HISTOPATHOLOGICAL AND PROPHYLACTIC EFFECT OF THE DANDELION ETHANOLIC EXTRACT AND LACTOBACILLUS ACIDOPHILUS ON PATHOGENIC BACTERIA *in vitro* AND *in vivo* STUDY.

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ABSTRACT

This study was designed to explore the pathological and prophylactic effect of the mix of Dandelion ethanolic extract with the bacterium *lactobacillus acidophilus in vitro* and *in vivo* using laboratory mice. Dandelion ethanolic extract, *lactobacillus acidophilus* and the mix of Dandelion ethanolic extract with the bacterium *lactobacillus acidophilus* were dealing in *in vitro* study against (*Salmonella typhimurium, Pseudomonas aerogenosa، Staphylococcus aureus and Escherichia coli*) using the concentration 100 mg/L for Dandelion extract, also dealing with the virulent bacteria in equal quantity and separately. *Lactobacillus acidophilus* had the highest inhibitory effect on the *Pseudomonas aerogenosa,* and the less inhibitory effect was by Dandelion extract, and the inhibitory effect of Dandelion extract on the pathogenic bacteria increased after mixed with *lactobacillus acidophilus* bacteria. Twenty four mice were randomly divided into six groups, each ghroup contain four animals. The first group infected with *Pseudomonas aerogenosa* and the dose $1x10^6$ cfu orally for 48 hours. The second group infected with *Pseudomonas aerogenosa* with dose $1x10^6$ cfu orally for two weeks. The third group infected with *Pseudomonas aerogenosa* and the dose $1x10^6$ cfu orally for 48 hours, they treated with the mix of Dandelion ethanolic extract and *lactobacillus acidophilus* orally (0.3 ml/mice) for two weeks. The fourth group were treated with the mix of Dandelion ethanolic extract and *lactobacillus acidophilus* orally (0.3 ml/mice) for two weeks, and then infected with *Pseudomonas aerogenosa* and the dose $1x10^6$ cfu orally for 48 hours. The fifth group administered Dandelion extract and concentration 100 mg/ml (0.3 ml/mice) orally for two weeks. The sixth group administered 0.3 ml/mice of normal saline as control group. The histopathological study showed pathological changes in the internal organs of the first and second groups that infected with *Pseudomonas aerogenosa* bacteria. The mix of Dandelion extract and *lactobacillus acidophilus* apparently has therapeutic effect more than prophylactic effect on the inhibition of *Pseudomonas aerogenosa* growth.

Key words: Dandelion ethanolic extract, *lactobacillus acidophilus, Pseudomonas aerogenosa,* pathogenic bacteria

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INTRODUCTION

Herbal medicines have been used very effectively for longer than synthetics, and many current pharmaceutical products have been derived from research on plants used as medicine by many cultures (Yarnell and Abascal, 2009). Dandelion (*Taraxacum officinalis*) is a member of the Asteraceae /Compositae family closely related to chicory. It is a perennial herb, native throughout the Northern hemisphere, found growing wild in meadows, pastures and waste grounds of temperate zones. Researchers suggest that dandelion root may possess anti-inflammatory properties (Hu and Kitts, 2005). Several laboratory studies report antioxidant properties of dandelion flower extract (Kim *et al.,* 2000). Also combination herbal preparation containing dandelion improved chronic pain associated with colitis ((Hu and Kitts, 2005).

