

## Effect of Leptin on Cold Restraint-Induced Gastric Lesions in Albino Rats

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### ABSTRACT

Leptin, 167 amino acid peptide hormone, is secreted by adipocytes, has been found to be present in the gastric mucosa. The locally secreted leptin was proved to have a cytoprotective effect. To investigate whether exogenous leptin may be implicated in gastric mucosal protection, male albino rats were randomly assigned to six groups of six rats each. The rats of control (C) group were left freely wandering in their cages. The rats of cold restraint stress (CRS) group were exposed to cold restraint stress for three hours. The rats of leptin (L) group were given leptin subcutaneously (SC) in a dose of 10 µg/kg/rat 30 minutes before subjection to CRS. The rats of famotidine (F) group were given famotidine SC in a dose of 50 mg/kg/rat just before subjection to CRS. The rats of L-NAME group were injected with N<sup>G</sup>-nitro-L-arginine methyl ester in a dose of 25 mg/kg/rat SC 15 minutes before giving leptin and 45 minutes before being exposed to CRS. The rats of indomethacin (I) group were injected with indomethacin SC in a dose of 10 mg/kg/rat followed 30 minutes later by leptin (10 µg/kg/rat, SC) and exposure to CRS. Pyloric ligation was done in all animals at the beginning of the experiment to collect the gastric juice for analysis. The juice was analysed to determine its volume, pH, free and total acid concentration (FAC and TAC), proteolytic activity and mucin concentration. Lesions of gastric mucosa were scored, the ulcer index (UI) and preventive index (PI) were calculated. Gastric mucosa was scrapped and stored at -80 °C until used for assay of gastric mucosal prostaglandin E<sub>2</sub> (PGE<sub>2</sub>). Exposure to CRS significantly reduced gastric juice volume, pH and mucin concentration and gastric mucosal PGE<sub>2</sub> and significantly increased gastric juice proteolytic activity and acidity (FAC and TAC) in CRS group compared with C group. CRS induced ulcerative lesions in all rats achieving an UI of 19.25. Administration of leptin or famotidine before exposure to CRS significantly increased gastric juice volume, pH and mucin concentration and gastric mucosal PGE<sub>2</sub> and significantly decreased gastric juice proteolytic activity in L and F groups compared with CRS group. Both leptin and famotidine exhibited profound protection of gastric mucosa against CRS-induced lesions achieving an UI of 9.5 and 9.75 in L and F groups respectively. This was evident from the PI which was 50.65 and 49.35 in L and F groups respectively. Administration of L-NAME or indomethacin before exposure to CRS aggravated CRS-induced gastric mucosal lesion achieving an UI of 10.75 and 14 in L-NAME and I groups respectively. It could be concluded from the present study that exogenous leptin has an ulcer preventing ability in case of CRS which is comparable to that of famotidine, the famous H<sub>2</sub> antagonist. The mechanism

of ulcer prevention of leptin may involve the cyclooxygenase and nitric oxide pathways. These results may have consequence for the clinical practice.

**Key Words:** Leptin, cold restrained stress (CRS), gastric mucosa, *N*<sup>G</sup>-nitroarginine methyl ester (L-NAME), famotidine, indomethacin, nitric oxide synthase (NOS).

## INTRODUCTION

Gastric ulcer disease remains wide spread; a stressful life style and non-steroidal anti-inflammatory drugs (NSAID) make significant contributions to that pathological situation<sup>1</sup>. Despite indubitable advances in elucidation of the pathogenesis of gastric ulceration, there are gaps in the understanding of ulcerogenesis. Development of a peptic ulcer depends on the balance between the known aggressive factors and mucosal defence mechanisms. Some of the aggressive factors are: gastric acid<sup>1,2</sup>, bile salts<sup>3</sup>, abnormal motility<sup>4</sup>, pepsin<sup>5</sup>, infection with micro-organisms as *H. pylori* and herpes simplex<sup>6</sup>. Among the protective factors are: mucus secretion, gastroduodenal bicarbonate production<sup>7</sup>, prostaglandin synthesis and normal tissue microcirculation<sup>8</sup>.

