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Original Research Article

Evaluation The Protective Effect of *Catharanthusroseus* Extract on Induced Peptic Ulcer in Male Rabbits

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Abstract

The goal of this study is to evaluate the role of *Catharanthusroseus* alcoholic extraction for treatment of induced peptic ulcer in male rabbits in which there was thirty male , the animals was divided in five groups normal control given only distal water, active control given 500 mg/kg acetyl salsylic acid orally, standard group received single dose 20mg/kg IP of omeprazole follow by 500mg/kg acetyl salsylic acid, the fourth and fifth group received 20, 30mg/kg subsequently of*C. roseus*extract orally by stomach tube one hour before acetyl salsylic acid 500mg/kg orally, macroscopically significant protective effect of *C.roseus* extract show in dose of 30mg/kg about 61.97%, microscopically in both 20,30mg/kg the sign of healing process were detected which was approximately same as that appear in the standard group, measuring serum level of pro-inflammatory cytokines (IL-4and IL-8),showed there was significant reduction in there level at same level in both doses ,so as conclusion the alcoholic extraction of *C.roseus* had an antiulcerognic effect with significant reduction in the both IL-4 and IL-8.

Key Words : peptic ulcer, *Catharanthusroseus*, protection,cytokines.

تقييم الفعالية الدفاعية لمستخلص نبات العناقية على القرحة المعوية المصطنعة في ذكور الارانب

الخلاصة

الهدف من الدراسة هو دراسة تأثير المستخلص الكحولي لأوراق نبتة العِناقِيَّة على قرحه المعويةالمحدثة في ذكور الارانب.تمتقسيم 30 ذكر من الارانب وتم تقسيمها الى 5 مجاميع الاولى طبيعة مجموعة السيطرة اعطيت ماء مقطر فقط اثناء التجربة لثانية مجموعة السيطرة الفعالة والتي اعطيت حامض الصفصاف لغرض استحداث قرحة معويه ,المجموعة الثالثة هي مجموعة النموذج علاجي والتي اعطيت عقار الامبرازول قبل جرعة حامض الصفصاف والتي تم من خلالها مقارنه مع باقي المجموعتين الرابعة والخامسة التي اعطيت جرعتان مختلفتان لمستخلص اوراق نبته العِناقِيَّة الجرعة الاولى كانت 20ملغم/كغم ,30ملغم/كغم وكانت النتائج العينية وجود تأثير وقائي ظاهري مهم ومميز للمستخلص وبجرعة 30ملغم/كغم حيث كانت نسبة الوقاية بحدود 61.97% .نسيجيا اظهرت النتائج المجهرية وجود تأثير وقائي لكلا الجرعتين 20و30 ملغم /كغم من المستخلص وبنفس التأثير من حيث تقليل نسبه الالتهاب النسيجي وقلة بعدد الخلايا الالتهابية وانحسار الوذمة في النسيج المعوي لوحظت وبنسبة تجعل التشريح النسيجي مشابه الى حد ما من المجموعة التي تم إعطاؤها الامبرازول كعلاج وقائي ,قياس نسبة الانترلوكين 4 و8 في مصل الدم لكل المجاميع واظهرت النتائج قدرة المستخلص وبجرعة 20ملغم /كغم على خفض نسبتهما في مصل الدم بالمقارنة بنسبتهما المنخفضة بمجموعة النموذج العلاجي .وبذلك يكون للمستخلص الكحولي اوراق نبته العِناقِيَّة فعالية على علاج القرحة المعوية .

الكلمات المفتاحية:قرحة المعدة, نبته العِناقِيَّة, وقائية ,سايتوكينات مناعية.

Introduction

Stomach ulcer is harmful sores present in the lining epithelium of the stomach or small intestine, this sores is visible landmark of peptic ulcer disease[1].

The etiology of peptic ulcer include:

1.infection with the bacterium *Helicobacter pylori* (*H. pylori*, long-term

2. Drugs such as use of nonsteroidal anti-inflammatory drugs (NSAIDs), such as aspirin and ibuprofen

3. hyperacidity in the stomach, which may be due to genetic factors.