Lactobacillus is a type of bacteria. There are lots of different species of lactobacillus. These are "friendly" bacteria that normally live in our digestive, urinary, and genital systems without causing disease. Lactobacillus is also in some fermented foods like yogurt and in dietary supplements. Medications that decrease the immune system (Yuan Wang *et al.*, 2004). The lactic acid bacteria preservative activity is due to their ability to produce a variety of antimicrobial substances as a natural competitive means to overcome other microorganisms sharing the same niche, among them, ethanol, hydrogen peroxide, diacetyl and bacteriocins (Al-Allaf *et al.,* 2009; Olivera *et al.,* 2008). The antimicrobial spectrum against competing natural flora was frequently includes spoilage bacteria and food-borne pathogens such as *Listeria monocytogenes* and *Staphylococcus aureus* (Lucke, 2000; Bromberg *et al.*, 2004). The antimicrobial activities of probiotics have been evaluated against *Escherichia coli*, *Salmonella*, *Listeria species*, *Helicobacter pylori* and *Candida albicans* (Puertollano1 *et al.,* 2008). But in digested form useful bacteria which are present in the digestive system, such as Lactobacillus which contain digestive enzyme and could be useful as a source of carbohydrate as a result of reproduction and increase of number and hence is composition with organism such as *E. coli* and *Salmonella*. Therefore, Dandelion could be used as a main source of inulin. And (*Taraxacum officinale*) could be used as a source of prebiotic (Al-Kassie *et al.,* 2008).

The aim of this study is evaluation the antibacterial activities, therapeutic and prophylactic effect of Dandelion ethanolic crude extract and the mixture (from Dandelion ethanolic extract with *lactobacillus acidophilus*) on pathogenic bacteria *in vitro* and *in vivo*.

MATERIALS AND METHODS:

1- Bacterial culture:

a- Bacterial isolates serotypes that used in vitro study were *Pseudomonous aerogenosa, Staphylococcus aureus*, *Salmonella typhimurium and E.coli*)were obtained from Zoonoses Unit/ Veterinary Medicine/ Baghdad University, and

the biochemical properties were tested depending on the method of (Quinn *et al.,* 1998).

b- The bacterial count of *Lactobacillus acidophilus* was ($1x10^8$) cfu.

c- The bacterial suspension ($1x10^6$) cfu of *Pseudomonous aerogenosa* was prepared as the method in (Quinn *et al.,* 1998).

2-Preparation of Dandelion extract - according to (Ahmed *et al.,* 2006).

3- Sensitivity test: as the following-

1- were taken 12 Petri dishes of agar –type Muller Hinton agar, which wiped every three dishes for one type of bacteria four drops of bacterial suspension that prepared and calculated manner according to M cFarland tube (first tube), and after that dried the dishes, punctured dishes by using the drilling cork (four holes /one dish).

2- each Petri dish contain one of the pathogenic bacteria has been injected into the four holes by Dandelion extract concentration 100 mg , *Lactobacillus acidophilus* suspension ($1x10^8$) cfu, mix of dandelion extract and Lactobacillus acidophilus and ethanol alcohol 70% as control respectively.

Then all the dishes were incubated at 37° C for 24 hours.

4- Experimental Design of *in vivo* study:

Twenty four white mice both sexes, 7-8 week olds and weight from 25-30 grams were randomly divided into six groups equally and treated as follows:

1- First group was administrated orally o.3 ml of *Pseudomonous aerogenosa* of bacterial suspension containing $1x10^6$ cfu orally as acute infection.

2- Second group was administrated orally with 0.3 ml of bacterial suspension containing $1x10^6$ cfu orally of *Pseudomonous aerogenosa* for 14 days.

3- Third group was administrated orally with 0.3 ml of bacterial suspension containing $1x10^6$ cfu orally *Pseudomonous aerogenosa* for 48 hours, then treated with o.3 ml of mix of *Lactobacillus acidophilus* and dandelion extract for 14 days daily.

4- Fourth group was administrated orally with o.3 ml of mix of *Lactobacillus acidophilus* and dandelion extract for 14 days daily, then infected with $1x10^6$ cfu orally *Pseudomonous aerogenosa* for 48 hours.

5- Fifth group was administrated orally with o.3 ml dandelion extract for 14 days daily.

6- Sixth group was administrated orally with o.3 ml of normal saline and served as control negative group.

All animals were sacrificed and pieces from internal organs were fixed in 10% formalin 72 hours for histopathological examination according to Luna, (1968).

