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Exploiting of *Cordia dichotoma* Aqueous-Methanol Extract to Reduce Effect of Aspirin Against Gastric Mucosa of Rabbit, *Oryctolagus cuniculus*

Mayada Nazar AL-Khafaji[*1] and Sura Hilal Abdul- Khaliq[2]

[1]Biology Department – College of Science – University of Diyala
[2]College of Veterinary Medicine – University of Diyala

Mamnazar83@gmail.com

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Abstract

Ulcer is an inflammatory break reaction in the mucous membrane of stomach. The results showed non- significant changes in most clinical, hematological parameters depended in the experiment. While the biochemical; parameters exhibited a significant change. Prevent index was (27.08%). The level of pH of stomach, volume of gastric fluid, free acidity and total acidity levels, showed a significant change. Gastric mucosa was more severely affected and showed more severe and numbers of lesion in those exposed to aspirin alone, then in aspirin and plant extract. Aqueous – methanol extract of *Cordia dichotoma* fruit showed mild gastric protection against aspirin induced ulcer.

Keywords: Rabbits, Cordia dichotoma, Gastric mucosa, aqueous- methanol extract.

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استغلال المستخلص المائي- الميثانول للبمبر *Cordia dichotoma* لتقليل تأثير الأسبرين على الغشاء المخاطي لمعدة الأرنب *Oryctolagus cuniculus*

ميادة نزار الخفاجي و[2] سرى هلال عبد الخالق[1]

[1]قسم علوم الحياة – كلية العلوم – جامعة ديالى
[2]كلية الطب البيطري – جامعة ديالى

الخلاصة

التقرح تفاعل التهابي تهتكي في الغشاء المخاطي للمعدة. اظهرت النتائج تغيرات غير معنوية في المعايير السريرية والدمية المعتمدة في التجربة. بينما اظهرت المعايير الكيميوحيوية تغيرات معنوية. مؤشر المنع كان 27.08%. مستوى حموضة المعدة، حجم سائل المعدة، مستويات الحامض الحر والكلي اظهرت تغيرات معنوية. تأثر الغشاء المخاطي للمعدة، واظهر عدد اكبر واشد من الآفات في مجموعة الاسبرين، ثم الاسبرين والمستخلص النباتي. اظهر المستخلص المائي – الميثانول لثمار البمبر حماية معتدلة للمعدة ضد القرحة المحدثة بالأسبرين.

الكلمات المفتاحية: الارنب، البمبر، مخاطية المعدة، المستخلص المائي الميثانول.

Introduction

Gastritis is an inflammation of the stomach lining, many kinds of agents may lead the stomach into an inflamed statement, in first place, non- steroidal- anti- inflammatory drugs (NSAID) such as aspirin, which is one of the most common cases, as they are widely used in pain control. The chronic use of NSAIDs is well known in causing gastroduodenal erosions and peptic ulcers [1]. Ulcer is the lesion that pierces the muscularis mucosa which does not show the tendency to heal. The etiology of gastro-duodenal ulcers can be due to imbalance of offensive - protective factors in lining of stomach such as their barriers, parietal cell, mucus secretion, cellular regeneration and endogenous protective agents (prostaglandins and epidemic growth factors [2 and 3].

Prostaglandins play an important role, as it stimulates the production and discharged of mucus and bicarbonate, enhancing blood supplied of mucous membranes, promoting epithelial proliferation. [4].

Several plant and their extracts have been used to treat gastric ulcers [5 and 6]. Flavonoids are phenolic compounds widely distributed in a wide variety of edible plants including leafy vegetables, fruits (Strawberry, apple, etc.) and beverages (tea, red wine, beer etc.), they have been reported to exert multiple biological effects, including antiviral, anti-thrombotic, anti-ischemic, anti- inflammatory, anti- histaminic, antioxidant and free- radical scavenging abilities [7]. The phenolic compounds, widely distributed in plants, are the major compounds associated to human health and beneficial effects on gastritis, ulcer and cancer. The gastroprotective effect seems to be related to increase in endogenous PG, reduction in histamine secretion, scavenging oxygen- derived free radicals, and even to gastric mucus stimulation [8 and 9]. Flavonoids has free radical scavenging and antioxidant activity. The important derivative of flavonoids is quercetin. Flavonoids increased the mucus production and also have antihistaminic properties which reduce the histamine production and reduction of mast cells which are produced by the aspirin. The main mechanisms of action for the gastro-protective effects of this flavonoid are its proton pump inhibitor and antioxidant properties. Flavonoids have anti- inflammatory properties without any ulcerogenic action as a side effect and thus show a great advantage in the treatment of peptic ulcers [10].