Leptin, a 167 amino acid peptide hormone, is secreted by the adipocytes into the circulation, lowers the body weight by decreasing appetite and increasing energy expenditure<sup>9-11</sup>. It has also been found to be present in the gastric mucosa<sup>12</sup>, and this has been thought to control food intake in humans. Another way of interpreting the presence of leptin in the gastric mucosa is to invoke its ability to function as a mucosal defence factor in the stomach. **Brzowski et al. (1999)**<sup>4</sup> showed that leptin has a gastro-protective effect in rats.

**Erkasap et al.**<sup>13</sup> showed that leptin has a gastro-protective effect on mucosal injury induced by ischemia reperfusion. To date, there is a dearth of information about the mechanism by which leptin prevents ulcer formation in the stomach.

The aim of the present study was to investigate the effect of leptin on ulcer formation induced by cold restraint stress (CRS) and to study the role of leptin in relation to the established anti-ulcer agents such as famotidine.

## MATERIALS & METHODS

Thirty six male albino rats weighing 150-200 grams were fed standard commercial rat chow with free access to water in mesh bottomed cages to minimize coprophagia and were left to acclimatize to laboratory conditions for 2 weeks prior to involvement in the experiment. Rats were fasted for 24 hours prior to the experiment. Except for the last hour, water was supplied *ad libitum*<sup>14</sup>. Rats were randomly classified into the following groups of six rats each:

1. **Control non-stressed group (C)**; in which rats were left freely wandering in their cages after being subjected to pyloric ligation.
2. **Cold-restraint-stressed group (CRS)**; in which rats were restraint by fixing the four limbs to a wooden board and placed in a refrigerator at 4°C for three hours<sup>2</sup>.

3. **Leptin-treated group (L)**; in which rats were given leptin (10 µg/kg/rat) subcutaneously (SC) 30 minutes before subjected to pyloric ligation and CRS exposure<sup>4</sup>.
4. **Famotidine-treated group (F)**; in which each rat was given famotidine (50 mg/kg) SC just before subjected to CRS and pyloric ligation<sup>15</sup>.
5. **N<sup>G</sup>-nitro L-arginine methyl ester - treated group (L-NAME)**; in which rats were given L-NAME (25 mg/kg SC) 15 minutes before giving leptin to inhibit nitric oxide synthase (NOS) activity. Rats were then subjected to pyloric ligation and CRS.
6. **Indomethacin-treated group (I)**; in which indomethacin was administered in a dose of 10 mg/kg SC. This dose inhibits prostaglandin synthesis but does not induce gastric ulceration. Leptin (10 µg/kg, SC) was given 30 minutes after indomethacin and rats were subjected to pyloric ligation and CRS.

Three hours after pyloric ligation, rats were decapitated and their stomachs were removed, opened along the greater curvature and the gastric juice was collected and centrifuged for 15 minutes at 3000 rpm to remove any solid debris. Finally, each stomach was rinsed in ice cold saline and scored for macroscopic gross mucosal lesions by an observer not aware of the nature of the experiment. Gastric mucosa was washed with indomethacin (10 µg/ml), scraped and stored at -80°C until used for determination of its prostaglandin (PGE<sub>2</sub>) content.

#### **Assessment of Gastric Mucosal Lesions:**

This was expressed in terms of ulcer index (UI) according to the method of **Robert et al.**<sup>16</sup>. The preventive index (PI) was calculated from the equation according to **Hano et al.**<sup>17</sup>.

#### **Analysis of Gastric Juice:**

The volume of clear supernatant was determined and analysed for these parameters:

1. Determination of pH according to the method described by **Moore**<sup>18</sup>.
2. Determination of gastric juice acidity, free acid concentration (FAC) and total acid concentration (TAC) as reported by **Hara et al.**<sup>19</sup>.
3. Determination of the proteolytic activity by a modified spectrophotometric method described by **Fouad**<sup>20</sup>.
4. Determination of mucin concentration by a colorimetric assay method described by **Bhavanadan et al.**<sup>21</sup>.
5. Determination of gastric mucosal prostaglandin E<sub>2</sub> (PG E<sub>2</sub>) level using PGE<sub>2</sub> enzyme immunoassay (EIA) kit (Assay Design Inc, MI, USA).

