

## Impact of Different Doses of Nicorandil-Induced Ulceration (Oral , Gastrointestinal Tract, and Anal) in Rats: Roles of Leptin and Prostaglandin E2

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### Abstract

Many reports confirm ulcers as an adverse effect of drugs such as nicorandil and aspirin. The exact responsible mechanisms of ulceration have until now not proved. Mucosal ulcers associated with the onset of ulcer are manifested by an increase in proinflammatory cytokine, excessive prostaglandin, and up-regulation of Endothelin-1 level, which directly impacts the release of leptin. These, released locally within mucosal tissues, have played a role in controlling the extent of local inflammatory responses and processes of mucosal repair.

This study was designed to find out the correlation of plasma leptin and prostaglandin levels as a possible mechanism of oral ulcer formation as an adverse effect of nicorandil. The effect of nicorandil for inducing ulceration was assessed. The plasma leptin and prostaglandin E2 for the tested groups in relation to the studied parameters (gender, and daily body weight change) were estimated in albino rats. Nicorandil causes mucous membrane damage, inflammation, and ulceration. A significant reduction of plasma leptin level, which was dose-dependent, and a non-significant reduction of serum prostaglandin E2 level. The mechanisms of ulcer induction as an adverse effect of nicorandil can be related to dose-dependant leptin and prostaglandin E2 levels, which affects on repair and healing process.

**Keywords:** Nicorandil, Leptin, Prostaglandin E2, Ulcer.

### تأثير الجرعة المختلفة في استحداث القرحة بواسطة عقار النيكوراندل ( الفم ، الجهاز الهضمي والشرج ) في الجرذان دور هرمون اللبتين والبروستاغلاندين E2 اسماء عوني حيدر\* و تغريد سهام الطائي\*\*1

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#### الخلاصة

تؤكد العديد من التقارير السريرية حالة قدرة بعض العقاقير في استحداث القرحة كتأثير ضار سلبي مثل عقار نيكوراندل والاسبرين. حتى الان لم تثبت الآليات الدقيقة المسؤولة للقرح . ان بداية تكون القرحة في الغشاء المخاطي مرتبطة مع زيادة في انتاج سايتوكاين الممهدة للالتهاب، والافراط في توليد البروستاجلاندين ، وزيادة انتاج ملحوظة في اندوثلين-1 ، والذي له تأثير مباشر في تنظيم تحرير مستوى الهرمونات مثل اللبتين. تلك التي تحررت موضعيا ضمن الانسجة المخاطية، لعبت دورا في السيطرة على مدى الاستجابات الالتهابية وعمليات إصلاح الغشاء المخاطي. الهدف من الدراسة الحالية لمعرفة وكشف العلاقة بين اللبتين و البروستاغلاندين E2 في مصل الدم كآلية مسببة ممكنة لاستحداث القرحة على انها التأثير السلبي لاستخدام قرص نيكوراندل وتمت مقارنة E2 مستويات اللبتين والبروستاجلاندين . تم تقييم تأثير النيكوراندل في استحداث القرحة. كذلك تمت دراسة الاختلافات بين المجاميع قيد الدراسة ومقارنتها مع مجموعة السيطرة وارتباطهم في التحريض على القرحة في الانسجة المختلفة من خلال المعلمات (الجنس، و تغير وزن الجسم يوميا) في الجرذان البيضاء. اظهرت النتائج ان فعالية عقار نيكوراندل تعتمد على كمية الجرعة المستخدمة ، وقد كشف الفحص المجهرى للانسجة ان عقار نيكوراندل يؤثر على بنية الانسجة ، مما يؤدي الى التهاب واحتقان في الاوعية الدموية في الغشاء المخاطي وفي تكون القرحة في انسجة الفم ، الجهاز الهضمي ، وفي انسجة الشرج للجرذان البيضاء. عقار نيكوراندل ادى الى انخفاض متوسط تركيز هرمون اللبتين في مصل الدم بنسبة عالية، يستنتج من هذه الدراسة ان الآليات استحداث القرحة باعتبارها التأثير السلبي لتعاطي عقار نيكوراندل يمكن اعتباره كآلية ثانية في استحداث القرحة وهو الانخفاض الغير ملحوظ احصائيا في مستوى البروستاجلاندين E2 في مصل الدم.

الكلمات المفتاحية: نيكوراندل ، اللبتين ، البروستاغلاندين E2 ، القرحة .

### Introduction

Ulcers are frequent lesions of the mucosa. Generally, they are circumscribed round or elliptical lesions surrounded by an erythematous halo and covered with an inflammatory exudate in their central portion, and follow with painful symptoms. Oral ulcers

are painful and may be single or multiple, symmetric or irregular in shape. Once an ulcer forms, it is subject to recurrent irritation from saliva and microflora, and the acute inflammatory stage may be followed by a pattern of chronic inflammation<sup>(1)</sup>.