4. stress or smoking main life style condition related to peptic ulcer.

5.zollinger-Ellison syndrome, condition associated with increase production of acid [2].

Treatment of peptic ulcer involve the treatment of causative factors which such as elimination of infection with *H-pylori*,avoid using of NSAIDS as well as using of antiseretory drugs such as proton pump inhibitors[3]. The prevalence in women is 8-11% and its lowerthan in men 11-14%. The rate of occurrence of ulcer (duodenal) decrease in young men and increase in old women [4]. *Catharanthusroseus* (L.) is medical plant their extract used in treatment of different of many disease related to family of Apocynaceae. *C. roseus* also play unimportant role against reactive oxygen species and act as antioxidant agents [5]. It also used internally for the treatment of gastritis, hypertension, cystitis the loss of memory, enteritis the raised blood sugar and diarrhea [6]. Some study showed that this plant composed of 70 type of biologically active alkaloids[7]. Detection of peptic ulcer as mucosal necrosis that revealed an abnormal abrasion about 0.5cm or more in diameter involving the acidic area in the gastrointestinal tract and the pain is piercing in nature[8].

Interleukin IL-8 isone of the strong neutrophil and lymphocyte-activating chemotactic cytokine (chemokine) is, and if there is infection with pathogenic bacteria gastric cells secreted activated IL-8 [9]. There is correlation between the level of IL-8 and various -markers, such as IL-,C3a, α1- antitrypsin, lactate[10]. The process of differentiation of Th0 cells into Th2 cells is mediated by interleukin -4,thus and as a positive feedback process more IL-4 will produce [11]. The releasing of many cytokines such as (IL-1,IL-8 and IL-6) was prevented by IL-4 which possess some anti-inflammatory [12]. IL-4 act as main controller for both type of immunity, it responsible for activation of B-cell to immunoglobulin type E and flaring the making of classII [13].

Materials and Methods

Choose of animals: A total of thirty male rabbits were used in the study. Their weight was between 1.25-2.5 kg .all the animals allowed to eat standard food (pellet) and also allowed to drink water.

Pilot study was done to determine the dose of acytel salsylic acid that induces peptic ulcer starting by 250 mg/kg, 500mg/kg and 750 mg/kg. Best sign of ulceration was seen in 500 mg/kg.

Acytel salsylic acid (ASA) preparation:

A dose of 500mg/kg was given by stomach tube to rabbit after suspended of pure powder of ACA in 5 ml of distal water [14].

Standard drug for treatment of ulcer is Omeprazole, dosage form tablet, make it as powder and dissolved in D.W. (5ml) and given by oral rout through stomach tube in dose of 20mg/kg.

the leafs of *Catharanthusroseus*were washed then after make it dry by exposure to air later on the leafs were crashed to obtained powder[15].

solvent extraction procedure:

About 10 g of dried leafs powder was put in 100 ml of ethanol for 48 Hrs. Then and by using filter paper number 1 this suspension were filter to get clear extracts which stored at 4°C until further use [16].

Active study design:

The thirty experimental animals (rabbits) were randomly classified into five groups each group contain six rabbits, the fourth and fifth group received the *C.roseus* in dose 20,30mg/kg respectively orally by

stomach tube for 15 days before starting the study.

The first group is control group received distal water (5ml) by stomach tube in same time of doing the experiment.

The second group received single dose of 500mg/kg ASA orally by stomach tube as positive control.

The third group given 20mg/kg IP of omeprazole one hour before given acetyl salsylic acid 500mg/kg orally by stomach tube.

The fourth group given 20mg/kg *C.roseus* extract orally by stomach tube one hour before acetyl salsylic acid 500mg/kg orally by stomach tube.

The fifth group given 30mg/kg of *C.roseus* extract orally by stomach tube one hour before acetyl salsylic acid 500mg/kg orally by stomach tube.

Histological analysis:

Small portion of stomach, involving the ulcers, were fixed in phosphate-buffered formaldehyde, then dehydrated, fixed in paraffin, and 5 mm- portion were obtained and stained with haematoxylin and eosin for light microscopic evaluation. Ulcers features and healing such as regeneration of the ulcerated mucosa, making of granulation tissue, and inflammatory infiltrate were detected.

