Obestatin Protective Role Against Stress Gastric Ulcer in Rat

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Abstract

Background: Acute hemorrhagic and erosive gastropathy may represent a potentially life-threatening condition. Obestatin hormone has been recognized as a key factor in regulating appetite and energy homeostasis. Obestatin is produced in the stomach, but little is known about his influence on gastric mucosal integrity.

Aim of Work: This study was designed to elucidate the effect of Intra-peritoneal "IP" administration of obestatin on acute gastric ulcer induced by 6 hours immobilization stress in rats, with or without suppression of nitric oxide "NO" by NG-Nitro-L-arginine methyl ester (L-NAME) in a trial to explore the possible involved mechanisms of action.

Material and Methods: The study had been carried out on 40 adult male albino rats. The animals were divided into three main groups: Group I (control group) (n=8), group II obestatin treated group "10, 30,100 µg/Kg. BW, IP (n=8 for each dose). Group III treated with L-NAME (10 µg/Kg. BW "Sc") 15 minutes before obestatin administration (30µg/Kg. BW "IP"). In all studied groups ulcer score, ulcer index and preventive index were evaluated, in addition, gastric superoxide dismutase (SOD), catalase (CAT) and glutathione peroxidase (GPX) activities were measured.

Results: IP administration of obestatin in a dose dependent manner, resulted in a significant decrease in ulcer score (p<0.001) and ulcer index with increase in preventive index together with increase activities of gastric SOD (p<0.001), CAT (p<0.001) and GPX activities (p<0.01, p<0.001 and p<0.001 with respect to the obestatin dose). In L-NAME pretreated animals, the ulcer prevention ability of obestatin was significantly reduced when compared to that of the same dose of obestatin in group II (30µg/Kg. BW) (p<0.001). However, there was still significant reduction in ulcer score and ulcer index when compared with that of the control group (p<0.001).

Conclusions: This study revealed that, potent dose dependent protective effect of exogenous obestatin against stress-induced gastric lesions accompanied by a significant increase in gastric antioxidant enzymatic activity. In addition, endogenous NO release is involved in the mechanism of obestatin action as pretreatment with L-NAME partially attenuated this protective effect indicating that nitric oxide release is not the only mechanism in obestatin action. Thus obestatin may deserve an attention as a new pharmacological tool for prevention of stress induced gastric ulcer.

Key Words: Stress gastric ulcer – Obestatin – Nitric oxide – Antioxidant.

Introduction

STRESS ulcers lie on the continuum of stress related mucosal disease, a term used to describe the range of changes seen in the gastrointestinal (GI) mucosa of individuals who are critically ill [1,2]. Stress ulcers, are discrete, deeper lesions that can extend into the submucosa, reaching significant blood vessels that can result in significant GI bleeding [3].

While the association between severe physiologic stress and GI ulceration is well established, the mechanism by which stress ulcers form is multifactorial and still incompletely understood [3]. Numerous publications addressing the overuse of stress ulcer prophylaxis both inside and outside of the intensive care unit (ICU) [3-5].

The surprising truth about stress ulcers is that acid, even excessive acid, is not the primary cause of ulcers [2]. Instead, acid is one of multiple pathogenic factors involved, and contributes to the persistence and worsening of already-formed ulcers [6]. The major cause of stress ulcers appears to be splanchnic hypoperfusion in critically ill patients [3,7], because of sympathetic nervous system activation, increased catecholamine release, vasoconstriction, and secretion of pro-inflammatory cytokines [8]. These effects are initially beneficial because they shift blood away from the GI tract to critical organs such as the brain [7], however, they cause break down of gastric mucosal defenses, leading to injury and ulceration. Drugs releasing the potent vasodilator nitric oxide (NO) protect against mucosal injury [9], so, an interactive role of NO in
controlling gastric mucosal blood flow is of great important and represent the main mechanism of leptin and ghrelin protective effect against gastric ulceration [10].

