

## Histopathological Study for the Effect of Ethanolic Extract of *Sonchus oleraceus* on *Escherichia coli* Bacteria in Mice

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### Summary

This study was designed to explore the effect of the ethanolic extract of *Sonchus oleraceus* on *Escherichia coli* infection in mice. The LD50 of the orally dosage ethanolic extract of *Sonchus oleraceus* was found to be 800gm/kg B.W for two weeks. Twenty five mice were randomly divided into five groups. The first group was given orally the ethanolic extract with dose 800 mg/kg B.W for two weeks, then infected with *Escherichia coli* bacteria with dose  $1 \times 10^8$  cfu for ten days. Second group was infected with *Escherichia coli* bacteria with dose  $1 \times 10^8$  cfu for ten days then treated with ethanolic extract of *Sonchus oleraceus* for two weeks. The third group was infected only with *Escherichia coli* bacteria with a dose  $1 \times 10^8$  cfu for ten days. Fourth group was given orally the ethanolic extract with a dose 800 mg/kg B.W orally for two weeks. Fifth group was given normal saline solution and served as control. The histopathological study showed pathological lesions in most of internal organs of the third group that infected with *Escherichia coli* bacteria specially liver, kidney and intestine. First group revealed lesions less than the third group, while the second group showed just aggregation of inflammatory cells in some internal organs and this refers that the ethanolic extract of *Sonchus oleraceus* have obvious inhibitory effect on *Escherichia coli* bacteria when compared with the infected group.

**Keywords:** *Escherichia coli* bacteria, *Sonchus oleraceus*, ethanolic extract.

### Introduction

*Escherichia coli* are a Gram-negative rod-shaped bacterium that commonly found in the lower intestine. Most *E. coli* strains are harmless, but some, can cause serious food poisoning in humans (1). *E. coli* is one of the six groups of *E. coli* recognized as aetiological agents of diarrhoea. It is now of public health importance as it is readily isolated from human and animal waste that pollute the environment (2). Therefore poses serious health hazard to the people especially infants (3). Traditional medicine is practiced and plants have been exploited for the treatment of many infections and diseases (4). The antimicrobial nature of these substances has been well documented. The extract of some plants, able to inhibit some intestinal pathogens including *E. coli* (5). Many plants extract their potency to the presence of substances such as tannins, phenolic compounds and so on. In recent times placed don use of natural materials in the control and treatment of various infections and diseases as some chemically synthesized drugs have undesirable side effects (6). *Sonchus*

*oleraceus* (Common sowthistle) is quite common; this plant is a winter to spring annual adaptable and found in full or partial sun, moist to slightly dry sites, and soil that is fertile. *Sonchus oleraceus* (Common sowthistle) constituents include vitamin C, carotenoids, mineral element, fibre, essential fatty acids (7). The seeds are used for medicine and young leaves are edible. It was suggested that the antioxidant activity in plants depend on environment factors such as growing season and location (8). The antioxidant activities of *S. oleraceus*, including the free radical scavenging activity, nitrite scavenging activity by total phenolic content, and flavonoid content (9). *S. oleraceus* might be a source of food and natural antioxidants (9). luteolin 7-O-glycoside, apigenin 7-O-glycoside was found to be the major flavonoid constituents of *Sonchus oleraceus* leaves (10). However, the aim of present study was the assessment of the possible antimicrobial action of the ethanolic extract of *Sonchus oleraceus* on *E. coli* in induced infected mice.

### Materials and Methods

*Escherichia coli* serotype was obtained from Zoonoses Unit/ Veterinary Medicine/ Baghdad University, and the biochemical properties were tested depending on the method of Quinn and his freinds (11).

The ethanolic of *Sonchus oleraceus* was Preparation according to Harborne (12). The determination of median lethal dose (LD50) was conducted according to Dixon (13) (up and down) method, LD50 of ethanolic extract was (8000 mg / Kg of B.W) and the treated dose was 800 mg / Kg of B.W orally.

Twenty five white mice both sexes, 7-8 week olds were randomly divided into five groups equally and treated as follows:

1- First group: Animals were administrated orally 0.2 ml of ethanolic extract of *Sonchus oleraceus* (concentration of extract was 0.125gm/1ml) daily for two weeks then administrated with 0.2 ml of bacterial suspension containing  $1 \times 10^8$  cfu.