RESULTS AND DISCUSSION

1- *In vitro* study:

Table (1) Shows that *Lactobacillus acidophilus* had a larger effect on the bacterium inhibition in diameter of 22 mm of *Pseudomonous aerogenosa*. While the Dandelion extract gives the less volume inhibition effect to *Salmonella typhimurium* in diameter of 11 mm when measured by a ruler. Similar study was carried out in Morocco by Kalalou whose studied the activity of LAB on some gram positive and negative pathogenic bacteria such as *E.coli, Pseudomonas aeroginosa, Klebsiella pneumoniae, Staphylococcus aureus* and *Bacillus cereus* and the inhibition zones were in the range of 1.4 to 2.8 cm (Kalalou *et al.*, 2004).

While the Dandelion extract gives the less volume inhibition effect to *Salmonella typhimurium* in diameter of 11 mm when measured by a ruler. It has been suggested that high resistant to plant extracts in gram negative bacteria is due to the outer membrane of their cell wall, acting as barrier to many substances including antibiotics (Marino *et al.*, 2011). *T. officnale* had a highly concentrated of some phytoco nstituents in the stem, root and flower such as saponins, flavonoids, alkaloids and phenols (Mir *et al.*, 2013). The low antioxidant activity of dandelion may be due to the presence of active scavenging compounds in other parts of plants such as flowers and roots more than leaves as in lutiolin and lutiolin-7-o-glycoside (Hu and Kitts, 2004).

Table 1: The sensitivity test results of *Lactobacillus acidophilus*, Dandelion extract and mix of Dandelion extract and Lactobacillus against some of pathogenic bacteria

Type of pathogenic bacteria	*Lactobacillus acidophilus* ($1x10^8$)			Dandelion extract (100 mg)			Mix of Dandelion extract and Lactobacillus		
	(mm)			(mm)			(mm)		
Pseudomonous aerogenosa	20	*22	20	13	11	11.5	16	16	15
Staphylococcus aureus	16	17	17	11	11	12	17	17	17
Salmonella typhimurium	13	14	14.5	*11	11	11	14	15	14.5
E.coli	17	18	18	13	14	13	18	16	18

Also notice that Lactobacillus acidophilus has higher inhibitory effect on all the bacterial growth than the Dandelion extract, but the mix between

Lactobacillus acidophilus and Dandelion extract shows more active and increase the inhibitory effect of Dandelion extract on the growth of the bacteria. Strain of Lactobacillus isolated to inhibit growth of some pathogenic bacteria; indicated the inhibitory effect on *E. coli* and *Pseudomonas aeruginosa* (Olanrewaja, 2007). The antagonistic activity of Lactobacilli may be due to production of organic acid resulting in pH decrease. Lactic acid bacteria have been shown to inhibit (*in vitro*) growth of many enteric pathogens and used in both humans and animals to treat a broad range of gastrointestinal disorders (Ouwenhand and Vesterland. 2004).

2- In *vivo* (Histopathological) study:

1- The first group-

a-Kidney- After infected with *Pseudomonous aerogenosa* orally as acute infection showing stenosis of renal tubules as star shape due to hyperplasia of renal tubular epithelium (fig: 1)**.**

b-Intestine- The first group that infected with *Pseudomonous aerogenosa* orally as acute infection showing hyperplasia of goblet cells and inflammatory cells in the lamina propria (fig: 2).

c-Spleen- After infected with *Pseudomonous aerogenosa* orally as acute infection showing infiltration of inflammatory cells &congestion of blood sinuses (fig: 3).

2- The second group-

a-Liver- The microscopic section revealed the central venule filled with inflammatory cells and infiltration of inflammatory cells in the liver parenchyma (fig: 4).

b- Kidney- The histopathological section showing dilatation of urinary space, infiltration of inflammatory cells in the renal parenchyma, stenosis of renal tubules and congestion of blood vessels (fig: 5) and in some sections there is atrophy of glomeruli and amyloid deposits in the interstitial peritubular tissue.

3- The third group-

a- Lung- The microscopic section revealed thickening of the interalveolar septa and infiltration with inflammatory cells (fig: 6).