#### **Statistical Analysis of Data:**

Data were expressed as mean ± standard error of the mean (M ± SEM). Statistical significance of differences between groups were evaluated by unpaired two-tailed student's "t" test. Values of p < 0.05 were considered statistically significant.

## RESULTS

### **Effect of CRS on Gastric Juice Parameters and Its Modulation By Famotidine, L-NAME And Indomethacin In Male Albino Rats:**

Data shown in table (1) clearly revealed that CRS significantly reduced gastric juice volume in CRS group compared with C group. Administration of leptin or famotidine significantly increased; while pre-treatment with indomethacin or L-NAME does not significantly change gastric juice volume compared with CRS group.

CRS significantly reduced the pH of gastric secretion in CRS group compared with C group. Administration of either leptin or famotidin significantly increased; while pre-treatment with L-NAME does not significantly change gastric juice pH in the treated groups compared with CRS group. Indomethacin significantly reduced gastric juice pH in I group compared with L group.

Both FAC and TAC were significantly increased by CRS group compared with C group. Administration of either leptin or famotidin significantly decreased both FAC and TAC in L and F groups compared with CRS group. Pre-treatment with indomethacin significantly decreased acidity in I group compared with L group.

CRS significantly increased the proteolytic activity of gastric juice. No significant changes in the proteolytic activity were observed in L, F and I groups compared with CRS group.

Gastric juice mucin concentration was significantly decreased in CRS

group compared with C group. Leptin or famotidine pre-treatment significantly increased mucin concentration compared with CRS group; while indomethacin pre-treatment significantly decreased gastric juice mucin concentration in comparison with L group.

### **Effect of CRS on Gastric Mucosal PGE<sub>2</sub> level And Its Modulation By Famotidine, L-NAME And Indomethacin In Male Albino Rats:**

Data in table (2) clearly demonstrated that CRS significantly reduced gastric mucosal PGE<sub>2</sub> compared with C group. Only leptin was able to increase the level of PGE<sub>2</sub> in the gastric mucosa in L group compared with CRS and C groups, while famotidine, L-NAME and indomethacin failed to produce significant change of PGE<sub>2</sub> in gastric mucosa of F, L-NAME and I groups in comparison to CRS group.

### **Effect of CRS on Gastric Mucosal Lesion Development And Its Modulation By Famotidine, L-NAME And Indomethacin In Male Albino Rats:**

CRS induced ulcerative lesions development in the gastric mucosa of all rats achieving an UI of 19.25 in CRS group. Both leptin and famotidine exhibited profound protection of the gastric mucosa achieving an UI of 9.5 and 9.75 in L and F groups respectively. This was evident also from the PI, which was 50.65 and 49.35 in L and F groups respectively. On the other hand, administration of L-NAME or indomethacin aggravated gastric mucosal ulcerative lesions achieving an UI of 10.75 and 14 in L-NAME and I groups respectively.

**Table (1): Effect of leptin on gastric juice parameters in CRS rats and its modulation by famotidine, L-NAME and Indomethacin**

Group Parameter	C	CRS	L	F	L-NAME	I
Volume (ml/3hours)	2.16±0.12	0.6 ± 0.03 <sup>a</sup>	1.8 ± 0.16 <sup>b</sup>	1.1±0.1 <sup>b</sup>	1.05±0.09	0.7±0.02
pH	3.07±0.12	2.48±0.18 <sup>a</sup>	3.8 ± 0.2 <sup>b</sup>	3.68±0.15 <sup>b</sup>	3.55±0.13	2.68±0.17 <sup>c</sup>
FAC (MEq/L)	46.6 ± 2.9	96 ± 4.0 <sup>a</sup>	44.6±1.8 <sup>b</sup>	44.7±1.8 <sup>b</sup>	48.5±2.06	95±4.0 <sup>c</sup>
TAC (mEq/L)	88.3±	137 ± 6.4 <sup>a</sup>	83.7± 3.2	84.7±3.3	85±2.47	127±6.4 <sup>c</sup>
Pepsin (mg/mL)	3.4 ± 0.2	5.2 ± 0.3 <sup>a</sup>	4.8 ± 0.38	4.7±0.24	4.8±0.28	5.1±0.3
Mucin (mg/mL)	39.2 ± 2.5	30.5± 4.4 <sup>a</sup>	59.4 ± 5.3 <sup>b</sup>	58±2.7 <sup>b</sup>	40±1.6	31.5±4.4 <sup>c</sup>

