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The Effect of Cold Stress on the Gastric Mucosa of Adult Male Albino Rats: A Light Microscope Study

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Abstract. Cold stress affects the human body systems especially respiratory, cardiovascular and gastrointestinal systems. An evaluation performed on the structural changes that occurred in the gastric mucosa of adult rat induced by cold exposure under laboratory conditions. In this study, twenty adult male albino rats were divided into two groups: A control group placed in room temperature (25°C), and an experimental group exposed for two hours to the cold weather (8°C). Results revealed that cold exposure induced shrinkage and increase of the mucosal folds, in addition to disruption, destruction of epithelial cells, which line these folds. Moreover, it resulted in increase secretions of gastric glands with loss of its normal architecture. Also notice, a marked congestion and a cellular infiltration of the mucosa. Hence, concluded that cold exposure affected gastric mucosa of the stomach that may lead to gastric ulcer on prolonged cold exposure.

Keywords: Cold stress, Rat, Stomach, Gastric mucosa

Introduction

Physical and psychological stresses are triggers or modifiers of the clinical course of gastrointestinal disorders, such as peptic ulcer, or irritable bowel syndrome. Stress can act synergistically with other factors, to produce gastrointestinal diseases[1] It has been demonstrated that restraint and cold, can induce ulceration[2,3]. The primary effect of

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cold stress is tissue cooling, the local type of which is the inhalation of cold air. Depending on the extent and intensity of tissue cooling, a sequel of effects develops[4]. Mental effects dominate with exposure to light cold stress, however, cold injuries occur with severe exposure to cold stresses[5]. Gastrointestinal and respiratory effects may be triggered by low levels of cold stress, partially as a function of poor individual protection[6].

Cold environments present a multi-factorial stress situation. General hypothermia is the imminent hazard with severe cooling conditions, but several types of local cold stress may develop under moderately cold conditions[7]. A rational approach to the assessment of cold stress must identify and quantify all types of effects of cold stress. The criteria associated with different levels of strain can be identified, nevertheless more research is needed to validate them and to quantify risk levels[8].

The stomach is a hollow, muscular organ of the gastrointestinal tract. It is involved in the second phase of digestion, following mastication. Histologically, the stomach consists of mucosa, submucosa, musculosa and serosa[9]. The clinical syndrome of gastric stress ulceration has been studied for years using rodent cold restraint stress models, although the pathogenesis of the characteristic focal gastric mucosal lesions produced in these models has been controversial[10,11]. Previous studies used gastric strain gauge to characterize fully the gastric motility effects of a 2-h cold restraint protocol, and determine the relationship of variations in gastric contents and in gastric contractions to the amount of gastric mucosal injury. They found a consistent relationship between the force of gastric contractions and gastric mucosal injury, in addition to a relationship between the initial duration of contractions during restraint and ultimate mucosal injury. Volume, acidity and mucus in the gastric contents were unrelated to mucosal injury[12,13]. Despite the availability of numerous gastric damage models, the field lacks a model showing the early events of superficial damage[14].

Consequently, the aim of the present study was to investigate the structural effects of cold stress on gastric mucosa of male albino rats by using the light microscope.

Materials and Methods

Twenty adult male albino rats weighing between (150-200 g) were purchased from the animal house at King Fahd Medical Research Center.

Animals were housed in a polycarbonate cage with stainless steel wire lids under constant conditions of temperature and humidity, with 12:12-h day/night cycles, with free access to water and a balanced diet. All experiments carried out with the consent of the animal ethics in accordance with the guidelines set out by the Canadian Council on Animal Care.

Animals were divided into two main groups: Control and experimental (10 rats each). Food and tap water were provided to both groups, and on the last day, animals were fasted for 6 hr. Then, the experimental group was exposed to cold stress by placing them in a refrigerator at 8ºC once for 2 hr for one day. Control rats stayed at laboratory room temperature at 25ºC.

On the next day, animals of both groups were fasted then sacrificed by decapitation. The anterior abdominal wall was opened for extraction of the stomach. Each stomach was opened, cleaned by tap water, and examined macroscopically for gastric mucosal damage then parts from the body were processed for light microscopy study. Specimens were immersed in 10% buffered formaldehyde for 24 hr, then in ethyl alcohol 70% (6 min), ethyl alcohol 90% (10 min), ethyl alcohol 100% (45 min), xylene (30 min), paraffin wax (45 min), and embedded in paraffin. Serial sections 5 µm thick were sliced and stained with Hematoxylin and Eosin (H&E) stain[15]. The specimens were examined and photographed with light microscope.

