STUDY THE EFFECT OF PROANTHOCYANIDIN AND RANITIDINE ON HAEMATOLOGY AND BIOCHEMICAL PARAMETERS IN ADULT FEMALE RABBITS WITH GASTRIC ULCERATION INDUCED BY INDOMETHACIN

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(Received 30 September 2015 , Accepted 2 November 2015)

Key words: Ranitidin, Gastric Ulceration,Rabbits.

ABSTRACT

The study was conducted in Collage of Veterinary Medicine \Basrah University,to evaluate the effect of proanthocyanidin and ranitidin on gastric ulcer, haematological and biochemical parameters changes by using female rabbits with acute gastric lesions induced by indomethacin.The study done on (30)adult female rabbits, their weight ranged between (1500-2000.0mg); divided into five groups, each group consist of six rabbits as the following: Group1:- healthy (negative control group) administrated normal saline (0.9 of normal saline) for 10 days; Group 2:-given indomethacin 75mg\kg B.W. for two days(positive control group); Group 3:- at first given indomethacin 75mg\kg B.W. for two days, then treated with proanthocyanidin(PA) 100mg\kg B.W. for 10 days; Group 4, initially given indomethacin 75mg\kg for two days, then treated with proanthocyanidin(PA) 200mg\kg for 10 days; Group 5, given indomethacin 75mg\kg for two days, then treated with ranitidin 50mg\kg for10 days.The results showed that proanthocyanidin(PA) and ranitidin caused significant reduction ($P\leq0.05$) in gastric volume, ulcer area, serum MDA, gastric tissue MDA while significant increase ($P\leq0.05$) in mucin and gastric pH. It also revealed significant decrease ($P\leq0.05$) in glucose concentration in rabbits treated with proanthocyanidin compared to positive control group while showedno-significant change in glucose concentration in rabbits treated with ranitidine compared with positive control group. It also, showed

significant increase (P≤0.05) in Red Blood Cell(RBC),Hemoglobin(Hb) andMean Corpuscle hemoglobin concentration(MCHC) in rabbit treated with proanthocyanidin or ranitidin, while there was significant decrease (P≤0.05) in Mean Corpuscle Volume(MCV) in rabbits treated with proanthocyanidin in dose of 100mg\kgand ranitidine in a dose of 50mg/kg with non-significant change of MCV in female rabbits with gastric ulceration treated with proanthocyanidin at dose 200 compared with positive control group.It showed non-significant changes in White Blood Cell (WBC) of female rabbits with gastric ulceration treated with proanthocyanidin compared with positive and negative control groups, while the results showed significant decrease (P≤0.05) in WBC of female rabbits with gastric ulceration treated with ranitidine group compared with positive and negative control groups and the other groups. The study revealed significant decrease (P≤0.05) in total cholesterol, triglyceride, Low Density Lipoprotein(LDL) and very Low Density Lipoprotein(VLDL) of female rabbits with gastric ulceration treated with proanthocyanidin and ranitidine group compared with positive control group while it showed significant increase (P≤0.05) in High Density Lipoprotein (HDL) in rabbits treated with proanthocyanidin and ranitidine group compared with positive control group. It is concluded that proanthocyanidin extract of the grape seeds(*Vitis vinifera*)displayed good antiulcer activity, hypoglycemia effect, amelioration of heamatological parameters and improve dyslipidemia corroborating the folk use of *Vitis vinifera* preparations, and contributing for its pharmacological validation.

INTRODUCTION

Gastric ulcer is an damage in the continuity of normal gastric mucosa that extends through the mucosa into the submucosa or deeper. It may be complicated by occurrence of hemorrhage, perforations, gastrointestinal obstruction, and malignancy. Therefore, this clinical condition represents a worldwide health problem because of its high morbidity, mortality and economic loss (1,2).

It occurs due to imbalance between aggressive and defensive factors of the gastric mucosa. Aggressive factors against gastric mucosa include acid, pepsin, infection by*Helicobacter pylori*, non-steroidal anti-inflammatory drugs (NSAIDs),ethanol, acetic acid, bile salt and even personal factors such as physical stress and

consumption of alcohol, tobacco and caffeine (3) while the local mucosal defensive factors include bicarbonate, mucus secretion, blood flow, cellular regeneration, and endogenous protective agents like prostaglandins (PG) and epidermal growth factors (4). the increasing oxidative stress is believed to be linked to the aggressive factors-induced gastric mucosal damage (5,6).

Non-steroidal anti-inflammatory drugs (NSAIDs) are common medication used for treating many conditions such as rheumatoid, musculoskeletal and cardiovascular disease. But because of its harmful effect on gastrointestinal tract, NSAIDs clinical use is restricted (7,8). The mechanism of NSAIDs by which causing gastric ulcer are through blocking of cyclooxygenase (COX) activity that leads to lowering mucus and bicarbonate secretion, decreased mucosal blood flow, neutrophils infiltration, alteration of micro vascular structures, an increase of acid and pepsinogen secretion. Indeed, increased production of reactive oxygen species (ROS), increased lipid peroxidation, and neutrophils infiltration have demonstrated to play important role in the pathogenesis of NSAIDs-induced ulcers, including the aspirin-induced ulcer (7,9).

Indomethacinis old non-steroidal anti-inflammatory drug which was synthesized in 1963 for the treatment of rheumatoid arthritis. It is an indolemathylate derivative that has anti-inflammatory, analgesic and anti-pyretic activities (10).

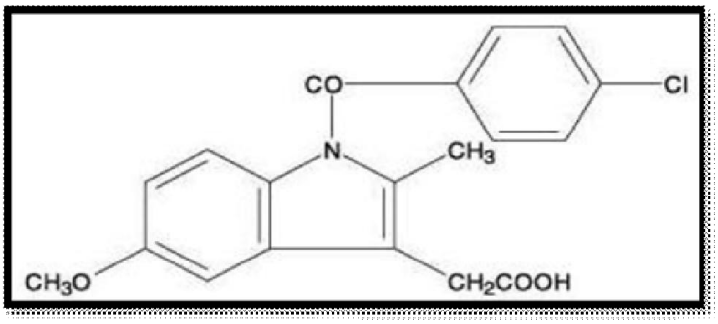

Fig. 1. Structural formula of indomethacin

It has ability to induce gastric ulcer by production of free radical, induce lipid peroxidation,inhibition of prostaglandinsand leukocyte infiltration(11)

In recent years, using of alternative therapies and natural products, especially those derived from plants attract attention of scientist (12,13).Grape seed proanthocyanidin extracts (GSPEs; Hanlim Pharm. Co., Ltd., Seoul, Korea), which was used in the current study, is a commercially available product of the substances that were extracted from European red grape seed, *Vitis vinifera*. The basic structure unitof proanthocyanidin is catechin. Proanthocyanidins are including monomer, dimer and trimer catechin, all of which are water-soluble molecules and contain a number of phenolic hydroxyls (14)The activity of proanthocyanidins oligomers is approximately fifty times greater than that of vitamin C and vitamin E, in terms of antioxidant action (15). Proanthocyanidins are powerful antioxidants(16) scavenger of free radicals and have many health-promoting effects (17).