The phytochemical studies on *Cordia dichotoma* showed presence of antiulcer compound as (Saponins, terpenoids, and flavonoids) [11]. Gastroprotective effect of flavonoid has been reported by [12]. Free radical scavenging ability of flavonoids has been reported to protect the gastrointestinal tract from ulcerative and erosion lesion [13].

The study was to demonstrate, the protective effects of aqueous- methanol extract of *C. dichotoma* in aspirin induced ulcer in rabbits.

Materials and Methods

Preparation of *C. dichotoma* extract

Ripe fruits of *C. dichotoma* obtained from the farms in Diyala province, Iraq, during April month. The aqueous: methanol extract (30:70%) of *C. dichotoma* fruits were prepared as described [14].

Determination of acute toxicity of *C. dichotoma* extracts (CDE)

Acute toxicity of *C. dichotoma* extract was performed as described [15].

Experimental animals: 15 New Zealand White male rabbits *(Oryctolagus cuniculus)* of, 1-1.900 kg body weight were utilized in conducting the study, after 2 weeks of adaptation, animals were divided, randomly into 3 groups (5 in each): Rabbits of group I, neither treated with plant extract, nor exposed to aspirin (control group), those of group II, exposed to aspirin, 300 mg / kg body Weight, in 2 ml water orally, meanwhile those of group III were treated with 300 mg /kg. B. wt. of *Cordia dichotoma* aqueous methanol extract, for 9 day, then exposed to aspirin, as in those of G II.

Design of the study

Twenty-four hours, before the experiment, the animals were fasted, but were add libitum to water. An hour before the experiment nothing allowed for them. Rabbits of G I (control group) not treated with extract, nor exposed to aspirin, those of G II, non- treated with extract, but exposed to aspirin. Meanwhile rabbits of G III were treated with plant extract for 9 day, orally before administration of aspirin, 300 mg / kg in 2 ml water orally.

Three-hour post exposures to aspirin in dependence of [16-19], all animals were sacrificed, and the stomachs were dissected out, opened. The lesions were counted and scored. The ulcer index / rabbits were taken. The percentage of ulcer protection (UP%) was calculated.

The clinical parameters which depended in experiment were done according to [20]. Body temperature (B. T.) by thermometer from rectum for 1 minute; Heart rates (H.R.) / minute through auscultation of heart by medicinal stethoscope; Respiratory rates (R.R.) / minute, through counting the respiration by direct auscultation of chest by stethoscope for 1 minute. Body weight by baby balance in kilogram. The hematological parameters (Blood picture; Bleeding and Clotting time) were done according to [21]. These parameters obtained in three times, before starting experiment (0 day), 5th day, and 9th day, the day post administration of aspirin. Estimation of biochemical parameters (Random Blood Sugar (RBS), Total Serum Protein (TSP), Alanine aminotransferase (ALT), Aspirtate aminotransferase (AST), Total serum bilirubin (TSB) were done according the kits on serum obtained at time of sacrificed of animals.

Assessment of gastric mucosal lesions was according to [22].

$$\textbf{Ulcer index (UI \%)} = \frac{Control\ Ulcer\ Index(CUI) - Treated\ Ulcer\ Index\ (TUI)}{Control\ Ulcer\ Index(CUI)}\ x\ 100$$

$$\textbf{Prevent index (PI)} = \frac{Ulcer\ Index\ (U.I.) of\ aspirin\ group - Ulcer\ Index\ (U.I.) of\ treated\ group}{Ulcer\ Index\ (U.I.) of\ aspirin\ group}\ x\ 100$$

Biochemical estimation

Volume and pH of gastric juice were estimated according to [23]. While total and free acidity were according to [24].

$$\textbf{Acidity} = \frac{Volume\ of\ NaOH\ x\ normality}{0.1}\ x\ 100$$

Histopathological studies according to [25].

Statistical analysis

Statistically the value of depended parameters were analyzed by ANOVA method and T- test. Values are represented as M ± S.E., with $P < 0.05$ as level of significant [26].

