

Determination of the pathological changes induced by *S. Typhimurium* in mouse against treatment with Olive's extracts.

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Abstract: This study was designed for examination pathological changes induced by *S. Typhimurium* and treated with Olive leaf alcoholic extract thirty mice mixed sexes were subdivided to Group 1 contained 10 mice which experimentally infected with *S. Typhimurium* 0.1ml intraperitoneally at (4×10^8 CFU) bacterial concentration and serve as control positive. Group 2 contained 10 mice submitted to pre and post treated with Olive leaf alcoholic extract by stomach tube at dose 0.3mg/kg.B.W orally one dose daily until 14 days as immune modulator after that injected with 0.1 ml (4×10^8 CFU/ml) *S. typhimurium*, followed by administration of 30mg/kg /0.3ml of Olive extract orally by stomach-tube daily for one month. Group 3 contained 10 mice given normal saline 0.3ml IP of and serve as control. Experiment's was ended at one month. Results of histopathological examination showed that 1st group or infected group was dead at percent 50% after infection about one week and as soon as death tissues samples were fixed and make routing histological processing and examined under light microscopy, necrosis was diffused in vital organs as liver and brain pattern of necrosis in liver shows bridge necrosis that means involvement from lobe to another sinusoids filled with inflammatory cells as well as dead cells and regenerated cells, 2nd group examination showed less or no pathological lesions, 3rd group showed normal appearance of cellular details. Conclusion: severe pathological lesions as necrosis with associated inflammatory reaction were absence in olive's leaf treated group that indicated permanent's antibacterial activity of olive. This observation will open the door for researchers who concerns with histopathology as well as plant industrial workers.

Keywords: Bacterial, Necrosis, Plant's extraction, Treatments.

1. Introduction

S. typhimurim is facultative intracellular zoonotic pathogens causes infection by ingestion of contaminated food and cause of foodborne outbreaks and infections in many countries [1]. *Salmonella typhimurium* members are gram negative microbes consisted bacilli with no sporulation beyond to general families of *Enterobacteriaceae* species that responsible for very contagious diseases called salmonellosis, this bacteria have very important capacity as extra and intra cellular survives ability for multiplying and reproduction or replication's which considers as the main feature for virulence's factors, so these bacteria easily caused inflammation for phagocytosis controlling cells such as macrophages and non-phagocytosis cells [2]. Characterized by general or systemic's disuses or gastrointestinal tract distress depending on bacterial vial ability and host factors [3]. Salmonellosis is common causes of gastrointestinal disease as food born enteritis [4]. And recurrently cause's in each year's highly and economically loss's [5].

Body offered immunity or defense's mechanism against salmonellosis represented by different cells, first one are neutrophil's fighting line, seconds are mononuclear's cells. Inflamed cells stimulated for secretion's cytokines as Interferon's type alfa and beta, Interleukin's types' 1,2,6,8 which produced inflammatory exudations [6]. Type 2 interleukin's as growing factors for T cells maturities Interleukins' 4 as cytokines stimulated maturation of native's T helper cells (th0) for th2 cells [7]. *Salmonella* pathogen in developing as well as developed countries and is responsible for morbidity and some death [8]. The most common sources responsible for outbreaks of salmonellosis are Poultry, meat products, and eggs [9] *Salmonella* can cause persistent infections, so hosts can remain as reservoir for a long time [10].

Salmonella antibiotic resistance is increasing, and treatment of invasive cases of infection is more difficult and risk of death has increased [11] olive's tree is mentioned in Holy Quran as a very beneficial plant. The main essential compounds in olives are Oleo europaea's. Highly production values of olives bi-products were obtained, olive leaves represent approximately 10 % of the weight of olive [12] olive

leaf believe to be as very useful because it's antibacterial and antifungal in addition antioxidant effect [13].

Olive leaf alcoholic extract rise level of energy levels, reduce the blood hypertension, elevation of immune response ;protection heart and circulatory apparatus [14] olive's leaf's alcoholic's extract's possess anti-inflammatory and antimicrobial activity against different pathogens as *L.monocytogene's Staphylococcus's aureus* and *E. coli's* (15). Commercial products for pharmaceutical uses and cosmetics as well as foods supplements. Olive leaves contain many potentially bioactive compounds that may have hypoglycemic, neurological protective, and anticancer properties [16].

There were limited study on the effective treatment of alcoholic Olive Leafs extracts against salmonella. The Aims of study: Histopathological investigation on the role of olive leaves alcoholic extract as antibacterial agents against *S.typhimurum* infection in the mice .