4- The fourth group-

a- Intestine- The section showing hyperplasia of goblet cells and inflammatory cells in the lamina propria of atrophic villi (fig: 7).

b- Spleen- The microscopic section revealed presence of amyloid in the interstitial tissue (fig: 8), and in some sections showed infiltration of inflammatory cells &congestion of blood sinuses with hyperplasia of lymphoid follicles.

c- Kidney- The histopathological section showing congestion of blood vessels, dilated of urinary space and amyloid precipitation in the interstitial peritubular tissue(fig: 9).**5- Fifth group-**No clear pathological changes were reported in other examined organs.

In vivo study- the histopathological study of the first and second groups that infected with *Pseudomonous aerogenosa* showed infiltration of inflammatory cells in some internal organs. 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 (Coussens and Werb, 2002). 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 (Belley *et al.,* 1999).

The virulence factors important for establishing acute infections are distinct from those critical for chronic infections. Chronic infections are minimally invasive and non cytotoxic. These infections involve the formation of biofilms, which in the context of human infection, protect against assault by the host immune system and provide resistance to antibiotics (Ryder *et al.,* 2007). Thus, chronic infections rarely result in systemic spread, but instead lead to unrelenting non-productive host inflammation that contributes to the resulting morbidity and mortality (Deretic *et al.,* 1995).

The third group less inflammatory infection due to the treatment by the mix of Dandelion extract with *Lactobacillus acidophilus.* Among the most important compounds in dandelion are sesquiterpene lactones (believed to have antiinflammatory and anti-cancer effects), phenylpropanoids (believed to have inflammation- modulating effects) .

Lactic acid bacteria secrets anti-inflammatory metabolites, such as lipoteichoic acids from *L. johnsonii* and *L. acidophilus* antagonize the responsiveness of human intestinal epithelial cell to lipopolysaccharide (Vidal *et al.,* 2002).

Several studies have demonstrated that *L. acidophilus* is able to boost the immunity of host by producing the strong colonies in the intestinal tract, so that pathogenic bacteria were not able to create any destruction in the host body (Perdigon *et al.,*1993).

The fourth group showed inflammation and infiltration of inflammatory cells with precipitation of amyloid in some organs. Amyloidosis is a systemic disorder characterized by the extracellular deposition of a protein-like material in multiple organs. The deposition of amyloid leads to Progressive organ dysfunction. Secondary (AA) amyloidosis is derived from the inflammatory protein serum amyloid A, and occurs with chronic inflammatorydisease . Amyloid deposits may occasionally occur in isolation without evidence of a systemic disease (Kumar *et al.,* 2007).

The mix of extract and *Lactobacillus* revealed no prophylactic effect against *Pseudomonas aeroginosa.* Studies on the effects of various dandelion extracts and compounds on the immune system are contradictory, some showing inhibition and some stimulation of tumor necrosis factor *(*Koo *et al.*, 2004). This may suggest that dandelion extract has various effects on different lymphocyte populations or body tissues, or it may indicate that dandelion can modulate immune reactions. The polysaccharides in dandelion, of which there are many, are often credited with being key inter-mediaries in immune interactions. More in-depth research is needed on dandelion's immune-system effects (Jeong *et al.*, 1991).

Fig1:Histopathological section in kidney of one animal that infected with *Pseudomonous aerogenosa* orally as acute infection showed stenosis of renal tubules as star shape(⟵).

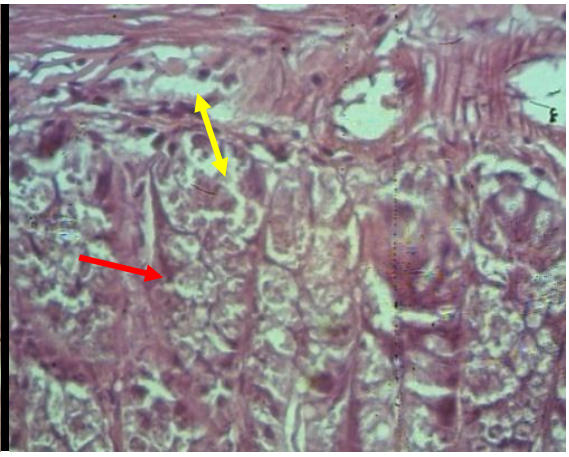

Fig2: Histopathological section in intestine of animal infected by *Pseudomonous aerogenosa* orally as acute infection showed hyperplasia of goblet cells (⟵)and inflammatory cells in the lamina propria (↔) (H&EX40).