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Numerous of the causes and factors involved in the formation of these lesions – including immunological alterations, infections, nutritional deficiency, food repetitive trauma to the mucosa, neoplasms, autoimmune diseases and contact allergies, as well as psychosomatic, genetic and environmental factors <sup>(2)</sup> were also observed after the use of many drugs such as labetalol, nicorandil, captopril, NSAIDs (aspirin, para-aminosalicylic acid, diflunisal, ibuprofen, indomethacin, naproxen, rofecoxib, sulindac), as well as after the use of mycophenolate, sirolimus, sodium lauryl sulfate, protease inhibitors (saquinavir, indinavir, ritonavir, lopinavir, nelfinavir, amprenavir), and sulfonamides, and alendronate <sup>(3)</sup>.

Nicorandil is a potassium channel activator used in the treatment of Angina pectoris <sup>(4)</sup>. Initial adverse reactions include headaches, nausea and cutaneous erythema. There is a 5% prevalence of oral ulceration in patients on nicorandil, compared with patients on various other anti-anginal medications <sup>(5)</sup>. Nicorandil is a cause of life threatening terminal ileum ulceration, while NSAID such as diclofenac and aspirin use has been linked with endoscopic ileitis <sup>(6)</sup>. It is important that clinicians elsewhere be made aware that nicorandil can be a potential inducer of ulcers that may mimic major aphthous ulcers or even carcinoma <sup>(7)</sup>, anal <sup>(8)</sup>, colonic <sup>(9)</sup>, vulval <sup>(10)</sup>, parastomal <sup>(11)</sup> and intestinal ulceration <sup>(12, 13)</sup>.

Aspirin could directly damage the gastric epithelium <sup>(14)</sup>. The breaking of the 'barrier' permitted the back-diffusion of acid into the mucosa, which eventually led to the rupture of mucosal blood vessels. These topical irritant properties were subsequently found to be predominantly associated with those NSAIDs with a carboxylic acid residue <sup>(15)</sup>. The ability of an NSAID to cause gastric damage correlates with its ability to suppress gastric prostaglandin synthesis; agents that are weak inhibitors of gastric prostaglandin synthesis are less ulcerogenic <sup>(16)</sup>.

Leptin is known to exhibit a variety of physiological actions on body weight homeostasis <sup>(17)</sup>, lipid metabolism <sup>(18)</sup>, hematopoiesis <sup>(19)</sup>, thermogenesis <sup>(20)</sup>, ovarian function <sup>(21)</sup>, bone formation <sup>(22, 23)</sup>, angiogenesis <sup>(24, 25)</sup> and wound healing <sup>(26-28)</sup>. The multifunctionality of leptin and the wide distribution of its receptor suggest that leptin plays a variety of physiological roles not only as a systemic hormone but also as a local growth factor. Leptin is present in human saliva as well as serum <sup>(29, 30)</sup>. Epithelial cells and vascular endothelial cells in oral mucosa are target cells for leptin <sup>(31)</sup>. Leptin promotes

wound healing by enhancing the epithelial cell proliferation <sup>(27, 28)</sup>.

Prostanoids, the E type prostaglandins, particularly PGE2 derived from arachidonic acid, are most widely produced in the body <sup>(32)</sup>. Molecular identification of the E type prostaglandins receptors was achieved by their cDNA cloning <sup>(33, 34)</sup>, which revealed that the receptors are G-protein-coupled receptors (GPCRs).

This study was designed to study the efficacy of nicorandil for inducing ulcers as adverse effects, by determining any possible correlation between, on one hand, plasma leptin and prostaglandin E2 levels and, on the other, the pathological causes of ulcers in albino rats, and compared to aspirin as a standard ulcerogenic agent. These were performed by:

1-The effect of 0.28, 0.4, 1, and 3 mg/kg of nicorandil on the microscopic appearance and histopathology features of albino rat tissues.

2-Effects of different doses of nicorandil mentioned above on the plasma leptin and prostaglandin E2 levels. 3-The correlation analysis of plasma leptin and prostaglandin E2 levels, and with the studied parameters (gender, and body weight).

## Materials and Methods

The study was carried out at Hawler Medical University in the Experimental Animal House of the College of Pharmacy, from February to July 2014. It was based on an ethical approved protocol (part of MSc study). Materials: Nicorandil tab 10 mg (Merk, Germany); soluble in water (4 mg/ml), aspirin effervescent 500 mg (Sanofi-Aventis, France); ether (diethyl ether=74.12g/mol) from England; normal saline (0.9% sodium chloride) from Pioneer Sulaymaniyah; formaldehyde, solution 37% w/w, extrapure, pHEur, BP, USP, stabilised with approximately 10% methanol (Scharlab S.L. Spain). For the enzyme-linked immunosorbent assay (ELISA), the following kits were used: rat leptin ELISA GWB-95C312 40-055-200005 (Genway Platinum, San Diego), and rat high sensitivity prostaglandin E2 enzyme immunoassay (Ann Arbor, Michigan).