Results

External Morphological Features

The present results showed that cold exposure increased mucosal folding in stomach of experimental group compared with control.

Light Microscopic Study of Rat Stomach

Control Group

Sections stained with H&E showed that body part of rat stomach in control group consists of mucosa, submucosa, muscular and serosa layers. Mucosa, the first main layer, appeared covered with columnar epithelium with no goblet cells. The surface epithelium was interrupted by the openings of the gastric pits that were uniformly arranged parallel

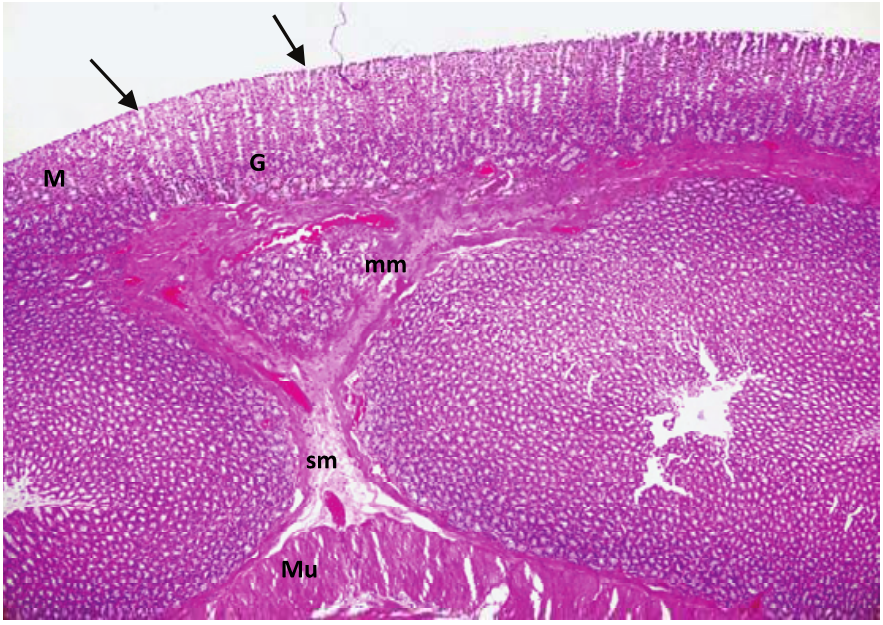

Fig. 1. **A control rat stomach (body part) showing the mucosa (M) with its lining epithelium. Gastric glands (G) are seen at the base of the mucosa, with their neck opening to the surface through gastric pits (arrow). These pits are uniformly arranged parallel to each other. Notice that the muscularis mucosa (mm) forms smooth muscle fibers and the submucosa (sm) forms a loose areolar connective tissue. Notice the musculosa (Mu). (H&E, X40).**