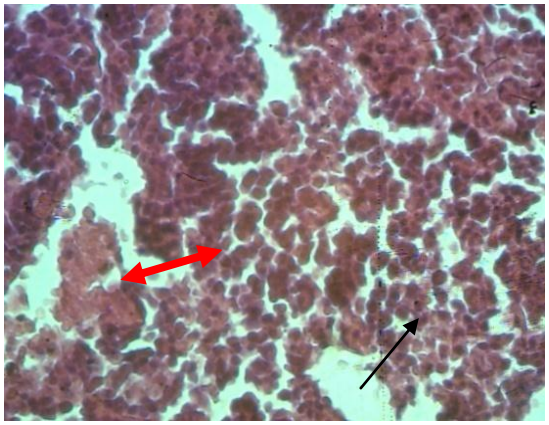

Fig 3 :Histopathological section in spleen of one animal infected by *Pseudomonous aerogenosa* orally as acute infection showed infiltration of inflammatory cells (⟶)&congestion of blood sinuses (↔) . (H&EX400

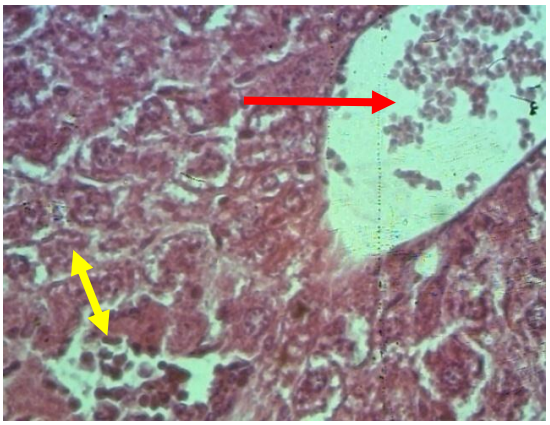

Fig4:Histopathological section of liver of one animal that infected with *Pseudomonous aerogenosa* orally for14 days showed the central venule filled with inflammatory cells (⟶) and infiltration of inflammatory cells in the liver paranchyma (↔). (H&EX400).

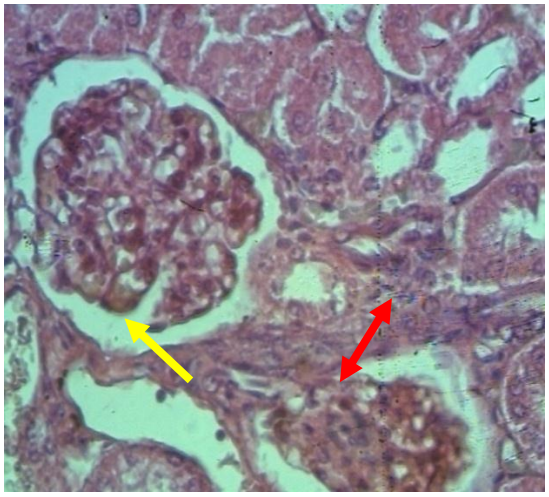

Fig5:Histopathological section of kidney of one animal that infected with *Pseudomonous aerogenosa* orally for14 days showed dilatation of urinary space (⟶), infiltration of inflammatory cells in the renal paranchyma (↔),stenosis of renal tubules and congestion of blood vessels ( ). (H&EX400).