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Results

The results did not showed any change in body weight and body temperature. While respiratory rates decreased non- significantly and heart rates were non- significantly increased in comparison with their pretreated values table 1

Table 1: Body Weight, Body temperature, Respiratory rate, and Heart rate of rabbits in experiment.

parameter	Group			
		0 day	5th day	9th day
Body Weight: Kg	GII	1.53 ± 0.19	1.54 ± 0.20	1.55 ± 0.15
	G I	1.50±0.10	1.52 ±0.20	1.53 ±0.30
Body temperature. ^{o}C	GII	37.95± 0.34	38.05± 0.39	38.20± 0.25
	G I	39.0 ± 0.15	38.9± 0.12	38.8 ± 0.20
Respiratory rates / min.	GII	151 ±21.99	142.5 ±14.36	140.0 ± 7.80
	G I	125.0± 5.50	125.0± 4.0	128.0±10.0
Heart rate / min.	GII	180.0 ± 8.16	197.5± 19.31	190 ± 5.40
	G I	171.5 ± 4.35	175.5 ± 10.0	170.0± 4.50

Values are M ±S.E.

The results of study indicated that bleeding time increased non- significantly. While clotting time decreased non – significantly in rabbits of treated group in comparison with their pretreated values table 2.

Table 2: Bleeding and clotting times of rabbits in experiment

Parameter	Group			
		0 day	5th day	9th day
Bleeding time / sec.	GII	28.75 ± 5.91	38.75 ± 3.15	40.25± 2.06
	G I	45.0 ± 2.04	40.0 ± 2.04	40.0± 1.78
Clotting time / sec.	GII	65.0 ± 10.41	50 .0 ±7.91	55.4 ± 8.90
	G I	45.20 ± 3.0	50.0 ± 3.0	48.0 ± 4.0

All values of erythrocytes count (TEC), Hemoglobin Concentration (Hb), Hematocrit (HCT%), and erythrocytes indices (Mean Corpuscular Hemoglobin Concentration (MCHC); Mean Cell Volume (MCV); Mean Corpuscular Hemoglobin (MCH) did not change significantly in treated group, in comparison with their pretreated values table 3.

Table 3: Total Erythrocytes Count, Hemoglobin Concentration, Hematocrit, and Erythrocytes Indices (Mean Cell Volume, Mean Corpuscular Hemoglobin, Mean Corpuscular Hemoglobin Concentration) of rabbits used in experiment

Parameter	Group			
		0 day	5th day	9th day
Erythro.x10^6/mm^3	GII	5.44± 0.38	5.23± 0.61	5.15 ± 0.50
	G I	5.70 ± 0.35	5.40 ± 0.08	5.50± 0.15
Hb g%	GII	9.58± 0.49	9.73± 0.31	9.20± 0.15
	G I	9.20± 0.25	8.85± 3.15	9.0 ± 0.28
HCT%	GII	28.0± 1.47	28.0 ±1.08	28.0± 1.0
	G I	35.7± 0.65	36.0 ± 0.90	36.2± 0.80
MCV ft	GII	52.25±6.78	57.23±3.83	55.26±0.22
	G I	55.40±3.15	49.6 ± 0.67	50.24± 1.85
MCHpg	GII	18.50±1.54	19.32± 2.16	19.20± 1.66
	G I	19.5± 0.90	18.19± 1.23	19.0 ± 0.85
MCHC%	GII	34.20±0.08	34.87± 1.07	35.20± 0.10
	G I	35.9 ± 0.25	34.9 ± 0.38	35.0 ± 0.18

Total leucocyte count and lymphocytes % were non- significantly increased. While Heterophil, Eosinophil %, Basophil % did not show any changes. Meanwhile monocytes % non- significantly decreased in treated group, in comparison with their pretreated values table 4.

Table 4: Total leucocytes count and differential leucocytes count (Lymphocyte %, Heterophil %, Eosinophil %, Basophil %, and Monocyte %) of rabbits used in experiment.