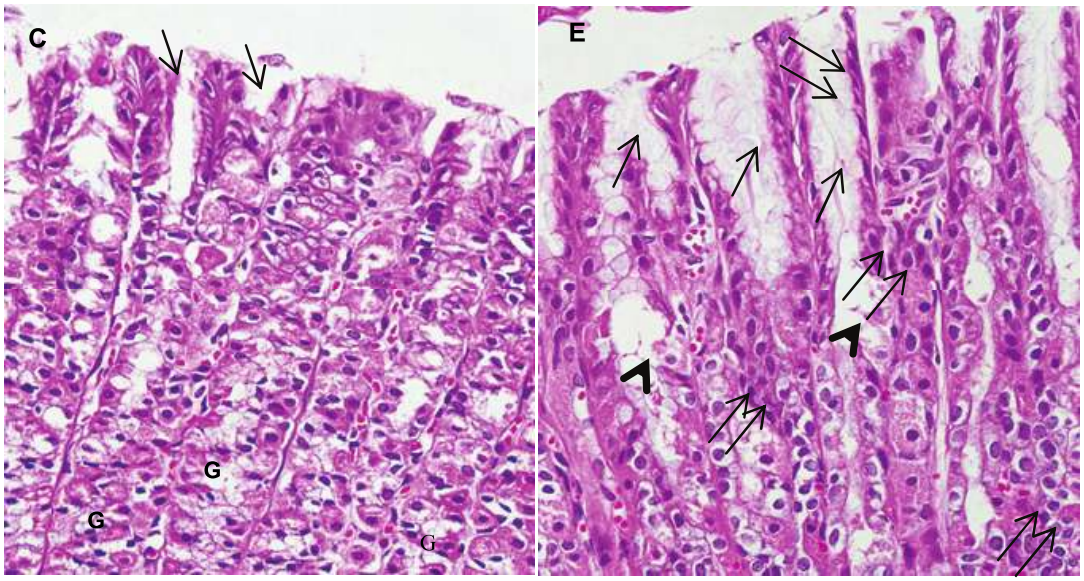

Fig. 2. **The control (C) rat stomach (body part) is showing the mucosa with its lining epithelium. Gastric glands (G) are seen at the base of the mucosa, with their neck opening to the surface through gastric pits (arrow). These pits are uniformly arranged parallel to each other. Experimental group (E) is showing: widening of spaces between mucosal folds (arrows) and increase secretions of gastric glands (arrowheads). The double arrows point at the shrunken, disrupted and destructed epithelial cells, which line the mucosal folds and gastric glands. (H&E, X400).**

to each other, as they conveyed secretion from the gastric glands (Fig. 1 and 2). These glands were present deep in the gastric mucosa and appeared rounded in the sections with normal architecture (Fig. 3). Deep in the mucosa was the muscularis mucosa formed of bundles of smooth muscles. Submucosa lies under the mucosa and consists of loose areolar tissue with occasional cellular infiltration and blood vessels. The mucosa and submucosa showed normal cellular infiltration (Fig. 1, 3). Muscularis externa lies under the submucosa. It has three layers of smooth muscle instead of two: inner oblique, middle circular and outer longitudinal layers (Fig. 1).

