STUDY THE EFFECT OF ISOLATED ALKALOID FROM *HALOXYLON SALICORNICUM* PLANT ON SOME HEMATOLOGICAL AND BIOCHEMICAL PARAMETERS IN GASTRIC ULCERATION RABBITS INDUCED BY INDOMETHACIN .

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ABSTRACT

This study was undertaken to isolate alkaloids from *Haloxylon Salicornicum* plant to show the curative effect after short-term daily orall administration for 10 days (300 mg/Kg B.W.) on gastric ulcer,hematological parameters and some biochemical parameters in gastric ulceration rabbits induced by indomethacin.Moreover,some qualitative chemical analysis,thin layer chromatography,UVspectroscopy and FTIR spectroscopy were used to identify alkaloid compound .Rabbits were divided randomly into three groups,6 rabbits in each.Group one received 3 ml normal saline orally (control group).Group two was treated with indomethacin (75 mg/Kg B.W.) (gastric ulceration group).Third group gastric ulceration rabbits post-treated with isolated alkaloid (300 mg/Kg B.W.).The results of the present study indicated that isolated alkaloid has Rf= 0.86,high significant decreased ($p \leq 0.001$) in RBC,Hb,PCVand mucin,($P \leq 0.01$) in MCHC and PH,no significant difference ($P \geq 0.05$) in MCH in gastric ulceration rabbits compared with control group and observed high significant increased ($p \leq 0.001$) in MCV, MDA and gastric juce volume,WBCcount appeared high significant increased ($P \leq 0.01$).While, observed high significant increased($P \leq 0.001$) in Hb,PCVand mucin,($P \leq 0.01$) in RBC,MCHC and pH.Significant increased($P \leq 0.05$) in MCH after treated with isolated alkaloid and high significantly decrease ($P \leq 0.001$)in MCV,gastric juce volume and MDA

and significant decrease ($p \leq 0.05$) inWBC count compared with gastric ulceration group .This study concluded that isolated alkaloid from *Haloxylon Salicoricum* plant can be used to treat gastric ulcer effectively .

INTRODUCTION

Peptic ulcer disease (PUD) is an illness characterized by lesions in the gastrointestinal mucosa that may penetrate the muscular layer [1]. PUD affects approximately 10% of the population worldwide [2] and represents not only costs to the individual, but also for public health [3]. The development of peptic ulcer is complex and involves a multifactorial process, which occurs by an imbalance between aggressive and defensive factors of the gastric mucosa [4]. The gastric mucosa is continuously exposed to harmful substances and factors, whether endogenous as acid secretion, peptic activity and biliary secretion or exogenous as the presence of *Helicobacter pylori*, the use excessive of alcohol, tobacco and non-steroidal anti-inflammatory drugs (NSAIDs), besides stressful life habits [4,5].

A natural products are a chemical organic substances which are produced by the living organisms found in the nature that are produced by the pathways of primary and secondary metabolism [6,7]. Among the natural products the alkaloids, biologically active secondary

metabolites, that can be found in plants, animals or microorganisms.Biosynthetically,the alkaloids are derived from amino acid biosynthesis or transamination processes, and they are classified according to the amino acid that yields the nitrogen atom as well as the part of its skeleton for the synthesis of the alkaloid [8].Thus, the alkaloids are compounds consisting of a basic nitrogen atom that may or may not be part of a heterocyclic ring [9].

Alkaloids are the oldest successfully used drugs throughout the historical treatment of many diseases[10].Alkaloids are known for a variety of biological activities such as antiviral and antimicrobial activities[11],anti-inflammatory

activity[12],Anticancer[13],Muscle relaxant [14].This study aimed to examine antiulcerogenic effect of isolated alkaloid from *Haloxylon salicornicum* plant on indomethacine induced gastric ulcer in male rabbits .