Obestatin is a novel 23-amino acid peptide first identified in the rat stomach as a ghrelin-accompanying peptide; it originates from the post-translational processing of preproghrelin gene, 117-amino acid precursor, and is secreted by gastric and intestinal cells in humans and several mammals [11].

In spite obestatin receptor, GPR39 belongs to the ghrelin family that includes the ghrelin receptor, neurotensin receptor, and motilin receptor [12]. obestatin opposes the effect of ghrelin, as it reduces food intake, body weight and intestinal motility [13-16].

It is also worth mentioning that a substantial amount of obestatin has been found in human colostrum and milk which supports the importance of obestatin (both endogenous and exogenous) in the regulation of gastrointestinal function [17], in vitro, obestatin influences fundus and antrum gastric smooth muscle exerting site-specific effects by inhibiting Ca \(^{2+}\) currents and decreases the amplitude of cholinergic contractile responses in mice [18], moreover, obestatin inhibited the jejunum contraction in vitro [11]. However, the effect of obestatin on gastric secretion has not been detected. Furthermore, the relationship between obestatin and the gastric mucosal integrity also is not mentioned yet.

On the basis of this background, this study was designed to elucidate the effect of IP administration of obestatin on acute gastric ulcer induced by immobilization stress in rats, with or without suppression of NO by NG-Nitro-L-arginine methyl ester (L-NAME) in a trial to explore some of its mechanism of action.

**Material and Methods**

40 healthy adult male albino rats (191 ± 16gm) were obtained from the animal house in Faculty of Veterinary Medicine, Zagazig University. The animals were kept in steel wire cages in the animal house in Faculty of Medicine of Zagazig University under hygienic conditions with an ambient temperature range of 22±2°C and a 12 hours light-cycle. All animals received care in compliance with the animal care guidelines in accordance with the guide for the care and use of laboratory animals according to Institute of Laboratory Animal Resources, 1996 [19]. The study protocol was approved by the Institutional Review Board (IRB) of Faculty of Medicine, Zagazig University. Rats kept one week without intervention for acclimatization. Experiments were performed between 7:00 AM and 4:00 PM (from the 4th to 23rd of February 2016).

The animals were divided into three main groups: Group I: (n=8 rats), is control group (IP saline treated group). Group II (obestatin treated): Consists of 24 rats (further subdivided into 3 equal subgroups n=8 for each dose) in which rats were treated with IP obestatin (10, 30 or 100mg/kg; Sigma-Aldrich Chemicals, St. Louis, Missouri, USA), dissolved in saline. Group III (L-NAME + obestatin treated): (n=8), L-NAME (10 µg/Kg . BW, Sc, Sigma-Aldrich Chemicals, St. Louis, Missouri, USA) [20] was injected 15 minutes before obestatin (30 µg/Kg . BW IP).

**Experimental model for immobilization stress induced gastric ulcer:**

Stress ulcer was produced by restraint immobilization as described by Brodie et al. [21]. Rats were housed separately deprived of food for 12 hours, but allowed water freely, then rats were immobilized in the supine position on a wooden board spaced 15cm apart from each other (because ulcer index drops by 34% when the animals were crowded [22]).

After a minor dosage of ether inhalation, the four limbs of the rats were fixed at the four corners of the wooden board in a manner sufficient to prevent the animals from turning or wedging itself, without hindrance of respiration for 6 hours.

Obestatin was dissolved in saline immediately before the experiment. The three subgroups of group II (obestatin treated) were injected IP with the obestatin (10, 30 and 100 µg/Kg . BW respectively) 30 minutes before immobilization. In group III (L-NAME treated) (L-NAME dissolved in saline) L-NAME was injected sc 15 minutes before obestatin.

After 6 hours restraint, the rats were scarified by over dosage of ether inhalation. Laparotomy was done and the stomachs were dissected out. The excised stomach was placed on an ice bearing surface and was opened along the greater curvature to be examined for ulceration [23].