Blood sample collection:

After anesthetization the animals with ether, 3 ml of blood was obtained directly from the heart of the rabbits after overnight fasting. by sterile syringes and put into sterilized plane glass tubes. Sera were obtained from blood samples by centrifugation for 15 min. at 3000rpm. Sera were placed then in another glass tubes which were sterilized previously by autoclaving and kept in a deep freeze at -20 ºC for measure serum level of IL8 and IL 4 by ELISA methods and the procedure was done according to the instructions (NeogenTest Kit is designed for quantitative determination of IL-4 and IL-8 levels in animal sera).

Ulcer index= total number of ulcers/ stomach + total severity of ulcers/ stomach. So the score can be determined depend on the below measures:

If the color of the stomach is normal so the score is 0.

Red coloration the score is 0.5.

Spot ulcers the score is 1.

Hemorrhagic streak the score is 1.5.

Ulcer the score is 2.

Perforation the score is 3.

"Ulcer index = Average number of ulcer per animal + Average number of severity score + Percentage of animal with ulcer $\times 10^{-1}$UI

%Protection = Control mean ulcer index – test mean ulcer index/ Control mean ulcer index [17] ".

Data Analysis:

Statistical analysis was carried out using SPSS version 17. Continuous variables were presented as (Mean ± SD). ANOVA test was used to compare means between three groups or more when study variable was normally distributed. Post Hoc test was used for multiple mean comparisons between groups. A p-value of ≤ 0.05 was considered as significant.

Results

Table 1 : Total protective effect of *C.roseus*extract on peptic ulcer in rabbit as compare with standard omeprazole control groups

Group	\Type of treatment	Mean of normal color stomach	Mean of red color stomach	Mean of Spot ulcer	Mean ofHemorrhagic streaks	mean of U≥3≤5	Mean of U>5	Mean of Total Score	Mean Ulcer ±SEM(Standard error of Mean)	Total protection (%)
1	Control	0	/	/	/	/	/	/	/	/
2	Positive control	/	0.5	1	1.5	1	0.33	3.083	4.33±2.866	/
3	Omprazole +ASA	/	0.5	1	0.75	/	/	2.25	4.13±3.093	77.9
4	*C. roseus*20 mg/kg+ASA	/	0.5	1	0.5	2	/	1.83	3.3±1.84	31.09
5	*C..roseus*30mg/kg+ASA	/	0.5	1	0.75	0.6	/	2.91	2.5± 1.58	61.97

In this study, the extract of *C.roseus*at dose of 20 and 30mg/kg showed significant gastro protective activity comparedwith omeprazole IP at dose of20mg/kg . Both doses of alcoholic extract of the leaf *C. roseus* at dose (20mg/kg and 30mg/kg) showed significant reducing in Ulcer index and the Ulcer protective effect of Catharanthusroseus was increase as we increase the dose.

Mean Differences of Interluken-8 of Animals according to type of treatment

Table(2) shows the mean differences of interluken-8 in animals group according to type of treatment. There were significant differences between means of interluken-8 by type of treatment.

Table 2: Mean differences of serum level of interluken-8 in animal groups according to type of treatment

Variable	Groups	N	Mean ± SD	F-test	P-value
IL- 8 (pg/ml)	Group1 (placebo)	6	21.25 ± 4.18	16.968	<0.001
	Group2 (Aspirin)	6	49.25 ± 9.12		
	Group3(Omeprazole and aspirin)	6	23.28 ± 7.38		
	Group4 (low dose of plant extractions and aspirin)	6	39.16 ± 8.86		
	Group5(high dose of plant extractions and aspirin)	6	24.63 ± 5.33		

*P value ≤ 0.05 was considered significant.

Table (3) shows mean differences of interluken-4 in animal group according to type of treatment. There were significant differences between means of interluken-4 by type of treatment.

