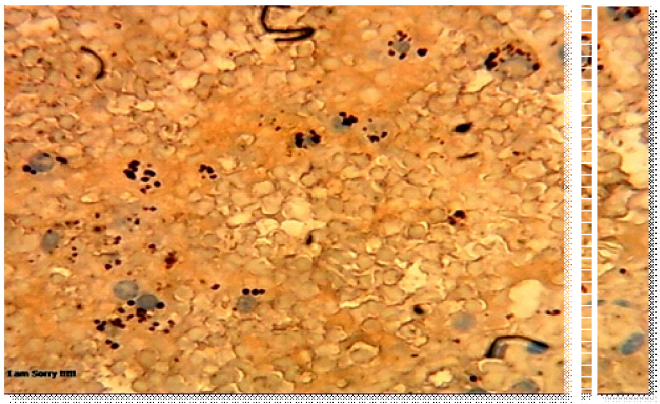

Fig. 3: Liver of mouse injected with bacterial suspension at 0.1 ml / $4\times10^{8.}$ CFU / ml I/P dose of *S. typhimurium* appear loading with brown deposition within interlobular space around hepatocytes (red arrow) Dap Stain immunohistochemistry staining, 40X.

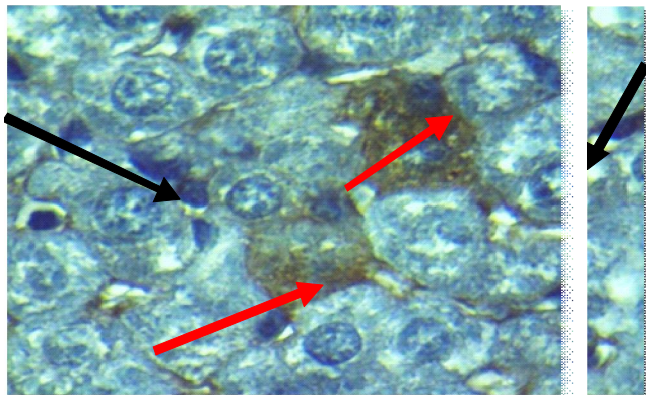

Fig. 4: Liver of mouse injected with bacterial suspension at 0.1 ml / $4\times 10^{8.}$ CFU / ml I/P dose of *S. typhimurium* appear loading with brown deposition within interlobular space around hepatocytes (red arrow) with necrotic area and lose of cellular arrangement (black arrow). Dap Stain immunohistochemistry staining, 100X.

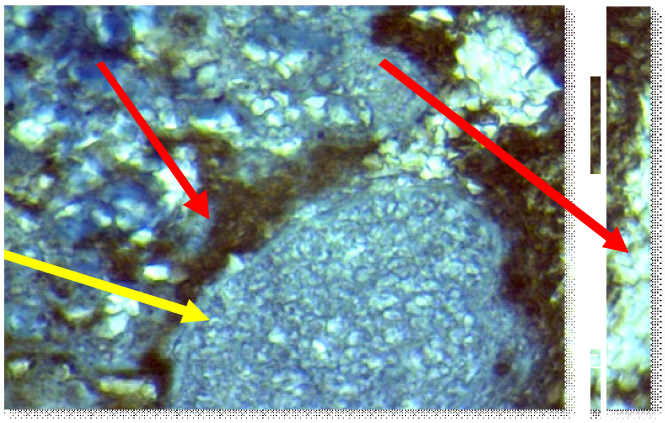

Fig. 5: Lung of mouse injected with bacterial suspension at 0.1 ml / $4\times10^{8.}$ CFU / ml I/P dose of *S. typhimurium* shows apoptosis marker appear very condensing brown discoloration within inter alveolar space (red arrow) and necrotic area around the depress tissues (yellow arrow). Dap Stain immunohistochemistry staining, 100X.