Rats and housing: In the current study, 42 local domestic albino rats, of both sexes weighing 169-409 g and aged 12-16 weeks were obtained and cared for in the Animal House of the College of Medicine/Hawler Medical University, Erbil, Kurdistan, Iraq. The animals were kept in polycarbonate cages on a layer of wood shavings, under standard laboratory conditions. The animals were housed in groups of four per cage. All rats used in this study were allowed to adapt to the housing

conditions for one week prior to commencement of the study. Rats were maintained on a 12-hour light/dark cycle at temperature range of 21-28 °C, humidity 10%-50%. The animals were kept in standard room conditions and supplied with rodent chow and free access to tap water, in compliance with the Institutional Animal Care and Use Committee.

**Experimental design:** A total of 42 local domestic albino rats, female and male, were randomly divided into six groups; study groups (1-4), and control groups (5, 6) as follows:

**Study groups:** Nicorandil 10 mg tablet was crushed into powder by mortar and pestle in the Biochemistry Laboratory of the College of Medicine, Hawler Medical University. The measured amount according to the rats' weight for each group was administered intraperitoneally (I.P.) as a solution of 1 ml [distilled water (D.W.) was used as a solvent].

Four doses of nicorandil were used. The animals were randomised into the following groups: G1: Nicorandil 0.28 mg/kg/day. G2: Nicorandil 0.4 mg/kg/day. G3: Nicorandil 1 mg/kg/day. G4: Nicorandil 3 mg/kg/day.

**Control groups:** Two types of control group were used:

- Positive control: an amount of aspirin measured according to the animal's weight was dissolved in 1ml D.W. The intraperitoneal dose used was 5 mg/kg/day, which was administered to 7 rats (three females: four males) for 10 days. Assigned as G5: Aspirin 5mg/kg/day.

- Negative control: Intraperitoneally normal saline was administered to 7 rats (three females: four males) for 10 days. Assigned as G6: Normal saline 1 ml/day.

**Samples preparation:** Rats were anaesthetized by diethyl ether solution (20 ml of diethyl ether on cotton in jar) to render them unconscious, which required approximately 5 minutes. Then intra-cardiac blood (2 ml) was drawn from rats as a baseline using EDTA tube<sup>(35)</sup>.

The blood was immediately centrifuged at 3500 RPM for 10 min and plasma was separated by pipette to Eppendorf covered by parafilm and stored at -20 °C<sup>(36)</sup>. Rats were weighed daily and then nicorandil was injected I.P. into each of them, except for the control groups which were injected with either aspirin (as +ve control) or normal saline (as -ve control) for 10 days.

Rats received the corresponding treatment drug according to the stated dose for each group. After 10 days, 24 hr. after the last dose, rats were anaesthetized, then intra-cardiac blood (2 ml) was drawn, and the plasma was separated as mentioned above for further

analysis. Albino rats were euthanized by 30 ml diethyl ether. After dissection of all animals' groups, the organs and tissues (Oral cheek pouches and tongue, GIT, anal) were removed, dried by filter paper, and fixed in 10% buffered formalin solution for histopathological sectioning.

#### **Enzyme linked immunosorbent assay**

**Rat leptin assay:** Rat leptin ELISA, standards, quality controls, and samples were incubated in microplate wells, which were pre-coated with anti-rat leptin antibody. After 60 minutes of incubation and washing, biotin-labelled polyclonal anti-rat leptin antibody was added to the wells and incubated with immobilised antibody-leptin complex for 60 minutes. After another washing, streptavidin-HRP conjugate was added. After 30 minutes incubation and the last washing step, the remaining conjugate was allowed to react with the substrate solution (TMB). The reaction was stopped by addition of acidic solution and absorbance of the resulting yellow product was measured spectrophotometrically at 450 nm. The absorbance was proportional to the concentration of leptin. A standard curve was constructed by plotting absorbance values against concentrations of standards, and concentrations of unknown samples were determined using this standard curve.

**Rat high sensitivity prostaglandin E2:** Plasma PGE2 was quantitatively measured. Standards or diluted samples were pipetted into a clear microtiter plate coated with an antibody Rat IgG. A PGE2-peroxidase conjugate was added to the standards and samples in the wells. After an overnight incubation at 4 °C, the plate was washed and substrate was added. The substrate was reacted with bound PGE2-peroxidase conjugate. After 30 minutes incubation, the reaction was stopped and the intensity of the generated colour was detected in a microtiter plate reader capable of measuring 450 nm wave length.

**Histopathology study: Fixation and Staining:** Organ specimens were collected from all animals in this study and fixed by immersion in 10% formalin. Xylene (clearing agent) was introduced to infiltrate the tissues, and finally paraffin was introduced to complete the tissues embedding process to produce paraffin block<sup>(37)</sup>, and then stained with hematoxylin and eosin (H&E) for microscopic examination<sup>(38)</sup>.