2. Methodology

Study design: Thirty (30) blab c laboratory white mice, mixed sexes, about 7-8 weeks aged with (20-25)g average weighing, were kept in plastic cages, fed commercial pellets with given water ad libitum and the temperature was controlled at $21 \pm 4^{\circ}\text{C}$ and were subdivided to Group 1 contained 10 mice which experimentally infected with *S.Typhimurium* 0.1ml intraperitoneally at (4×10^8 CFU) concentration as control positive. Group 2 contained 10 mice submitted to pre and post treated with Olive leaf alcoholic extract by stomach tube at dose 0.3mg/kg. B.W orally one dose daily until 14dayes as immune modulator then injected with 0.1 ml (4×10^8 CFU/ml) *S. typhimurium*, followed by administration of 30mg/kg /0.3ml of Olive extract orally by stomach-tube daily for one month. Group 3 contained 10 mice given normal saline 0.3ml IP of and serve as control.

Plant extract prepares

Olive leaves were collected from Baghdad Garden City, Iraq. Make washing and weighting and dried then put in dark place at ($27 \pm 2^{\circ}\text{C}$) for several weeks (3-4) for removing moisture's. Draying leaves were weighted, and grounded to forming tiny powered at weight (50g) intake's and macerated in 95% ethanol's at 37°C

temperature for 48hr, produced drakes green – brown liquid, this compound was filtered using, putting in remarrying evaporator's r at 200RPM,50°C for 30 minute to separate the ethanol from the Olive leaf extract [17].

Bacterial suspension preparation and cultivation

S. typhimurium's cultured on Simon Striates media using transporting single purring colonies sterling methods, then incubated at 37°C/ 24 hours until the appearance and fully growing bacterial colonies was seen, then sub culture on nutrient's media's at 37°C/ 24 hrs to activates bacterial growth. Stimulation of bacteria doing by injection of bacterial suspensions at dose (0.2 - 0.3ml).I.P /mouse and then waited 24 hours if mice is die immediately resulted this means that bacterial was activated and when no died animals occurs we killed the mouse for make culturing from internal organ on selective media Simon citrate media for identified bacteria then made harvesting occurs in twenty four hours at 37°C, then new-culture on nutrient's brothing was prepared for again harvesting [18].

3. Results and discussion

Histopathological Examination First group:

This group was injected 0.1.ml(I/P) at concentration.(4×10^8 bacteria's, histopathological examination of tissues samples that, brain sections shows neutrophils and monocytes infiltration with hemorrhagic and necrotic foci with proliferation of astrocytes or glial cells and peri neuronal edema, Figure (1: A,B,C). Examined livers appeared have dilated central vein which congested at the same time with cuffing of inflammatory reaction adjacent to the portal area, mononuclear cells in sinusoidal lobules and others have necrosis (Figures 2, 3). Examined lungs shows severe inflammatory cells aggregation around congested blood vessels bronchioles (Figure 4).

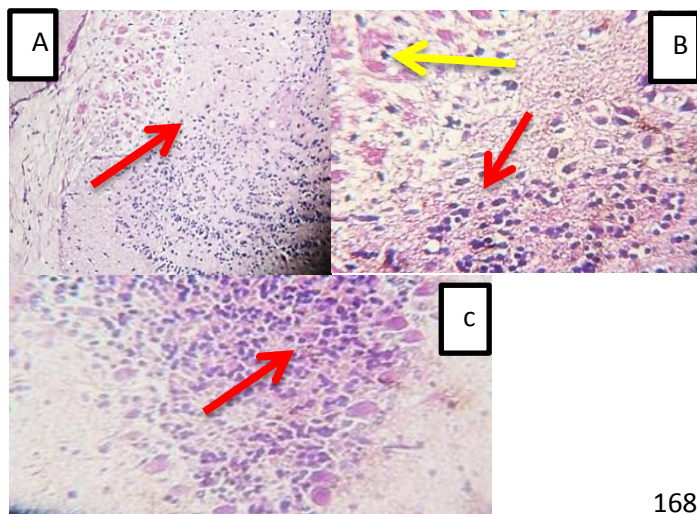


Fig 1: A Histopathological changes in cerebellum of infected mice injected 0.1 ml (4×10^8 CFU/ (I/P) of bacteria. **(A)** Neutrophils and monocytes infiltration in necrotic area in the brain parenchyma (red arrow)(H&E stain 10X). **(B)** Proliferation of astrocytes or glial cells (red arrow) and hemorrhagic foci in the brain parenchyma (yellow arrow). (C) edema around blood vessel perineuronal edema (red arrow) in pia mater of meninges (H&E, 40X).

Fig 2: Gross appearance of liver from infected mice show abscesses foci as white nodules (arrow).