*C* = control non-stressed, *CRS* = cold restraint stressed, *L* = leptin treated, *F* = famotidine treated, *L-NAME* = *N*<sup>G</sup>-nito *L*-arginine methyl ester treated and *I* = Indomethacin treated CRS groups. *FAC* = free acid concentration, *TAC* = total acid concentration. Data are expressed as mean ± standard error of the mean of six observations. Values of *p* < 0.05 was considered statistically significant. *a* = significant compared with *C*, *b* = significant compared with *CRS* and *c* = significant compared with *L* group.

**Table (2): Effect of leptin on gastric mucosal PGE<sub>2</sub> level in CRS rats and its modulation by famotidine, L-NAME and Indomethacin**

Group	Gastric Mucosal PGE <sub>2</sub> Level (pg/g wet tissue)
C	345 ± 30
CRS	230 ± 21 <sup>a</sup>
L	500 ± 24 <sup>a,b</sup>
F	210 ± 40
L-NAME	225 ± 19
I	200 ± 50

*C* = control non-stressed, *CRS* = cold restraint stressed, *L* = leptin treated, *F* = famotidine treated, *L-NAME* = *N*<sup>G</sup>-nito *L*-arginine methyl ester treated and *I* = Indomethacin treated groups. Data are expressed as mean ± standard error of the mean of six observations. Values of *p* < 0.05 was considered statistically significant. *a* = significant compared with *C*, *b* = significant compared with *CRS*.

**Table (3): Effect of leptin on gastric mucosal ulcer development in CRS rats and its modulation by famotidine, L-NAME and Indomethacin**

Parameter Group	% Incidence	MSS	MUS	UI	PI
C	0	0	0	0	0
CRS	100	2.50	6.75	19.25	-
L	075	0.75	1.25	09.50	50.65
F	075	0.75	1.50	09.75	49.35
L-NAME	100	1.25	1.50	10.75	48.05
I	100	1.25	2.75	14.00	27.28

CRS = cold restraint stressed, L = leptin treated, F = famotidine treated, L-NAME = N<sup>G</sup>-nitro L-arginine methyl ester treated and I = Indomethacin treated groups. MSS = mean severity score, MUS = mean ulcer score and PI = preventive index.

## DISCUSSION

Leptin is a peptide produced by adipocytes and regulates the function of the satiety center in the hypothalamus<sup>9</sup>. Although it has also been found in stomach<sup>12</sup>, its function in the stomach is not clear. In the current study, the property of leptin as an ulcer preventing agent was investigated. The results of the present study showed that exogenous leptin demonstrated profound ulcer preventing ability in rats when their gastric mucosa was exposed to ulcer inducing agents, such as stress. The basal leptin concentration did not prevent by itself the ulcerogenic effect of stress exposure. However, the exogenously administered leptin prevents that effect, indicating a pharmacological action of leptin.

Cold restraint stress (CRS) is a well-known ulcerogenic procedure. In the present study, CRS produced hemorrhagic lesions in the gastric mucosa three hours after its induction, which is in agreement with previous reports<sup>22,23</sup>. The pathogenic

mechanisms responsible for stress-induced gastric mucosal lesions include disturbance of gastric mucosal microcirculation and abnormal gastric motility<sup>24</sup>.

The rate of gastric secretion is linearly correlated with mucosal blood flow<sup>25</sup>. In the current investigation, CRS reduced significantly the volume of gastric secretion. This is likely to be due to reduced gastric mucosal blood flow, as reported by **Murakami et al.**<sup>2</sup> rather than direct inhibition of secretion.

CRS was observed, in the present study, to increase significantly both FAC and TAC with subsequent decrease in the pH of gastric juice that may be explained by the increased vagal discharge to the stomach induced by exposure to stress<sup>2</sup>. Leptin demonstrated profound ulcer prevention ability, which was compared well with that of famotidine when CRS was employed as an ulcerogenic model. Such finding is in line with **Brzozowski et al.**<sup>4</sup> who reported that leptin has gastroprotective effect on mucosal

injury induced by topical application of 75% ethanol.

