ROLE OF GHRELIN IN THE REGULATION OF GASTRIC ACID SECRETION IN NORMAL AND EXPERIMENTAL DIABETIC RATS

By

Ahmad Bahaa El-Deen Abdallah, Akmal Ahmad Hasan Diab, Salah Ibrahim Zakhlol and Doaa Attia Abdel Moeity

Physiology Department - Faculty of Medicine – Zagazig University

ABSTRACT

Aim: To clarify the role of ghrelin in the regulation of gastric acid secretion in both normal and experimental diabetic rats. **Material and Methods:** A total number of 140 adult male albino rats were included in this study. All animals were fed the same type of food to avoid the effect of diet on the experiments.. They had free access to water and kept at room temperature. The animals were divided into two main groups: Group A (Normal rats) , Group B (Streptozotocin –induced diabetic rats) . Each group was subdivided into four subgroups : Subgroup 1 : control group : injected with saline 0.5 ml i.v. in the tail vein ,Subgroup 2 : injected with Ghrelin 20µg /Kg i.v. ,Subgroup 3 : injected with Ghrelin 20µg /Kg i.v. + L-NAME 70 mg /Kg s.c.,Subgroup 4 : injected with L-NAME 70 mg /Kg ,Gastric secretions were collected and gastric secretion volume , both free and total acidity, pH were measured.

Results: The study reported that ghrelin significantly enhanced the secretion of both gastric acid and mucus in both normal and diabetic rats, and these effects inhibited by the nitric oxide synthase inhibitor, L_NAME. Also, there was a significant reduction in gastric acid secretion in STZ – induced diabetic rats in comparison with that in normal rats**.**

Conclusions: Ghrelin plays a stimulatory role on gastric acid secretion and mucus secretion in both normal and STZ-induced diabetic rats and the nitric oxide synthase inhibitor L-NAME blocks this effect. So, this role is performed via nitric oxide as a mediator.

Key words: Gastric secretion, Ghrelin and Diabetes.

INTRODUCTION

Ghrelin is a 28 amino acid motilin-related hormone mainly secreted by X/A-like cells of gastric mucosa .It is the endogenous ligand for the growth – hormone secretagogue receptor (GHS-R type1a) and a potent releaser of GH. In addition, actively participate in controlling energy balance and the regulation of food intake[1,2] **.**

Most of the circulating plasma ghrelin originates from ghrelin-secreting cells in in the stomach and these cells were charachterized by round, compact, electron dense secretory granules of P/D(1) type in humans (mean diameter 147+/-30nm), A-like type in rats (183+/-37nm) and X-like type in dogs (273+/-49nm). Ghrelin cells are mainly located in the acid secreting mucosa between neck and base of oxyntic glands, and fewer in glands of pyloric mucosa[3]**.** Lower concentrations have also been reported at various regions in the body[4]**.**

Ghrelin has been proposed to stimulate gastric acid secretion[5-7]**.**while ghrelin administered subcutaneously exerted no effect on gastric acid secretion [8]**.** Thus, it is possible to speculate that ghrelin may play a role in the regulatory mechanism of acid secretion in the stomach. The role of ghrelin in the mechanism of gastric secretion and gastroprotection has been little investigated. However [9]showed that central administration of ghrelin exerts gastroprotection and this effect is attenuated by the blockade of nitric oxide synthase (NOS) activity with L-NAME (N-nitro-L-arginine methyl ester) suggesting that NO may play an important role in this protection. Recently,the involvement of nitrergic mechanisms in ghrelin regulation of gastric acid secretion

and mucus secretion have been demonstrated by [10]who concluded that, ghrelin plays a stimulatory role in the secretion of both gastric acid and mucus in rats and these effects can be abolished by a NOS inhibitor,L-NAME.

MATERIAL &METHODS

A total number of 140 adult male albino rats weighing from 180 to 220 gm were enrolled in this study. They were bred in the animal house and kept in steel wire cages (10/cages).

All animals were fed the same type of food to avoid the effect of diet on the experiments. Their diet consisted of mixed commercial rat laboratory chow and supplied in separate clean containers. They had free access to water and kept at room temperature.