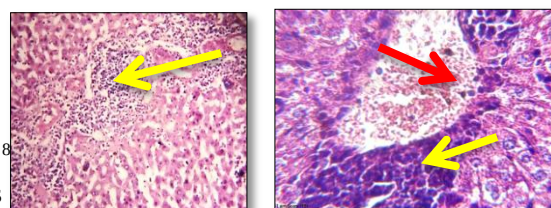


Fig 3: Histopathological two section of liver from infected mice shows mononuclear cells aggregation in necrotic area (yellow arrow) and dilated congested central vein (red arrow) (H&E, 40X)

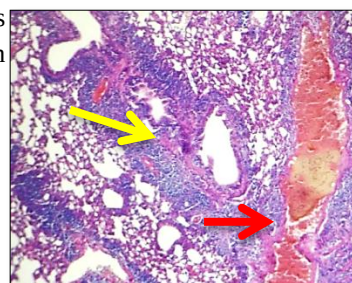


Fig 4: Histopathological section of lung from infected mouse shows interstitial pneumonia appears as thick inflammatory cells and red blood cells exudation mainly around dilated and congested blood vessels (red arrow) and bronchioles (yellow arrow) (H&E, 10X).

Second group

This group was pretreated with plants extract by stomach gavage for two weeks and followed by giving of intraperitoneal $0.1 \text{ ml } /4 \times 10^8 \text{ CFU/ml } S. typhimurum$, later treating with $30 \text{ mg/kg } /0.3 \text{ ml}$ Olive leaf extract orally by stomach-gavage for 30 days. Liver Histopathological section from mouse infected and pre and post -treated shows moderately hepatocyte degeneration with apoptosis due to regeneration appear as apoptotic bodies with lowering inflammatory response and congested blood vessels and less inflammatory reaction with keeping of normal tissue architectures response to treatment (Figures 5,6), with evidences of hepatocytes regeneration referring to fast response to treatment with olive leave extract (Figure 7). Lung tissues also shows no clear pathological changes after the same treatment (Figure 8).

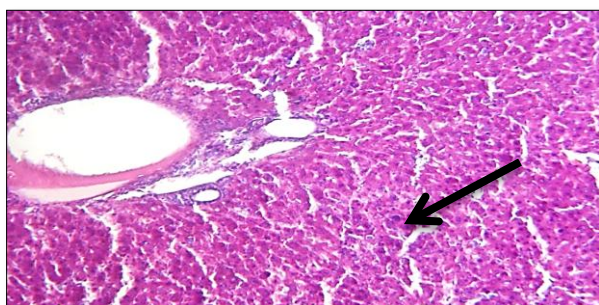


Fig 5: Histopathological section of Liver from mouse infected and treated shows absence of necrosis and lowering inflammatory reaction appear as few inflammatory cells at portal area (red arrow) 10X.H&E.

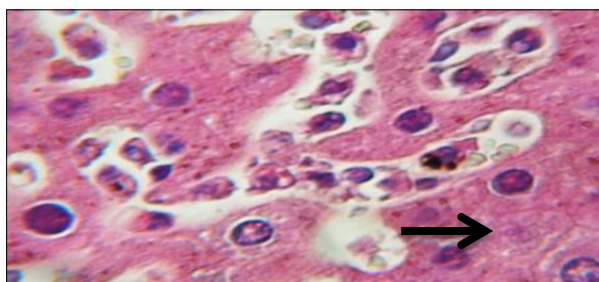


Fig 6: Liver Histopathological section from mouse infected and pre-treated shows moderately hepatocyte degeneration with regeneration in others hepatocytes (arrow) due to regeneration appear as apoptotic bodies with lowering inflammatory response.40X.H&E.

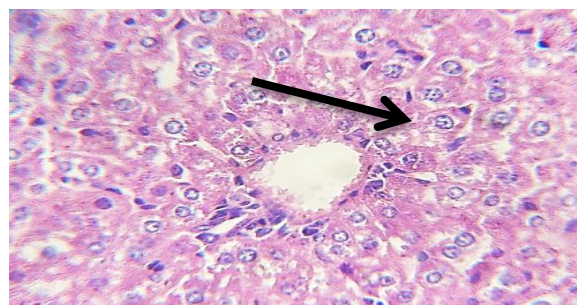


Fig 7: Histopathological section of Liver from mouse infected and treated shows regeneration of hepatocytes appears on its cytoplasm as more than one nucleus (arrow) (H&E, 10X).

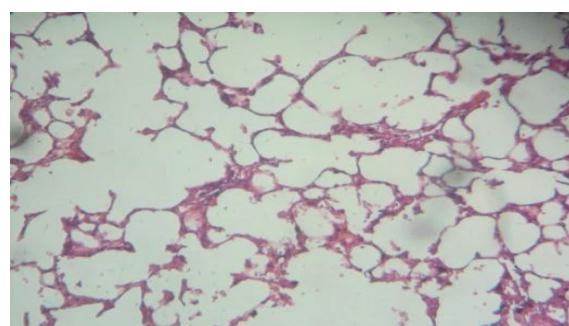


Fig 8: Histopathological section of lung from mouse infected and treated shows no clear lesions (H&E, 10X).

