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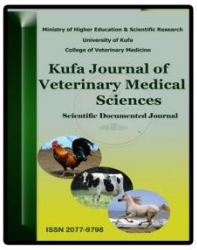

Pathological study of alcoholic *plantagolanceolata* crude extracts effectonsome Salmonella species *in vitro* and *in vivo*

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Summary

This study was designed to explore the pathological effect of alcoholic *plantagolanceolata* crude extracts effect on some Salmonella species *in vitro* and *in vivo* study by using laboratory mice. *plantagolanceolata* crude extracts were dealing in *in vitro* study against some virulent bacteria including *Salmonella typhimurium*, *Salmonella typhi and Salmonella hadar*. And using the concentrations (100,150,200,250 and 300) mg for *plantagolanceolata* crude extract, concentration at 300 mg had the highest inhibitory effect on the three types of salmonella, when compared with the other concentrations. The pathological study of *plantagolanceolata* crude extract was done against the infection of *Salmonella typhimurium*as *in vivo* study by using white laboratory mice.

Twenty four mice were randomly divided into six groups, each group contain four animals. First group was administrated orally o.3 ml of *Salmonella typhimurium* of bacterial suspension containing $1x10^6$cfu orally for one week of infection. Second group administrated orally with 0.3 ml of bacterial suspension containing $1x10^6$cfu orally of *Salmonella typhimurium* for two weeks as infection. Third group administrated orally with 0.3 ml of bacterial suspension containing $1x10^6$cfu orally *Salmonella typhimurium* for three weeks as infection. Fourth group was administrated orally with o.3 ml of *plantagolanceolata* extract for one week daily after infection with *Salmonella typhimurium*. Fifth group was administrated orally with o.3 ml of *plantagolanceolata* extract for two weeks daily after infection with *Salmonella typhimurium*. And sixth group was administrated orally with o.3 ml of *plantagolanceolata* extract for three weeks daily after infection with *Salmonella typhimurium*.

The histopathological study showed pathological changes in the intestine of the first , second and third groups that infected with *Salmonella typhimurium* bacteria, (sixth group) no clear pathological changes were reported except some lesions. For all, the *plantagolanceolata* extract apparently has therapeutic effect when used in high concentration *in vitro* and for long time *in vivo*.

دراسة مرضية لتاثير المستخلص الكحولي لاوراق لسان الحمل على بعض عتر السالمونيلا داخل وخارج الجسم الحي

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الخلاصة

صممت هذه الدراسة للتحري عن التأثيرالمرضي للمستخلص الكحولي لنبات لسان الحمل الخام على بعض جراثيم السالمونيلا المرضية خارج الجسم وداخل الجسم في الفئران المختبرية. تم معاملة مستخلص لسان الحمل الخام خارج الجسم الحي على أنواع من الجراثيم المرضية وهي *Salmonella typhimurium* و*Salmonella typhi* و*Salmonella hadar*وذلك باستخدام ترا كيز (100,150,200,250 and 300) ملغرام لمستخلص لسان الحمل الخام, ويمتلك التركيز 300 ملغرام التاثير التثبيطي الاعلى على الانواع الثلاثة من جرثومة السالمونيلاعند مقارنته مع التراكيز الاخرى, و قد أجريت الدراسة المرضية لمستخلص لسان الحمل ضد الاصابة بجرثومة *Salmonella typhimurium* داخل الجسم الحي باستخدام الفئران المختبرية البيضاء, حيث قسمت مجاميع الفئران عشوائيا (24 فارة) الى ستة مجاميع كل مجموعة اربعة حيوانات,المجموعة الاولى اصيبت بجرثومة ال*Salmonella typhimurium*وبجرعة1× 10^6 0.3 مل عن طريق الفم لمدة اسبوع من الاصابة ,المجموعة الثانية تم اصابتها بجرثومة ال*Salmonella typhimurium*وبجرعة1×10^6 0.3 مل عن طريق الفم لمدة اسبوعين, المجموعة الثالثة اصيبت بجرثومة ال *Salmonella typhimurium* وبجرعة1×10^6 0.3 مل عن طريق الفم لمدة ثلاثة اسابيع من الاصابة,المجموعة الرابعة اعطيت مستخلص لسان الحمل وبجرعة1×10^6 0.3 مل عن طريق الفم لمدة اسبوع بعد الاصابة بجرثومة السالمونيلا, المجموعة الخامسة اعطيت مستخلص لسان الحمل وبجرعة1×10^6 0.3 مل عن طريق الفم لمدة اسبوعين بعد الاصابة بجرثومة السالمونيلا, اما المجموعة السادسة اعطيت مستخلص لسان الحمل وبجرعة1×10^6 0.3 مل عن طريق الفم لمدة ثلاث اسابيع بعد الاصابة بجرثومة السالمونيلا.أظهرت الدراسة المرضية النسجية تغيرات مرضية في الامعاء في المجموعة الاولى والثانية و الثالثة المصابة ويشيرذلك الى ان مستخلص لسان الحمل الخام يمتلك تاثيرا علاجيا واضحا عند استخدامه بتركيز عالي في الزجاج, ولمدة طويلة داخل االجسم الحي.