The animals were divided into two main groups as follows :

(1)Group A (Normal rats):

(II)Group B (Streptozotocin –induced diabetic rats):

Each of the two main groups:

Further subdivided into four subgroup :

*Subgroup 1 : control group : injected with saline 0.5 ml i.v. in the tail vein .

*Subgroup 2: injected with Ghrelin 20µg /Kg i.v.

*Subgroup 3: injected with Ghrelin 20µg /Kg i.v. + L-NAME 70 mg /Kg s.c.

*Subgroup 4 : injected with L-NAME 70 mg /Kg

Dosage and routes of administration, [10]

Induction of experimental diabetes

Insulin dependent diabetes was induced by single intra-peritoneal injection of freshly prepared solution of streptozotocin 50 mg/kg of body weight dissolved in 0.2 mmol/L sodium citrate, at PH 4.5 and the rats were provided with 10% glucose solution after 6 hours of streptozotocin administration for the next 48 hours and maintained for 30 days [11]Three days later, diabetes induction was confirmed through measurement of blood glucose level in each animal (from blood sampled from the tail vein) with the One Touch Ultra Glucometer [12]and rats with blood glucose levels more than 250 mg/dl were selected for experiments[13]. Rats with STZ –induced insulin dependent diabetes exhibit reduced body weight, glucosuria ,hyperphagia ,hyperglycaemia ,hypoinsulinaemia [14]

For all studied groups, the following investigations were done :

I.Collection of Gastric Secretion :

Precautions for the collection of gastric juice :

Animals were allowed to fast for 12 hours, as this period ensures complete gastric emptying [15]The animal was anaesthetised, using ether, only-during the operation

The operation of pyloric ligation was fully described by [16]

At the end of collection period, the animal was ana- esthetized again with ether, the laporatomy wound was opened and a ligature was tied around the oesophago-gastric junction to prevent reflux and spilling of gastric secretion. The gastric juice was allowed to flow out into a calibrated centrifuge tube and centrifuged at approximately2000 rpm for 15 minutes. The supernatant was decanted and measured for volume. Samples were discarded if they tinged with blood [17,18]

II.Determination of pH of Basal Gastric Secretion : pH of the gastric secretion was determined by using PH meter

III.Determination of Free Acidity of Gastric Juice : Free acidity (HCl) of the gastric secretion was determined by titration of a given volume of gastric juice with NaOH, 0.1 mol/**L** using Toepfer's reagent (0.5 gm Diethylaminoazobenzene/ 100 ml ethanol) as an indicator with a PH range from 2.9 to 4 [19]

IV. Determination **of the Total Acidity of Gastric Secretion:** Total acidity was determined by titration of a given volume of gastric secretion with NaOH, 0.1 mol/L employing 1% alcoholic phenolphathalein as an indicator with PH range from 8.3 to

10.0 phenophthalein is used as an indicator because it shows the color change after neutralization of total acidity [19]

V. Alcian Blue staining protocol of gastric mucosa Method of staining according to [20]

Slides examination

The slides were examined by oil emersion technique at power 100X and 400 X to detect the mucin content of gastric mucosa according to the density of the blue colour of alcian blue stain as mild intenisty (+) , moderate intensity (++) and marked intensity (+++) [21]

RESULTS

The study showed the gastric secretion volume (ml/3h/100 gm body weight) in normal and STZ – induced diabetic rats between different subgroups . There was a significant increase in gastric secretion volume in ghrelin subgroups when compared with that in control subgroups ($P < 0.001$). While there was a significant reduction in gastric secretion volume in ghrelin+L-NAME subgroups ($P < 0.01$) and L-NAME subgroups ($P < 0.001$) when compared with that in control subgroups in both normal and diabetic.

The study recorded the pH of gastric secretion in normal and STZ – induced diabetic rats between different subgroups. There was a significant decrease in pH in ghrelin subgroups when compared with that in control subgroups ($P < 0.001$). While there was a significant increase in pH in ghrelin+L-NAME subgroups ($P < 0.001$) and L-NAME subgroups ($P < 0.001$) when compared with that in control subgroups in both normal and diabetic.