**Gastric mucosal gross examination:**

The mucosal surface was gently rinsed by cold saline solution. Stomach was stretched over the ice. The severity of mucosal lesions were inspected using a magnifier and rated for gross pathology.
according to the scale of ulcer score described by Dekansky et al. [24].

0 = No damage.
1 = Blood at the lumen.
2 = Pin point erosions.
3 = 1-5 small erosions <2mm.
4 = >5 small erosions <2mm.
5 = 1-3 large erosions >2mm.
6 = >3 large erosions >2mm.

The modification induced by Martin et al. [25] on Dekansky et al. [24] scoring system by getting the mean of two trained observers. The average scores for each group were calculated and expressed as the ulcer index. The data were expressed in terms of: Number of ulcers per stomach, percentage incidence of ulceration per group and severity of ulceration (ulcer score).

The ulcer index (U.I) was calculated by the following equation: U.I = Mean ulcer score of similarly treated group X percentage of ulcerated animals of the same group [26].

The preventive index of obestatin against ulceration was calculated according to the method of Hano et al. [26]. Preventive index (P.I):

\[
P.I = \frac{U.I \text{ control} - U.I \text{ treated}}{U.I \text{ control}} \times 100
\]

Preparation of gastric homogenate: The stomachs were sliced and homogenized in cold 50mM phosphate buffer (pH 7.0) containing 0.1mM EDTA to give 10% homogenate (w/v). The homogenates were centrifuged at 1000 r.p.m. for 10min. The supernatants were separated and used for enzymes activity assays [27].

Gastric antioxidant evaluation: Assay of superoxide dismutase (SOD) activity: According to the method described by Kakkar et al. [28]. Assay of catalase (CAT) activity: According to the method described by Luck [29]. Assay of glutathione peroxidase (GPX) activity: According to the method described by Reddy et al. [30]. All are measured by using spectrophotometer (spectronic 3000 Array, Germany) at 560, 240, 430, 535nm respectively.

Statistical analysis:

Results were presented as mean ± standard deviation (SD). Statistical analysis was performed using the Statistical Package for the Social Sciences (SPSS), version 20.0 (SPSS Inc., Chicago, IL, United States). Repeated measures of analysis of variance (ANOVA) were applied followed by the Student-least significant deference (LSD), post hoc test to compare means of each two different groups. Pearson's correlation analysis was performed to screen potential relations between the dose of obestatin used and ulcer severity and antioxidant enzymatic activity. For all statistical tests done, p-value <0.05 was considered to be statistically significant.

Results

Table (1) and Fig. (1) show the mean values of gastric ulcer score (severity) of immobilized stressed male albino rats in all studied groups. There was a significant decrease in ulcer score in obestatin treated rats (group II) in a dose dependent manner ”(10 µg/Kg.BW, 30 µg/Kg.BW, 100 µg/Kg.BW) (2.12±0.64, 1.37±0.74 and 0.50±0.42 respectively) when compared with that of the control ”group I” (4.00±1.30, p<0.001). In group III (obestatin “30 µg/Kg.BW”) pretreated with L-NAME (10 µg/Kg.BW), there was a significant decrease in the mean value of ulcer score (2.5 ±0.53, p<0.05) when compared with that of control group (p<0.001). Furthermore, this group had a significant increase in ulcer score when compared with that of group IIB (30 µg/Kg.BW obestatin treated) (p<0.001). However, it revealed non-significant difference when compared with the group treated with 10 µg/Kg.BW obestatin (p>0.05). There was a significant negative correlation between the dose of obestatin and the ulcer score ( \( r = -0.694, \ p < 0.001 \)).

The calculated ulcer index in all studied groups:

- Ulcer index (UI) = The mean ulcer score X % of ulcerated animals of the same group.
- UI of group I (control) = 4.00 X 100 (all ulcerated) = 400.
- UI of group II A = 2.12 X 100 (all ulcerated) = 212.
- UI of group IIB = 1.37X 100 (all ulcerated) = 137.
- UI of group IIC = 0.50X 75 (6 ulcerated) = 37.5.
- UI of group III = 2.5X 100 (all ulcerated) = 250.