MATERIALS AND METHODS

Plant material

Haloxylon Salicornicum was brought from local market.The aerial parts of *Haloxylon Salicornicum* were washed with tap water and dried at room temperature (25 ˚ C) , then ground to fine powder using an electric grinder.

Isolation of alkaloid

A (60 g) of *Haloxylon Salicornicum* powder was extracted with n-hexan using soxhlet extraction method to remove the oil . The defatted powder was dried at room temperature then treated with 300 ml of (10% acetic acid in ethanol) by using magnetic string for 4 hrs .The suspension was filtered through Whatman filter paper No. 41 under vacuum .The filtrate was concentrated by rotary evaporator at 50° C then basified with ammonium hydroxide to pH= 9 and extracted with chloroform (3*20ml).The chloroform layer was concentrated by rotary evaporator and dried at room temperature [15] .

Phytochemical Analysis :

Some chemical tests were carried out on isolated alkaloid by using standard procedures to identify the constituents, by characteristic colour changes as described by [16] ,[17], [18] ,[19] and [20] .

Thin Layer Chromatography

Thin layer chromatography (TLC) was performed on precoated silicagel (2 x 10) cm plates , 0.2 mm thick with MeOH : Conc.NH_4OH (10 : 1) as mobile phase and spot was detected by spraying with Dragendoroff reagent,Folin reagent, 40% H_2SO_4, iodine and UV light [15] .

Experimental Animals

Adult male domestic rabbits weighing (1000- 2000)g were used and housed in cages in the animal house of the veterinary medicine college –Basra university and fed with bran,alfalfa and tap water.The animals were randomly divided into three groups , six rabbits in each group .

Group I : was given 3 ml of normal saline (0.9 % NaCl) orally by gastric tube served as control group .

Group II and Group III : were treated with a dose of indomethacin 75 mg / Kg (B.W.)initially for 3 days to induce gastric ulcer then group II was treated with normal saline for 10 days while,group III was treated with isolated alkaloid (300 mg/ Kg B.W.) for 10 days.Blood samples were taken from the heart of overnight fasted animals after ten days using plastic syringes and divided into two parts,the first part was deposited into tube with anticoagulant which used for heamatological analysis.Another part was allowed to clot and centrifuged at (3000 rpm) for 10 minutes and serum samples were stored in polyethylene eppendroff tubes at(– 20)° C until analysis . [21]

Then the animals were scarified .A mid-line abdominal incision was performed .Stomach rapidly removed and gently washed with normal saline

Hematological analysis :

Blood parameters were immediately determined by using count 60 apparatus (Genex laboratories , Germany) for determinig (RBC, Hb, MCV, MCH, MCHC, PCV and WBC)

Determination of gastric juice volume

The volume of the gastric juice was measured by graduated cylinder . [22]

Determination of gastric juice acidity :

Acidity of gastric juice was determined by using pH meter apparatus (HI 9021) .

Mucin assay :

The free mucin in the gastric tissue was estimated by measuring the amount of Alcian blue dye (Ab) bound to mucin [23].

Determination of serum malondialdehyde (MDA)

The principle of assay was based on the reaction of MDA with thiobarbituric acid (TBA) , forming an MDA-TBA2 adduct (pink color) that absorb at(532) nm [24].

Identification of isolated alkaloid

UV spectra were recorded by using Spectro Scan 80D (200-1000) nm- UK and using 1 cm pathway quartz cell and FT-IR spectra were recorded by using a Shimdzu FTIR (8400s-Japan) spectrometer. [25].

Statistical Analysis

The results of the present study were analyzed by univarate analysis of variance .The data were expressed as mean ±standard deviation (Mean±SD) . Least significant different test (LSD) was used to test the difference between means (groups) by using statistical program for social science SPSS. $p \leq 0.05$ was considered significant.