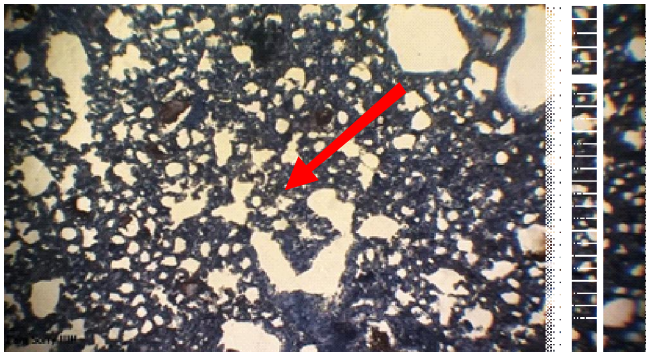

Fig. 6: Lung of mouse injected with bacterial suspension at 0.1 ml / $4\times10^{8.}$ CFU / ml I/P dose of *S. typhimurium* and treated with Ciprofloxacin I/M injection 30mg/kg / 0.3ml ,showes moderat depositions of appoptosis marker as dark brown color in interalveolar wall (red arrow). Dap Stain immunohistochemistry staining, 40X.

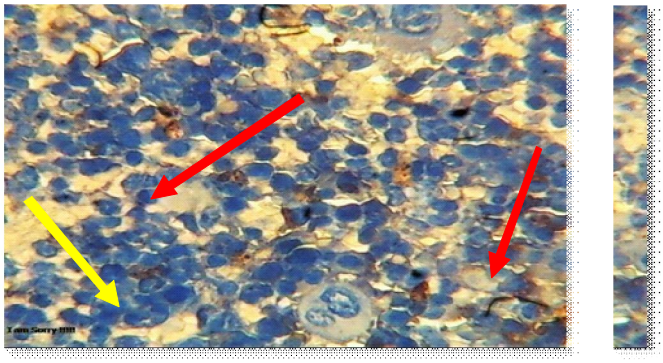

Fig. 7: Spleen of mouse injected with bacterial suspension at 0.1 ml / $4\times10^{8.}$ CFU / ml I/P dose of *S. typhimurium* and treated with Ciprofloxacin I/M injection 30mg/kg / 0.3ml shows hyperplasia of whit pulp increase namber of lymphocyts (yellow arrow) there is deposition of dark brown material (red raws) refers to appoptosis (red arrow). Dap Stain immunohistochemistry staining, 100X.

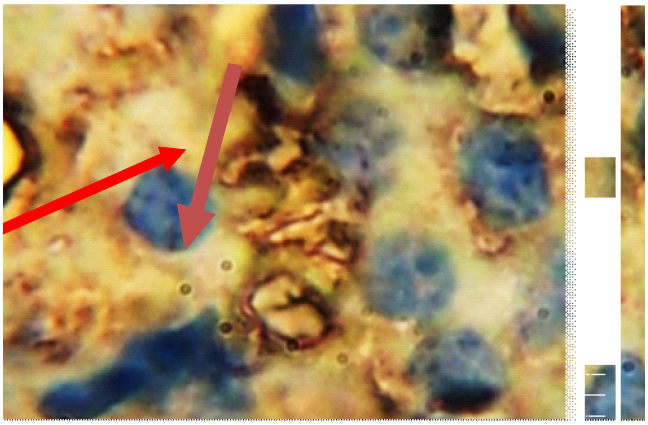

Fig. 8: Liver of mouse injected with bacterial suspension at 0.1 ml / $4\times10^{8.}$ CFU / ml I/P dose of *S. typhimurium* and treated with Ciprofloxacin I/M injection 30mg/kg / 0.3ml shows hepatocytes's atrophied due to covering with heavy material appear as brown color (red arrow) Dap Stain immunohistochemistry staining, 100X.

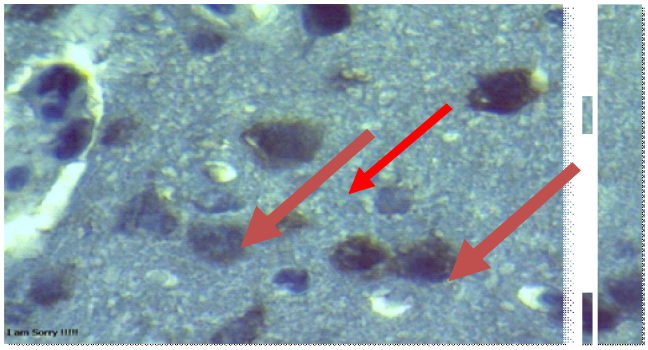

Fig. 9: Brain of mouse injected with bacterial suspension at 0.1 ml / 4×108. CFU / ml I/P dose of S. typhimurium and treated with Ciprofloxacin I/M injection 30mg/kg / 0.3ml shows neurons degeneration and small in size surrounding with dark brown coloration refer to apoptosis (red arrows). Dap Stain immunohistochemistry staining, 100X.