The profound effect of inhibiting CRS ulcer formation exhibited by leptin was maintained, even when the animals were pre-treated with low dose of indomethacin. However, a competition in the production of gastric mucosal PGE<sub>2</sub> between indomethacin (inhibitor) and leptin (inducer) can not be ruled out. In CRS-induced ulceration, evaluation of the effect of leptin on gastric mucosal PGE<sub>2</sub> synthesis revealed an increase of gastric mucosal PGE<sub>2</sub> concentration, as reported in the present study and supported by another study<sup>26</sup>. This finding suggests that leptin might act, at least in part, via the cyclooxygenase pathway in preventing gastric CRS-induced ulceration. Furthermore, the results of the current investigation that leptin stimulated mucus secretion support the notion that this peptide acted by stimulating PGE<sub>2</sub> production.

It has been reported previously that PGE<sub>2</sub> stimulate mucus secretion<sup>26-28</sup>. In contrast to the results of the present investigation, **Brzozowski, et al.**<sup>4</sup>, reported that PGE<sub>2</sub> is not involved in the gastroprotective effect of leptin. The contradictory finding observed in the present results could be due to methodological difference in ulcer induction.

The results of the current study clearly demonstrated that leptin at dose level of 10 µg/kg inhibited gastric acid secretion (both FAC and TAC), a result which is in accordance with that of **Konturak et al.**<sup>29</sup>, who have investigated the effect of leptin on gastric acid secretion using *H. pylori* positive and negative patients

and reported that gastric meal and CCK-induced leptin is capable of inhibiting basal and meal-stimulated gastric acid secretion in *H. pylori*-positive patients. But in *H. pylori* negative patients, the release of leptin was reduced in response to CCK and meal.

Pre-treatment of animals with L-NAME (25mg/kg, SC), an inhibitor of nitric oxide synthase before exposure to stress, reduced peptic ulcer prevention ability of leptin significantly. This finding suggests that peptic ulcer preventing ability of leptin does not depend solely on the nitric oxide pathway. This again is in line with observation of **Brzozowski et al.**<sup>4</sup>, that L-NAME reduced the ulcer preventing ability of leptin against acidified ethanol. **Brzozowski et al.**<sup>30</sup> have also investigated the effect of centrally and intra-peritoneal administered leptin and reported that its gastro-protective action, accompanied by increased gastric blood flow and increased plasma gastrin levels, depends on vagal activity and involves hyperemia-mediated by NO.

The results of the current study may have consequences for the clinical practice. Based on these data, one would want to recommend the use of leptin SC in a pilot study in humans, in order to access its efficacy as an ulcer prevention drug in patients in the intensive care units, who are more prone to develop stress ulcer<sup>31</sup>.

**In conclusion**, leptin at a dose of 10 µg/kg, could have an ulcer preventing ability, which is comparable to that of famotidine, an H<sub>2</sub> antagonist. The ulcer prevention ability of leptin on CRS-induced ulcer

may involve the cyclooxygenase and nitric oxide pathways.

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## تأثير الليبتين علي إصابات الغشاء المخاطي للمعدة المستحدثة بالتبريد والتثبيت في الفئران البيضاء

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تم في هذا البحث دراسة تأثير هرمون الليبتين علي قرح الغشاء المخاطي للمعدة نتيجة تعرضها للإجهاد بالتثبيت والتبريد في الفئران ومقارنة ذلك التأثير بتأثير الفاموتيدين. تم تقسيم ٣٦ فأراً ذكراً يزن كل منها 150-200 جراماً عشوائياً إلي ٦ مجموعات بكل مجموعة ٦ فئران علي الوجه التالي:-