Parameter	Group			
		0 day	5th day	9th day
WBC x10^3/mm^3	GII	14.28 ±1.03	15.25 ±3.43	15.24± 2.40
	G I	20.0± 1.50	19.8 ± 0.6	18.8 ±0.5
Heterophil%	GII	67 ± 1.53	61.25 ± 2.18	65.5 ± 3.0
	G I	48.0 ± 2.5	50.5± 6.7	49.0± 2.5
Lymphocyte%	GII	24.67± 6.12	32.5 ± 2.21	30.2 ± 4.60
	G I	46.8 ± 3.5	48.0± 4.0	48.0± 2.80
Eosinophil%	GII	4.67± 0.67	3.75 ± 0.25	3.5 ± 0.35
	G I	3.9 ± 0.7	3.5 ± 0.5	3.5 ± 0.5
Basophil%	GII	0.67 ± 0.34	1.0 ± 0.41	1.0 ± 0.25
	G I	2.8 ±0.25	1.5 ± 0.5	1.8 ± 0.15
Monocyte%	GII	4.67 ± 2.40	1.75 ± 0.63	3.6 ± 0.52
	G I	1.5± 0.5	1.5± 0.5	1.8 ± 0.5

The experiment indicated that levels of Random Blood Sugar (RBS) and Total Serum Protein (TSP) were significantly decreased in rabbits exposed to aspirin in comparison with those of control non- exposed to aspirin and those exposed to plant extract and aspirin. In addition to RBS showed a significant high level in those exposed to plant extract and aspirin in comparison with those exposed to aspirin. Alanine Aminotranferase (ALT) level significantly increased in those exposed to aspirin alone and those exposed to plant extract and aspirin in comparison with those not exposed to aspirin. Aspirtate Aminotransferase (AST) and Total Serum Bilirubin (TSB) levels were significantly increased in those exposed to aspirin in comparison with those none exposed to aspirin table 5.

Table 5: Random Blood Sugar (RBS), Alanine Aminotransferase (ALT), Aspirtate Aminotransferase (AST), Total Serum Bilirubin (TSB), Total Serum Protein (TSP) levels of rabbits in experiment.

parameter	group		
	Plant + Aspirin (G II)	Aspirin (G III)	Normal (G I)
RBS mg/dl	120.63± 3.09bc	35.0 ± 0.5a	100.5 ± 4.5b
TSP g /dl	8.9 ± 0.71b	6.2 ± 0.20a	8.0 ± 0.10b
ALT U/L	80.67±3.54b	85.3± 0.30b	40.8 ± 2.65a
AST U/L	55.23 ± 3.49a	70.55 ± 5.65bc	60.5 ± 2.8b
TSB mg/dl	0.6± 0.12a	0.73 ± 0.09b	0.6±0.05a

Activity of *C. dichotoma* fruit extract against ulcer:

Group III exposed to aspirin No of lesion 8;

4=lesions 2-4 mm; and 4 lesions > 4

Lesion scores = 24

Ulcer Index (UI) = 24 x 4

Group II treated with *Cordia* and exposed to aspirin

Number of 7 lesions 4 lesions = 2-4 mmm; 3 lesions > 4 mm.

Lesions scores 20

UI = 20 x4

Prevent index (PI) $= \frac{Ulcer\ index(U.I.)of\ aspirin\ group - U.I.of\ treated\ group}{U.I.of\ aspirin\ group}\ x\ 100$

$==\frac{8x24x4-7x20x4}{8x24x4}\ x\ 100$ = **27.08%**

The level of pH of stomach was the lowest in those exposed to aspirin, followed by those exposed to plant extract and aspirin, which was significantly different from the level in those non- exposed to aspirin table 6.

Volume of gastric fluid was the highest in those exposed to aspirin, followed by those exposed to plant extract and aspirin, which was significantly different in comparison with those of rabbits non- exposed to aspirin table 6. Free acidity and total acidity levels were higher in those exposed to aspirin which was significantly differ from those exposed to plant extract and aspirin and those non- exposed to aspirin. In the same time this value was significantly lower in those exposed to plant extract and aspirin in comparison with those exposed to aspirin or those none-exposed to aspirin table 6.