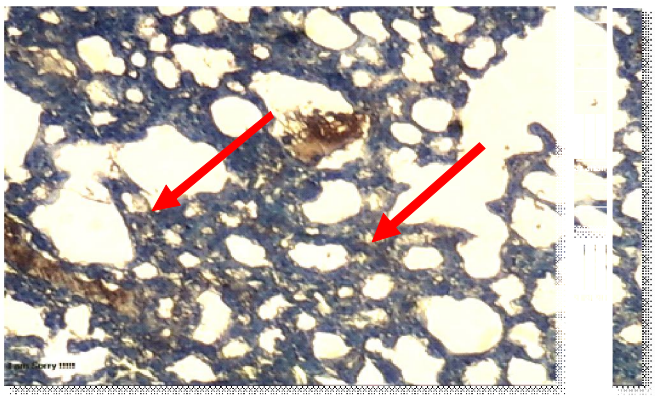

Fig. 10: Lung of mouse injectede with *S. typhimurium* 0.1 ml / 4×10^{8} CFU/ ml and treated with 30mg/kg / 0.3ml of Olive leaf alcoholic extract orally shows fewer depositions of appoptosis marker as dark brown color in interalveolar wall (red arrow)also there is emphysematous changes (blue arrow) Dap Stain immunohistochemistry staining, 40X.

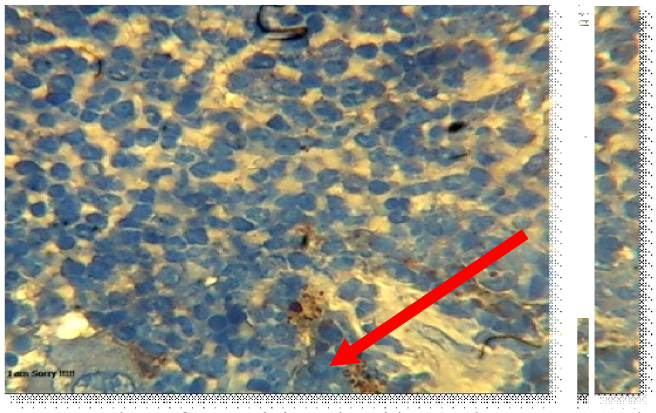

Fig. 11: Spleen of mouse injectede with *S. typhimurium* 0.1 ml / 4×10^{8} CFU/ ml and treated with 30mg/kg / 0.3ml of Olive leaf alcoholic extract orally shows fewer dark-brown discoloration (red arrows) as aresults of appoptosis marker. Dap Stain immunohistochemistry staining, 100X.

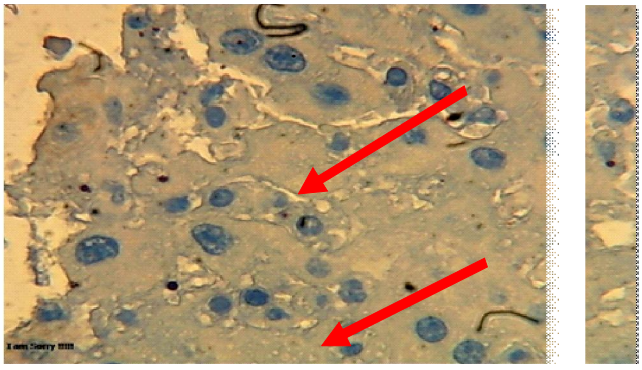

Fig. 12: Liver of mouse injectede with *S. typhimurium* 0.1 ml / 4×10^{8} CFU/ ml and treated with 30mg/kg / 0.3ml of Olive leaf alcoholic extract orally shows fewer deposition of apoptosis marker(red arrow). Dap Stain immunohistochemistry staining, 100X.