RESULTS

Photochemical Analysis

Phytochemical screening for isolated alkaloid revealed the presence of alkaloids and phenols while, the test showed negative results of carbohydrate,Tannins,amino acids and saponins as shown in table 1 :

Table 1 : The phytochemical analysis of isolated alkaloid

Phytochemical	Isolated alkaloid
Alkaloids	+
phenol	+
carbohydrates	-
Tannins	-
Saponins	-
Amino acids	-

The result of the thin layer chromatography of isolated alkaloid revealed the presence of one component in Rf (0.86) as shown in figure 1 and table 2 .

Figure 1: Thin layer chromatography for isolated alkaloid

Table 2 : Thin layer chromatography for isolated alkaloid

Dragendroff reagent	Folin reagent	40% H_2SO_4	I2	UV
0.86	0.86	-	0.86	0.86

Hematological analysis :

The results from table (3) showed that high significantly decreased($p \leq 0.001$) in RBC count, Hb concentration,PCV,($p \leq 0.01$) in MCHC percentage of gastric ulceration group compared with control group and high significant increased($p \leq 0.01$) in count RBC and percentageMCHC,($p \leq 0.001$) in Hb and PCV of gastric

ulceration treated with isolated alkaloid (300mg / Kg) group compared with gastric ulceration group while,observed high significant increased ($p \leq 0.001$) in MCV of gastric ulceration group compared with control group and high significant decreased ($p \leq 0.001$) in MCVof gastric ulceration treated with isolated alkaloid (300mg / Kg) group compared

with gastric ulceration group while,observed significant increased($p \leq 0.05$) in MCH of gastric ulceration treated with isolated alkaloid(300mg/Kg) group compared with gastric ulceration group .

Table 3 : Effect of isolated alkaloid on RBC counts and RBC index in rabbits with gastric ulceration induced by indomethacin .

Parameters / Treatments	N	RBC x 10 6/µL	Hb g /dl	PCV %	MCV fL	MCH Pg	MCHC %
Control (Normal saline)	6	7.22±1.21	12.81± 2.12	45.11 ± 3.44	59.54 ± 3.90	17.70 ± 2.22	28.25 ± 3.65
Gastric ulceration (Indomethacin75mg/ Kg)	6	5.68 ± 1.36 ***	9.18 ± 1.59 ***	40.11 ± 1.16 ***	68.43 ± 2.73 ***	14.76 ± 1.56	23.43 ± 2.70 **
Indomethacin75 mg / Kg) + Isolated alkaloid (300 mg/Kg)	6	6.32 ± 1.50 **	10.20 ± 1.30 ***	44.18± 2.92 ***	60.01 ± 2.99 ***	18.18 ± 2.15 *	27.98± 3.55 **

Mean ±SD , N= number of animals , * p ≤0.05 ,P≤ 0.01 ,*** P≤ 0.001, RBC=Red Blood Cell , Hb= Hemoglobin,PCV=Packed cell volume , MCV=Mean corpuscular volume , MCH=Mean corpuscular Hemoglobin MCHC= Mean corpuscular Hemoglobin concentration**

Effect of isolated alkaloid on WBC counts

The results in table (4) showed a significant increased($p \leq 0.01$) in WBC count of gastric ulceration group when compared with control group,while significant decreased($p \leq 0.05$) in WBC count of gastric ulceration treatment with isolated alkaloid (300 mg/Kg) group compared with gastric ulceration group .

Table 4: Effect of isolated alkaloid on WBC count in rabbits with gastric ulceration induced by indomethacin.

Parameters / Treatments	N	WBC x 103/µL
Control (Normal saline)	6	8.58±2.32
Gastric ulceration (Indomethacin 75 mg / Kg)	6	11.80±1.70**
Indomethacin75 mg/Kg + Isolated alkaloid (300 mg/Kg)	6	9.66±2.02 *

Mean ± SD , N= Number of animals , WBC=Wight Blood Cell ,*p≤0.05 , ** p ≤ 0.01 , ***p≤0.001

Effect of isolated alkaloid on gastric juice volume and gastric PH :

The results in table 5 revealed that a high significant increased($p \leq 0.001$) in gastric juice volume of gastric ulceration group compared with control group while,observed high significant decreased($p \leq 0.001$) in gastric juice volume of gastric ulceration treatment with isolated alkaloid (300 mg/Kg) compared with gastric ulceration group .