Table 6: Gastric PH, juice volume, gastric lesion number of lesions, and ulcer protection in rabbits of experiment

Groups	pH	Volume	Free acidity	Total acidity	Gastric ulcer lesion (No.)	% of ulcer protection
control GI	2.6 ± 0.08c	1.5 ± 0.06a	250 ± 18.9b	280± 12.5b	0.0 ± 0.00	-
Aspirin G III	1.33 ± 0.12a	2.67 ± 0.03b	325 ± 7.5c	450 ± 5.0c	8.0± 0.50a	-
Plant + aspirin G II	1.94 + 0.12b	1.8 ± 0.0a	175.0 ± 7.37a	252 ± 10.63a	7.0 ±0.15a	27.08

The gastric mucosa was more severely affected and showed more sever and more numbers of lesions in those exposed to aspirin (Picture 1-2), and of less severity and less numbers of lesions in those exposed to aspirin and plant extract (Picture 3) in comparison with those of none exposed to aspirin alone or with plant extract.

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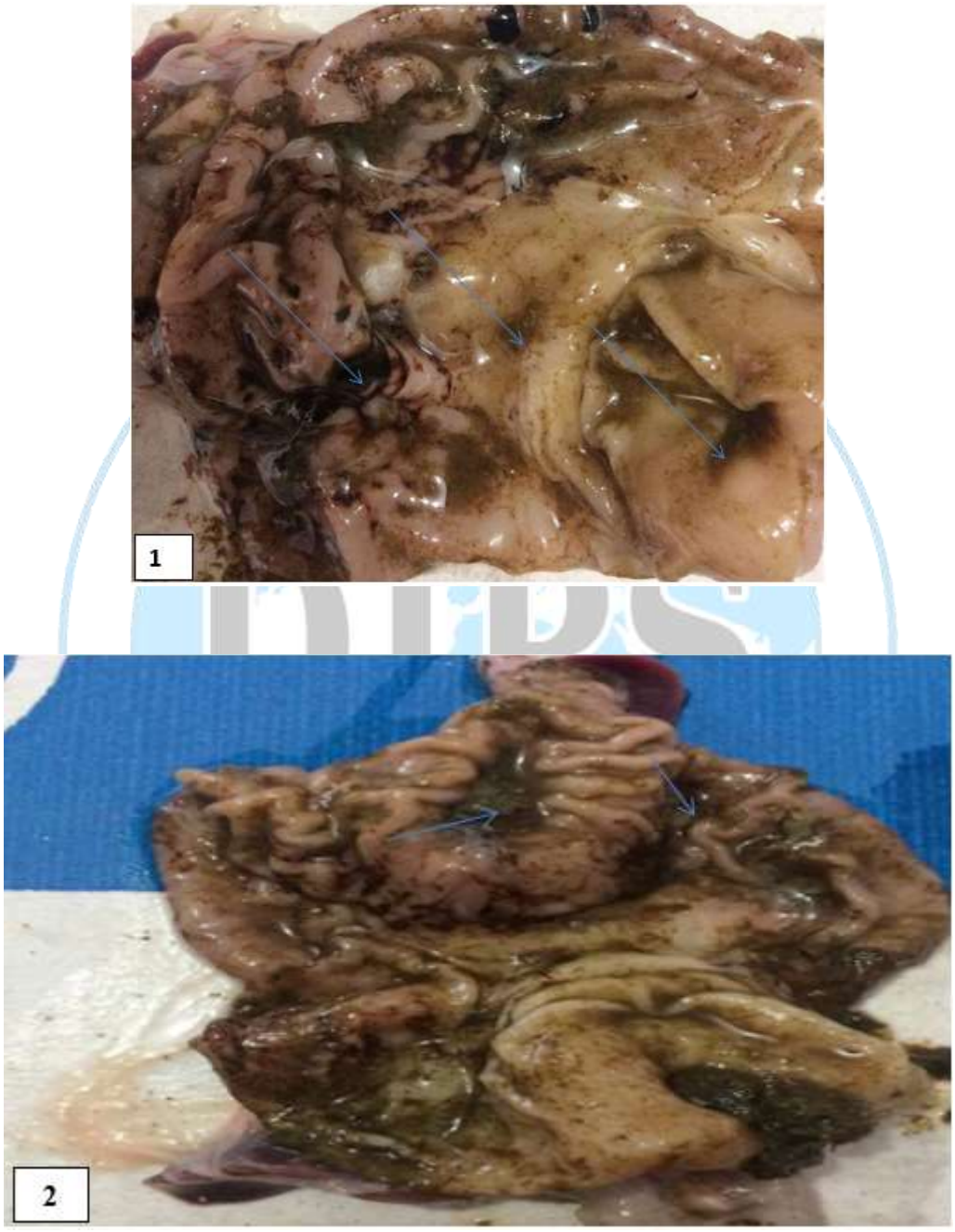