According to the above data, the ulcer index of obestatin treated subgroups was less than that of the control group. Furthermore, ulcer index of L-NAME pretreated group (group III) was markedly increased after pretreatment with L-NAME.

The preventive index in all studied groups:

- PI of group IIA (obestatin “10 µg/Kg.BW”) = 47.
- PI of group IIB (obestatin “30 µg/Kg.BW”) = 65.75.
• PI of group IIC (obestatin “100 µg/kg BW”) = 90.62.
• PI of group III (obestatin + L-NAME) = 37.5.

From the previous data it is clear that this preventive index was markedly decreased after pretreatment with L-NAME.

Table (2) shows the gastric antioxidant enzymatic activity of immobilized stressed male albino rats in all studied groups. Regarding obestatin treated (10 µg/kg BW) (group IIA), there was a significant increase in the main values of SOD activity (U/mg protein) (89.72±11.8), CAT activity (U/mg protein) (26.11±4.29) and GPX activity (U/mg protein) (32.71±2.08) when compared with that of control group (59.37±6.34, 18.51±2.07 and 23.82±2.15 respectively). In the same context, group IIB (obestatin; 30 µg/kg BW) the mean values of SOD activity (120.72±16.8), CAT activity (39.11±3.91) and GPX activity (53.51±5.43) were significantly higher than that of group I (p<0.001) and group IIA (p<0.001, p<0.01 and p<0.05 respectively). Moreover, in group IIC (obestatin; 100 µg/kg BW) the mean values of SOD activity (157.72±14.8), CAT activity (56.33±8.62) and GPX activity (71.3±6.03) were significantly higher than that of group I (p<0.001), group IIA (p<0.001) and group IIB (p<0.01, p<0.05 and p<0.01 respectively). Moreover, there was a positive correlation between the dose of obestatin used and the levels of the activity of SOD (r=0.963, p<0.001), CAT (r=0.965, p<0.001) and GPX (r=0.939, p<0.001) in gastric tissues.

Regarding the L-NAME pretreated groups “group III”, the mean values of SOD activity (79.72±9.42), CAT activity (31.09±5.32) and GPX activity (39.39±4.91) were significantly higher than that of group I (p<0.001), but they were significantly lower than that of group group IIIB (the group treated by the same dose of obestatin (p<0.01, p<0.01 and p<0.05 respectively). In addition, the L-NAME pretreated groups “group III”, showed non-significant difference in SOD, CAT and GPX activities compared with that of group IIA (obestatin treated (10 µg/kg BW) (p>0.05).

Table (1): Gastric tissue SOD activity (U/mg protein), CAT activity (U/mg protein) and GPX activity (U/mg protein) of immobilized stressed male albino rats in all studied groups.

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Group I (Control) (n=8)</th>
<th>Group II Obestatin treated</th>
<th>Group III Obestatin (30 µg/kg BW) + L-NAME (10 µg/kg BW) (n=8)</th>
</tr>
</thead>
<tbody>
<tr>
<td>X ± SD</td>
<td>4.00±1.30</td>
<td>2.12±0.64</td>
<td>1.37±0.74</td>
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<td>F</td>
<td>16.893 (p&lt;0.001)</td>
<td>16.893 (p&lt;0.001)</td>
<td>16.893 (p&lt;0.001)</td>
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<tr>
<td>p-value VS group I</td>
<td>p&lt;0.001</td>
<td>p&lt;0.001</td>
<td>p&lt;0.001</td>
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<tr>
<td>p-value VS group IIA</td>
<td>p&lt;0.001</td>
<td>p&lt;0.001</td>
<td>p&lt;0.001</td>
</tr>
<tr>
<td>p-value VS group IIB</td>
<td>p&lt;0.001</td>
<td>p&lt;0.001</td>
<td>p&lt;0.001</td>
</tr>
</tbody>
</table>

Table (2): Gastric tissue SOD activity (U/mg protein), CAT activity (U/mg protein) and GPX activity (U/mg protein) of immobilized stressed male albino rats in all studied groups.