The results indicated that high significant decreased($p \leq 0.01$) in pH of gastric content of gastric ulceration group as compared with control group and significant increased($p \leq 0.01$) of gastric ulceration treatment with isolated alkaloid (300mg/Kg)group as compared with gastric ulceration group .

Table 5 : Effect of isolated alkaloid on gastric juice volume and gastric PH in rabbits with gastric ulceration induced by indomethacin.

Parameters / Treatments	N	Gastric Volume (ml)	PH of gastric content
Control (Normal saline)	**6**	14.75±2.69	2.00±0.14
Gastric ulceration (Indomethacin 75 mg / Kg)	**6**	30.15±6.12 ***	1.58±0.26 **
Indomethacin75 mg / Kg)+Isolated alkaloid (300mg/Kg)	**6**	19.61±1.85 ***	2.00±0.16 **

Mean ±SD , N= number of animals ,* p ≤ 0.05 , p ≤ 0.01,***p≤ 0.001**

Effect of isolated alkaloid on serum MDA and mucin

The results indicated that high significant increased(p≤ 0.001)in serum MDA of gastric ulceration group compared with control group and high significant decreased(p≤ 0.001) of gastric ulceration treatment with isolated alkaloid (300mg/Kg) group compared with gastric ulceration and observed non-significant chang compared with control group as shown in table 5 .

The results revealed high significant decrease(p≤ 0.001) in mucin of gastric ulceration group compared with control group and high significant increased

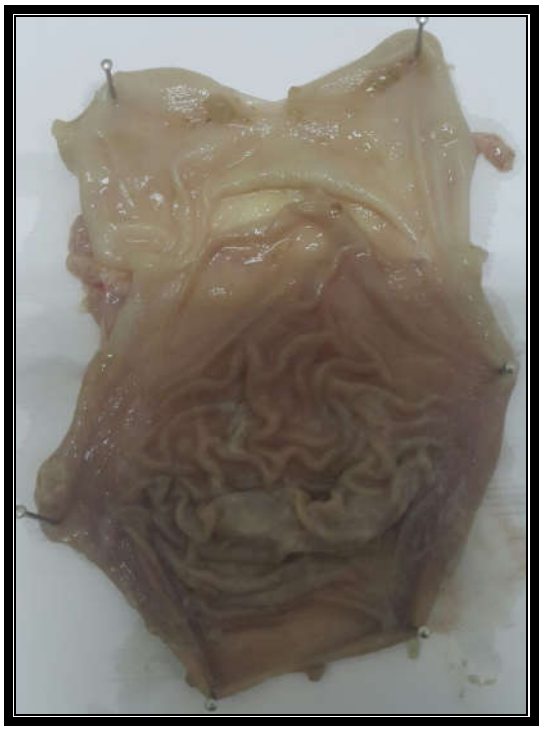
(p≤ 0.001) of gastric ulceration treatment with isolated alkaloid (300mg / Kg) group compared with gastric ulceration as shown in table 6

Table 6: Effect of isolated alkaloid on serum MDA and mucin in rabbits with gastric ulceration induced by indomethacin.