The study measured the free acidity (mmol/L/3h/100 gm body weight) in normal and STZ – induced diabetic rats between different subgroups . There was a significant increase in the free acidity in ghrelin subgroups when compared with that in control subgroups ($P < 0.001$). While there was a significant reduction in the free acidity in ghrelin+L-NAME subgroups ($P < 0.05$) and L-NAME subgroups ($P < 0.01$) when compared with that in control subgroups in both normal and diabetic.

The study measured the total acidity (mmol/L/3h/100 gm body weight) in normal and STZ – induced diabetic rats between different subgroups . There was a significant increase in the free acidity in ghrelin subgroups when compared with that in control subgroups ($P < 0.001$). While there was a significant reduction in the total acidity in ghrelin+L-NAME subgroups ($P < 0.05$) and L-NAME subgroups ($P < 0.05$) when compared with that in control subgroups in both normal and diabetic.

The study showed also, significant increase in the intensity of the density of blue color of alcian blue dye in ghrelin subgroups when compared with that in control subgroups. While there was a significant reduction in intensity of the density of blue color of alcian blue dye the in ghrelin+L-NAME subgroups and L-NAME subgroups when compared with that in control subgroups in both normal and diabetic.

Table 1 & Figure 1: :**Normal rats**

N= 10	Gastric secretion volume			PH of gastric secretion			Free acidity			Total acidity		
	Mean ± SD	**F**	P (VS group1)	**Mean ± SD**	**F**	**P (VS group1)**	**Mean ± SD**	**F**	**P (VS group1)**	**Mean ± SD**	**F**	**P (VS group1)**
Group 1 (Control)	1.44 ± 0.36			2.4 ± 0.699			13.92 ± 3.67			28.4 ± 7.32		
Group 2 (Ghrelin)	1.93 ± 0.28	< 0.001	< 0.001	1.2 ± 0.42	P<0.001	<0.001	23.91 ± 3.35	<0.001	<0.001	48.26 ± 6.87	<0.001	<0.001
Group 3 (Ghrelin+ L-name)	1.01 ± 0.15		< 0.01	3.8 ± 0.42		<0.001	11.18 ± 1.39		<0.05	22.31 ± 5.8		<0.05
Group 4 (L-name)	0.73± 0.189		< 0.001	4 ± 0.667		<0.001	9.84 ± 1.49		<0.01	21.48 ± 5.34		<0.05