2- Second group: Animals were administrated orally with 0.2 ml of bacterial suspension containing  $1 \times 10^8$  cfu of *E. coli* for 10 days, then treated with ethanolic extract of *Sonchus oleraceus* (concentration of extract was 0.125gm/1ml) daily for two weeks.

3-Third group: Animals were administrated orally with 0.2 ml of bacterial suspension containing  $1 \times 10^8$  cfu of *E. coli* for 10 days according to the method (14).

4-Fourth group: Animals were administrated orally with 0.2 ml of ethanolic extract of *Sonchus oleraceus* as the first group.

5- Fifth group: Animals were administrated orally with 0.2 ml of normal saline and served as control negative group.

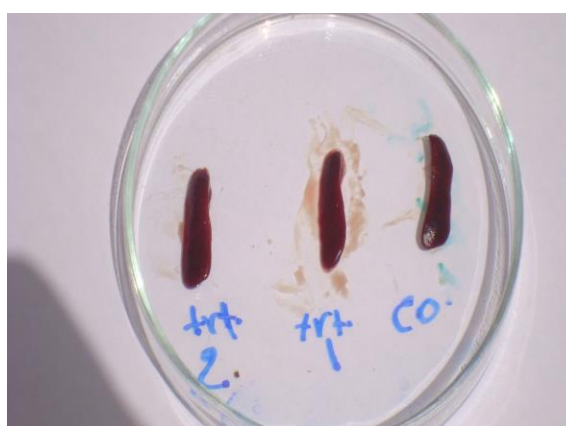
At day10 post challenge all animals were sacrificed and pieces from internal organs were taken for bacterial isolation and other pieces were fixed in 10% neutral buffer saline 72 hours for histopathological examination according to (15).

### Results and Discussion

Chemical detection indicated the presence of glycosides in the extraction of *Sonchus oleraceus* as seen in (Table, 1), flavonoid, and glycosides, tannins and phenols presence in ethanolic extract.

**Table,1: The results of some active compounds detection for ethanolic extract of *sonchus oleraceus***

Chemical compounds	Ethanolic extract
Tannins	+
Saponins	-
Flavonoids	+
Glycosides	+
Terpenes	-
Alkaloids	-
Phenols	+



**Figure, 1:** showed the increased in the size of spleen of the group infected by *Escherichia coli* bacteria (co) then treated with the ethanolic extract of *Sonchus oleraceus* (trt. 1, trt. 2)



**Figure, 2:** Increased size of spleen of the group that treated with the ethanolic extract of *Sonchus oleraceus*, then infected by *Escherichia coli* bacteria as control group (co.1, co.2).

Table, (1) showed the presence of glycosides, flavonoids and tannins in the ethanolic extract of *S. oleraceus*. Camparoto and his team (16) suggest that plant infusions are phyto-complexes of varying composition and contain alkaloids, flavonoids, tannins and other complex compounds that are produced by plants as protective mechanisms and which may be toxic or non-toxic when isolated in pure form.

The higher content of total phenolic and flavonoid compounds in *S. oleraceus* may account for the antioxidant effect (9). Fig. 3 showed hypertrophy of spleen of *Escherichia coli* infected group treated with the ethanolic extract of *Sonchus oleraceus*.

The same was found also in the spleen of the group that treated with the ethanolic extract of *Sonchus oleraceus*, then infected by *Escherichia coli* bacteria (Fig. 4) when compared with control group. The components of *Sonchus oleraceus* extracts may have an activation and anti-inflammatory effect on immune system. This may explain the enlargement of spleen in the first and second groups when compared with the third group. During a wound healing response, inflammation occurs because immune cells accumulate at the site of tissue damage (17).

The immune cells try to repair the damaged tissue by releasing various chemical signals (cytokines/chemokines) to create an environment that promotes cellular proliferation (growth). The immune cells maintain this enriched environment until the damaged tissue has been repaired or replaced with healthy tissue (18).

Histopathological study of the first group, the Liver was showed multiple granulomatous lesions consist of aggregation of macrophages scattered throughout the liver paranchyma (Figure, 3) and mononuclear cells aggregated around congested central veins which contain inflammatory cells in their lumen. In other sections, megakaryocytes are seen.