Parameters / Treatments	N	Serum MDA mmol/L	Mucin µg Ab /cm2
Control (Normal saline)	6	1.94±0.19	0.48±0.14
Gastric ulceration (Indomethacin 75 mg / Kg)	6	4.73±1.50***	0.02±0.006***
Indomethacin75 mg / Kg)+ Isolated alkaloid (300 mg/Kg)	6	2.31±0.41***	0.33±0.04***

Mean ±SD , N= number of animals , MDA= Malondialdehyde , * p ≤ 0.05 . ** p ≤ 0.01*p≤ 0.001**

Gross appearances of stomach :



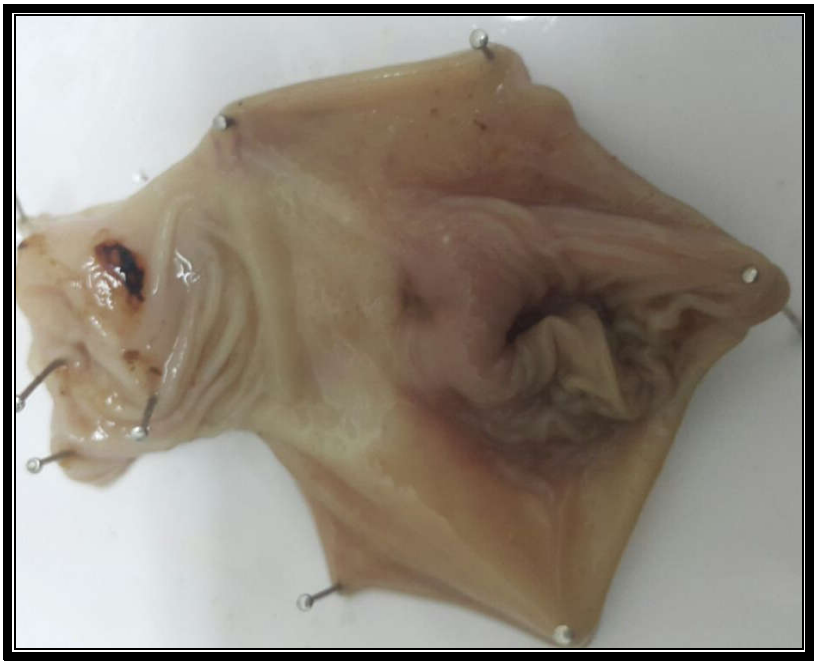


Figure 3: Stomach of male rabbits (treated for 3 days) indomethacin for induced gastric ulcer Showed gastric damage including gross mucosal lesion .

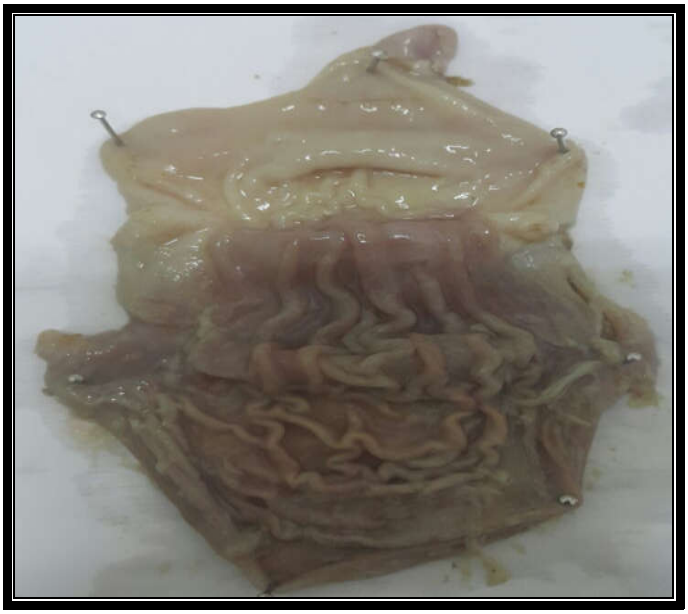


Figure 4- Stomach of male rabbits treated with(indomethacin 75mg⁄Kg for3 days + isolated alkaloid 300mg⁄Kg for 10days) .showed no lesionat all tissue of stomach

Identification of isolated alkaloid

The UV spectrum of isolated alkaloid indicated bands in 230 nm, 272 nm and 385 nm. The FTIR spectrum indicated the results as shown in table 7