**Statistical analysis:** Data were analysed using the Statistical Package for Social Science (SPSS) software version 18 (SPSS Inc., 2010). The data represent quantitative observations and were summarized using means  $\pm$  standard deviations ( $M \pm SD$ ). Statistical analysis with t test was used to compare between means of

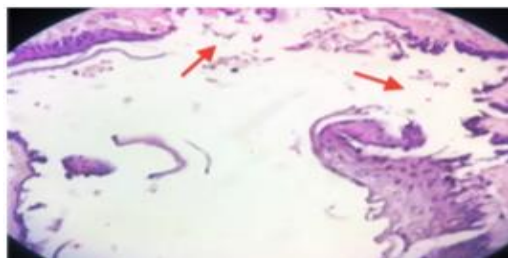
two groups (comparing means of two different groups or comparing means of one sample in two different occasions), and independent sample t test was used to compare the different average values between two groups. Also, one-way analysis of variance (ANOVA) was performed to compare the differences in the means among groups. Pearson correlation coefficient was used to assess the correlation between two numerical variables.  $P \leq 0.05$  was considered statistically significant in all the results of the current study.

## Results

The effects of nicorandil for ulceration adverse effect: The effect of 0.28, 0.4, 1, 3 mg/kg/day nicorandil was studied, to explore its ability to induce ulcers as an adverse effect in different tissues of albino rats via the examination of the microscopic features, and pathological findings of the oral, gastrointestinal, and anal tissues, as discussed in this section:

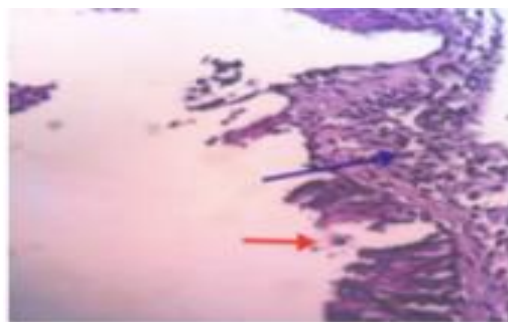
Microscopic appearance and histopathology study:

Oral tissues of the albino rats: Histopathological section of the oral tissues showed that different doses of Nicorandil on tongue mucous produced sloughing of the mucous, ulceration with moderate inflammatory cell infiltration, as shown in Figure (1).



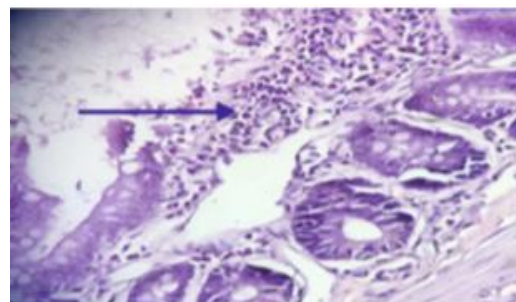
**Figure (1):- Section of rat's tongue treated with 3 mg/kg/day nicorandil showing surface ulceration and sloughing, with inflammatory cells infiltration and vascular congestion of mucosal tongue (H&E 400X).**

Gastrointestinal tract tissues of the albino rats: Histopathological section of rat's gastrointestinal tract showed different response in females rather than males group; section from stomach showed gastritis, surface ulceration, granulation tissue formation and mononuclear inflammatory cell infiltration as shown in figure (2).



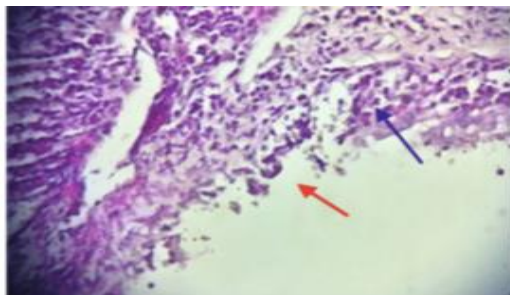
**Figure ( 2 ) :- Section of male rat's stomach treated with 3 mg/kg/day nicorandil showed surface ulceration, granulation tissue and moderate mononuclear inflammatory cell infiltration of lamina propria (H&E: 400X).**

In small intestine section showed sloughing, erosion and heavy mononuclear inflammatory cell infiltration of lamina propria as shown in figure (3).