Ranitidine introduced in 1981 and was found to have a far better tolerability profile (fewer adverse drug reactions), longer-lasting action, and ten-times the biochemical activity of cimetidine. Ranitidine HCl is a competitive, reversible inhibitor of the

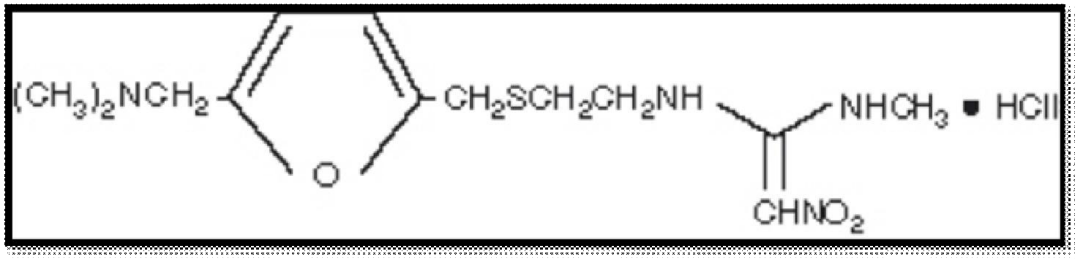

Fig.2 Chemical Structure of Ranitidine.

action of histamine at the histamine H2-receptors, including receptors on the gastric cells which decreases the amount of acid produced in the stomach.

It is used to treat ulcer, gastroesophageal reflux disease (a condition in which a backward flow of stomach acid causes heartburn and injury of the esophagus), and conditions where the stomach produces too much acid, such as Zollinger-Ellison syndrome. Ranitidine is available over-the-counter used to prevent and treat symptoms of heartburn associated with acid indigestion and sour stomach (18).

MATERIALS AND METHODS

Drugs and Chemicals:

Indomethacin obtained from Safa co. Diala-Iraq, and ranitidine provided from GlaxoSmithKline, S.A. Aranda de Duero, Spainwere suspend in 2ml of normal saline.

Plant Material:

Proanthocyanidin had been extracted from black Grape seeds that were used in this study. The black grape was hand-picked from local market with full skin intact. It was washed with tap water,the skin and fleshes were removed and the seeds are dried.The seeds of the grape were turned to powder with the help of an electric grinder and kept in dark container at 25C°.

Preparation proanthocyanidin extract from grape seeds

Fifty grams of dried grape seeds powder was defatted with (500 ml) of n-hexane for 2hours by soxhlete. The combined n-hexane extract was concentrated below 50°C under reduced pressure in a rotary evaporator to get 7ml of yellow oily mass. This mass was dried at room temperature and further (40 gm.) was refluxed in (500ml) methanol (80%) in water with 3% hydrochloric acid for one hour then filtered by Buchner funnel and filter paper (Wattman No.185).The filtrate was extracted with an equal volume of chloroform to remove pigments. The alcoholic layer was extracted with an equal volume of ethyl acetate treated with 2% of hydrochloric acid ,the ethyl acetate layer was concentrated by rotary evaporator at 45˚C and dried at room temperature (19,20). The resultant extract (2.5gm) was pink color and dry material .The extract was kept in dark glass container at 4°C.

Experimental Animals

Thirty adult female rabbits weight ranged between (1500-2000.0mg) kept for an adaptation period for 1 month in the animal house of Veterinary Medicine College / Basrah University. The experimental animals were kept in individual cages, provided with standardration in addition to green alfalfa (Medicago *sativa*) and tap water *ad libitum* and given a prophylaxis drug against coccidiosis (Amprollium 1g/L of drinking water).

Experimental design:

The rabbit divided into five groups of comprising 6 animals in each group as the following:

Group1:- healthy (negativecontrol group)oral administration 3ml of normal saline (0.9 of NaCL) for 10 days.

Group 2:- oral administration with indomethacin 75mg\kg B.W.dissolved in3ml of normal saline two days(positive control group) and remain without treatment for10 days.

Group 3:- treated with indomethacin 75mg\kg B.W. dissolved in 3ml of normal saline for two days, then treated with proanthocyanidin 100mg\kg B.W.dissolve with 3ml of normal saline for 10 days.

Group 4:- treated with indomethacin 75mg\kg B.W. dissolved in 3ml of normal saline for two days, then treated with proanthocyanidin 200mg\kg B.W. dissolve with 3ml of normal saline for 10 days.

Group 5:- treated with indomethacin 75mg\kg B.W. dissolved in 3ml of normal saline for two days, then treated with ranitidine 50mg\kg B.W. dissolved in3ml of normal saline for 10 days.

Induction of gastric ulcer:

Gastric ulcers were induced in twenty four non starved rabbits by giving indomethacin (Safa co. Diala-Iraq) orally by one ml size syringe and in dose 75mg\kg for two days.

Collection of Blood Samples

Blood samples (15ml) were collected from each animals at end of experiment by the heart (cardiac puncture). The (10ml) of blood was deposited into tube without anticoagulant and then the blood samples were centrifuged at (3000 rpm) for 15 minutes and serum samples stored in polyethylene eppendorff tubes at (-20ºC),which then used to study hormonal ; (HDL, Triglyceride, total cholesterol, serum glucose).The remaining (5ml) of blood was deposited into tube with anticoagulant which used for heamatological analysis (RBC,WBC, Hb, MCV, MCH, MCHC, PCV, differential WBC)

Study parameter:-

Gastric ulcer index:

The method described by(21)was employed in the present study stomachs were opened along the greater curvature, washed with saline and examined by magnifying class for gastric ulcers observation .The sum of length for all lesions area for each animal was measured and served as the ulcer index . The curative ratio was calculated for each group using following equation:

Curative ratio (CR) = (LC-LT/LC) x100.

LC: The length of gastric ulcer in positive group.

LT: The length of gastric ulcer in treated group.

Determination of Gastric Juice Volume:

Gastric juice collected from each animal was centrifuged at 3000 rpm for 10 minutes to remove any solid debris, the volume of the supernatant was measured by graduated cylinder.