In spleen histopathological showed lymphocytic proliferation in the periarteriolar sheath with congestion of red pulp (Figure, 4).

While the intestine was showing hyperplasia and hyperatrophy of goblet cells with

mononuclear cells infiltration in the lamina propria of intestinal mucosa.

Histopathological of heart appeared congestion of blood vessels with inflammatory cells in their lumen were reported between muscular fibers of the heart (Fig. 5). Also Kidney showed congested blood vessels between renal tubules with inflammatory cells in their lumen together with acute cellular degeneration (Fig. 6).

Lung was showed increased thickness of intra alveolar septa of the lung due to congested capillary blood vessels and inflammatory cells infiltration (Fig. 7).

Histopathological results of second group, the liver revealed proliferation of kupffer cells and mononuclear cells aggregation around central veins as well as vaculation and fatty degeneration in the hepatic cells (Fig. 8).

Intestine was showed few mononuclear cells infiltration in the lamina propria of intestinal mucosa (Fig. 9). While spleen showed marked hyperplasia of white pulp of the spleen was seen (Fig.10)

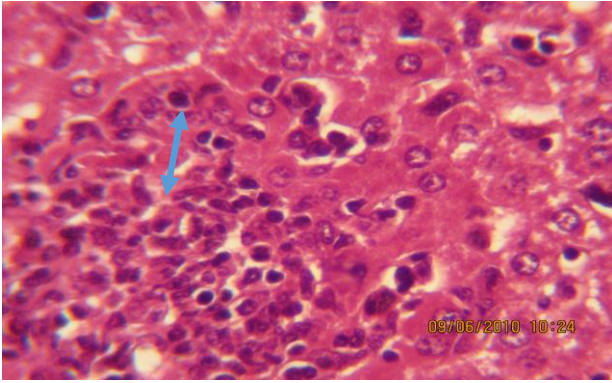
No clear pathological lesions were reported in the kidney, lung and heart.

Histopathological results of third group, The Liver was showed multiple variable size granulomatous lesions scattered throughout the liver (Fig. 11), with congested central veins and neutrophils in their lumen.

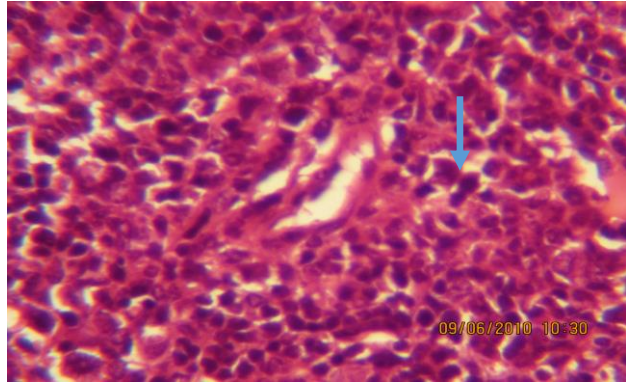
Kidney revealed acute cellular degeneration of the epithelial lining cells of the renal tubules with mononuclear cells aggregation around the blood vessels between renal tubules (Fig.12).

The lesions of the intestine characterized by hyperplasia of goblet cells, and inflammatory cells mainly neutrophils and macrophages in the lamina propria of atrophic villi (Fig.13). While heart showed inflammatory cells aggregation around blood vessels between cardiac muscle fiber (Fig.14).

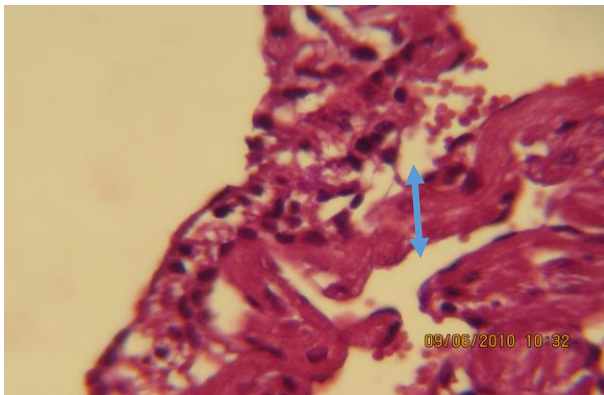
Histopathological results of fourth group were showed in Liver marked mononuclear cells aggregation around central veins (Fig. 15). While Spleen showed moderate hyperplasia of lymphocytic cells in the perarteriolar sheath of the spleen (Fig. 16). No clear pathological changes were reported in other examined organs.