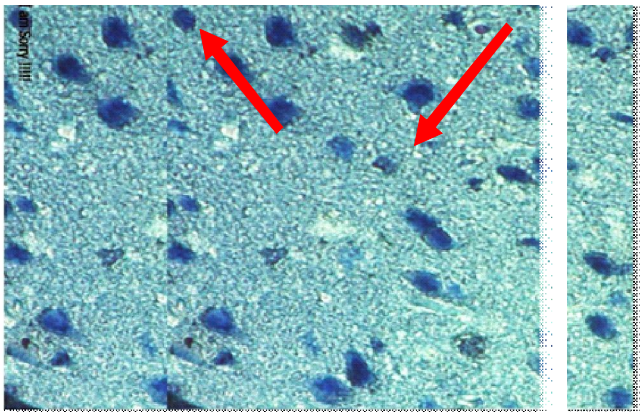

Fig. 13: Brain of mouse injected with *S. typhimurium* 0.1 ml / 4×10^8 CFU/ ml I/ P and treated with 30mg/kg / 0.3ml of Olive leaf alcoholic extract orally ,nuerons appeare marging with light dark brown appoptosis marker. Dap Stain immunohistochemistry staining, 100X.

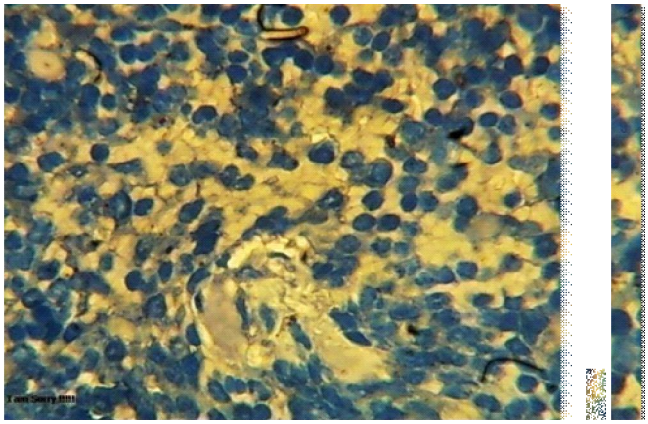

Fig. 14: Spleen of mouse treated with with 30mg/kg / 0.3ml Olive leave alcoholic extract orally, shows simple deposition of apoptosis marker in some fields and absence in others's. Dap staine immunohistochemistry, 40X .

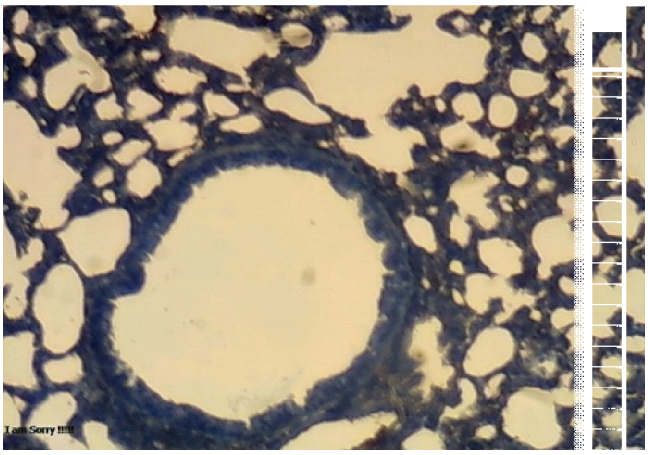

Fig. 15: Lung of mouse treated with with 30mg/kg / 0.3ml Olive leave alcoholic extract orally showes simple deposition of apopptosis markers'. Dap staine immunohistochemistry, 100X.

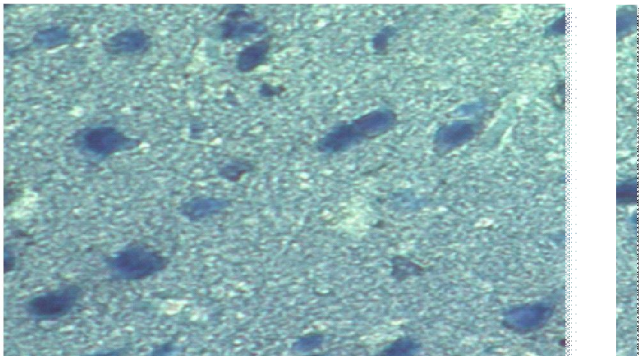

Fig. 16: Brain of mous treated with with 30mg/kg / 0.3ml Olive leave alcoholic extract orally, shows very little deposition of apopptosis marker or absence. Dap staine immunohistochemistry, 100X.