Determination of Gastric Juice Acidity

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

measurement of serum glucose:

Serum glucose is measured by using RANDOX\GLUC-PAP, United Kingdom.

measurement of MDA

Serum MDA was measured by using thiobarbituric acid assay(22).Gastric tissue MDA was measured by using method (23) .The free mucin in the gastric tissues was estimated by measuring the amount of Alcian blue dye (Ab.)

Heamatological analysis

RBC,WBC, Hb, MCV, MCH, MCHC, PCV, differential WBC were measured by count 60 (Genex laboratories, Germany) apparatus.

Measurement of total cholesterol:

Total cholesterol measured by using method CHOD-POP,France.

Measurement of triglycerides:

Triglycerides measured by using Triglycerides–liquizyme\GPO-POP, Germany.

Measurement of High Density lipoprotein–cholesterol (HDL-c)

HDL-C measured by CHOLESTEROL liquicolor test kit.

Measurement of serum Low–density Lipoprotein Cholesterol (LDL):-

Serum LDL-C concentration was calculated according to (24).

LDL-C=Total cholesterol-[(HDL-C) + Triglyceride /5].

Measurement of serum very low- density Lipoprotein (VLDL):-

The serum very low-density lipoprotein concentration was calculated by dividing serum triglyceride by five (25).

VLDL=Triglyceride/5.

Histopathological processing:

Stomach samples which had been immersed in 10% formalin were used. the damaged, ulcerated as well as normal tissue parts were cut selectively then washed with normal saline and immersed in serial dilutions of ethanol 50% ,70%, 80% for once while 90% and 100% twice; two hours for each step. After the dehydration step the specimens were immersed in xylene for half to one hour to become ready to be embedded in the paraffin wax at 60°C, left overnight in the oven.Five microns thickness sections of paraffin-embedded tissue were mounted on glass slides and stained with Hematoxyline and Eosin stain (H & E stain)(26,27)

Statistical Analysis:

The results of the present study were analyzed by using two-way covariance (ANOVA) test in all study. All statistical calculations were carried out by the aid of the statistical package SPSS V. 11 (SPSS Inc.). The data were expressed as means ± standard deviation (X ± SD). Least significant different test (LSD) was calculated to test difference between means of groups and subgroups(28).

RESULTS

Grossly examination

Indomethacin induced gastric damage showed marked gross mucosal lesion, including hemorrhagic and petechial lesion.

On gross examination these hemorrhagic bands were characterized by different sizes along the longitudinal axis of the glandular part of stomach

Animals treated with PA showed no lesion at all (Fig.3).

Fig.4 reveals the degree of recovery of indomethacin-induced ulcerations when the animals were treated with PA, There was a profound inhibition of ulcer formation than ranitidine treated rabbits which also showed necrosis and more hyperemic areas(Fig.5).

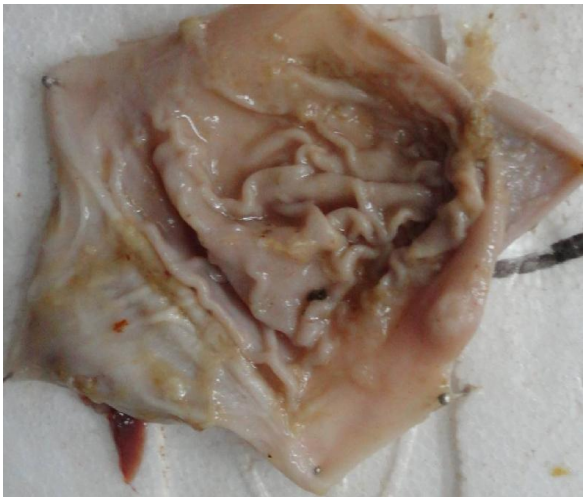

Fig.1:-Stomach of female rabbits (-ve) (Normal control). Showed normal gastric mucosa.

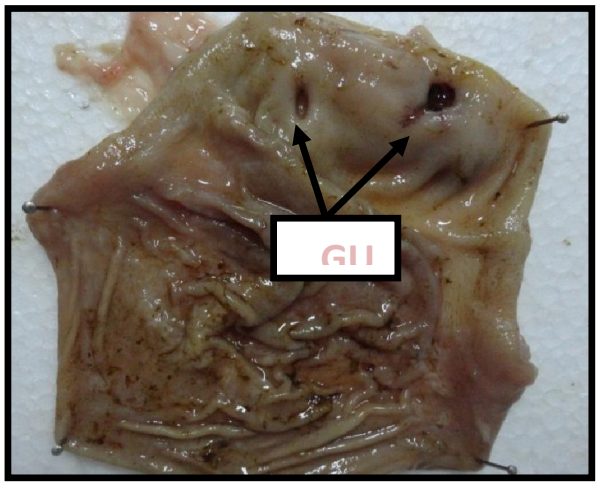

Fig.2:-Stomach of female rabbits (+ve control) treated with Indomethacin for 2 days causing gastric ulcer(GU). Showed gastric damage including gross mucosal lesion and haemorrhagic lesion.

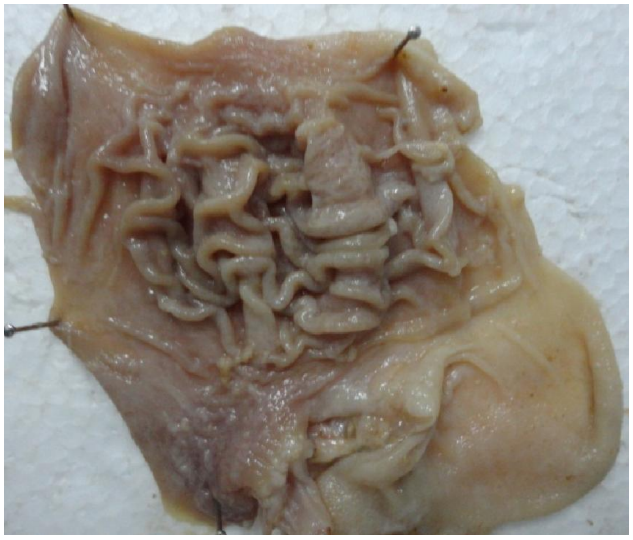

Fig.3:-Stomach of female rabbits treated with (PA)at a dose 100mg/kg) for 10 days of treated. Showed no lesion at all tissue of stomach.