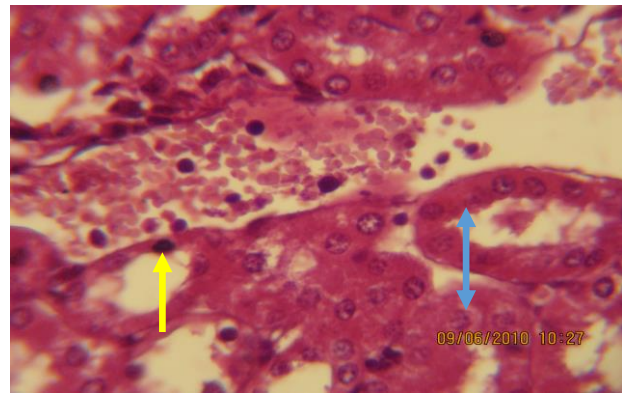
Figure,3: Section in liver shows multiple granulomatous lesion consist from aggregation of macrophages scatter throughout the parenchyma, (H&E X40 ).



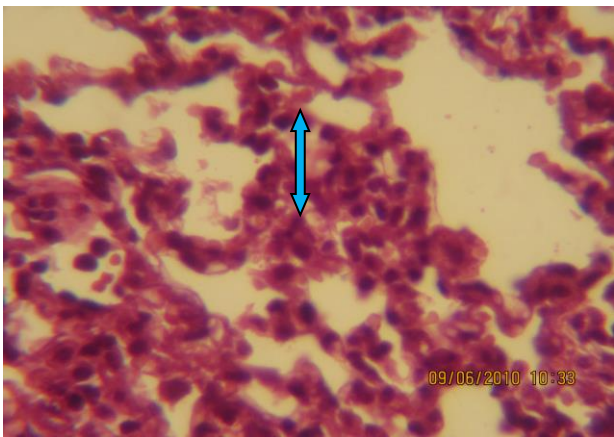
Figure, 4: Section in liver shows lymphocytic proliferation in the perarteriolar Sheath with congestion of red pulp (H&E X40).



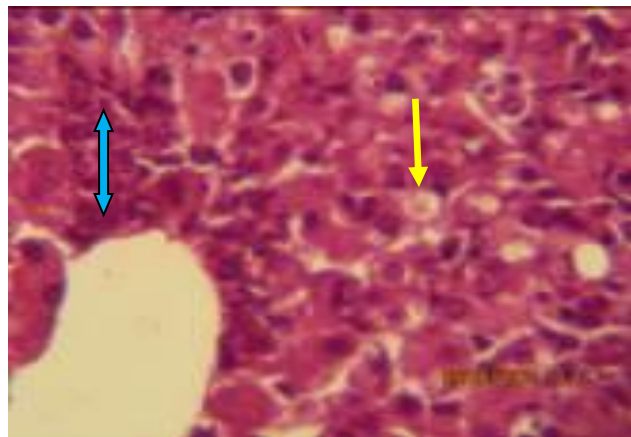
Figure, 5: Section in heart shows congestion of blood vessels with inflammatory cells in their lumen between muscular fiber (H&EX40).



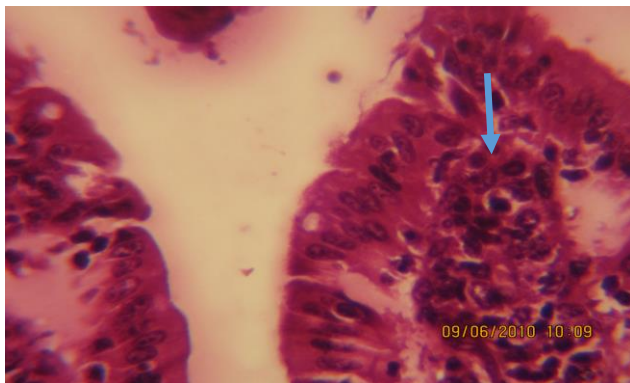
Figure, 6: Section in kidney shows showed congested blood vessels between renal tubules (  $\longleftrightarrow$  ) with inflammatory cells in their lumen (  $\rightarrow$  ) together with acute cellular degeneration (H&EX40).