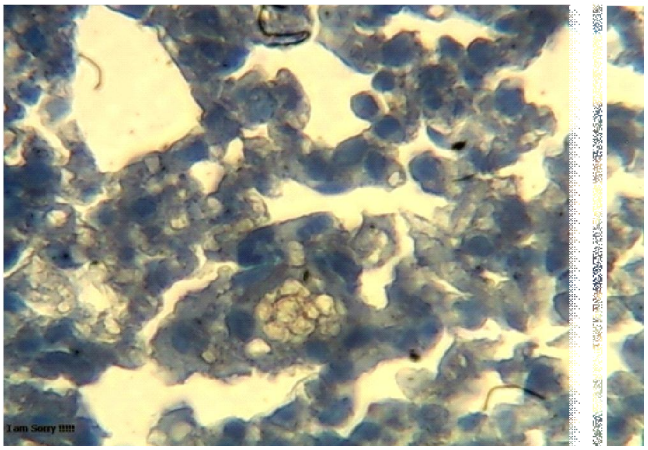

Fig. 17: Lung on mouse treatment with 0. 3ml of Ciprofloxacin intra muscular injection shows no deposition of apoptosis marker Dap. Staining immunohistochemistry,100X.

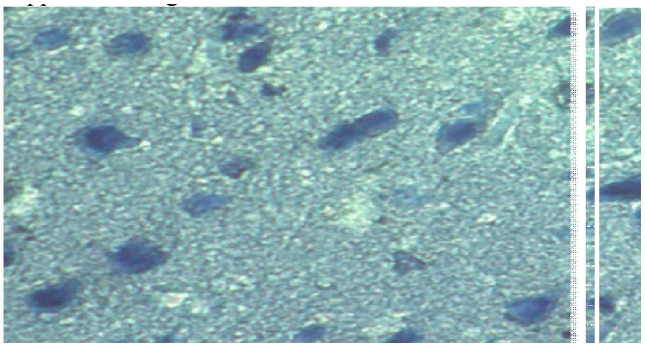

Fig. 18: Brain of mouse treated with normal saline, shows no deposition of apoptosis marker. Dap. Staining immunohistochemistry .100X.

tissues sections of spleen; lung; brain and liver appeare have very low or absence apopptosis marker deposition's as showes in (Fig. 14), (Fig. 15) and (Fig. 16).

Fifth group: This group was treatment with 0. 3ml of Ciprofloxacin intra muscular injection. As control antibiotic. All tissues specimens have no deposition of apoptosis marker after application immunohistochemistry staining due to there is no inflammatory reaction. (Fig. 17).

Six group: This group was treated with normal saline. As control negative, examination of tissues sections showed that no apopptosis marker deposition in all organs. As appeard in (Fig. 18).

Scoring of marker deposition:

Examined deposition of apoptosis marker doing by observation for 10 field for each slides of each organs per/ animal, reported data in group 1 (infected group) occur at score 1 (10%) of apoptosis marker deposition intracellular component's and at score 2(30%) and at score 3(60%). Group 2 (infected and Ciprofloxacin treated) appear at score 1(50%) and score 2(30%) and at score 3(20%).Group 3 (infected and olive leaf extracts treated) occur mainly at score 1(45%) and score2 (40%) and low at score3 (15%).Group 4(olive leaf treated only) mainly appear at high reaction score1(80%) and score2(20%) but reports (0%) at score 3.Group 5(Ciprofloxacin treated only) reports(30%) at score1 and (40%) at score2 and (20%) at score3..Group 6(control negative) occur at 1st score (100%).as reported in table (4-5).

Discussion

Bacterial infectioin

Salmonella *typhimurium* isolated from animals and experimentally use in induction salmonellosis in chicken, our results are in agree with (Buthianah, 2020, Kareem, 2018; Mahdi; 2019, Abouzeed; 1998).