Pictures 1-2: Stomach mucosa of rabbits exposed to aspirin without treatment with plant extract

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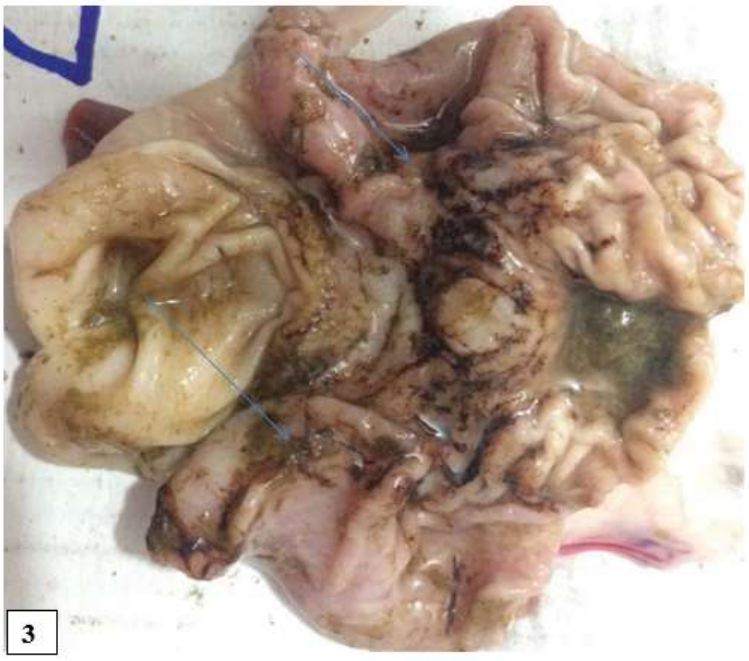

Picture 3: Stomach mucosa of rabbit exposed to aspirin after treatment with plant extract.

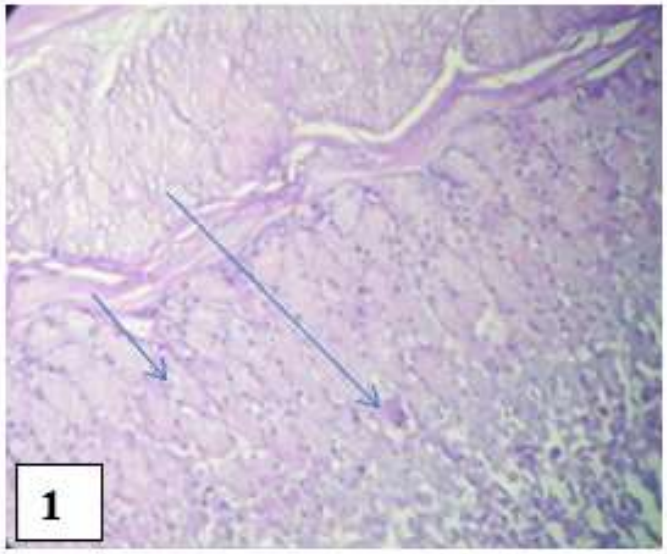

Figure 1: Histopathological changes in gastric mucosa of rabbit exposed to aspirin and treated with *C. dichotoma* extract. Few sloughing in the mucosal epithelium surface of the stomach (in the pits) also present few inflammatory cells (H & E 10X).