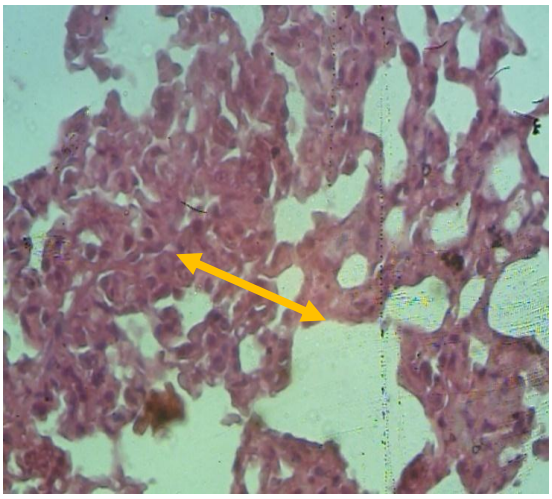

Fig6: Histopathological section in lung of animal infected by *Pseudomonous aerogenosa* , then treated with mix of *Lactobacillus acidophilus* and dandelion extract showed thickening of the interalveolar septa and infiltration with inflammatory cells (↔) (H&EX40).

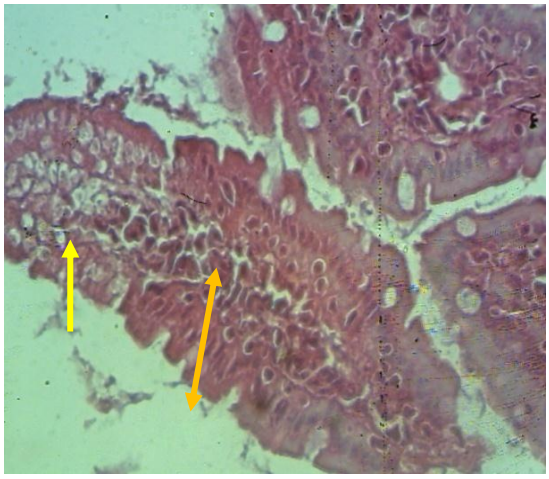

Fig7:Histopathological section of intestine of one animal that treated with mix of *Lactobacillus acidophilus* and dandelion extract for 14 days daily, then infected with *Pseudomonous aerogenosa* showed hyperplasia of goblet cells(⟶) and inflammatory cells in the lamina propria of atrophic villi (↔). (H&EX400).

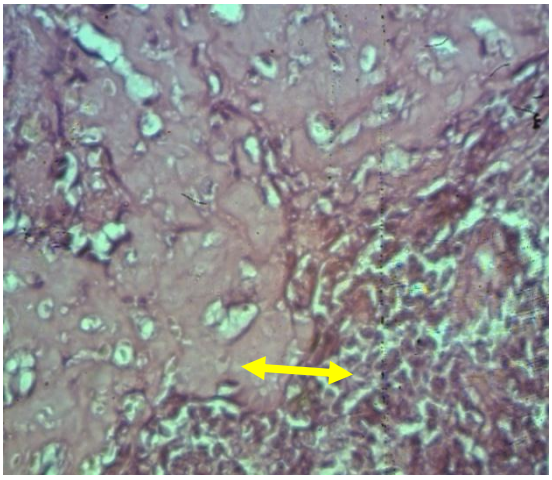

Fig8:Histopathological section of spleen of one animal that treated with mix of *Lactobacillus acidophilus* and dandelion extract for 14 days daily, then infected with *Pseudomonous aerogenosa* showed amyloid in the interstitial tissue (↔) (H&EX400).

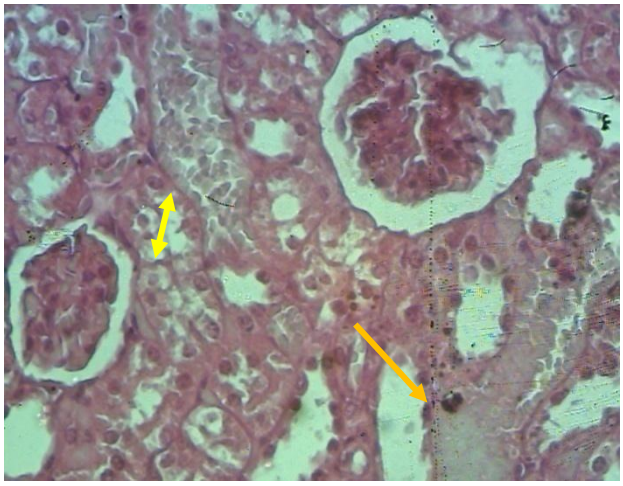

Fig9:Histopathological section of kidney of one animal that treated with mix of *Lactobacillus acidophilus* and dandelion extract for 14 days daily, then infected with *Pseudomonous aerogenosa* showed congestion of blood vessels (↔), dilated of urinary space and amyloid precipitation in the interstitial tissue(⟶) (H&EX400).