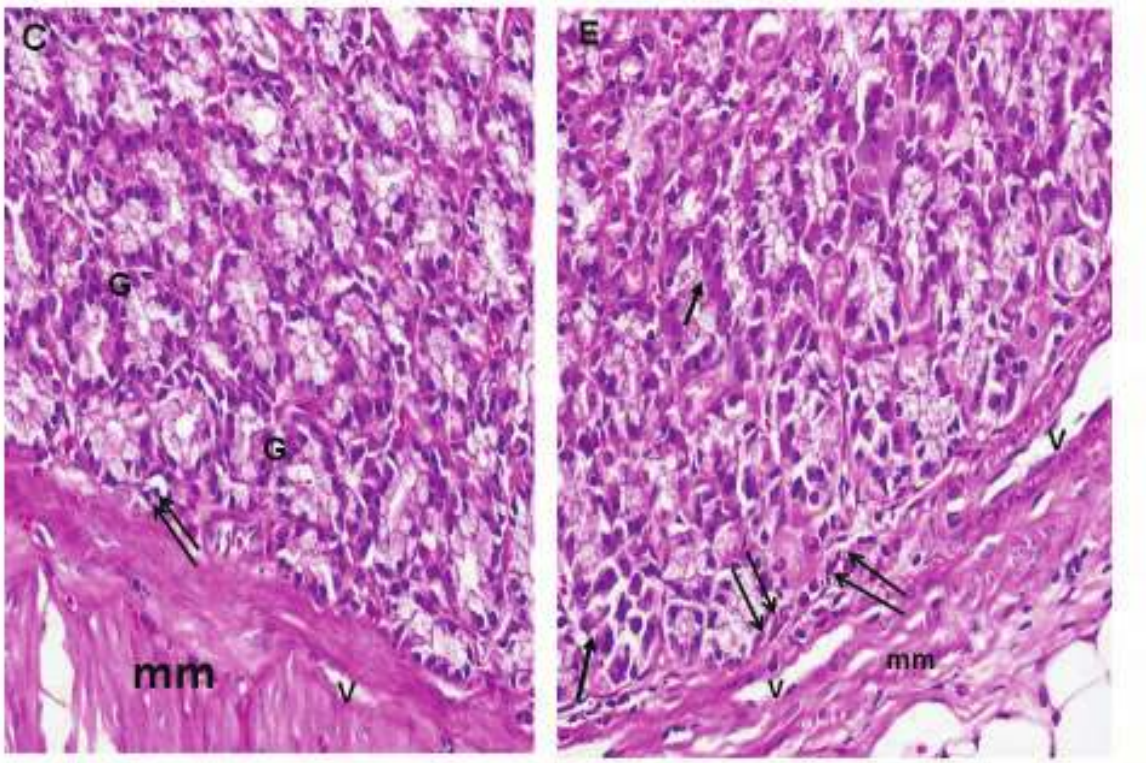

Fig. 3. The Control (C) rat stomach (body part) is showing the gastric glands (G) at the base of the mucosa where they appear circular in cross-section. Notice that the muscularis mucosa (mm) forms of smooth muscle fibers. The double arrows point at the cellular infiltration normally present in the mucosa. Blood vessels (v) are also seen. Experimental group (E) shows the mucosa with loss of normal architecture of gastric glands (arrows) and shrinkage and destruction of muscularis mucosa (mm). Notice the increased vasculature (v) and cellular infiltration (double arrows) in this group. (H&E, X400).

Experimental Group

The normal architecture of the mucosal cells appeared completely lost after exposure to cold for two hours. There was widening of the spaces between mucosal folds and some of these spaces connected with

the gastric pits. The gastric mucosa showed shrinkage, disruption and destruction of the epithelial cells lining the mucosal folds and gastric glands (Fig. 2). Also observed, increase secretions of gastric glands, a marked congestion, and an increased cellular infiltration of the mucosa. The loss of normal architecture of the gastric glands with shrinkage of the muscularis mucosa (Fig. 3) was also noticed in the same group.

Discussion

Workers, who exposed to extreme cold or work in cold environments, may be at risk of cold stress. Extreme cold weather is a dangerous situation that can affect respiratory, cardiovascular and gastrointestinal systems[4]. The stomach is designed to protect itself from a wide variety of intrinsic and exogenous stress factors or irritants[16]. Several mucosal defense mechanisms protect the stomach against cold stress. The pre-epithelial protection is made up of mucus-bicarbonate barrier creates a near neutral pH[9,16,17]. Rapid cell turnover and the process of restitution contribute to an intact epithelial lining. For the subepithelial protection, the mucosal blood flow is essential for supplying the epithelium with nutrients and oxygen, and for disposal of hydrogen ions and noxious agents permeating the mucosa[17].

The present work studied the effect of cold stress only on the gastric mucosa of rats. Animal models, specially rats, are favored for studying gastric processes as they can provide responses of physiological significance for understanding similar events in human ulcer disease[14]. Previous studies declared that the most imperiled component of the gastric mucosa is the layer of epithelial cells directly exposed to the gastric lumen. Thus, injury and repair of this epithelium was studied *in vivo* and *in vitro*[14,18-20].

During the current work, the experimental group subjected to cold stress for 2 hrs, resulted in an increased mucosal folding in stomach of experimental group compared with control. This was in accordance with previous work[13,21]. However, prior studies which subjected the animals for cold stress for 3h, observed gastric mucosal ulceration[22-24]. A former study reported that exposure of rats to water-immersion and restraint stress for 6 h, resulted in severe gastric mucosal lesions[25]. No lesion or few lesions were observed in rats subjected to 2 h of preceding stress[25]. The development of the gastric mucosal lesions in cold stress

could be attributed to the following sequence: stress decreases gastric mucosal blood flow, therefore, weakens the resistance of the gastric mucosal barrier, back diffusion of acid which causes intramucosal acidosis, increases mucosal permeability and finally destroys cell membranes[23].

In this study, mucosal layer showed large spaces between the mucosal folding which may be due to increase secretions from gastric glands as a reaction to cold exposure. In favor with this suggestion, is that some of these spaces connect with the gastric pits. The results obtained in the present study clearly showed that the normal architecture of the mucosal cells appeared completely lost in all rats exposed to cold stress. There was shrinkage, disruption, and destruction of epithelial cells lining the mucosal folds and gastric glands. These findings are in accordance with former researches[21,22]. Previous studies reported change in volume and shape of deteriorating gastric mucosal cells, in addition to stretching, shrinking, and detaching which resulted in increased spaces between these cells[24].

In the current work, no gastric ulcer was observed. Former study[26] hypothesized that cells keep the same position within the surface monolayer initially during injury, presumably because tight junctions and the basolateral membrane of affected mucous cells remain intact in the damage expansion phase. Damage then expands to surrounding cells that also exfoliate. The authors also added that exfoliation occurs in very tight time synchrony with restoration of the epithelial layer, and somewhat less tightly with restoration of normal surface pH control. Most importantly, repair of the epithelial layer started while only a few injured cells lifted off, not after basal lamina seemed totally denude.