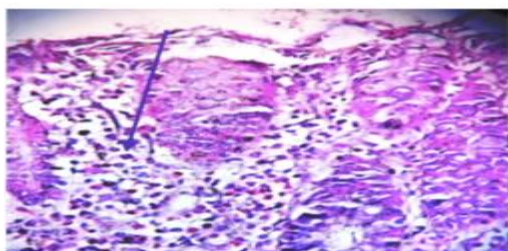


**Figure ( 3 ):- Section of rat's small intestine of male treated with 3 mg/kg/day nicorandil showing sloughing, erosion and heavy mononuclear inflammatory cell infiltration of lamina propria (H&E: 400X).**

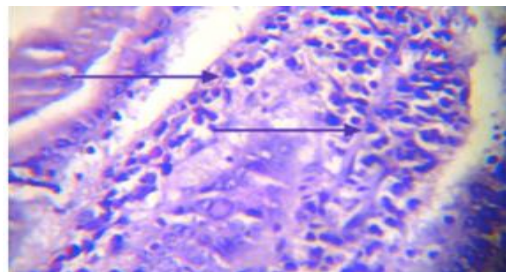
Higher dose of nicorandil (3 mg/kg/day) showed a large size ulcer in the mucosa of the small intestine with heavy mixed inflammatory cell infiltration, granulation tissue formation and fibrosis as shown in figure (4). While section from small intestine of female rats showed heavy mixed inflammatory cell infiltration including eosinophil with erosion and cryptitis as shown in figure (5). The section of the positive control group (G5) treated with aspirin showed gastritis with heavy mixed inflammatory cell infiltration of lamina propria and granulation tissue formation as shown in figure (6).



**Figure (4):-** Section of rat's small intestine of male showing surface ulceration of small intestine, heavy mononuclear inflammatory cell infiltration with lamina propria (H&E: 400X).

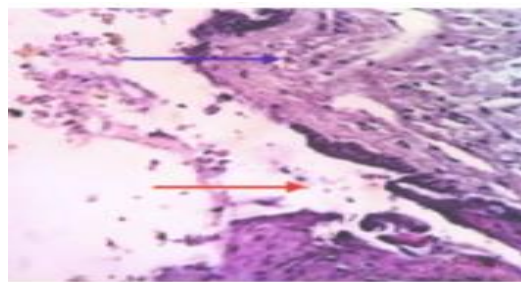


**Figure (5):-** Section of rat's small intestine of female treated with 3 mg/kg/day nicorandil has heavy mixed inflammatory cell infiltration include eosinophil with erosion cryptitis (H&E: 400X).

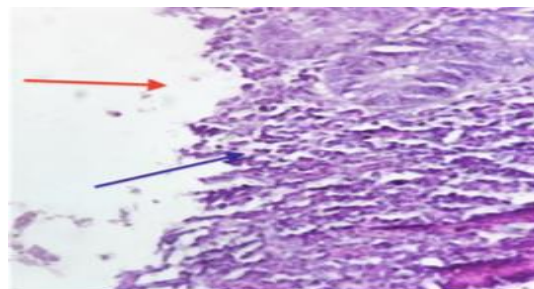


**Figure (6):-** Section of rat's stomach treated with 3 mg/kg/day nicorandil showing inflammation (Gastritis) H&E: 100X.

Anal tissues of the albino rats: The histopathological sections showed the damage of anal tissues. There were different responses in the tested groups; in first group; females showed relatively normal, while in male there was erosion of mucosa anal tissue. Group 2 showed sloughing, erosion and exudation as shown in figure (7). In higher dose of Nicorandil as in groups 3 & 4 section from rectal anal mucosa shows surface ulceration, heavy mononuclear inflammatory cell infiltration, cryptitis and granulation tissue formation as shown in figure (8). Control groups (5 and 6) showed non-effect on the anal tissues in both genders.



**Figure (7):-** Section of female rat treated with 3 mg/kg/day nicorandil; anal tissues with sloughing, erosion and exudation (H&E: 400X).



**Figure (8):-** Section of rat's male anal tissue treated with 3 mg/kg/day nicorandil appeared with surface ulcer, heavy mononuclear inflammatory cell infiltration and cryptitis (H&E: 400X).

The effects of nicorandil on plasma leptin and prostaglandin E2:

#### **Estimation of plasma leptin:**

Table (1) showed the effects of treatment with different doses 0.28, 0.4, 1, or 3 mg/kg/day of nicorandil (G1-G4) compared to 5 mg/kg aspirin (G5), and N.S. (G6) groups. The administration of different doses of nicorandil produced a highly significant reduction in plasma leptin concentration; in G2 from  $110.81 \pm 31.58$  to  $62.91 \pm 46.88$ , in G3 from  $153.86 \pm 33.27$  to  $48.09 \pm 16.17$ , and in G4 from  $149.41 \pm 58.46$  to  $56.09 \pm 44.43$ , while in G1 there was a decreased level of plasma leptin from  $118.13 \pm 31.58$  to  $62.91 \pm 46.88$ , but a non-significant difference ( $P=0.07$ ). The comparison between baseline and after 10 days of treatment in the aspirin group showed a non-significant increase of plasma leptin levels from  $94.01 \pm 50.25$  to  $135.03 \pm 88.41$ ,  $p=0.161$ , while the negative control showed a non-significant decrease in plasma leptin levels from  $86.33 \pm 42.30$  to  $68.38 \pm 57.03$ , with a P value of 0.234. There are significant differences within groups (G1-G6); at baseline  $P=0.051$ , and P value within groups after 10 days of treatment was 0.055.