Introduction

PlantagoLanceolata is a perennial plant from Plantaginaceae family, *P. Lanceolata* has also been used as an anesthetic, antiviral, anti-inflammatory, astringent, anti-helmintic, analgesic, analeptic, antihistaminic, anti-rheumatic , antitumor, anti-ulcer, diuretic, expectorant and hypotensive in traditional medicine (Kobeasy*et al.*, 2011). *P. lanceolata* is species belongs to family Plantaginaceae, genus plantago and native in Europe and Asia, which has introduced to other temperate zones (Now naturalized throughout the world), in addition to that antimicrobial potentials of extracts of *P. lanceolata* against standard and drug resistant human bacterial pathogens and fungus (Taskova*et al.*, 2002). Plantain (*Plantagolanceolata L.*) is one of the perennial herbs having some bioactive compounds such as acteoside, aucubin, and catalpo, which have anti-oxidative activity (Wang *et al.*, 1996) and anti-inflammatory effects (Marchesan*et al.*, 1998).

The species Salmonella enterica comprises a group of gram-negative bacteria that are important pathogens for humans and livestock (Ma ¨kela ¨ and Hormaeche, 1997). In humans, ingestion of various Salmonella serovars gives rise to infection of the small intestine and to gastro enteritis. A small number of Salmonella serovars can lead to systemic infection and enteric fever. In contrast to the severe outcome of disease in humans, *S. typhi* is avirulent in most animals, including mice. However, in mice, infection with *S. typhimurium* gives rise to enteric fever, with symptoms similar to those observed in humans after infection with *S. typhi* (Eisenstein, 1999).

The aim of this study is evaluation the antibacterial activities, therapeutic effect of *PlantagoLanceolata* crude extract on some pathogenic salmonella species *in vitro* and *in vivo*.

Materials and Methods

1- Bacterial culture:

- Bacterial isolates serotypes that used *in vitro* study were *Salmonella typhimurium, Salmonella typhi and Salmonella hadar* were obtained from Zoonoses Unit/ Veterinary Medicine/ Baghdad University, and the biochemical properties were tested depending on the method of (Quinn *et al.,* 1998).

2- Preparation of *plantagolanceolata* 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 McFarland tube (first tube), and after that dried the dishes, punctured dishes by using the drilling cork (six holes /one dish).

2 –Each Petri dish contain one of the pathogenic bacteria has been injected into the four holes by *plantagolanceolata* extract concentrations (100,150,200,250,300) mg and distilled water as control group 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 *Salmonella typhimurium* of bacterial suspension containing $1x10^6$cfu orally for one week of infection.

2- Second group was administrated orally with 0.3 ml of bacterial suspension containing $1x10^6$cfu orally of *Salmonella typhimurium* for two weeks as infection.

3- Third group was administrated orally with 0.3 ml of bacterial suspension containing $1x10^6$cfu orally *Salmonella typhimurium* for three weeks as infection.

4- Fourth group was administrated orally with o.3 ml of *plantagolanceolata* extract for one week daily after infection with *Salmonella typhimurium*.

5- Fifth group was administrated orally with o.3 ml of *plantagolanceolata* extract for two weeks daily after infection with *Salmonella typhimurium*.

6- Sixth group was administrated orally with o.3 ml of *plantagolanceolata* extract for three weeks daily after infection with *Salmonella typhimurium*.

All animals were sacrificed and pieces from intestine 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 the effect of *plantagolanceolata* extract against some Salmonella species had a same effect to the different concentrations of the extract, and the largest the bacterium inhibition in diameter of 29 mm against *Salmonella typhi* in the concentration 300 mg of *plantagolanceolata* extract. While the extract gives the less volume inhibition effect to *Salmonella hadar* in diameter of 19 mm when measured by a ruler.

Similar study was carried out *In vitro* investigations with pressed juice and aqueous extracts of Plantagolanceolata showed antibacterial effects against Salmonella species and other pathogenic bacteria (Cáceres et al. 1987).