In the present investigation, increased secretions of gastric glands and loss of their normal architecture were observed, which is in accordance with many other research findings[23,27-29]. The mechanism of increase in acid secretion during exposure to cold may be correlated to the increased vagal effect on the stomach[30], which promote secretion of thyrotropin-stimulating hormone[30] and thyrotropin-releasing hormone in rats[31-33].

In the present work, it was noticed marked congestion and cellular infiltration of the mucosa with shrinkage of muscularis mucosa. Reduced mucosal blood flow due to hypothermic stress, increased sympatho-adrenal activity and increased gastric motility, thus limit the ability of the

mucosa to wash the back-diffused acid. The acid accumulates more, attacking submucosal capillaries with consequent endothelial swelling and more reduction of mucosal blood flow. The condition is worsened when acid attacks mast-like cells, de-granulating them and releasing histamine and leukotrienes, and increase capillary permeability[28]. This leads to infiltration and activation of phagocytes (especially neutrophils) brought about by proinflammatory cytokines[34].

In conclusion, the present study confirms the cold stress response in rats for limited time can induce superficial gastric mucosal lesions. Accordingly, recurrent exposure to cold stress for longer periods may induce stress gastric ulcer. Further studies are required to clarify the harmful effect of cold stress relative to period of stress exposure, and the prospective protective agents against gastrointestinal disorders initiated by cold stress.

References

[1] **Caso JR, Leza JC, Menchén L.** The effects of physical and psychological stress on the gastro-intestinal tract: Lessons from animal models. *Curr Mol Med* 2008; **8**(4): 299-312.

[2] **Kleiman-Wexler RL, Adair CG, Ephgrave KS.** Pharmacokinetics of naloxone: an insight into the locus of effect on stress-ulceration. *J Pharmacol Exp Ther* 1989; **251**(2): 435-438.

[3] **Savran B, Görgün CZ, Zengil H.** Circadian reactivity rhythm of rat gastric mucosa to restraint-cold stress and indomethacin: Temporal variation in the protective effect of iloprost. *Chronobio Int* 1997; **14**(6): 575-583.

[4] **Holmér I.** Work in the cold. Review of methods for assessment of cold stress. *Int Arch Occup Environ Health* 1993; **65**(3): 147-155.

[5] **Keatinge WR, Donaldson GC, Bucher K, Cordioli E, Dardanoni L, Jendritzky G, Katsouyanni K, Kunst AE, Machenbach JP, Martinelli M, McDonald C, Näyhä S, Vuori I.** Cold exposures associated with winter mortalities from ischaemic heart disease, cerebrovascular disease, respiratory disease, and all-causes, in warm and cold regions of Europe. *Lancet* 1997; **349**(9062): 1341-1346.

[6] **Keatinge WR, Donaldson GC.** Time courses of mortalities after cold weather. *Proceedings of the 33rd International Congress of Physiological Sciences,* June 1997, St. Petersburg.

[7] **Lassvik C**. Angina pectoris in the cold. Effects of cold environment and cold air inhalation at exercise test. *Acta Med Scand Suppl* 1981; **644**: 21-22.

[8] **Lovallo W.** The cold pressor test and autonomic function: a review and integration. *Psychophysiology* 1975; **12**(3): 268-282.

[9] **Sherwood L**. Human physiology: From cells to Systems, 6th ed. Belmont CA: Thomson Brooks/Cole, 2007. 530-601.

[10] **Glavin GB, Szabo S.** Experimental gastric mucosal injury: laboratory models reveal mechanisms of pathogenesis and new therapeutic strategies. *Faseb J* 1992; **6**(3): 825-831.

[11] **Weischer CH, Thiemer K.** A method for induction of cold, indomethacin and restraint ulcers in rats. *Methods Find Exp Clin Pharmacol* 1983; **5**(5): 315-319.

[12] **Garrick T, Buack S, Bass P.** Gastric motility is a major factor in cold restraint-induced lesion formation in rats. *Am J Physiol* 1986; **250** (2 Pt 1): G191-199.

[13] **Brzozowski T, Konturek PC, Konturek SJ, Kwiecien S, Drozdowicz D, Bielanski W, Pajdo R, Ptak A, Nikiforuk A, Pawlik WW, Hahn EG.** Exogenous and endogenous ghrelin in gastroprotection against stress-induced gastric damage. *Regul Pept* 2004; **120**(1-3): 39-51.