١. المجموعة الضابطة وفيها تركت الفئران تتجول بحرية في أقفاصها بعد ربط فتحة البواب لجمع العصارة المعدية.
٢. مجموعة الإجهاد بالتثبيت والتبريد وفيها تم تثبيت الفئران بربط الأطراف الأربعة ووضعها في درجة حرارة ٤ مئوية لمدة ٣ ساعات.
٣. مجموعة الليبتين وفيها تم حقن الليبتين تحت الجلد (١٠ مكجم/كجم) قبل ربط فتحة البواب وتعرض الفئران للإجهاد بالتثبيت والتبريد مباشرة.
٤. مجموعة الفاموتيدين وفيها تم إعطاء عقار الفاموتيدين تحت الجلد (٥٠ ملجم/كجم) قبل التعرض للإجهاد بالتثبيت والتبريد.
٥. مجموعة L-NAME وفيها تم حقن (L-NAME) تحت الجلد (٢٥ ملجم / كجم) قبل إعطاء الليبتين ثم تم تعريض الفئران للإجهاد بالتثبيت والتبريد.
٦. مجموعة الإندوميتالين وفيها تم حقن الإندوميتالين تحت الجلد (١٠ ملجم/كجم) وهي جرعة تثبط تخليق البروستاجلاندين دون إحداث قرح في المعدة. وبعد ٣٠ دقيقة تم إعطاء الليبتين كما سبق ثم تم تعريض الفئران للإجهاد بالتثبيت والتبريد.

وقد تم ربط فتحة البواب في كل الفئران قبل بدء التجربة لجمع العصارة المعدية في نهاية التجربة لتحليلها لتحديد الحجم ، pH ، الحموضة الحرة والكلية، النشاط المحلل للبروتين، وتركيز الميوسين. وتم فحص الغشاء المخاطي للمعدة لبيان ما به من قرح وإصابات وحساب معامل القرحة ومعامل الحماية ثم كحت الغشاء المخاطي للمعدة وحفظه بالتبريد العميق - ٨٠ درجة مئوية حتي تم قياس البروستاجلاندين ٢٥ (PGE<sub>2</sub>).

وقد أدي التعرض للإجهاد بالتثبيت والتبريد إلي نقص ذي دلالة إحصائية في الحجم، pH وتركيز الميوسين في العصارة المعدية وكذلك في مستوي PGE<sub>2</sub> في الغشاء المخاطي للمعدة ؛ بينما زاد زيادة ذات دلالة إحصائية النشاط المحلل للبروتين وكذلك الحموضة الحرة والكلية في العصارة المعدية للفئران التي تعرضت للإجهاد بالمقارنة مع المجموعة الضابطة. وقد حدثت قرح في كل الفئران التي تعرضت للإجهاد بالتثبيت والتبريد حيث وصل معامل القرحة إلي ١٩.٢٥ .

وقد ادي إعطاء الليبتين أو الفاموتيدين قبل التعرض للإجهاد بالتثبيت والتبريد إلي حدوث زيادة ذات دلالة إحصائية في حجم، pH ، تركيز الميوسين في العصارة المعدية وكذلك في مستوي PGE<sub>2</sub> في الغشاء المخاطي للمعدة في فئران مجموعتي الليبتين والفاموتيدين بالمقارنة مع مجموعة الإجهاد بالتثبيت والتبريد. وقد أحدث كل من الليبتين والفاموتيدين حماية كبيرة ضد القرح وانخفض معامل القرحة إلي ٩.٥ و ٩.٧٥ وكان معامل الحماية ٥٠.٦٥ و ٤٩.٣٥ في مجموعتي الليبتين والفاموتيدين علي الترتيب.

وقد أدى إعطاء L-NAME أو الإندوميثاسين قبل التعرض للإجهاد بالتثبيت والتبريد إلي تفاقم آثار الإجهاد وزاد معامل القرحة إلي ١٠.٧٥ و ١٤ في مجموعتي L-NAME والإندوميثاسين علي الترتيب.

ومن نتائج هذا البحث يمكن استنتاج أن حقن الليبتين له اثر مضاد للقرح المستحدثة في العشاء المخاطي للمعدة عند التعرض للإجهاد بالتثبيت والتبريد في الفئران البيضاء. وهذا التأثير المضاد للقرحة الإجهادية يمكن مقارنته بالفاموتيدين المضاد الشهير لمستقبلات الهيدوجين  $H_2$ . ومن الممكن أن تشمل آلية منع القرح الإجهادية التي يتمتع بها الليبتين التأثير علي تخليق أكسيد النيتريك والبروستاجلاندين. وقد يكون لهذه النتائج مردودا علي الممارسة الطبية.