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Group I (Control) (n=8)</th>
<th>Group II Obestatin treated (10 µg/kg BW) (n=8)</th>
<th>Group II Obestatin treated (30 µg/kg BW) (n=8)</th>
<th>Group III Obestatin treated (30 µg/kg BW) + L-NAME (10 µg/kg BW) (n=8)</th>
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<tr>
<td>SOD activity (U/mg protein):</td>
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<tr>
<td>X ± SD</td>
<td>59.37±6.34</td>
<td>89.72±11.82</td>
<td>120.72±16.82</td>
<td>157.72±14.82</td>
</tr>
<tr>
<td>p-value</td>
<td>p&lt;0.001 a</td>
<td>p&lt;0.001 a,b</td>
<td>p&lt;0.001 a,b</td>
<td>p&lt;0.001 a,b</td>
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<tr>
<td>CAT activity (U/mg protein):</td>
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<tr>
<td>X ± SD</td>
<td>18.51±2.07</td>
<td>26.11±4.29</td>
<td>39.11±3.91</td>
<td>56.33±8.62</td>
</tr>
<tr>
<td>p-value</td>
<td>p&lt;0.001 a</td>
<td>p&lt;0.001 a,b</td>
<td>p&lt;0.001 a,b</td>
<td>p&lt;0.001 a,b</td>
</tr>
<tr>
<td>GPX activity (U/mg protein):</td>
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<tr>
<td>X ± SD</td>
<td>23.82±2.15</td>
<td>32.71±2.08</td>
<td>53.51±5.43</td>
<td>71.3±6.03</td>
</tr>
<tr>
<td>p-value</td>
<td>p&lt;0.01 a</td>
<td>p&lt;0.001 a,b</td>
<td>p&lt;0.001 a,b</td>
<td>p&lt;0.001 a,b</td>
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a = Significant VS group I, b = Significant VS group IIA, c = Significant VS group IIB.
Discussion

The association between severe physiologic stress and GI ulceration is well established; however, the exact mechanism by which stress ulcers form is multifactorial and still incompletely clarified [3]. The overuse of stress ulcer prophylaxis both inside and outside of the ICU was reported in many studies, most of them act directly against gastric acid [4,5]. Thus the trial to find other prophylaxis against stress ulcer is mandatory.

The present study demonstrates that intraperitoneal administration of obestatin exerted a significant protective effect against stress-induced gastric lesions in rats in a dose dependent manner; this protective effect is accompanied by a significant increase in gastric antioxidant activities. In addition, NO is involved in the protective mechanisms produced by obestatin against stress-induced mucosal lesions, since this effect was significantly reduced by L-NAME-pretreatment. However, it did not blot it out completely.

The obestatin protective effect against stress induced gastric ulceration can be explained by a variety of mechanisms. First is increasing the production of nitric oxide (NO), this possibility is proved in the present work as L-NAME which is blocker of NO synthesis partially attenuated the
obestatin beneficial action on the gastric mucosa. It is known that NO plays a pivotal role in the regulation of gastric mucosal integrity via action on the microcirculation and on the surface mucus bicarbonate barrier [31,32]. So it can be stated that NO plays a role in the ulcer repair process [32].

On line with this possibility Agnew et al. [33] have proved that obestatin has an effect on vascular relaxation in the isolated aorta and mesenteric artery of rats by activating the endothelium-dependent signaling of NO. Moreover, obestatin mediates blood flow indirectly via inhibiting somatostatin and gastrin, as somatostatin inhibits blood flow to the organs [34], gastrin in chronic ulcer of nephrectomy-uremic rat model, contributes to mucosal trophic and blood flow deficient [35,36].