Apoptosis's induction

Infected organs with *S.typhimurium* in this experiments, show sever deposition of apoptosis marker due to massive infection.Necrosis and apoptosis that were seen in the present study may be due to release of ROS which associated with releasing apoptotic factors from mitochondria agreement with (Tonnus & Linkermann, 2017) who reported that GSH play essential role in control of mitochondria pore which influenced by oxidation of protein thiol group of inner mitochondria. This lead to open mitochondrial permeability transition that facilitated the released of cytochrome C from mitochondria, cytochrome C activated caspases cascade that lead to cell death either by apoptosis or necrosis (Ou *et al.,*2017 Thurston, *et al*, 2016). Scoring results were agree with (Janke *et al.,* 2019; Al-Aamery, 2013) who reported scoring marker within tissues and quantification of cell death can be used either as part of a larger scoring scheme or as the final point of a study,analysis and description of the appearance of cell death in tissue sections can add useful information to research.

Conclusions

1. *Salmonella typhimurium* infection induce apoptosis reaction very prominent in brain, lung, liver and spleen of infected group
2. *Olive leaves extracts* make prominent reduction for inflammatory processes by depressed the apoptosis values in infected and then treated mic.

References

Al-Aamery, M.S.H. (2013). Molecular, Pathological and ImmunologicalStudy of Vincristine Sulfate Effects in White Male Mice and Rats.Ph.D thesis/Coll. Of Vet. Med. University of Baghdad.

Alakomi, H.L. and M. Saarela (2009). *Salmonella* importance and current status of detection and surveillance methods. *Qual. Assur. Saf. Crops & Foods*, **1:** 142-152.

Table 1: Shows results of scoring after application of Tunel procedure and immunohistochemistry staining on tissues specimens.

Group/ number	Treatment	Mouse/Score 1 (0-25%)	Mouse/ Score 2 (26-75%)	Mouse/ Score 3 (76-100%)
1st /10	0.1ml IP salmonella typhimurium(control Positive)	1(10%)	3(30%)	6(60%)
2nd /10	0.1ml IP salmonella typhimurium+0.3mlCiprofloxacin IM	8(50%)	1(30%)	1(20%)
3rd /10	0.1ml IP salmonella typhimurium+0.3 Olive leaf extracts orally	1(45%)	8(40%)	1(15%)
4th /10	0.3 ml Olive leaf extract orally	6(80%)	4(20%)	0(0%)
5th / 10	0.3 ml Ciprofloxacin IM	2(30%)	6(40%)	2(20%)
6th /10	0.1 ml normal saline(control negative)	10(100%)	0(0%)	0(0%)

Scoring apoptosis marker within tissues in infected group (1st group) appears mainly at severity value (76%-100) score3, while non-infected group (6th group) appears at lower value or normal condition (0-25%) score 1.

AL-Karawiy, H.A. (2008). Isolation and identification of *Salmonella Typhimurium* and detection of gene encoded Type-1- fimbriae by using Polymerase Chain Reaction. MSc. thesis, Vet. Med. University of Baghdad.

Al-saadi, H.G. (2013). Study the immune response of *salmonella Hadar* Antigens in Mice. M.S.C. Thesis Vet.College. Baghadad University.

Ansari, B., P.J. Coates, B.D. Greenstein and P.A. Hall (1993). In situ end-labelling detects DNA strand breaks in apoptosis and other physiological and pathological states. *The Journal of Pathology*, **170:** 1–8.

Banesaru, S. (2015). *Chemical genetics and immunomodulation in Salmonella enterica serovar typhimurium infection*. Inst för mikrobiologi, tumör-och cellbiologi/Dept of Microbiology, Tumor and Cell Biology. *Biochemistry*, **18:** 427–442.

Bisbini, P., E. Leoni and A. Nanetti (2010). An outbreak of *Salmonella Hadar* associated with roast rabbit in a restaurant. *Eur. J. Epidemiol*., **16:** 613-618.

Buthianah, J.J. (2020). Patho-toxicological study of mycotoxin in chicken immunized with a whole sonicated *Salmonella Typhimurium* Antigen Ph.D. Thesis. College. Of Veterinary Medicine University of Baghdad.

Fierer, J. and D.G. Guiney (2001). Diverse virulence traits underlying different clinical outcomes of *Salmonella* infection. *J. Clin. Invest*., **107:** 775-780.