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دراسة مرضية وقائية لتاثير مستخلص الهندباء الكحولي مع ال *lactobacillus acidophilus* على بعض البكتيريا المرضية خارج الجسم وداخل الجسم

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المستخلص

صممت هذه الدراسة للتحري عن التأثيرالمرضي والوقائي لمزيج المستخلص الكحولي لنبات الهندباء Dandelion مع جرثومة ال *lactobacillus acidophilus* على بعض الجراثيم المرضية خارج الجسم وداخل الجسم في الفئران المختبرية. تم معاملة مستخلص الهندباء الكحولي و جرثومة ال *lactobacillus acidophilus* و مزيج مستخلص الهندباء مع جرثومة ال *lactobacillus acidophilus* خارج الجسم الحي على أنواع من الجراثيم المرضية وهي *Salmonella typhimurium* و *Pseudomonas aerogenosas* و *Staphylococcus aureus* و *Escherichia coli* وذلك باستخدام تركيز 100 ملغم/لتر لمستخلص الهندباء وكذلك معاملة الجراثيم

المرضية بمقادير متساوية كل على حدة, وكان أعلى تأثير لجرثومة ال *lactobacillus acidophilus* على جرثومة ال *Pseudomonas aerogenosa* واقل تاثير تثبيطي لمستخلص الهندباء على جرثومة ال *Salmonella typhimurium* ، وقد ازداد التاثير التثبيطي لمستخلص الهندباء على الجراثيم المرضية بعد مزجه بجرثومة ال *lactobacillus acidophilus* .

قسمت مجاميع الفئران عشوائيا (24 فارة) الى ست مجاميع كل مجموعة اربعة حيوانات،المجموعة الاولى اصيبت بجرثومة ال*Pseudomonas aerogenosa* وبجرعة 1×10^{6} عن طريق الفم لمدة 48 ساعة ،المجموعة الثانية تم اصابتها بجرثومة ال *Pseudomonas aerogenosa* وبجرعة 1×10^{6} عن طريق الفم لمدة اسبوعين، المجموعة الثالثة اصيبت بجرثومة ال *Pseudomonas aerogenosa* وبجرعة 1×10^{6} لمدة 48 ساعة ثم عولجت بمزيج مستخلص الهندباء مع جرثومة ال *lactobacillus acidophilus* وبحجم 0.3 مل/فارة عن طريق الفم لمدة اسبوعين،المجموعة الرابعة جرعت مستخلص الهندباء مع جرثومة ال *lactobacillus acidophilus* وبحجم 0.3 مل/فارة عن طريق الفم لمدة اسبوعين ثم اصيبت بجرثومة ال *Pseudomonas aerogenosa* وبجرعة 1×10^{6} لمدة 48 ساعة، المجموعة الخامسة جرعت بمستخلص الهندباء الكحولي و بتركيز100 ملغم/مللتر وبحجم 0.3 مل/فارة عن طريق الفم لمدة اسبوعين، اما المجموعة السادسة اعطيت المحلول الملحي المتعادل وبحجم 0.3 مل/فارة كمجموعة سيطرة. أظهرت الدراسة المرضية النسجية تغيرات مرضية في الاعضاء الداخلية في المجموعة الاولى والثانية المصابة بجرثومة *Pseudomonas aerogenosa*، ويشيرذلك الى ان مزيج مستخلص الهندباء مع جرثومة ال *lactobacillus acidophilus* يمتلك تاثيرا علاجيا واضحا اكثر من امتلاكه تاثيرا وقائيا على تثبيط نمو جرثومة *Pseudomonas aerogenosa*.