The inhibition of the growth of the *in vitro* with the superiority of the concentration 200mg / ml of alcoholic

extract gave the highest inhibition by *plantagolanceolata* extract against other pathogenic bacteria (Abdul – Ratha and Mohammad, 2012) .Crude extracts of *P. lanceolata* showed various degrees of antimicrobial activity towards each standard and drug resistant microbial pathogen with mean zone of inhibition ranges up to 26±3.60 mm against multidrug resistant (Fisseha and Berhanu, 2014).

extract Table 1:The total sensitivity test results of *plantagolanceolata* against some Salmonella species

Type of pathogenic bacteria	100 mg A (mm)	150 mg B (mm)	200 mg C (mm)	250 mg D (mm)	300mg E (mm)	Control D.W (F) (mm)
Salmonella typhi	**20**	**22**	**24**	**25**	**29**	**0**
Salmonella hadar	**19**	**21**	**25**	**26**	**27**	**0**
Salmonella typhimurium	**21**	**23**	**24**	**26**	**27**	**0**

Picture (1): showed the effect of *plantagolanceolata* extract with different concentrations (A,B,C,D,E) and F as control on *Salmonella typhi* .

Picture (2): showed the effect of *Plantagolanceolata* extract with different concentrations (A,B,C,D,E) and F as control on *Salmonella typhimurium*.

2- In *vivo* (Histopathological) study:

-Intestine-The first group that infected with of *Salmonella typhimurium* orally of bacterial suspension one week of infection and second group that infected with *Salmonella typhimurium* for two weeks showing infiltration of inflammatory cells in the lamina propria of atrophic villi (fig: 1,3) respectively. While third group that infected for three weeks showing hyperplasia of goblet cells **(**fig: 5).

Fourth and Fifth group were treated of *plantagolanceolata* extract for one and two weeks respectively group after infection with *Salmonella typhimurium* showing hyperplasia of goblet cells (fig: 2, 4), where as sixth group that administrated *plantagolanceolata* extract for three weeks after infection with *Salmonella typhimurium* showing hyperplasia of goblet cells less than other groups (fig: 6).

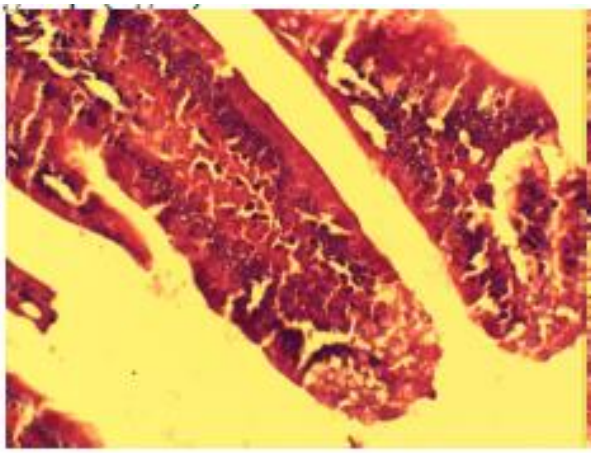

Fig1:Histopathological section of intestine of one animal that infected with*Salmonella typhimurium* after one weekshowed infiltration of inflammatory cells in the lamina propria of atrophic villi(H&EX400).

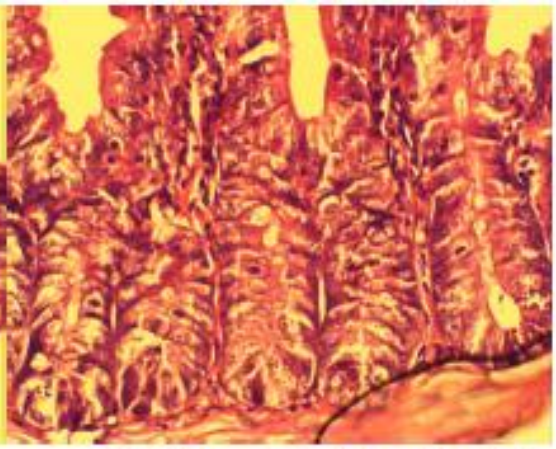

Fig2:Histopathological section in intestine of animal infected by *Salmonella typhimurium*orally after one week treated with *plantagolanceolata*extract showed hyperplasia of goblet cells (◄—) (H&EX40).