Estimation of plasma prostaglandin E2: There was non-significant reduction of plasma PGE2 in all tested groups. Comparison of significance within baseline, and after 10 days of treatment of all groups showed a non-

levels (baseline and after 10 days of treatment)

significant difference, with P values of 0.349 and 0.354, respectively, as shown in Table (1).

**Table (1):- The plasma leptin, and prostaglandin E2 concentrations of all treated groups (baseline and after 10 days of treatment) of albino rats, with P values.**

Group	Plasma leptin concentrations			Plasma prostaglandin E2 concentration		
	Baseline (pg/ml)	After 10 days of treatment (pg/ml)	P-value	Baseline (pg/ml) M± SD	After 10 days of treatment (pg/ml) M± SD	p-value
G1	118.13± 31.58	62.91± 46.88	0.017*	32.99±12.89	34.11±22.68	0.401
G2	110.81± 59.04	53.071± 52.40	0.017*	32.99±12.89	34.82±15.22	0.219
G3	153.86± 33.27	48.09± 16.17	0.000*	45.47±4.54	39.49±9.41	0.060
G4	149.41± 58.46	56.091± 44.43	0.002*	44.11±65.78	42.42±59.69	0.573
G6 – N.S.	86.33± 42.30	68.38± 57.03	0.234*	74.11±75.07	31.24±22.56	0.211
<b>P value Within group</b>	0.051	0.051		0.349	0.354	

Note: G1: 0.28 mg/kg/day nicorandil , G2: 0.4 mg/kg/day nicorandil , G3: 1 mg/kg/day nicorandil , G4: 3 mg/kg/day nicorandil, G5: 5 mg/kg/day nicorandil , G6: normal saline nicorandil

Correlation of plasma leptin and PGE2 analysis: The correlation of plasma leptin and prostaglandin E2 levels were estimated and analysed according to the studied parameters, gender, and body weight of the albino rats of all tested groups.

Analysis of leptin, and PGE2 with gender: The ratio of female: male albino rats were 18:24, as shown in Table (2). The mean plasma baseline leptin (LP-B) concentration of females was 89.31 ± 43.05 pg/ml, while for males it was 140.84 ± 45.87 pg/ml; this represented a highly significant difference of P=0.001. The mean plasma LP-A (after 10 days of treatment) concentrations were 45.02± 37.50 pg/ml, 89.77 ± 66.48 pg/ml for females and males, respectively, which is significantly different (P=0.014). Females showed significantly less plasma leptin concentrations (pre and post values). The mean baseline plasma PGE2-B concentration was 66.60 ± 56.72 pg/ml, 26.83 ± 13.59 pg/ml, for females and males, respectively, which was more significant in female than male rats (P=0.002). The mean plasma concentrations of PGE2 after 10 days of treatment were 39.38 ± 36.38 pg/ml, 23.70 ± 19.92 pg/ml for females and males, respectively (P= 0.08), as shown in Table (2).

Analysis of leptin, and PGE2 with body weight of rats: All animals were weighed daily, from day 0 till the end of 10 days of treatment in all

studied groups. Table (3) showed the mean weight of treated groups, compared to positive and negative controls. The elevation of body weight of albino rats (250.85±59.20 to 265.57± 64.22) that received 3 mg/kg/day nicorandil (G4) was highly significant (P=0.008), and a significant increase of rats' body weight (260±75.52 to 276.14±87.52) was seen with administration of 1 mg/kg/day nicorandil (G3) (P=0.024), while the second and N.S. group showed a non-significant increase in the rats' body weights. The aspirin control group showed a significant reduction of albino rats' body weights (301.28±68.17 to 279.57±69.68) after 10 days of treatment (P= 0.036). There was a weak, non-significant positive correlation between weights and plasma leptin concentrations after treatment; r = 0.272, P=0.08. Also there is a weak, non-significant negative correlation between rats' body weights after treatment and plasma prostaglandin E2 concentrations; r = -0.215, P=0.172.

The correlation of plasma leptin and prostaglandin E2 levels in albino rats after 10 days of treatment: There was a non-significant negative correlation between plasma leptin and prostaglandin E2 concentrations after 10 days of treatment in all studied groups (G1-6); r = -0.252, P= 0.108.

**Table (2):-The relation of plasma leptin and prostaglandin E2 (baseline and after 10 days of treatment) to the rat's gender.**

	Sex	No.	Mean Conc. (pg/ml)	SD	P-value
Leptin _B	M	24	140.8417	45.8725	0.001*
	F	18	89.3167	43.0592	
Leptin _A	M	24	89.7708	66.4848	0.0014*
	F	18	45.0267	37.5048	
Leptin _B	M	24	26.83	13.5974	0.002*
	F	18	66.6	56.7200	
Leptin _A	M	24	23.70	19.9254	0.081*
	F	18	39.38	36.3802	

Note: Leptin \_B : Baeline plasma Leptin concentration . Leptin \_A : plasma Leptin concentration after 10 days of treatment .PGE2 – B : Baeline plasma prostaglandin E2 concentration. PGE2 – A : plasma prostaglandin E2 concentration after 10 days of treatment .