Brain histopathological section of infected and pre and posted-treated mice showing normal neurons appearance with no clear pathological changes and no inflammatory cells (Figure 9).

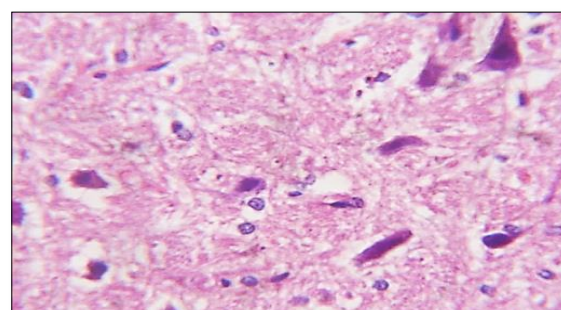


Fig 9: Histopathological section in brain of infected and treated mice shows normal neurons and no inflammatory cells infiltrations (H&E, 40X).

Third group

This group was treated with normal saline only. Routine histopathological examination for each tissues sample in this group showed no lesion as appeared in brain (Figure 10).

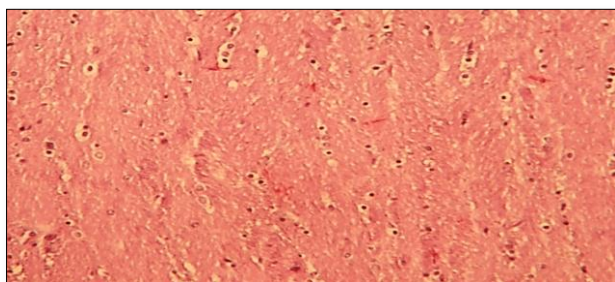


Fig 10: Brain section of mice treated with normal saline only shows normal cellular details (H&E, 40X).

Histopathological examination showed severe pathological lesions in mouse infected with *S. Typhimurium* in compare to control group. Our findings improved that bacteria was strongly virulence for stopping immunity attempts of host against infection so easily spreading for all tissues and organs [19]. Argued that *S. Typhimurium* is a virulent intracellularly pathogen's which districted defense mechanism factors enabling itself for surviving and colonization in different part of digestive system mainly small intestine, make injury in mucosa layer, and sub mucosa and may be penetrating into epithelial lining cells, if there's good host immunity facilitative putdown of infection through engulfed by macrophages and neutrophils .Intracellular *S. Typhimurium* toxins spreading in infected host caused elevation of free radicals toxic compounded induction as peroxidase hydrogen's. There is moderate pathological lesion in regeneration in the infected organs by *S. typhimurum* and treated by olive leaf extract this result was agreement with [19] who said that olive leaf extract rich with phenolic compound mainly oleuropein possess medical properties as antibacterial ; prevent oxidants free radicals roles reduced virulence ability of pathogen's as *Salmonella's enterica's* . These activities of plants as antioxidants due to presence of phenolic materials which a natural antioxidanting materials have free radical scavenging activity [22]. *Salmonella typhimurium* and others bacteria can be found as natural environment's pathogens infected many animals and caused severe pathological lesions mainly

necrosis in domestic and laboratory animals that what be founds by many researcher's results [22, 23]. Neutrophil's have inducing ability for stimulation cellular immunity for production of Interferon Alfa secretion from activated immune's cells at the site of infection [24]. In our experiments we found that infected mice with *S.typhimurum* and treated with olive's extracts have lowering or no pathological changes that's agrees with. [25] Who reported olive leaf is rich with Phenolic compounds that have been recorded as a strong anti-free radicals scavengers agents. Antioxidants factor against oxidations after metabolism's processes and can prevents H_2O_2 attached or products . Olive leaf was rich with GSH that play essential role in control mitochondria pore through limitation of oxidative processes of protein groups thiol inside mitochondrial inner membranes lead to open mitochondrial permeability transition that facilitated releasing of cytochrom C from mitochondria, cytochrome C activated caspases cascade that lead to cell apoptosis [26].Other author found similar pathological lesions in liver infected with parasite associated with secondary bacterial infection. [27] And others found that induced salmonellosis in mouse can be caused marked elevated in apoptosis level [28].

Conclusion

Olive's leaf extracts has very effective antibacterial activity against experimental salmonellosis in mice.

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Author's Contributions

All authors had an equal contribution in the preparation and of the manuscript.

Ethics

Current experiment was completed under supervision of ethics committee in the College of Veterinary medicine of University of Baghdad (2020).Others associated

scientific finding and reported pictures doing by pathologist's who participating researcher's.

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