Figure, 7: Section in lung shows increased thickness of intra alveolar septa of the lung due to congested capillary blood vessels and inflammatory cells infiltration (  $\longleftrightarrow$  )(H&EX40 ).



Figure, 8: Section in liver shows proliferation of kupffer cells and mononuclear cells aggregation around central veins (  $\longleftrightarrow$  ) as well as vacuolar and fatty degeneration in the hepatic cells(  $\rightarrow$  ) (H&EX40 ).



Figure, 9: section in intestine of animal infected by *Escherichia coli* , and then treated with the ethanolic extract of *Sonchus oleraceus* shows few mononuclear cells infiltration in the lamina propria of intestinal mucosa (H&EX40 ).

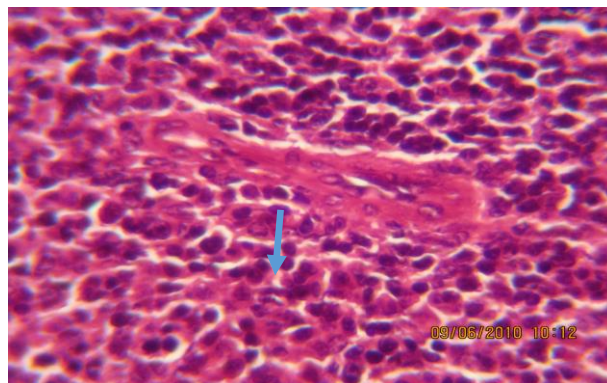
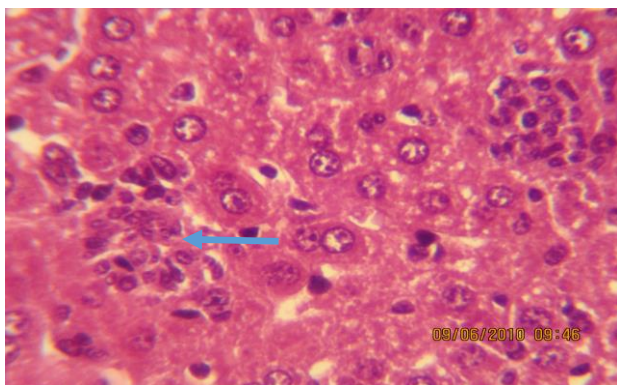


Figure10: Section in spleen showed marked hyperplasia of white pulp of the spleen (H&EX40).



Figure, 11: Section in liver shows multiple variable size granulomatous lesions scatter throughout the liver (H&EX40 ).

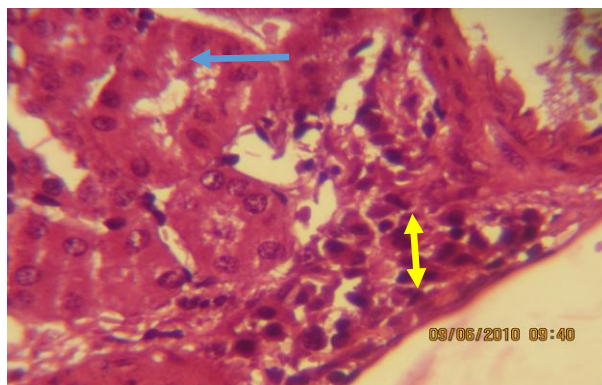
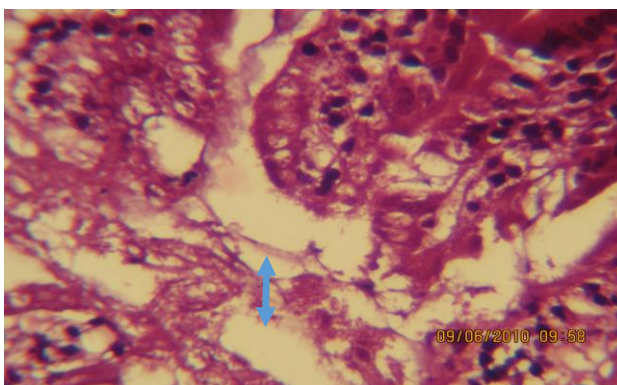
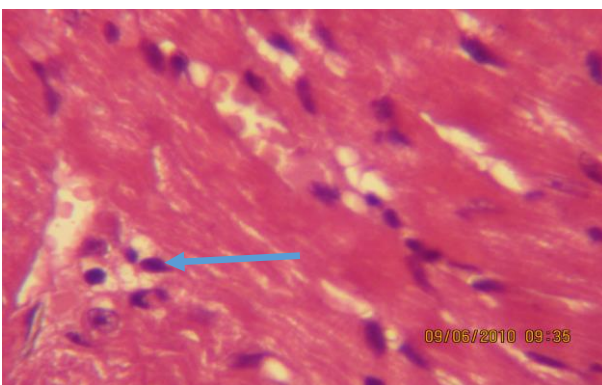