Table 7: The locations of important bands in isolated alkaloid

Range $(CM)^{-1}$	Assignment & remarks
1735.99 s	C=O stretch.
1068.60 s	C-O stretch
1627.97 s	C=C stretch
1334.78 m	C-N stretch
3320 w	CH aromatic stretch
2924.14-2854.74 s	CH aliphatic stretch
3410.26 s	OH phenol
898.86 m	CH aromatic bend

DISCUSSION

In the present study ,the qualitative chemical analysis and thin layer chromatography of isolated alkaloid indicated that the component of alkaloid is present [26] and [27] mentioned that the Haloxylon salicornicum contains alkaloids .

Hematological analysis evident that the administration of indomethacin caused decrease in hematological parameters RBC, Hb, PCVand MCHC compared with control group this result agreement with [28] this result may be due to effects of indomethacin including gastric and peptic ulceration .Red blood cell deficiency is common in patients with ulcers, not only due to deficient nutritional status ,but also due to effects of inflammatory cytokines which consequent to the lesion on erythroid progenitor cell [29].Cytokines play an important role in acute inflammation and in the maintenance and

regulation of gastric mucosal damage [30]and [31]. While, a high significant increase was observed after treatment with isolated alkaloid compared with gastric ulceration group this may be due to effect of alkaloid by modulating the levels of pro-inflammatory cytokines and decreasing the activity of myeloperoxidase (MPO) Almeida and colleagues [32] .

High significant increased($p \leq 0.01$)in the number of WBCs after administration of indomethacin as compared with control group may be due to stress that induced by indomethacin and stimulation of immune system.This result agreed with[33] and [28] who refers that indomethacin increase number of WBCs .While,significant decreased ($p \leq 0.05$) was observed after treatment with alkaloid this due to antiinflammatory properties [34] of alkaloid and consequently have anti-ulcer effects since peptic ulcer is an inflammatory phenomenon. The results indicated that changes in the gastric juice volume and the acidity after administration of indomethacin compared with control group this may be due to promotes destabilization of mucus-bicarbonate-phospholipid layer, leading to retrodiffusion of H+ ions, with consequent damage to epithelial cells and mast cell activation inducing the release of inflammatory mediators, that allow the migration of neutrophils to the injured area, releasing reactive oxygen species that elevate the cellular damage and result in tissular necrotic damages and treatment with alkaloid resulted in a high significant increase of pH when compared with gastric ulceration group. The mechanism of action of the gastroprotective activity of, alkaloid reduced gastric acid secretion, inhibited activity of the proton pump,reduced gastric juice volume because of a reduction in the overproduction of mucus [35]

In the present study, isolated alkaloid caused a high significant decreased in the concentration of malondialdehyde, an indicator of lipid peroxidation, lipid peroxidation is a wellknown example of oxidative damage that affects cell membranes lipoproteins, and other lipid-containing structures under conditions of oxidative stress.Therefore, the fact that isolated alkaloid decreased lipid peroxidation might mean that alkaloid has an antioxidant effect[36]. Isolated alkaloid has effective antiulcer activity against indomethacin -induced ulcerogenesis and it can be used for therapy of ulcerogenesis and gastric mucosal injury.

In our study MDA was high significantly increased in gastric ulceration group compared with control group, isolated alkaloid treatment decreased the elevated MDA as compared with gastric ulceration group our results indicate that the preventive effects of isolated alkaloid may be due to inhibition of lipid peroxidation by its antioxidant nature.

Mucus ,the first line of defense of the mucous membrane,is being secreted by epithelial cells .It's consisting of 95% water and 5% mucin, a polymerized glycoprotein that forms a gel.

The gastric mucosa is constantly assaulted by various endogenous and exogenous factors [37], however it has several defense mechanisms, such as the mucus layer that acts as a physical barrier [38] and reduces the damage caused by oxidative stress [39] in the present observed that after administration of isolated alkaloid, an increase of mucus production[40] compared with gastric ulceration group .