**Table ( 3 ):-The effect of different doses of nicorandil on rats' body weights (baseline and after 10 days of treatment) with its relation to plasma leptin and**

Group	Baseline (day 0) M± SD	After 10 days of treatment M± SD	P-value
G1	250.42± 53.10	242.14± 43.17	0.598
G2	269.57± 75.52	276.14± 87.52	0.024*
G4	230.85± 59.20	265.57± 64.22	0.008*
G6 – N.S.	270.71± 76.73	274.42± 80.08	0.531
P value Within group	0.738	0.913	

## Discussion

All drugs can produce untoward consequences, even when used according to standard or recommended methods of administration. Adverse drug reactions can involve every organ and system of the body and are frequently mistaken for signs of underlying disease. Systemic medication is also known to have potential adverse side effects on the oral mucosa and the mouth, and many drugs or chemicals can affect associated structures. The results of this study showed for the first time that the dose-dependent effects of nicorandil have the ability to cause ulcerations in oral, GIT, and anal tissues, which correlated with the plasma levels of leptin and PGE2. Ulceration has many etiological factors; the oral mucosa is affected by many factors including systemic diseases / conditions such as vascular disease, infection, immunosuppression, and chemotherapy for malignancies. In the presence of these factors, oral lesions often fail to heal adequately, resulting in chronic ulcer formation followed by serious systemic infections<sup>(39, 40)</sup>.

Nicorandil is generally well tolerated, but more specific adverse effects such as oral ulceration and stomatitis were first reported in 1998<sup>(41)</sup>, which was subsequently followed by reports of anal ulceration<sup>(42, 8)</sup>. It has emerged

that nicorandil can cause very painful chronic ulceration of the colon and small intestine<sup>(43, 44)</sup>. Painful parastomal ulceration has been reported in patients with ileostomies or colectomies<sup>(10)</sup>. Gastrointestinal ulceration has been reported from the mouth to the perineum, and it may also associate with skin, peri-vulvar and penile ulcers<sup>(45-47)</sup>. Oral ulceration is known to occur with an aspirin-like chemical burn if it left to dissolve whilst in contact with the oral mucosa<sup>(48)</sup>. The anti-anginal drug nicorandil is increasingly being recognised as a causative factor for mucous membrane ulceration. The pathophysiology of the ulceration remains unclear. It has been postulated in this study that leptin and prostaglandin E2 may play a role in the formation of ulcers by the administration of nicorandil, and compared to standard ulcerogenic agent aspirin. This may explain the mechanism of mucous membrane damage that led to ulceration. Oral ulceration may be due to a more local toxic effect and therefore be dose-related<sup>(49)</sup>. This study agrees with the above-mentioned study, and the effect of nicorandil for inducing ulcers was dose-dependent.

The evidence of histopathological examination showed that different doses of

nicorandil have an effect of mild inflammation and inflammatory cells infiltration, and vascular congestion on buccal mucosa, and mucosa of the tongue. Stomach section showed moderate inflammatory cell infiltration with vascular congestion of mucosa, gastroenteritis, and small intestinal ulceration. The response of females and males to a dose of 0.28-mg/kg/day nicorandil differed, with a mild erosion in females' small intestines and gastritis in males. The other tested doses of 0.4 and 1 mg/kg/day showed the same response in both sexes; where, moderate ulceration in the small intestine was observed. The group treated with 3 mg/kg/day nicorandil showed a moderate inflammatory cell infiltration with vascular congestion of mucosa, small intestinal ulceration in oesophagus and stomach for both sexes. The aspirin positive control group showed gastro-intestinal erosion in both sexes. Microscopic appearance of anal tissues showed that the damage produced ulcers as well as inflammatory cell infiltration, with different gender responses in the tested groups; females showed normal anal, while there was a basal cell degeneration and erosion in males of the 0.28 mg/kg/day nicorandil treated group. The dose of 0.4 mg/kg/day nicorandil showed anal ulcer in both sexes. Increasing doses to 1 and 3 mg/kg/day of nicorandil produced a small anal ulcer in females, and large anal ulceration in males. Positive and negative control groups showed no effect on the anal tissues in both sexes of albino rats.