Table 3: Mean differences of interluken-8 in animal group according to type of treatment (multiple comparisons LSD)

Group	Multiple comparisons	P-value
Group 1 (placebo)	Group2	**<0.001**
	Group3	0.631
	Group4	**<0.001**
	Group5	0.426
Group 2 (Aspirin)	Group1	**<0.001**
	Group3	**<0.001**
	Group4	**0.024**
	Group5	**<0.001**
Group 3 (omeprazole and aspirin)	Group1	0.631
	Group2	**<0.001**
	Group4	**0.001**
	Group5	0.750
Group 4 (low dose of plant extraction and aspirin)	Group1	**<0.001**
	Group2	**0.024**
	Group3	**0.001**
	Group5	**0.002**
Group 5 (high dose of plant extraction and aspirin)	Group1	0.426
	Group2	**<0.001**
	Group3	0.750
	Group4	**0.002**

*P value ≤ 0.05 was considered significant.

Mean Differences of Interluken-4 of Study Animals according to type of treatment

Table 4: Mean differences of interluken-4 of study animals according to type of treatment

Variable	Group	N	Mean ± SD	F-test	P-value
IL-4 (pg/ml)	Group1 (placebo)	6	12.98 ± 1.08	**33.023**	**<0.001**
	Group2 (Aspirin)	6	29.55 ± 4.98		
	Group3(Omeprazole and aspirin)	6	14.53 ± 2.19		
	Group4 (low dose of plant extractions and aspirin)	6	15.41 ± 3.45		
	Group5(high dose of plant extractions and aspirin)	6	13.18 ± 1.36		

*P value ≤ 0.05 was considered significant.

Table 5: Mean differences of interluken-4 of study animals according to type of treatment (multiple comparisons LSD)

Group	Multiple comparisons	P-value
Group 1 (placebo)	Group2	**<0.001**
	Group3	0.378
	Group4	0.171
	Group5	0.909
Group 2 (Aspirin)	Group1	**<0.001**
	Group3	**<0.001**
	Group4	**<0.001**
	Group5	**<0.001**
Group 3 (omeprazole and aspirin)	Group1	0.378
	Group2	**<0.001**
	Group4	0.613
	Group5	0.441
Group 4 (low dose of plant extraction and aspirin)	Group1	0.171
	Group2	**<0.001**
	Group3	0.613
	Group5	0.207
Group 5 (high dose of plant extraction and aspirin)	Group1	0.909
	Group2	**<0.001**
	Group3	0.441
	Group4	0.207

*P value ≤ 0.05 was considered significant.

Microscopical examination of thegastric mucosa:

A.Normal Control Group:

The mucosal cell show intact appearance and there is no abnormal changes seen as shown in figure A

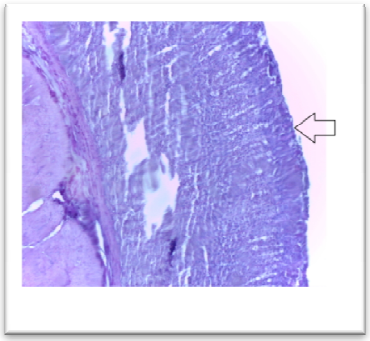

Figure A

B. **Acetylsalicylic AcidTreated Group:** Acetylsalicylic Acid causes damage and necrosis of the epithelial mucosa as well as neutrophil cells infiltration with edema at the sub mucosal region as shown in Figure B.

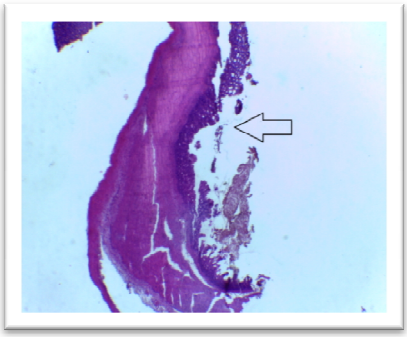

Figure B

C. Omeprazole treated Group:
Omeprazole in a dosage of 20 mg/kg obviously decrease the changes that have been observed in ASA treated group, as shown in Figure C.