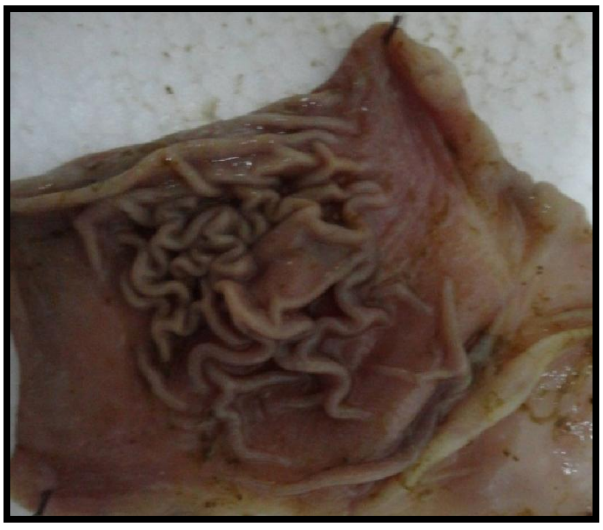

Fig.4:-Stomach of female rabbits treated with (PA at a dose 200mg/kg) for 10 days of treated. Showed no lesion at all tissue of stomach.

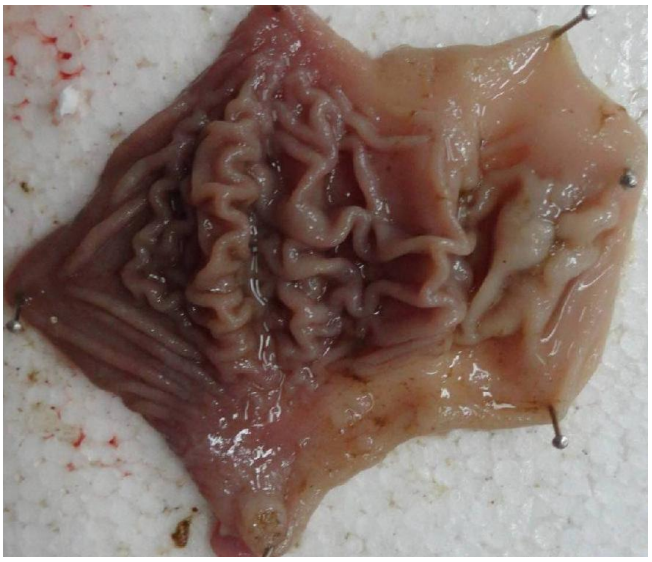

Fig.5:-Stomach of female rabbits treated with Ranitidin at a dose 50mg/kg) for 10 days of treated. Showed redness at all tissue of stomach indicate that the presence of inflammation.

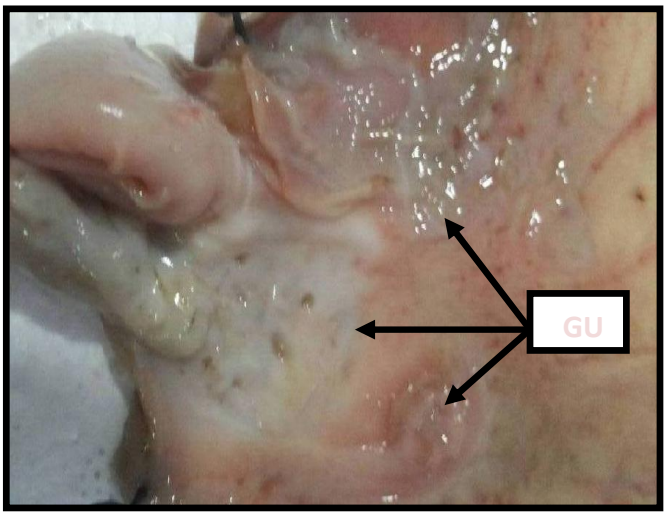

Fig.6:-Stomach of female rabbits (+ve control) treated with Indomethacin induced gastric ulcer (GU) for 10 days of treated. Showed gastric damage including gross mucosal lesion and petical lesion.

The obtained results in Table (1) revealed significant decrease ($P \leq 0.05$) of gastric volume in female rabbits with gastric ulceration treated with (PA at a dose 100mg/kg), (PA at a dose 200mg/kg) and Ranitidine at a dose 50mg/kg compared with(+ve) group. the gastric volume in female rabbit with gastric ulceration treated with (PA at dose 200) revealed a significant decrease ($P \leq 0.05$) compared with (-ve) control and another groups while gastric volume in female rabbit with gastric ulceration treated with (PA at dose 100mg/kg) and Ranitidine revealed non-significant changes compared with (-ve) control group.

It also showed significant increase ($P \leq 0.05$) in gastric pH in female rabbit with gastric ulceration treated with (PA at a dose 100mg/kg), (PA at a dose 200mg/kg) and Ranitidine at a dose 50mg/kg a compared with (+ve) control group.

The results of ulcer area showed significant reduction ($P \leq 0.05$) in gastric ulceration female rabbits treated with(PA at dose 100mg/kg), (PA at dose 200mg/kg) and Ranitidine compared with (+ve) control group, and it inhibition 100% in gastric ulceration female rabbits treated with (PA at dose 100mg/kg), (PA at dose 200mg/kg) and showed non-significant change compared with control group while the ulcer area showed 71.09% in gastric ulceration female rabbits treated with Ranitidine and showed significantincrease ($P \leq 0.05$) compared with (-ve) control group.

The obtained results in Table (2) revealed significant decrease (P≤0.05) of glucose concentration in female rabbits with gastric ulceration treated with (PA at dose 100) and (PA at dose 200) compared with positive group and non-significant change in glucose concentration compared with negative control group while the results showed non-significant change of glucose concentration in female rabbits with gastric ulceration treated with Ranitidine compared with (+ve) control group but the results showed significant increase (P≤0.05) in glucose concentration in female rabbits with gastric ulceration treated with Ranitidine compared with (-ve) control group.

The results revealed significant decrease (P≤0.05) in serum MDA of gastric ulceration female rabbits treated with (PA at a dose 100),(PA at a dose 200) and Ranitidine at a dose 50mg/kg compared with (+ve) control group. The results revealed non-significant in serum MDA of gastric ulceration female rabbits treated with (PA at a dose 100),(PA at a dose 200) and Ranitidine at a dose 50mg/kg compared with (-ve) control group.

The results of MDA Mucin revealed significant increase (P≤0.05) in gastric ulceration female rabbits treated with (PA at a dose 100) and (PA at a dose 200) compared with (+ve) control group while the results revealed non-significant in MDA Mucin of gastric ulceration female rabbits treated with Ranitidine at a dose 50mg/kg compared with (+ve) control group.

The results of gastric tissue MDA revealed significant decrease (P≤0.05) of gastric ulceration female rabbits treated with (PA at a dose 100),(PA at a dose 200) and Ranitidine at a dose 50mg/kg compared with positive group. The results revealed non-significant in gastric tissue MDA of gastric ulceration female rabbits treated with (PA at a dose 100),(PA at a dose 200) and Ranitidine at a dose 50mg/kg compared with (-ve) control group.