Serum level of obestatin has an evident increase after 8 wk treatment in patients with helicobacter pylori [37] raising the possibility that decreased obestatin was involved in the pathogenesis of peptic ulcer. Therefore, in terms of ulcer healing, it is suggested that correction of the level of obestatin in ulcer model rats can accelerate the healing of chronic gastric ulcers, by increasing the flow of blood and promoting cell proliferation in the gastric mucosa [38].

In the same context, Koc et al. [20] concluded possible use of obestatin against ischemic renal injury as obestatin attenuates renal ischemic reperfusion injury via its anti-inflammatory and anti-apoptotic properties, which appear to involve the modulation of NO metabolism through the suppression of neutrophil accumulation.

The pathologic changes affect blood flow and ulcer healing because of inflammatory stimuli in the bottom of ulcer scar tissues, the small arteries is often accompanied by endarteritis proliferans, which promotes stenosis and thrombosis. Interestingly, obestatin in rats showed a clear effect on inhibiting platelet aggregation in vitro and in vivo [39], thus the score of obestatin beneficial effect on blood flow is elevated.

At cytokine levels, obestatin significantly decreases the expression of nuclear transcription factor (NF-kB) which is an important nuclear transcription factor mediating the expression of TNF-a, interleukin (IL)-1 β, and IL-6, which contribute to gastric ulceration [40-43]. This data provides evidence for the antiinflammatory role of obestatin in cells, [44-46]. In chronic atrophic gastritis, the level of plasma obestatin is decreased. The correlation between the degree of atrophy and the level of plasma obestatin was proved [47].

Second possibility of obestatin gastro-protective role is anti-oxidative activity of obestatin as ip obestatin treatment in the present work associated with a significant increase in antioxidant SOD, CAT and GPX activity in gastric tissue in a dose dependent manner. In support of these results, the function of obestatin in antioxidative stress has been reported in diabetes or ischemia/reperfusion-induced inflammatory models. Sen et al. [40] stated that, treatment with obestatin attenuates ischemia/reperfusion-induced oxidative injury in the ileum of rats by alleviation of the degeneration of surface epithelium and inflammatory cell infiltration and limitation of the elevated malondialdehyde (MDA), and opposing, myeloperoxidase (MPO) activity. These molecular, especially MDA and MPO, have a tight relationship with oxidative stress [48]. Furthermore, obestatin can inhibit oxidative stress-induced damage or apoptosis by activating phosphatidylinositol 3-kinase (PI3K)/Akt, extracellular signal-regulated kinase (ERK) 1/2 and protein kinase C (PKC) in many kinds of tissues and cells [41,49,50].

Obestatin restores oxidative balance and increases the phosphorylation levels of AKT, ERK1/2, and glycogen synthase kinase (GSK)3 b in myocardiastissue in diabetic Wistar rats, both in vivo and H9 c2 cell line [41]. Phosphorylation of GSK3 b, which acts as substrate of PI3K/Akt, ERK1/2 and PKC [51], plays an essential role in antioxidative stress [48,52].

Matuszyk et al. [42] reported that intraperitoneal injection of obestatin improves the amelioration of oxidative stress-related to acetic acid-induced colitis and an improvement in mucosal damage with increases mucosal blood flow. Moreover, Jung et al. [53] reported that in ulcerative colitis (UC), the ratio of ghrelin to obestatin has a lower statistic in active UC and a negative relation to the level of C-reactive protein. Also, Obestatin play an immunomodulatory role and it protects against the development of pancreatitis in a curulein-induced rat model [54].

In conclusion, the results of the present study proved that obestatin has a preventive effect against stress induced gastric ulcer, in a dose dependent manner. Moreover, this protective effect proved to be mostly through NO pathway as L-NAME reduced their effects on ulcer score and ulcer index. In addition, the significant increase in gastric antioxidant enzymatic activity may be another mechanism to explain obestatin gastro-protective effect.
Finally, the present results may have consequences for the clinical practice since acute stress ulcers may represent a potentially life-threatening condition, obestatin may deserve an attention as a new pharmacological tool for the prevention of such case like in intensive care unit patients who are prone to develop stress ulcer.