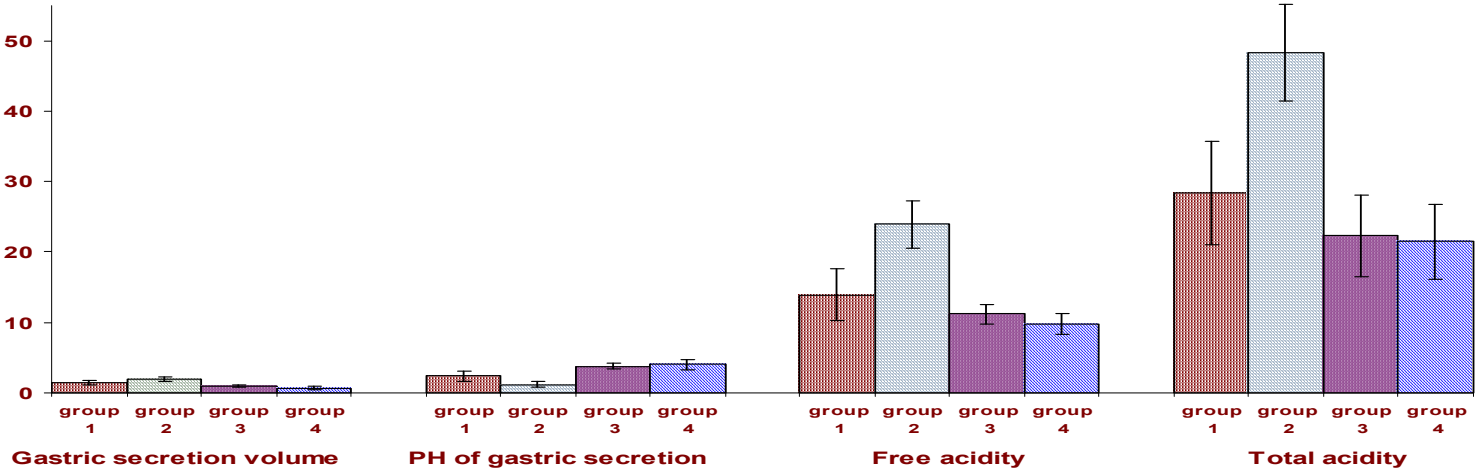
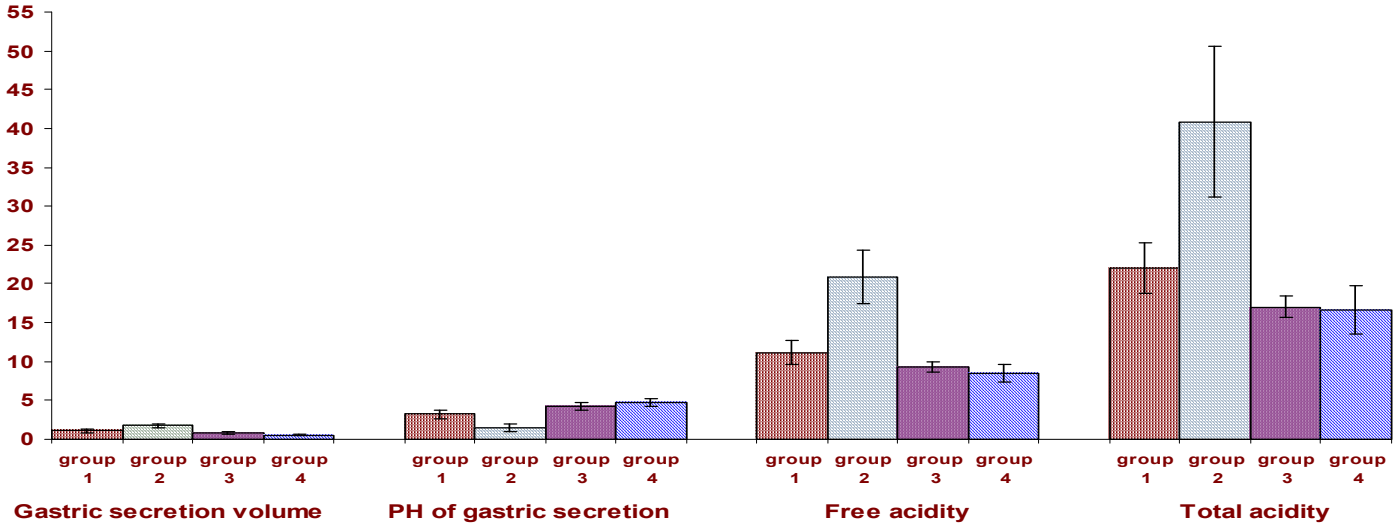


Table 2& Figure 2: STZ Induced Diabetic Rats.

N= 10	Gastric secretion volume			PH of gastric secretion			Free acidity			Total acidity		
	Mean ± SD	F	P (VS group1)	Mean ± SD	F	P (VS group1)	Mean ± SD	F	P (VS group1)	Mean ± SD	F	P (VS group1)
Group 1 (Control)	1.07±0.189			3.2± 0.633			11.16±1.51			22.05±3.32		
Group 2 (Ghrelin)	1.74±0.222	< 0.001	< 0.001	1.5 ± 0.527	P<0.001	<0.001	20.94±3.45	<0.001	<0.001	40.86±9.66	<0.001	<0.001
Group 3 (Ghrelin+ L-name)	0.76±0.17		< 0.01	4.3±0.483		<0.001	9.28±0.647		<0.05	17.04±1.40		<0.05
Group 4 (L-name)	0.57±0.134		< 0.001	4.7 ± 0.483		<0.001	8.44±1.15		<0.01	16.6±± 3.13		<0.05


55
50
45
40
35
30
25
20
15
10
5
0
group 1
group 2
group 3
group 4
Gastric secretion volume
PH of gastric secretion
Free acidity
Total acidity

DISCUSSION

Ghrelin was shown to enhance the gastric motility and gastric acid secretion [22]Also, the role of nitric oxide as a mediator in the regulation of the secretion of both gastric acid and mucus has been recorded by [10]

The present study examined the effects of ghrelin on gastric acid secretion and mucus secretion in both normal and STZ-induced diabetic rats aimed to evaluate the role of NO in this mechanism. So, the study used L-NAME for this purpose.