The obtained results in Table (3) revealed significant increase (P≤0.05) in RBC of gastric ulceration female rabbits treated with (PA at dose 100) , (PA at dose 200) and Ranitidine at a dose 50mg/kg compared with (+ve) control group and non-significant change in RBCs count compared with (-ve) control group while the results showed significant increase (P≤0.05) in RBC of gastric ulceration female rabbits treated with (PA at a dose 100) compared with (PA at dose 200)group and non-significant change in RBCs count compared Ranitidine group.

The results of Hb revealed significant increase (P≤0.05) in gastric ulceration female rabbits treated with (PA at dose 100), (PA at dose 200) and Ranitidine at a dose

50mg/kg compared with (+ve) control group and non-significant change in Hb compared with (-ve) control group.

The results of PCV% revealed significant increase (P≤0.05) in gastric ulceration female rabbits treated with (PA at dose 100), (PA at dose 200) and Ranitidine at a dose 50mg/kg compared with (+ve) control group and non-significant change in RBCs count compared with (-ve) control group.

The results of MCV revealed significant decrease (P≤0.05) in gastric ulceration female rabbits treated with (PA at dose 100) and Ranitidine at a dose 50mg/kg compared with positive control group and non-significant change of MCV in gastric ulceration female rabbits treated with (PA at dose 200) compared with (+ve) control group.

The results of MCH revealed non-significant changes between treated groups (PA at dose 100), (PA at dose 200) and Ranitidine at a dose 50mg/kg compared with (+ve) control group and (-ve) group.

The results of MCHC revealed significant increase (P≤0.05) in gastric ulceration female rabbits treated with (PA at dose 100), (PA at dose 200) and Ranitidine at a dose 50mg/kg compared with (+ve) control group and non-significant change in MCHC compared with (-ve) control group.

The obtained results in Table (4) revealed non-significant changes in WBC of gastric ulceration female rabbits treated with (PA at a dose 100) and (PA at a dose 200) compared with (+ve) control group and (-ve) control group while the results showed significant decrease (P≤0.05) in WBC of gastric ulceration female rabbits treated with Ranitidine group compared with(+ve) control group, (-ve) control group and another groups.

The results of neutrophils% showed significant increase (P≤0.05) in gastric ulceration female rabbits treated with (PA at a dose 200) compared with (+ve) control group, (-ve) control group and another treated groups while the result of neutrophils% showed significant decrease (P≤0.05) in gastric ulceration female rabbits treated with Ranitidine group compared with(PA at a dose 100), (PA at a dose 200) and (-ve) control group but this results showed non-significant compared with (+ve) control group. The results of neutrophils% showed significant increase (P≤0.05) in gastric ulceration female rabbits treated with(PA at a dose 100) compared with Ranitidine group and(+ve) control group but this results showed non-significant compared with (-ve) control group.

The results of eosinophile % showed significant decrease (P≤0.05) in gastric ulceration female rabbits treated with (PA at a dose 100) and Ranitidine group compared with (+ve) control group and non-significant change compared with(-ve) control group.
The results of basophile % showed significant decrease (P≤0.05) in gastric ulceration female rabbits treated with (PA at a dose 100), (PA at a dose 200) and Ranitidine group compared with (+ve) control group and non-significant change compared with(-ve) control group.

The results of lymphocyte % showed significant decrease (P≤0.05) in gastric ulceration female rabbits treated with (PA at a dose 100) and (PA at a dose 200)compared with (+ve) control group and Ranitidine group and this results of lymphocyte % showed non-significant change in gastric ulceration female rabbits treated with (PA at a dose 100) compared with(-ve) control group but this results of lymphocyte % showed significant decrease (P≤0.05) in gastric ulceration female rabbits treated with(PA at a dose 200) compared with(-ve) control group.

The results of monocyte % showed significant decrease (P≤0.05) in gastric ulceration female rabbits treated with (PA at a dose 100), (PA at a dose 200) and Ranitidine group compared with (+ve) control group.

The obtained results in Table (5) revealed decrease significant(P≤0.05)in total cholesterol, triglyceride, LDL and VLDL of gastric ulceration female rabbits treated with (PA at a dose 100), (PA at a dose 200) and Ranitidine group compared with (+ve) control group and this result showed non-significant changes compared with(-ve) control group while the results of HDL showed significant increase (P≤0.05) in gastric ulceration female rabbits treated with(PA at a dose 100), (PA at a dose 200) Ranitidine group compared with(+ve) control group, and non-significant changes compared with (-ve) control group.

Table (1) Effect of Proanthocyanidin and Ranitidine on Gastric Volume, Gastric PH, Ulcer area and Inhibition Percentage in Gastric Ulceration Female Rabbits induced by Indomethacin (Mean±SD) (n=6)

Parameters / Treatment	Gastric Volume (ml)	pH of Gastric Content	Ulcer area	Inhibition %
Control (-ve) Normal Saline(0.9% NaCl)	12.6±2.30 BC	2.42±0.30 A	0.00±0.00 C	-
Control (+ve) Indomethacine(75mg/kg)	22.08±3.42 A	1.40±0.22 B	7.37 ± 2.35 A	-
Indometh+PA(100mg/kg)	13.91±1.90 B	2.67±0.28 A	0.00 ± 0.00 C	100
Indometh+PA(200mg/kg)	9.00±1.04 D	2.70±0.41 A	0.00±0.00 C	100
Indometh+Ranitidine (50mg/kg)	11.25±1.54 C	2.46±0.12 A	2.13±1..17 B	71.09

N=number of animals., Capital letters denote differences between groups, P≤0.05 vs. control.

Table (2) Effect of Proanthocyanidin and Ranitidine on Serum of Glucose Concentration, Serum MDA, MDA Mucin and Gastric tissue MDA in Gastric Ulceration Female Rabbits Inducedby Indomethacin (Mean±SD) (n=6)

Parameters / Treatment	Glucose mg/dL	Serum MDA (nmol/L)	MDA Mucin (µg Ab./cm2)	Gastric Tissue MDA(nmol/ mg)
Control (-ve) Normal Saline(0.9% NaCl)	97.66±14.12 B	0.26±0.017 B	0.53±0.044 A	4.50±1.67 B
Control (+ve) Indomethacine(75mg/kg)	174.17±35.42 A	1.03±0.042 A	0.04±0.001 B	14.50±2.67 A
Indometh+PA(100mg/kg)	115.50±13.35 B	0.24±0.030 B	0.44±0.015 A	3.79±1.19 B
Indometh+PA(200mg/kg)	121.13±43.26 B	0.23±0.015 B	0.32±0.014 A	3.09±0.71 B
Indometh+Ranitidine (50mg/kg)	154.33±18.50 A	0.28±0.011 B	0.22±0.010 AB	3.93±1.07 B

N=number of animals., Capital letters denote differences between groups,P≤0.05 vs. control.