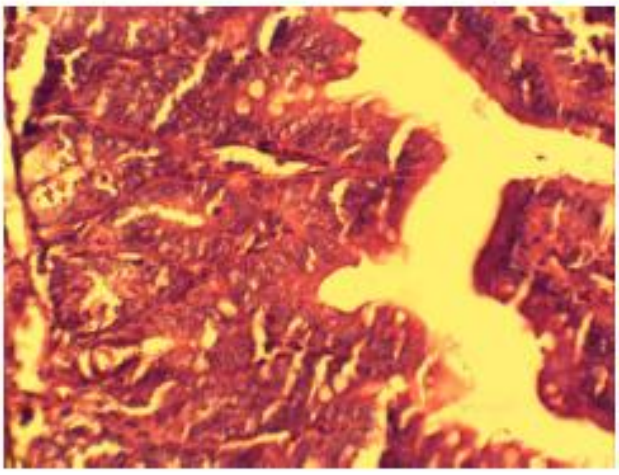

Fig3:Histopathological section of intestine of one animal that infected with*Salmonella typhimurium*after two weeksshowed infiltration of inflammatory cells in the lamina propria of atrophic villi(H&EX400).

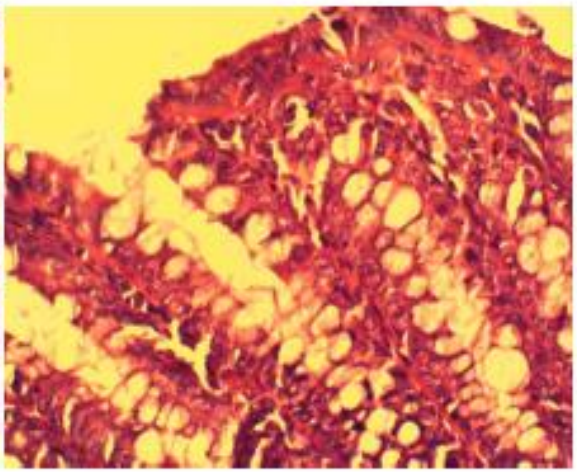

Fig4:Histopathological section in intestine of animal infected by *Salmonella typhimurium*orally after two weeks treated with *plantagolanceolata*extract showed hyperplasia of goblet cells (H&EX40).

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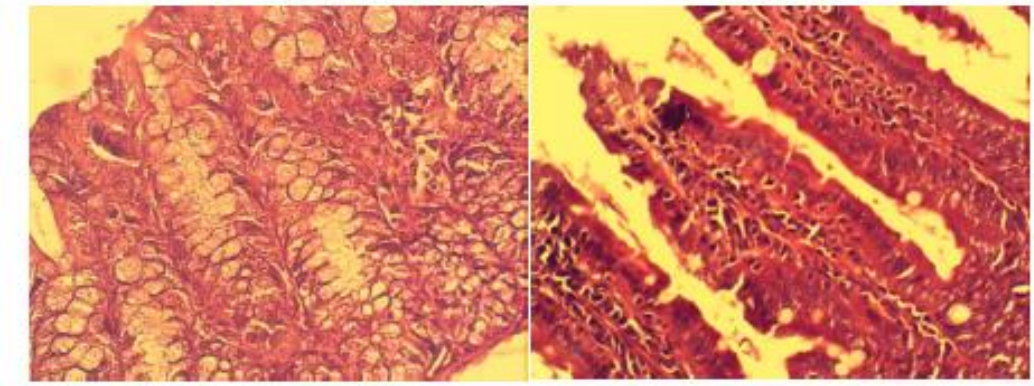

Fig5:Histopathological section in intestine of animal infected by *Salmonella typhimurium*orally after three weeks showed hyperplasia of goblet cells (H&EX40).

Fig6:Histopathological section in intestine of animal infected by *Salmonella typhimurium*orally after three weeks treated with *plantagolanceolata*extract showed hyperplasia of goblet cells less than other groups (H&EX40).

After oral ingestion and colonization of the small intestine *S. typhimurium* penetrates the intestinal epithelium and enters the Peyer's patches, lymphoid structures that line the intestine (Carter and Collins, 1974). For *S. typhimurium*, the main entrance into the Peyer's patches appears to be M cells, a specialized cell population overlaying the Peyer's patches and involved in antigen sampling from the intestinal lumen into these lymphoid follicles (Neutra*et al.*,1996). *Salmonella typhimurium* causes gastroenteritis in humans and other mammals. When the bacterial cells enter epithelial cells lining the intestine they cause host cell ruffling which temporarily damages the microvilli on the surface of the cell (McCormick, 1995). Mucociliary transport was investigated by viscosimetry using a ciliated epithelium preparation of a frog. A 4.6% extract from *Plantagolanceolata* did not increase mucociliary activity (Müller-Limmroth and Fröhlich 1980).

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