The results of secretory studies performed on pylorus ligated rats were evaluated. The present study demonstrated that intravenous injection of ghrelin in normal and STZ-induced diabetic rats produced a significant increase in gastric secretion volume, both free and total acidity , a significant decrease in pH and a significant increase in the intenisity of the denisty of blue color of alcian blue indicating that ghrelin stimulates gastric acid secretion and mucus secretion in stomach of both normal and diabetic rats .However there was a significant reduction in gastric secretion volume, both free and total acidity , a significant increase in pH and a significant decrease in the intenisity of the denisty of blue color of alcian blue in both ghrelin+L-NAME and L-NAME subgroups indicating that L-NAME blocks the stimulatory effect of ghrelin on gastric acid secretion and mucus secretion in stomach of both normal and diabetic rats either when used alone or with ghrelin.

These results revealed that exogenous administration of ghrelin stimulates both gastric acid secretion and mucus secretion and these effects of ghrelin were inhibited by applying the nitric oxide synthase inhibitor, L-NAME indicating the role of NO as a mediator in ghrelin action.

These observations are in keeping with the findings of previously reported studies [22-24]which also demonstrated an increase in the gastric acid secretion after administration of ghrelin and that the secretory effect of ghrelin was abolished by an inhibitor of NOS, LNAME.

And this hypothesis supported by two factors:

Firstly**:** The relationship between ghrelin and nitric oxide release that was proved by many arguments:

[25]demonstrated that ECL cells contain NOS. Also, the expression of a neuronal isoform of NO synthase (nNOs) in rat parietal cells has been demonstrated by [26]suggesting the possibility that NO derived from parietal cells may act on adjacent endocrine cells like ECL cells regulating gastric acid secretion. In addition, [27]revealed that ghrelin induces the release of nitric oxide in rat stomach by acting directly on enteric neurons .

Secondly: The role of nitric oxide in the regulation of many cellular functions in the body, namely, the regulation of gastric acid secretion and mucus secretion:

[28]demonstrated that nitric oxide was involved in the regulation of acid and alkaline secretion and, it was reported also, that endogenous NO is involved in an increase of postprandial acid secretion in humans [29] Also, [30]showed that NO donors enhanced acid secretion via release of endogenous histamine in isolated bullfrog fundic mucosa preparations.

In contrast, the inhibitory effect of ghrelin on acid secretion at pylorus-ligated rats by intracerebroventricular application was also observed [8]This seems reasonable as in this setting the route of ghrelin administration was

different. It was also demonstrated that ghrelin constitutes no effect on gastric acid output [23]and on acid secretion [24] in rats. These different results could be related with the existence of different methods as the studies of gastric acid secretion began 7-10 days after surgery, pentobarbital and isoflurane was used to perform anesthesia and the acid was collected in 30 minutes by using a plastic gastric fistula.

CONCLUSION

Ghrelin plays a stimulatory role on gastric acid secretion and mucus secretion in both normal and STZ-induced diabetic rats and the nitric oxide synthase inhibitor L-NAME blocks this effect. So, this role is performed via nitric oxide as a mediator.

REFERENCES

1- Kojima M, Hosoda H, Date Y, Nakazato M, Matsuo H, and Kangawa K: Ghrelin is a growth-hormone-releasing acylated peptide from stomach. Nature 402: 656–660, 1999.

2- Tomasetto C, Wendling C, Rio MC, and Poitras P: Identification of cDNA encoding motilin related peptide/ghrelin precursor from dog fundus. Peptides**22**: 2055–2059, 2001

3-Rindi G, Torsello A, Locatelli V & Solcia E (2004): Ghrelin expression and actions: a novel peptide for an old cell type of the diffuse endocrine system. Experimental Biology and Medicine **229** 1007–1016.