Table (3)Effect of Proanthocyanidin and Ranitidine on RBC counts and RBC Index in Gastric Ulceration Female Rabbits Induced by Indomethacin (Mean±SD) (n=6)

Parameters / Treatment	RBC × 10^6/µL	Hb g/dl	PCV%	MCV Fl	MCH pg	MCHC %
Control (-ve) Normal Saline(0.9% NaCl)	5.46±0.27 AB	11.38±1.59 A	33.11±2.5 A	60.66±5.53 AB	20.76±2.61 NS	34.28±3.54 AB
Control (+ve) Indomethacine(75mg/kg)	4.03±0.72 C	8.13±0.99 B	27.56±1.48 B	70.28±13.91 A	20.81±5.36 NS	29.61±4.89 B
Indometh+PA(100mg/kg)	5.76±0.68 A	11.95±0.97 A	32.43±1.32 A	56.57±7.42 B	20.86±1.62 NS	37.70±4.70 A
Indometh+PA(200mg/kg)	5.02±0.35 B	11.03±0.71 A	31.86±2.16 A	63.77±7.06 AB	21.86±0.97 NS	34.68±3.12 A
Indometh +Ranitidine (50mg/kg)	5.20±0.44 AB	10.68±1.34 A	30.90±2.11 A	59.44±2.89 B	20.40±1.19 NS	34.46±2.97 A

N=number of animals., Capital letters denote differences between groups,P≤0.05 vs. control.
NS=non-significant.

Table (4) Effect of Proanthocyanidin and Ranitidine on WBC Counts and Percentage of Differential Count of WBC in Gastric Ulceration Female Rabbits Induced by Indomethacin (Mean±SD) (n=6)

Parameters / Treatment	WBC× 10^3/µL	Neutro %	Eosino %	Baso %	Lymph %	Monocyte %
Control (-ve) Normal Saline(0.9% NaCl)	8.67±1.99 A	38.0±4.38 B	2.33±0.81 AB	1.0 ±0.63 AB	57.66±5.81 B	2.83±0.75 AB
Control (+ve) Indomethacine(75mg/kg)	10.34±1.35 A	23.0±2.44 C	3.16±0.75 A	1.5±0.54 A	69.50±2.16 A	3.33±0.51 A
Indometh+Proantho (100mg/kg)	9.53±1.49 A	39.33±5.20 B	2.0±0.89 B	0.66±0.05 B	56.33±3.72 B	2.16±0.75 B
Indometh+Proantho (200mg/kg)	7.76±2.29 AB	49.0±2.09 A	2.66±1.03 AB	0.66±0.05 B	47.16±3.54 C	1.50±0.54 BC
Indometh+Ranitidine (50mg/kg)	6.84±2.16 B	28.16±3.43 C	1.50±0.83 B	0.66±0.05 B	68.0±3.57 A	1.16±0.40 C

N=number of animals., Capital letters denote differences between groups,P≤0.05 vs. control.

Table(5)Effect of Proanthocyanidin and Ranitidine on Serum of Lipid profile in Gastric Ulceration Female Rabbits induced by Indomethacin
(Mean±SD) (n=6)

Parameters / Treatment	Total Cholesterol mg/dl	Triglyceride mg/dl	HDL mg/dl	LDL mg/dl	VLDL mg/dl
Control (-ve) Normal Saline(0.9% NaCl)	138.33±26.39 B	68.65±14.12 B	50.66±3.55 AB	29.66±2.58 B	13.50 ±1.87 B
Control (+ve) Indomethacine(75mg/kg)	325.00±93.54 A	123.67±25.96 A	38.00±5.89 C	117.17±5.56 A	39.16±2.22 A
Indomethacin+Proantho (100mg/kg)	80.00± 8.94 B	55.99±15.57 B	55.00±2.60 A	20.16±2.48 C	10.33± 1.63 C
Indometh+Proantho (200mg/kg)	84.33±7.73 B	53.94±23.61 B	53.16 ±4.91 AB	21.00±3.40 C	12.16±3.18 BC
Indometh+Ranitidine (50mg/kg)	100.00±14.14 B	91.97±34.44 B	48.00±8.85 B	28.16±3.06 B	14.66±2.87 B

N=number of animals., Capital letters denote differences between groups,P≤0.05 vs.

DISCUSSION

An ulcer results from an imbalance between aggressive and defensive factors of the gastric mucosa. Pepsin and gastric acid make up the offensive factors, whose proteolytic effect is buffered by mucin secretion, mucosal glycoprotein, cell shedding, cell proliferation and prostaglandins(29).To regain the balance, different therapeutic agents including plant extracts are used to inhibit the gastric acid secretion, or to stimulate the mucosal defense mechanism by increasing the mucus production protecting the surface epithelial cells or interfering with the PGsynthesis(30). Stress-induced ulcers are probably mediated by histamine release with enhancement in acid secretion, a reduction in mucus production and generation of free radicals etc., mast cell activation, alterations in prostaglandin generation, cytokine liberation and breakdown of normal cytoprotective mechanism.

The exact mechanism of pathogenesis in the indomethacin induced gastric ulcer model has not been fully known, but hypersecretion of gastric acid, deterioration of the mucosal resistance and promotion of gastricemptying are among the possible mechanisms. Indomethacin is inhibition synthesis of prostaglandin coupled with formation of free radicals(31-33). All animals administered with indomethacin showed reduced feed intake, sluggishness, unthrifty appearance with some mortalities. Indomethacin caused gastric damage was further confirmed by the gross section and

histopathologic lesions produced. Mortality occur may be due to gastric bleeding and perforation or may be due to hepatotoxicity of indomethacin (34).

In present study, oral administration of indomethacin caused remarkably significant decreased in gastric pH, increased in gastric volume, total acidity and ulcer score. The ulceration induced by indomethacin is attributed mainly to different processes which include generation of reactive oxygen species, initiation of lipid peroxidation, infiltration of leukocytes, induction of apoptosis and inhibition of prostaglandin synthesis(35). These findings are in agreement with (32). The significant increase in gastric volume following oral administration of indomethacin may be attributed to either free radicals formation or inhibition of prostaglandin synthesis which lead to increase gastric acid secretion(36).this result is agrees with(35,37,38) where they indicated that indomethacin have caused alterations in gastric secretions of rats. The significant decrease in gastric pH and significant increase in total acidity may attribute to decrease in mucin secretion allows hydrogen ions and pepsin to diffuse into the mucosa from lumen. So, back diffusion of acid and pepsin into the tissue stimulate more acid and pepsin secretion to cause more damage.Low pH value is a manifestation of increase hydrogen ion concentration in gastric juice (32). This finding is on line with(36).