Acknowledgment:

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References


إن هرمون الأوبستانتين يعتبر من الهورمونات المنظمة القابلة للتنقل للطعام وتحولات الطاقة في الجسم، وقد اكتشف حديثا أنه يوفر بواسطة خلايا خاصة بالعدة ولكن دوره في تنظيم وتقلص العدة غير كامل الوضوح وبناءً على ذلك قد صممت هذه الدراسة استكشاف تأثير هرمون الأوبستانتين على قرحة العقدة العصبية التاجية عن منع الحركة لمدة 3 ساعات متتالية في نواع الجرذان البيضاء بالفأرة وقد قسمت هذه الجرذان (العدد: 40) إلى ثلاث مجموعات رئيسيّة:

- مجموعة ضابطة (العدد: 8): تم فيها إعداد قرحة العقدة العصبية تم هزتها ببطء داخل فص الجرذن 20 دقيقة قبل تثبيت الجرذان.

- المجموعة الثانية: مجموعة دراسة تأثير هرمون الباريبريسين (العدد: 24) حيث قسمت إلى ثلاث مجموعات متساوية (العدد: 8):
  1- أولى تم إعطاؤها هرمون الأوبستانتين (10 ميكروجريام / كجم من وزن الجسم) بالفماء البريبريسين قبل منع الحركة بـ ½ (نصف) ساعة.
  2- تم إعطاؤها هرمون الأوبستانتين (20 ميكروجريام / كجم من وزن الجسم) بالفماء البريبريسين قبل منع الحركة بـ 2/1 (نصف) ساعة.
  3- تم إعطاؤها هرمون الأوبستانتين (100 ميكروجريام / كجم من وزن الجسم) بالفماء البريبريسين قبل منع الحركة بـ 2/1 (نصف) ساعة.

- المجموعة الثالثة: (العدد: 8) تم إعطاؤها مقارنة لتشبيط تتثبيت النيتريك أوكسيد (L-NAME) تحت الجلد بجرعة 10 ميكروجريام / كجم من وزن الجسم قبل إعطاؤها الأوبستانتين (30 ميكروجريام / كجم من وزن الجسم) بالفماء البريبريسين بـ 0/2 (ربع) ساعة.

وقد أسفرت النتائج عن:

- حدوث نقص في قرحة العقدة العصبية في قياس ومؤشر النقل مع حربق زيادة في المؤشر المائع للحرارة مصحوبةً برفع مستوى الانزيمات
- مضادة للأكسدة (تفاعلي الأكسدة)، والكازيبرين (GPX) والكازيبرين (CAT) والكازيبرين (SOD) وكما ذات الحركة ذات تأثير هرمون الباريبريسين من الخلاف.
- وقد أصبح هذا النبأ الوقائي لأوبستانتين أقل فاعلية بعد المعالجة المسبقة بعقار مثبط لتصنب النيتريك أوكسيد (L-NAME) مقارنة بالجموعة المعالجة نفس الجرعة من الأوبستانتين فقط ولكنها مازلت نداً للإحصاءية مقارباً بالجموعة الضابطة.

ومع ذلك يمكن استنتاج التالي:

1- أن هرمون الأوبستانتين قد تكون قد قرحة للبطانة المعدية من الإصابة بالحرارة نتيجة لتثبيت الحركة عن منع الحركة في نواع الجرذان

2- أن هذه الحماية تتأقث العديد من الميكانيكيات وخاصة زيادة إفراز النيتريك أوكسيد وارتفاع مستوى مضادات الأكسدة بالمخاوف والذات ثباً بالدليل الإحصائيا في هذه الدراسة.

ومع هذا يقترح أن استخدام الميكاتيفات لهرمون الأوبستانتين من الممكن أن يكون له دورًا فعالًا في الحماية من قرحة المعدية العصبية والتي أصبحت متطرفةً انتشارًا واسعاً في عصرنا هذا.