4-Gaytan, F., Barreiro, M.L., Chopin, L.K., Herington, A.C., Morales, C., Pinilla, L., Casanueva, F.F., Aguilar, E., Dieguez, C. & Tena-Sempere, M: (2003): Immunolocalization of ghrelin and its functional receptor, the type 1a growth hormone secretagogue receptor, in the cyclic human ovary. Journal of Clinical Endocrinology and Metabolism, **88**, 879–887.

5- Date Y, Nakazato M, Murakami N, Kojima M, Kangawa K, and Matsukura S: Ghrelin acts in the central nervous system to stimulate gastric acid secretion. Biochem Biophys Res Commun 280: 904–907, 2001.

6- Yakabi K, Kawashima J, Kato S. (2008): Ghrelin and gastric acid secretion.WorldJ Gastroenterol:14(41):6334-8,

7- Sakurada T, Ro S, Onouchi T, Ohno S, Aoyama T, Chinen K, Takabayashi H, Kato S, Takayama K, Yakabi K (2010): Comparison of the actions of acylated and desacylated ghrelin on acid secretion in the rat stomach. J Gastroenterol. 45(11):1111-20.

8- Sibilia V, Pagani F, Guidobono F, Locatelli V, Torsello A, Deghenghi R, Netti C(2002): Evidence for a central inhibitory role of growth hormone secretagogues and ghrelin on gastric acid secretion in conscious rats. Neuroendocrinology 75: 92- 97.

9-Sibilia V, Rindi G, Pagani F, Rafetti D, Locaelli V, Torsello A(2003): Ghrelin protects against ethanol-induced gastric ulcers in rats: studies on the mechanisms of action. Endocrinology **144**(1): 353-359.

10-Bilgin HM, Tumer C, Diken H, Kelle M, Sermet A. Role of ghrelin in the regulation of gastric acid secretion involving nitrergic mechanisms in rats. Physiol Res.;**57**(4):563-8,2008.

11-Toba H., Sawai N., Morishita M., Murata S., Yoshida M., Nakashima K., Morita Y., Kobara M., Nakata T. (2009): Chronic treatmeant with recombinant erythropoietin exerts renoprotective effects beyond hematopoiesis in streptozotocin-induced diabetic rat. Eur. J. Pharmacol., **12**:106-114.

12-Yves M.H. and Theo F.M. (2007): The effect of low dose insulin on mechanical sensitivity and allodynia in type1 diabetes neuropathy. Neurosc. Letters., 417:149-154.

13-Coskun O., Ocakci A., Bayraktaroglu T., Kanter M. (2004): Exercise training prevents and protects streptozotocin-induced oxidative stress **and** β-cell damage in rat pancrease. Tohoku J. Exp. Med., 203:145-154.

14-Granneman JG, Stricker EM: Food intake and gastric emptying in rats with streptozotocin-induced diabetes.

15-Menguy, R. (1960): Effect of restraint stress on gastric secretion. Indian, J. Physiol. & Pharmacol. **2** : 165.

16-Alumets J, Ekelund M, Hakanson R, Hedenbro J, Rehfelt J.F, Sundller F,Vallgren S: Gastric acid response to pylorus ligation in rats: Is gastrin or histamin involved?. J Physiol **323**:145 -156, 1982.

17-Shay, H., Sun, D.C. and Gruenstein, M (1954): A quantitative method for measuring spontaneous gastric secretion in the rat. Gastroenterology**, 26**: 906-913.

18-Lee, Y.H. and Thompson, J.H. (1967): Effect of anaesthetic agents on maximal histamine-induced gastric secretion in shay rats. Am. J. physiol., **213** (5) :1331.

19-Oser, B.L.(1965): Gastric digestion and analysis. In Hawk's physiological chemistry. **14th** ed., New York and London. McGraw-Hill book Co. Ch. **16**, pp.466-487.