The epithelial cells and vascular endothelial cells in oral mucosa are target cells for leptin, which stimulates angiogenesis in the connective tissue beneath the ulcer, and promotes wound healing in the oral mucosa by accelerating the supply of nutrients, oxygen and even some bioactive substances. Leptin promotes wound healing in the oral mucosa by accelerating epithelial cell migration as well as enhancing angiogenesis around the wounded area. This explains the physiological function of leptin in saliva promoting wound healing<sup>(31)</sup>. The increased mucosal level of leptin accompanies acute gastric mucosal injury and also characterizes mucosal inflammatory responses to bacterial infection, and exogenous leptin has been demonstrated to exert protective effects against gastric injury induced by ischemia-reperfusion as well as to accelerate the healing of experimentally induced gastric ulcers<sup>(50-54)</sup>. Studies with other tissues indicate that the release of adipose-derived hormones such as leptin, adiponectin and resistin is regulated by vascular factors such as ET-1, a potent vasoconstrictor recognized for its role in normal tissue repair,

and play a role in mediation of local leptin release<sup>(55-57)</sup>. This study showed that plasma leptin levels were decreased significantly with the administration of 0.4 mg/kg/day, and highly significantly by the administration of 1 and 3 mg/kg/day, which explains the dose-dependent effect of nicorandil. The aspirin positive control group showed a non-significant elevation of the plasma leptin concentration. The lowest used dose (0.28 mg/kg/day) and negative control group showed a non-significant reduction of plasma leptin levels. There is a significant difference within groups before and after 10 days of treatment by nicorandil.

The ulcer repair process, both in humans and in experimental ulcer models, is mediated by the secretion of growth factors, enzymes and extracellular matrix components<sup>(58)</sup>, which is delayed if prostaglandins are depleted<sup>(59)</sup>. Prior work has demonstrated that ulcer induced by 50 mg aspirin showed a notable reduction in the serum levels of TGF- $\beta$ , while the group in which ulcer was induced by 10 mg nicorandil showed a very slight reduction in the serum levels of TGF- $\beta$ . This was a non-significant statistical difference, when compared to the controls that showed a slight increase in this cytokine level<sup>(60)</sup>.

Prostaglandins do not represent a unique pathway to protect the mucosa. Nitric oxide (NO) has the potential to counteract potentially noxious effects of inhibition of prostaglandin synthesis<sup>(61)</sup>. NO has well characterized inhibitory effects on neutrophil activation/adherence demonstrated in various tissues<sup>(62)</sup>. Agents that are weak inhibitors of prostaglandin synthesis are less ulcerogenic<sup>(63)</sup>. The mean plasma prostaglandin E2 concentrations assessed in this study through tested doses of nicorandil compared to the positive control (aspirin) and N.S. negative control showed that there was non-significant reduction of plasma PGE2 levels when comparing concentrations of baseline and after 10 days of treatment. It may be the case that the short period of observation played a role in this finding.

The mean plasma baseline leptin concentration was highly significant ( $P=0.001$ ), and after 10 days of treatment was significantly ( $P=0.014$ ) less in female than male albino rats. Comparison of pre and post plasma leptin values was significantly reduced in both sexes of albino rats. This study agrees with that studied by Landt et al. 1998<sup>(64)</sup>; sex difference is reversed in rats, with male rats having higher leptin concentrations than female rats. This difference is likely to be due to the greater amount of body fat in male rats.



The mean baseline plasma PGE2 concentration was highly significantly higher ( $P= 0.002$ ), and after 10 days of treatment showed non-statistically significantly higher PGE2 levels in female than male albino rats. The findings of this study agree with those of Paul et al. (2011)<sup>(65)</sup>, which found that the mean plasma leptin concentration was significantly higher in males than in females when compared to males before and after treatment. The serum leptin level increased as body mass index (BMI) increased, irrespective of gender. The elevation of body weights of albino rats that received 3 mg/kg/day nicorandil was highly significant ( $P= 0.008$ ), and a statistically significant increase of rats' body weight was seen with albino rats administered 1 mg/kg/day nicorandil ( $P= 0.024$ ), while 0.4 mg/kg/day and negative control groups showed a non-significant difference in the rats' body weights. Comparison with the aspirin positive control group, there was a significant reduction of albino rat's body weights after 10 days of treatment ( $P= 0.036$ ).

The correlation analysis of this study showed that there was a weak, non-significant positive correlation between body weights and plasma leptin concentrations after treatment. For PGE2 concentration, there was a weak, non-significant negative correlation between rats' body weights and plasma prostaglandin E2 concentrations after treatment. Salivary leptin may have a role in the maintenance of oral health<sup>(66)</sup>. The nicorandil-induced ulceration pathogenesis is unclear. This study showed that plasma leptin concentration was found to play a role in the dose-dependent nicorandil-induced ulceration effects. Leptin has emerged as an important regulator of mucosal inflammatory response that may lead to ulceration after administration of different doses of nicorandil in a dose-dependent effect. Understanding the method by which nicorandil causes mucous membrane damage, inflammation, and ulceration will help in the development of a prophylactic agent that reduces its toxicity. In conclusion, the present study, clearly demonstrated for the first time that highly significantly decreased plasma leptin level might be one of the mechanisms behind the adverse ulceration effects of nicorandil administration. Also, it has been suggested that there is a minimal dose required to induce ulceration, which would point to a dose-dependent effect of nicorandil administration inducing adverse ulceration effects.

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