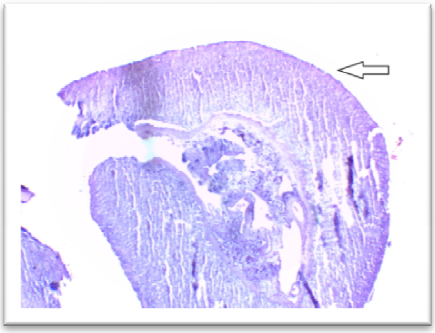

Figure C

D.Low dose *C.roseus* extract Pretreated Group:

Given 20 mg/kg P.Oextraction of *C.roseus* of showed decrease in the pathological changes of the gastric mucosa but less than that of omeprazole as show in Figure D.

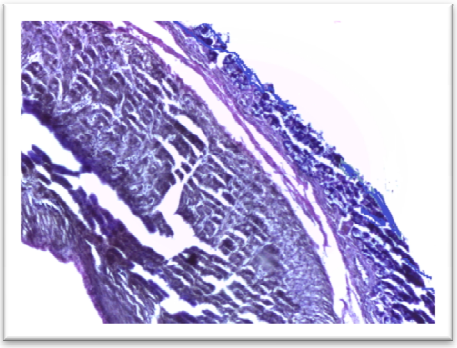

Figure D

E. High dose *C.roseus* extract Pretreated Group:

Figure E, revealed perfect look of histological structure same asnegative control group.

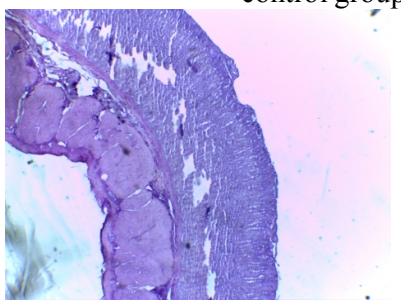

Figure E

Discussion

According to the results of this study ,the protection percentage was increase by increasing the dose of *C. roseus* extract so it has antiulcer genic effect this is due to the phytochemical studies showed the presence of Alkaloids, Carbohydrates, Saponins, Flavonoids, and Steroids in the entire plant[18].In dose of 30mg/kg of the extraction the protection percentage rise about double than that of 20mg/kg. The outcome with 30mg/kg dosage reach the protection percentage with standard drug for peptic ulcer (omeprazole).Histopathological study show features of acute ulceration of gastric mucosa ,hemorrhagic appearance and deepithelial-zation with edema and congestion in the induction group ,indicated the effect of salicylic acid on the gastric mucosa [19]. Omeprazole treatment showed the hemorrhage subside with good healing in the macerated mucosa and decrease inflammatory reaction[20]. In both groups treated with *C. roseus* extract (20mg/kg and 30 mg/kg) histopathological study show significant (P value $\leq$ 0.05)reduction in the inflammation and also significant reduction in the neutrophil infiltrations but the higher dose of extract approximately near the appearance of omeprazole treated group, whichhadsignificant(P value $\leq$ 0.05), graded and dose dependent anti-ulcer activity and anti-secretory activity when compared to control group [21] .Regarding serum level of IL-8 there was no significant difference between both doses of *C. roseus* extract and the standard (omeprazole treated group), which means even in low dose (20mg/kg) of *C. roseus* extract can reduced the serum level of IL-8,one of the essential component which has important effect on the gastritis and its act as chemotactic and activator for neutrophils is IL-8 [22].In chronic gastritis in which *H. pylori* is positive, there is rise in neutrophil infiltration in associated with increase the level of IL-8 is in the mucosa of the body,. While and regardless increases in body IL-8 level in DU patients ; neutrophil infiltration is decrease and the gastritis may be limitedto the antrum [23].

About serum IL-4, there was no significant difference between both doses of *C. roseus* extract and the standard (omeprazole treated group), which means even in low dose (20mg/kg) of *C. roseus* extract can reduced the serum level of IL-4,one of the important factors that causes damage to the gastric mucosa is the reactive oxygen species which was produced from activated neutrophils, so infiltration of the neutrophils is responsible for gross lesion, as result of damaging musosal this will lead to attraction and activation of neutrophils[24].

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