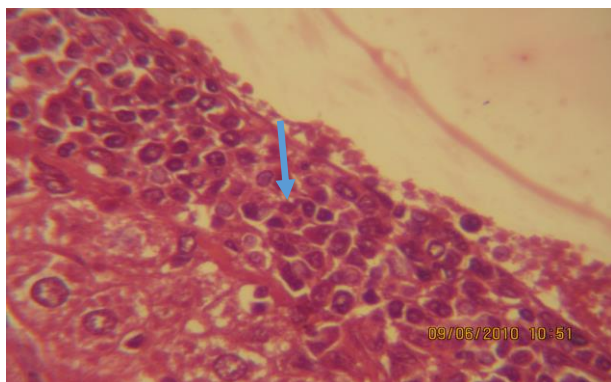
Fig12: Section in kidney cells of the renal tubules (→)with mononuclear cells aggregation around the blood vessels between renal tubules(↔) (H&EX40 ).



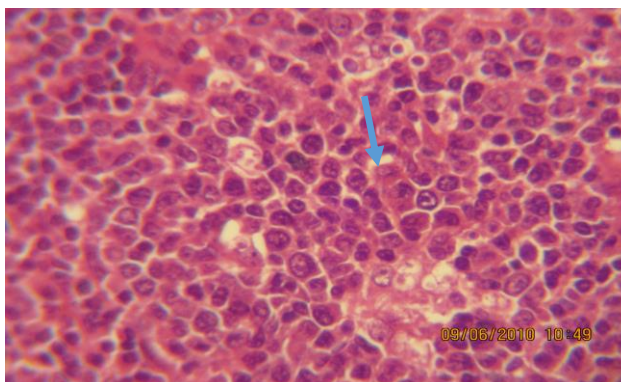
Figure, 13: Section in intestine bacteria shows hyperplasia of goblet cells, mucin in the lumen and inflammatory cells mainly neutrophils and macrophages in the lamina propria of atrophic villi (H&EX40).



Figure, 14: Section in heart shows inflammatory cells aggregation around blood vessels between cardiac muscle fiber (H&EX40).



**Figure, 15:** Section in liver shows marked mononuclear cells aggregation around central veins (H&EX40).



**Figure, 16:** Section in spleen shows moderate hyperplasia of lymphocytic cells in the periarteriolar sheath of the spleen (H&EX40).

The present study showed supportive inflammation and small multiple granulomatous lesion in some internal organs of infected animals with *E.coli*. These results indicated that the bacteria used in this study were highly virulence. Invasion of the epithelial cells of intestinal mucosa and spread to blood stream of internal organs and induced suppurative inflammation, these evidence was agreement with Conour and his team (19) who explained that, the lesions of the intestine due to *E. coli* infection characterized by hyperplasia of goblet cells, mucin in the lumen and inflammatory cells mainly neutrophils and macrophages in the lamina propria of atrophic villi. Inflammation is a common host response to this pathogen. Increased numbers of goblet cells may represent an intestinal epithelial response to the inflammation. Increased numbers of goblet cells have been shown in other instances of injury, leading some to propose that this adaptive response allows goblet cell-secreted mucin to form a viscous gel that traps microorganisms and irritants and limits their access to the epithelium (20). In chemically induced intestinal inflammation, the expression and secretion of mucin increased with disease progression (21). Furthermore, it has been reported that the overproduction and secretion of mucin are associated with inflammation caused by bacterial infection (22).