Oral administration of ranitidine significantly increase gastric pH , and decrease in gastric volume, total acidity and ulcer score. However, ranitidine decimate gastric acidity and increasethe gastric pH due to its ability to block binding of histamine to H_2 receptor on parietal cell (39). Therefore ranitidine can counter the effect of indomethacin on gastric acid secretion. These result is cited by (40,41)

Oral administration of proanthocyanidin(100mg\kg and 200mg\kg) significantly increase in gastric pH, and decreased in gastric volume, total acidity and ulcer score. Increasing gastric pH and decreasing total acidity proving anti-secretory activity of proanthocyanidin grape seed extract(42).in addition to, grape Seed Extract has anti-histamine properties (it stabilizes the release of histamine from mast cells)(43-45) .Proanthocyanidin decrease the gastric volume because proanthocyanidin is antioxidant, cause lowering in the gastric secretion by acting on the gastric mucosa and inhibiting the generation of reactive oxygen species that initiate the oxidative stress in the gastric lumen (42).Number of ulcers score decreased due to decreased in gastric volume and total acidity, and increase the gastric pH (42). This findings are in agreement with (46).

From result, it has been conclude that oral administration of indomethacin caused significantly increased in MDA and decreased in mucin. Gastric ulceration induced by indomethacin cause severe oxidative stress in gastric tissue that lead to detriment of lipid and stimulation of lipid oxidation which leading to increase accumulation of MDA (32). Decreasing of mucin secretion (gastric mucus) in the indomethacin – administration referred to suppress production of prostaglandin and damage of surface epithelial cells and mucus neck cells, where one function of prostaglandin is regulating of mucus secretion((32). These result seem to be accordant with study made by(40).

This study revealed that oral administration of proanthocyanidin cause significant reduction in MDA level along with significant increase in mucin. This due to proanthocyanidins are powerful antioxidants(16) and scavenge of free radicals (17). This result is agreement with(47).

This study revealed that oral administration of ranitidincause significant reduction in MDA level along with non- significant increase in mucin. Indeed, this reduction in MDA level proposed the decrease in the lipid peroxidation level and total oxidant capacity by ranitidine. In addition to ranitidine caused significant reduction in neutrophils infiltration which cause decreased in source of reactive oxygen species during healing process (41). This result is agreement with(40,48). Insignificant increase in mucin may be due to decrease of prostaglandin secretion as result of incomplete healing of ulcer where the percentage of inhibition was 71.09

Oral administration of indomethacin caused significant increase in serum glucose. This finding may be due to indomethacin directly or indirectly play a specific role in pancreatic secretions, gluconeogensis process, glycogen metabolism or glucose oxidation. It means that there was a disturbance in carbohydrate metabolism of rabbits administered indomethacin (49). This result is cited by (50) who indicated indomethacin inhibits insulin secretion stimulates endogenous glucose production(EGP) in type 2 diabetes.

Oral administration of proanthocyanidin caused marked improvement in the serum glucose. This effect due toproanthocyanidins, a major component in GSE, potent inhibits intestinal α-glucosidase and pancreatic α-amylase(51). These are key enzymes of dietary carbohydrate digestion(52). Nowadays, it has been found that the treatment with an α-glucosidase inhibitor (acarbose) particularly decrease postprandial hyperglycemia, reduced the risk of type 2 diabetes (53). GSPE

decreased glucose levels in diabetic rats by increasing circulating insulin levels (It is possible that consumption of GSE may prevent or delay developing type 2 diabetes in healthy people(54). In addition to,grape seeds extract decrease serum glucose level at 28 days because plant extract with high flavonoid have potent inhibition of renal glucose re-absorption through inhibition of sodium- glucose symporters locate in proximal renal tubule(55). This result on line with (56) who study the effect of proanthocyanidin(100mg and300mg) in postprandial plasma glucose in healthy participants.

Oral administration of ranitidin caused significant increase in serum glucose. This finding due to inability of ranitidin to healing destruction of β- cell of the islet of Langerhans induced by indomethacin that lead to decrease of insulin secretion and increase serum glucose level. This result is disagreement with(57 who indicated that acute administration of H_2- receptor antagonist cimetidine and ranitidine produces marked reduction in blood glucose level in diabetic patient receiving glipizide

From result, it has been conclude that oral administration of indomethacin caused reduction in haematological parameters such as RBC, Hb, PCV, MCV, MCH and MCHC. These result explained by(58) who indicated that decreased of hemoglobin, blood cell count and hematocrit is due to blood loss particularly from stomach because of the ability of indomethacin to inhibition of cyclo oxygenase enzyme, mitochondrial dysfunction and oxidative stress which leads to hemorrhagic anemia in agreement with (59). Another cause may be due to kidney damage induced by indomethacin (60,61) that lead to ability of kidney to decrease secretion of erythropoietin hormone which stimulates the bone marrow to produce more red blood cells. Indomethacin cause non-significant increase in number of WBC which were (10.34) compared with (-ve) which were (8.67), this increase due to increase percentage of lymphocyte, monocyte, basophil and eosinophil. Number of WBC increase due to stress induced by indomethacin and stimulation of immune system. This result is on line with (58). also, there is significant in percentage of neutrophil due to infiltration of neutrophile into inflammatory organ, that lead to decrease number of blood stream neutrophil. This result is agreement with (62) who indicated that meloxicam which is one of NSAIDs cause decrease in neutrophil.

The present study revealed use of proanthocyanidin cause significant increase in RBC counts and RBC Index compared with (+ve) group. This result may be due to antioxidant and scavenger of oxygen free radical effect of proanthocyanidin which

lead to healing of gastric ulcer and stopping of hemorrhage, that lead to improvement of RBC counts and RBC Index. Another cause may be due to ability of proanthocyanidin to healing kidney damage induced by indomethacin that lead to ability of kidney to secretion sufficient amount of erythropoietin hormone which stimulates the bone marrow to produce more red blood cells. This result is cited by (63) who indicated that dose of 100mg\kg of proanthocyanidin cause increase in RBC count while dose of 200and 300mg\kg of proanthocyanidin cause decrease of RBC count. Oral administration of proanthocyanidin caused non-significant increase in number of WBC, this indicate ability of proanthocyanidin in healing of inflammation inflation and decrease effect of indomethacin. This result is cited by (63) who found that dose of 100mg\kg cause non-significant increase in WBC count compared with control group, while dose of 200mg\kg and 300mg\kg cause non-significant decrease in WBC count. Also, there is significant increase in percentage of neutrophil at dose 200mg\kg and non-significant increased at dose 100mg\kg compared with control group, this result indicate decrease inflammation and infiltration of neutrophil. In addition to, there is decrease in percentage of eosinophil, basophil, lymphocyte and monocyte, this indicate decrease of stress. This result is on line with (63).