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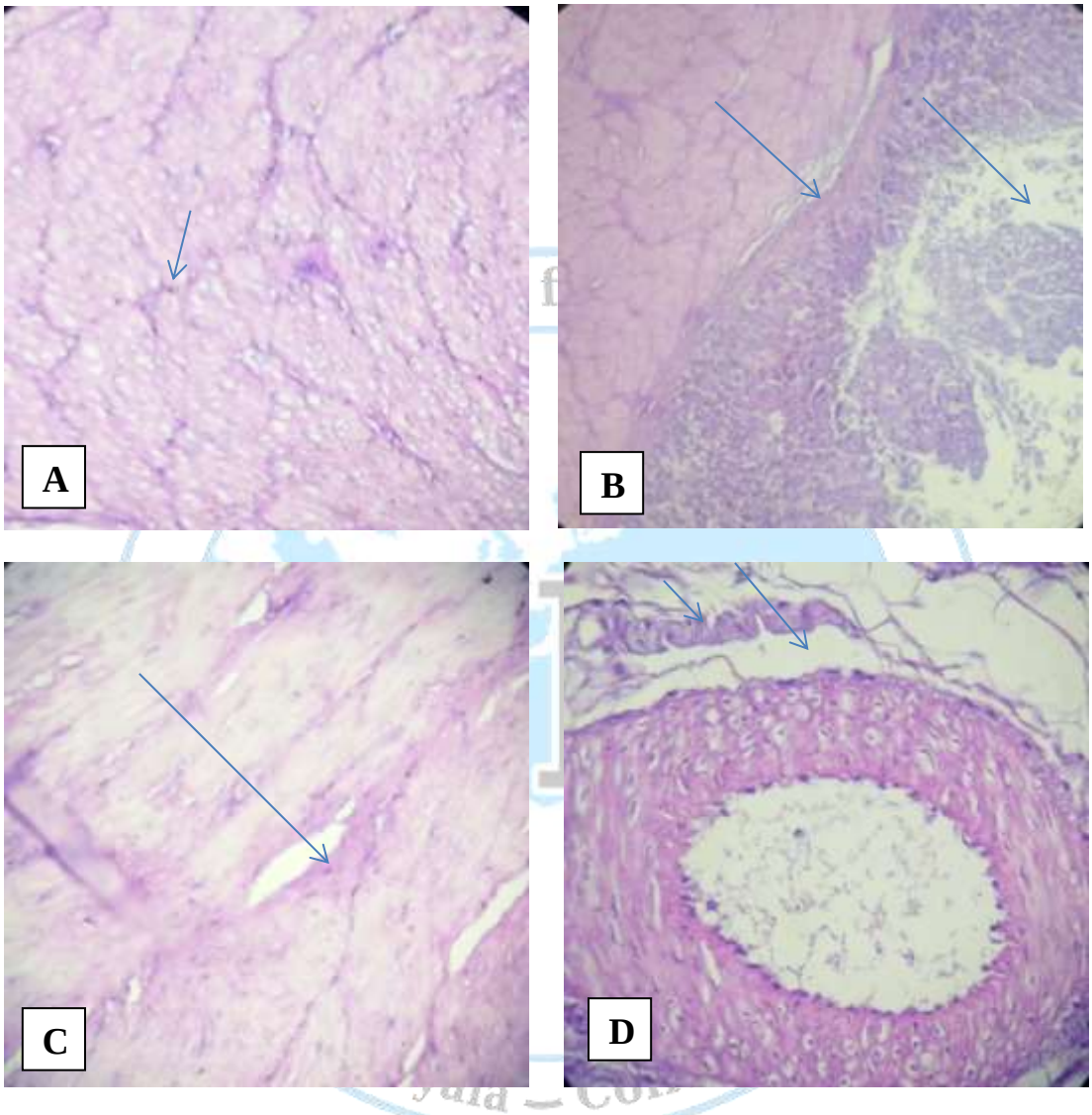

Figure 2: Histopathological changes in gastric mucosa of rabbit exposed to aspirin without treatment with plant. Showed degeneration and atrophy in the pyloric glands in the mucosa of stomach (A), some granulation tissue in the lamina muscularis layer of the stomach with sloughing (B), Presence of granulation tissue in the wall of artery that enriched the stomach (C), presence of necrosis and granulation tissue in the lamina muscularis with degeneration in the surface epithelium (D) (H & E 10X and 20X).