Fournier, J.B., K. Kimberly, H. Maureen and N. Michael (2015). Family Outbreaks of Nontyphoidal Salmonellosis Following a Meal of Guinea Pigs. *Case Reports in Infectious Diseases* (March): e864640. doi:10.1155/2015/ 864640.

Glynn, J.R. and S.R. Palmer (1992). Incubation Period, Severity of Disease, and InfectingDose: Evidence from a Salmonella Outbreak. *American Journal of Epidemiology,* **136(11):** 1369–77.

Guiney, D.G., F.C. Fang, M. Krause, S. Libby, N.A. Buchmeier and J. Fierer (1995). Biology and clinical significance of virulence plasmids in *Salmonella* serovars. *Clin. Infect. Diseas*., **21:** 146-151.

Hurtado, A., M. Ocejo and B. Oporto (2017). Salmonella spp. and Listeria monocytogenes shedding in domestic ruminants and characterization of potentially pathogenic strains. *Veterinary microbiology*, **210:** 71-76.

Janke, L.J., J.M. Ward and P. Vogel (2019). Classification, scoring, and quantification of cell death in tissue sections. *Veterinary pathology*, **56(1):** 33-38.

Kareem, A.A. (2018). Synergistic effect of sonicated *Salmonella typhimurium* and *lactobacillus acidophilus* antigens and immune response against *Salmonella typhimurium* infection in the rabbits. MSc. thesis, Vet. Med. University of Baghdad.

Mahdi, Z.S. (2019). Pathological and experimental study of the main Pulmonary infections isolated from sheep in Holy Karbala province.MSc. thesis, Pathology.Vet. Med. University of Baghdad.

McEvoy, J.M., A.M. Doherty, J.J. Sheridan, I.S. Blair and D.A. McDowell (2003). The prevalence of *Salmonella* spp. In bovine fecal, rumen and carcass samples at a commercial abattoir. *J. Appl. Microbiol*., **94:** 693-700.

Miles, A.A. and S.S. Misera (1938).The estimation of bactericidal power of blood. *J. Hyg*., **38:** 732-749.

Nakazato, G., F.L. Paganelli, J.C. Lago, F.H. Aoki, C. Mobilon, M. Brocchi and E.G. Stehling (2011). *Lactobacillus acidophilus* decreases *Salmonella typhimurium* invasion in vivo. *J. food Saf*., **31:** 284-289.

Nilsson, O.R., L. Kari and O. Steele-Mortimer (2019). Foodborne infection of mice with Salmonella Typhimurium. *PloS one*, **14(8):** e0215190.

Ou, Q., J. Fan, D. Duan, L. Xu, J. Wang, D. Zhou, H. Yang and B. Li (2017). Involvement of cAMP receptor protein in biofilm formation, fimbriaproduction, capsular polysaccharide biosynthesis and lethality in mouse of *Klebsiella* pneumoniae serotype K1 causing pyogenic liver abscess. *J. Med. Microbiol.*, **66(1):** 1–7.

Pereira, C.S., L.M. Medeiros, R.G. Costa, M.L. Festivo, E.M.F. Reis, L.M. Seki and D.P. Rodrigues (2007). Phage Typing and Multdidrug resistance profile in *S. Typhimurium* isolated from different sources in Brazil from 1999 to 2004. *Braz. J. Microbiol*., **38:** 385-390.

Polo, F., M.J. Figueras, I. Inza, J. Sala, J.M. Fleisher and J. Guarro (1999). Prevalence of Salmonella serotypes in environmental waters and their relationships with indicator organisms. *Antonie von Leeuwenhoek*, **75:** 285-292.

Shen, S., D. Chen , X. Li , T. Li , M. Yuan , Y. Zhou and C. Ding (2014). Optimization of extraction process and antioxidant activity of polysaccharides from leaves of Paris polyphylla, Carbohydr. *Polym*., **1016:** 104 80–86.

Thurston, T.L., S.A. Matthews, E. Jennings, E. Alix, F. Shao, A.R. Shenoy and D.W. Holden (2016). Growth inhibition of cytosolic Salmonella by caspase-1 and caspase-11 precedes host cell death. *Nature communications,* **7:** 13292.

Tonnus, W. and A. Linkermann (2017). The in vivo evidence for regulated necrosis. *Immu-nol Rev*., **277(1):** 128–149.