Indomethacin is a nonselective cyclooxygenase inhibitor that impairs the synthesis of prostaglandins,as a result of the inhibition of PGE2 synthesis there is a decrease in gastric mucus ,bicarbonate production and changes in blood flow, thus, reducing the protective barrier of the normal mucosa and also the ability to repair mucosal cells [41].

The isolated alkaloid has been characterized by using UV spectroscopy.The compound was measured at a concentration of $1*10^{-4}$ mole/L in the methanol. The spectra of the compound showed two bands in the region (230 and 272) nm for [aromatic ring, C=C, C=O] groups which are due to the electronic transitions of $\pi \rightarrow \pi^*$ and a band appeared in the region (385) nm due to the electronic transition $n \rightarrow \pi^*$ that are related to the carbonyl group C=O [25].

The FTIR spectrum of isolated alkaloid showed a stretching vibration band in the region (1334.78) cm^{-1} due to (C-N) group and stretching vibration bands due to (C=O), (C-O), (C=C) (CH aromatic), (CH aliphatic) and (OH) groups [42].

دراسة تأثير القلويد المعزول من نبات الشنان على بعض المعايير الدموية والبايوكيميائية في الارانب المصابة بالقرحة المعدية المستحدثة بواسطة الاندوميثاسين

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الخلاصة

أجريت هذه الدراسة لعزل القلويدات من نبات الشنان لمعرفة التأثير العلاجي بعد الاعطاء قصير المدى (١٠ ايام) للقلويد المعزول بجرعة يومية (٣٠٠ ملغم / كغم) فمويا على قرحة المعدة حيث تم قياس معايير الدم وبعض المعايير البايوكيميائية في الارانب المصابة بالقرحة المعدية المستحدثة بواسطة الاندوميثاسين (indomethacin) بالاضافة لاجراء بعض الكشوفات الكيميائية النوعية كما استخدمت كروموتوغرافيا الطبقة الرقيقة ومطيافية الاشعة فوق البنفسجية ومطيافية الاشعة تحت الحمراء لتشخيص القلويد المعزول .تم تقسيم الارانب عشوائيا الى ثلاثة مجموعات (٦ لكل مجموعة) ،المجموعة الاولى هي مجموعة السيطرة جرعت ٣ مل من المحلول الملحي الفسيولوجي ، المجموعة الثانية المصابة بالقرحة المستحدثة بواسطة indomethacin(٧٥ ملغم / كغم)والمجموعة الثالثة المصابة بالقرحة المعالجة بالقلويد المعزول (٣٠٠ ملغم / كغم) . اوضحت نتائج الدراسة ان للقلويد المعزول معدل سريان (Rf=0.86) كما اوضحت نتائج الدراسة حصول انخفاض عالي المعنوية ($p\leq0.001$) في عد RBC ومستوى Hb،PCV وبفرق عالي المعنوية ($p \leq 0.01$)في مستوى PH و MCHC وعدم حدوث فرق معنوي في pH، mucinو وبفرق عالي المعنوية ($P\leq 0.01$) في عد WBC في المجموعة المصابة بالقرحة المعدية مقارنة بمجموعة السيطرة وبعد المعاملة بالقلويد المعزول لوحظ ارتفاع عالي المعنوية($P\leq 0.01$) في عد RBC و نسبةMCHC وpH وبفارق عالي المعنوية ($P\leq 0.001$) في مستوى PCV, Hb وmucin وانخفاض عالي المعنوية ($P\leq 0.001$) في حجم سائل المعدة ومستوى MDA وانخفاض معنوي ($p\leq0.05$) في عد WBC وارتفاع معنوي($P\leq0.05$)في مستوى MCH مقارنة بالمجموعة المصابة بالقرحة المعدية وتدل نتائج الدراسة الى امكانية استخدام القلويد المعزول من نبات الشنان كعلاج للقرحة المعدية وبشكل فعال .

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