20-Ellis R .: Alcian blue staining protocol , prepared by Roy Ellis , IMVS Division of pathology , The Queen Eliazabeth Hospital , Australia , Kiva Microfinance, **2008.**

21-Ganesh I. M. , Subramani D. and Halagowder D: Mucin glycoarray in gastric and gall bladder epithelia , J.Carcinog :12, 6-10**, 2007.**

22-Masuda Y, Tanaka T, Inomata N, Ohnuma N, Tanaka S, Itoh Z, Hosoda H, Kojima M, and Kangawa K: Ghrelin stimulates gastric acid secretion and motility in rats. Biochem Biophys Res Commun 276: 905–908,**2000.**

23-Brzozowski T, Konturek PC, Konturek SJ, Kwiecien S, Drozdowicz D, Bielanski W, PajdoR, PtakA, Nikiforuk A, Pawlik WW, Hahn EGl: Exogenous and endogenous ghrelin in gastroprotection against stress-induced gastric damage. Regul Peptides 120: 39-51, **2004.**

24-Mori M, Suzuki H, Masaoka T, Imaeda H, Nomoto Y, Hosoda H, Nishizawa T, Kangawa K, Hibi T: Intravenous ghrelin administration enhances gastric acid secretion – evaluation using wireless pH capsule. Alimentary Pharmacology & Therapeutics 24: 96 -103, **2006.**

25-Furness J. B., Types of neurons in the enteric nervous system.J. Auton. Nerv. Syst., 81, 87—96,**2000.**

26-Premaratne S, Xue C, McCarty JM, Zaki M, Mccuen RW, Johns RA: Neuronal nitric oxide synthase: Exparession in rat parietal cells. J. Physiol. Gastrointest. Lliver Physiol,280 (2):G308-3, **2001.**

27- Tanaka T, Uneyama H, Yoshie S. Nitric oxide generation by ghrelin and localization of GHS-R in the rat stomach. Gastroenterology 126 (suppl 2) :A410,**2004.**

28-Takeuchi K, Kato S, Yasuhiro T, Yagi K: Mechanism of acid secretory changes in rat stomach after damage by taurocholate: Role of nitric oxide, histamine and sensory neurons. Dig Dis Sci **42**: 645–653,**1997.**

29-Konturek JW, Fischer H, Gromotka PM, Konturek SJ, Domschke **W:** Endogenous nitric oxide in the regulation of gastric secretory and motor activity in humans. Aliment. Pharmacol. Ther. **13**: 1683–1691,**1999.**

30-Kawauchi S., Sugamoto S., Furukawa O., Mimaki H., Takeuchi K., Stimulation by nitric oxide of gastric acid secretion in bullfrog fundic mucosa in vitro. J. Physiol. Pharmacol., **52**, 93—105 (2001).

الملخص العربي

يعتبر هورمون الجريلين الذى يفرز من جدار المعدة من الهورمونات المنظمة لافراز العصارة المعدية والذى ثبت ايضا وجوده فى مناطق أخرى بالجسم وكذلك ايضا يلعب اكسيد النيتريك دورا مساعدا فى افراز المخاط من جدار المعدة وتنظيم افراز العصارة المعدية ، وقد لاحظت الدراسات انخفاضا فى افراز العصارة المعدية بعد المعالجة بدواء مانع لتكوين اكسيد النيتريك.

تناول عدد قليل من الدراسات العلمية العوامل والطرق المنظمة لعمل هورمون الجريلين والتى أثبتت تأثره بموانع تكوين اكسيد النيتريك التى تؤدى الى انقاص افراز العصارة المعدية فى الانسان والحيوان .

أثبتت بعض الدراسات على حيوانات التجارب المصابة بمرض السكر قلة افراز العصارة المعدية والتى ارجعت اسبابها الى ضمور خلايا جدار المعدة او الى التهاب العصب الحائر السكرى .

تهدف الدراسة الحالية الى دراسة تاثير هورمون الجريلين وكذلك موانع تكوين اكسيد النيتريك على افراز العصارة المعدية فى حيوانات التجارب الطبيعية وكذلك المصابة بمرض السكر التجريبى .

وقد خلصت الدراسة الى ان هورمون الجريلين يزيد من افراز العصارة المعدية فى فئران التجارب المصابه والغير مصابة بالسكر مما يفتح المجال الى تطبيق الدراسة على الانسان وخاصة لمرضى السكر لتحسين اداء وكفاءة العصارة المعدية لهؤلاء المرضى.