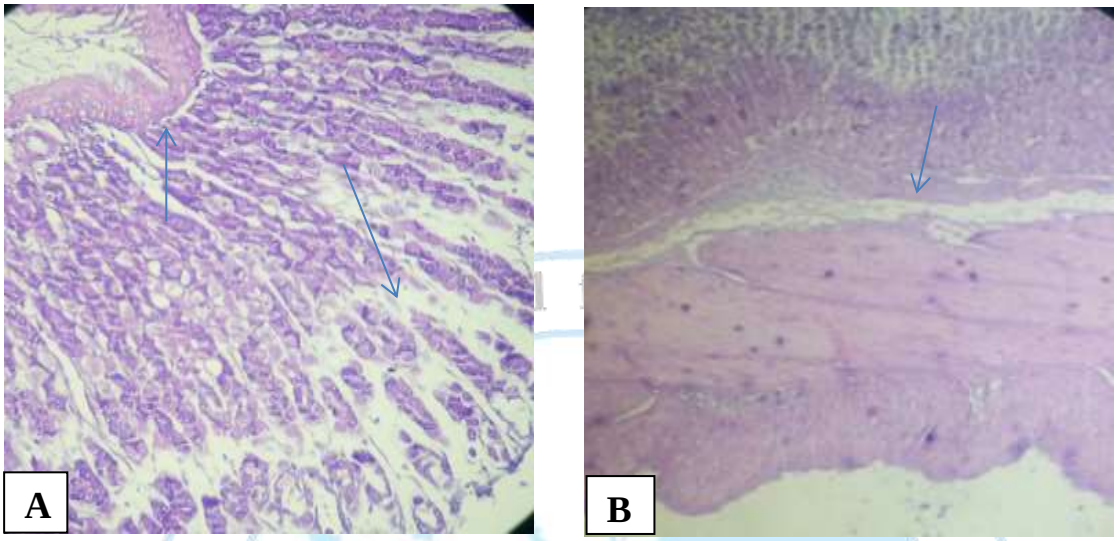

Figure 3: Histopathological changes in gastric mucosa in rabbit not exposed to aspirin, not treated with plant extract showed sloughing in the pyloric glands (A), slight granulation tissue in the lamina muscularis, slight presence of fibrin in the muscular lamina (H & E 20X).

Discussion

In current experiment, administration of *Cordia dichotoma* significantly increased gastric mucus secretion, while reduce in the acidity of the gastric juice in rabbits, therefore, the enhanced mucous secretion may help to protect against the aspirin induced damage by preventing the action of acid on the stomach mucous epithelium.

The phytochemical studies on *Cordia dichotoma* showed presence of antiulcer compound as (Saponins, terpenoids, and flavonoids) [11]. Gastroprotective effect of flavonoid has been reported by [12]. Free radical scavenging ability of flavonoids has been reported to protect the gastrointestinal tract from ulcerative and erosion lesion [13].

Flavonoids are phenolic compounds. The phenolic compounds widely distributed in plants, which are the major compounds associated to human health and beneficial effects on gastritis, ulcer and cancer. The effects seems to be related to increase in endogenous PG, reduction in histamine secretion, scavenging oxygen – derived free radicals and even to gastric mucus

stimulation [8 and 9]. In present investigation, Cordia dichotoma possess mild gastro-protective properties as evident by its significant inhibition of the formation of gastric lesion (In term of number and space) induced by aspirin.

Gastritis is an inflammation of the stomach lining, have different causes, in the first place, the non- steroidal anti- inflammatory drugs (NASIDs) such as aspirin. Aspirin, a famous NSAID, is thought to cause gastric damage by both, topical irritant effects on the gastric epithelium and systemic effects related to suppression of mucosal prostaglandin synthesis. Inhibition of prostaglandin synthesis reduces mucosal defenses, including mucus and bicarbonate secretion, blood flow, epithelial turnover and repair, and mucosal immunocyte function. NSAIDs can interfere with the healing of preexisting lesions and cause a fast drop in pH within mucus cap [1]. The etiology of gastroduodenal ulcers is influenced by various aggressive and defensive factors, from which mucus secretion, blood flow and endogenous protective agents (prostaglandins) [3]. According to [27] excessive ingestion of non- steroidal anti- inflammatory agents may be one of responsible factors for the development of peptic ulcer.

The use of non- steroidal anti-inflammatory drugs NSAIDs is considered to be the major risk factor in gastric ulcer. The mechanisms are inhibition of prostaglandin synthesis and inhibition of epithelial cell proliferation in the ulcer margin, which is critical for the reepithelization of the ulcer crater [28].

The results of our investigation revealed that the Cordia dichotoma did not affected on the clinical and hematological parameters depended in study, as they did not show significant changes. This may be due to the duration of study is short, in addition to the non- toxic effects of the plants.

Conclusion

We can conclude that aqueous – methanol extract of *Cordia* dichotoma fruits has a mild gastroprotective in gastritis induced by aspirin administration, and further study had to be carrying to explore more about this.

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