Oral administration of ranitidin cause significant increase in RBC count and RBC index. This result may due to oxygen free radical scavenger of ranitidin and its ability to healing gastric ulcer and stopping of hemorrhage. this result is agreement with(64). Oral administration of ranitidin cause significant decrease WBC count and percentage of neutrophil while it cause significant increase in percentage of lymphocyte, this indicate partial healing of infection. Also there is decrease in basophil and monocyte, this indicate to suppression of stress. This result is accordance with (64).

Oral administration of indomethacin result in significant hyperlipidemia (increase VLDL, LDL,TC, TG AND decrease HDL). Indomethacin increased triglyceride in all the indomethacin treated group, this may be due to increase in fatty acid synthesis, enhanced catabolism of LDL, activation of Lipid catabolism and tissue lipase and or increased of acetyl-CoA carboxylase and production of triglycerides precursors such as acetyl-CoA and glycerol phosphate. More so, the significant increase in serum cholesterol may be attributed to an increased concentration of acetyl-CoA is a key substrate in the biosynthesis of cholesterol or increase in absorption from the intestine by binding with bile acid within the intestine and increasing bile acid secretion due to the drug(65). This finding is cited by(66).

Oral administration of proanthocyanidin caused marked improvement in the lipid profile. The hypolipidemic effect of proanthocyanidin may occur due to a reduction in the bioavailability of lipids, mainly TG or the reduction in the availability of free fatty acids to the liver that lead to inhibition of VLDL-C secretion by the liver (67). Another suggestion is the delay and inhibition of dietary fat and cholesterol absorption and a reduction in chylomicron secretion by enterocytes (68,14), since proanthocyanidin inhibits the absorption of TC and bile acids by decreasing micellar cholesterol solubility(Blade *et al*., 2010). The ability of proanthocyanidin to trap reactive oxygen species in aqueous series such as plasma and interstitial fluid of the arterial wall produce diminish lipid concentrations by administration of proanthocyanidin(69).

Oral administration of ranitidine caused marked improvement in the lipid profile. The hypolipidemic effect of ranitidin may occur due to antioxidant effects of H2-receptor antagonists, such as cimetidine, ranitidine, famotidine(70) and scavenger of oxygen free radicals. This result is accordance with (48) who indicated that radioprotective effect of H2-receptor antagonists on lipid profile and lipid peroxidation.

دراسة تأثير بروانثوسياندين و الرانتدين على المعايير الدمويه والكيموحيويه في اناث الارانب البالغة المستحدثه القرحه بواسطه الاندوميثاسين

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الخلاصه

تمت الدراسه في كليه الطب البطري/جامعه البصره وكان الغرض منها هو تقيم تأثير البروانثوسياندين و الرانتدين على القرحه المعده، المعايير الدمويه والحيويه عن طريق استحداث قرحه حاده بواسطه الاندوميثاسين في اناث الارانب البالغة. استخدمت الدراسه ثلاثون انثى بالغه بلغت اوزانها (1500-2000ملغم) قسمت الى خمسه مجاميع،كل مجموعه تتكون منستة ارانب وكما يلي:المجموعه الاولى اعطت المحلول الفسلجي الطبيعي(نورمل سَلاين) لمده عشره ايام وأُعتبرت مجموعهالسيطره السالبه ،المجموعه الثانيه اعطيت الاندوميثاسين بجرعه 75ملغم /كغم لمده يومين وأعتبرت مجموعه السيطره الموجبه، المجموعه الثالثه اعطيت اولاً الاندوميثاسين بجرعه 75ملغم /كغم لمده يومين ثم جُرعت البروانثوسياندين بجرعه 100ملغم \كغم لمده عشرة ايام، المجموعه الرابعه اعطيت الاندوميثاسين بجرعه 75ملغم /كغم لمده يومين ثم جُرعت

البروانثوسياندين بجرعه 200ملغم \كغم لمده عشرة ايام، المجموعه الخامسه اعطيت الاندوميثاسين بجرعه 75ملغم /كغم لمده يومين ثم الرانتدين 50ملغم\كغم لمده عشره ايام. وقد اظهرت النتائج ان البروانثوسياندين والرانتدين سببا نقصاناً معنوياً في حجم المعده ومساحه القرحه وزياده معنويه في معامل الحموضه للمعده .ولوحظ حصول نقصان معنوي في تركيز السكر بالدم في المجاميع التي اعطيت البروانثوسياندين مقارنه مع مجموعه السيطره الموجبه ونقصان غير معنوي لسكر الدم للمجموعه التي اعطيت الرانتدين مقارنه مع مجموعه السيطره الموجبه. اظهرت ايضا نقصان معنوي في MDAالمصل ونسيج المعده و زياده معنويه فيالماده المخاطيه للمعده للمجاميع التي اعطيت البروانثوسياندين والرانتدين مقارنه مع مجموعهالسيطره الموجبه. كان هناك زياده معنويه لعدد كريات الدم الحمراء والهيموكلوبين وحجم الخلايا المتراصه و متوسط تركيز الهيموكلوبين للمجاميع التي اعطيت البروانثوسياندين والرانتدين ونقصان معنوي لمعدل حجم كريات الدم الحمراء للمجموعه التي اعطيت البروانثوسياندين بجرعه 100ملغم/كغم والرانتدين ونقصان غير معنوي لمعدل حجم كريات الدم الحمراء للمجموعه التي اعطيت البروانثوسياندين بجرعه 200ملغم/كغم. ولوحظ هناك تغير غير معنوي لكريات الدم البيضاء للمجاميع التي اعطيت البروانثوسياندين بينما لوحظ نقصان معنوي للمجموعه التي اعطيت الرانتدين مقارنه مع مجموعه مجموعه السيطره الموجبه والسالبه. واظهرت نقصان معنوي لجميع انواع الدهون عدا الدهون الجيده(عالية الكثافه) للمجاميع التي اعطيت البروانثوسياندين والرانتدين مقارنه مع مجموعه السيطره الموجبه. نستنتج مما سبق ان البروانثوسياندين المستخلص من بذور العنب لهُ فعاليه جيده في شفاء قرحة المعده، مخفض لسكر الدم، ومحسن لمعايير الدمو الدهون الضاره.

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