The first group show multiple granulomatous lesions consist from aggregation of macrophage and mononuclear cells in the liver around congested central

veins which contain inflammatory cells in their lumen, lymphocytic proliferation in the periarteriolar sheath in the spleen, hyperplasia and hyperatrophy of goblet cells with mononuclear cells infiltration in the lamina propria of intestinal mucosa and infiltration of inflammatory cells in other organs, and this may explain due to the innate immunity characterized by the establishment of chemotactic gradients redirecting normally circulating phagocytes into the sites of infection takes place immediately after introduction of an infectious agent into the host environment (23). The accumulation of circulating cells at the site of infection appears to be mediated by the localized release of chemokines that are produced by a variety of cell types (23). Also the present study explained that the animals administrated with ethanolic extract of *Sonchus oleraceus* expressed granulomatous lesions with internal organs that investigation may be indicated that the plant extract stimulated immune cells particularly macrophages and lead to aggregate activated macrophages around invasion pathogen and stimulation granuloma in order to destroyed the pathogen these evidence was agreement with (24), who explained that many plants contain nontoxic glycosides which can get hydrolyzed to release phenolics which are toxic to microbial pathogens. Antimicrobial properties of substances are desirable tools in the control of undesirable microorganisms especially in the treatment of infections and in food spoilage. The active components usually interfere with

growth and metabolism of microorganisms in a negative manner and is quantified by determining the minimum inhibitory concentration and minimum bacteriocidal activity. These values are used as guide for treatment of most infections. These results suggest that the ethanolic extract of *Sonchus oleraceus* stimulated immune response against bacterial infection that needs more investigations.

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### دراسة مرضية لتأثير المستخلص الكحولي لنبات المرير على الإصابة بجراثيم *E. coli* في الفئران

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#### الخلاصة

صممت هذه الدراسة للتحري عن تأثير المستخلص الكحولي لنبات المرير *Sonchus oleraceus* على جرثومة الايشيريشيا القولونية *Escherichia coli* في الفئران. و قد أجريت دراسة تقييم تأثير المستخلص الكحولي، و كانت الجرعة المميتة الوسطية (LD<sub>50</sub>) للمستخلص (8غم/كغم من وزن الجسم)، و قد أعطيت الجرعة العلاجية للمستخلص عن طريق الفم يومياً و لمدة أسبوعان. قسمت مجاميع الفئران عشوائياً (25 فارة) الى خمسة مجاميع كل مجموعة خمسة حيوانات، المجموعة الاولى جرعت بالمستخلص الكحولي بجرعة 800ملغم/كغم من وزن الجسم عن طريق الفم لمدة اسبوعين ثم اصيبت بجرثومة الايشيريشيا *Escherichia coli* و بجرعة 10<sup>8</sup>×1 لمدة عشرة ايام، المجموعة الثانية تم اصابتها بجرثومة الايشيريشيا *Escherichia coli* و بجرعة 10<sup>8</sup>×1 لمدة عشرة ايام ثم عولجت بالمستخلص الكحولي لمدة اسبوعين، المجموعة الثالثة اصيبت بجرثومة الايشيريشيا *Escherichia coli* و بجرعة 10<sup>8</sup>×1 لمدة عشرة ايام فقط، المجموعة الرابعة جرعت بالمستخلص الكحولي بجرعة 800ملغم/كغم من وزن الجسم عن طريق الفم لمدة اسبوعين فقط، المجموعة الخامسة حقنت بالمحلول الملحي المتعادل كمجموعة سيطرة. أظهرت الدراسة المرضية النسجية تغيرات مرضية في معظم الاعضاء الداخلية في المجموعة المصابة بجرثومة الايشيريشيا *Escherichia coli* (المجموعة الثالثة) وخاصة الكبد والكلية والامعاء، اما المجموعة الاولى فقد اظهرت اعراضاً اقل حدة من المجموعة المصابة (المجموعة الثالثة)، في حين لوحظ في المجموعة الثانية فقط انتشار للخلايا الالتهابية في بعض الاعضاء الداخلية، ويشير ذلك الى ان المستخلص الكحولي لنبات المرير يمتلك تأثيراً واضحاً على تثبيط نمو جرثومة الايشيريشيا القولونية مقارنة بالمجموعة المصابة.

الكلمات المفتاحية:- جرثومة الايشيريشيا، نبات المرير، المستخلص الكحولي.