[14] **Silen W.** Experimental models of gastric ulceration and injury. *Am J Physiol Gastrointest Liver Physiol* 1988; **255**(4 Pt 1): G395–G402.

[15] **Drury RAB, Wallington EA.** Carleton's histological techniques.5th ed. London: Oxford Univ. Press 1980.

[16] **Allen A, Flemstrom G.** Gastroduodenal mucus bicarbonate barrier: protectionagainst acid and pepsin. *Am J Physiol Cell Physiol* 2005; **288**(1): C1-C19.

[17] **Forssell, H.** Gastric mucosal defense mechanisms: A brief review. *Scandinavian Journal of Gastroenterology* 1988; Supplement **155**: 23-28.

[18] **Gronbech JE, Lacy ER.** Role of gastric blood flow in impaired defense and repair of aged rat stomachs. *Am J Physiol Gastrointest Liver Physiol* 1995; **269**(5 Pt 1): G737-G744.

[19] **Gronbech JE, Lacy ER.** Role of sensory afferent neurons in hypertonic damage and restitution of the rat gastric mucosa. *Gastroenterology* 1996; **111**(6): 1474-1483.

[20] **Lacy ER.** Epithelial restitution in the gastrointestinal tract. *J Clin Gastroenterol* 1988; **10** Suppl 1: S72-77.

[21] **Saravanan S, Dhasarathan P, Indira V, Venkatraman R.** Gastro Protective and antioxidant of Solanum nigrum Linn. Against Aspirin and cold Restraint Stress induced Ulcerated Rats. *Res J Immunol* 2011; **4**(1): 1-11.

[22] **Guzel C, Kurt D, Sermet A, Kanay Z, Denli O, Canoruc F.** The Effects of Vitamin E on Gastric, Ulcers and Gastric Mucosal Barrier in Stress Induced Rats. *Tr J Med Sci* 1998; **28**: 19-21.

[23] **Khalifa MM, Abdel-Al MK, El-Moselhy MA, Fadel AF.** Evaluation of the effects of rofecoxib and nitric oxide-releasing aspirin on stress-Induced gastric ulcers in rats. *Saudi Pharmaceutical J* 2008; **16**(3-4): 203-213.

[24] **Alsarra IA, Ahmed MO, Alanazi FK, El Tahir KEH, Alsheikh AM, Neau SH.** Influence of cyclodextrin complexation with NSAIDs on NSAID/Cold Stress-Induced gastric ulceration in rats. *Int J Med Sci* 2010; **7**(4): 232-239.

[25] **Lou LX, Geng B, Yu F, Zhang J, Pan CS, Chen L, Qi YF, Ke Y, Wang X, Tang CS.** Endoplasmic reticulum stress response is involved in the pathogenesis of stress induced gastric lesions in rats. *Life Sci* 2006; **79**(19): 1856-1864.

[26] **Starodub OT, Demitrack ES, Baumgartner HK, Montrose MH.** Disruption of the Cox-1 gene slows repair of microscopic lesions in the mouse gastric epithelium. *Am J Physiol Cell Physiol* 2008; **294**(1): C223-C232.

[27] **Takeuchi K, Nishiwaki H, Niida H, Okabe S.** Body temperature-dependent action of baclofen in rat stomach. Relation to acid secretion and ulcerogenicity. *Dig Dis Sci* 1990; **35**(4): 458-466.

[28] **Pilchman J, Lefton HB, Braden GL.** Cytoprotection and stress ulceration. *Med Clin North Am* 1991; **75**(4): 853-863.

[29] **Khalifa MMA, Hassan MK, Ashour OM, Heeba GH.** Evaluation of the anti-ulcer activity of pibutidine hydrochloride (IT-066), the new histamine H2 receptor antagonist, in cold-restraint stress- and ethanol-induced ulcer models in rats. *Alazhar Med J* 2002; **31**: 33-47.

[30] **Murakami M, Lam SK, Inada M, Miyake T.** Pathophysiology and pathogenesis of acute gastric mucosal lesions after hypothermic restraint stress in rats. *Gastroenterology* 1985; **88**(3): 660-665.

[31] **Tuomisto J, Ranta T, Mannisto P, Saarinen A, Leppalioto J.** Neurotransmitter control of thyrotropin secretion in the rat. *Eur J Pharmacol* 1975; **30**(2): 221-229.

[32] **Tache Y, Lesiege D, Vale W, Collu R.** Gastric hypersecretion by intracisternal TRH: dissociation from hypophysiotropic activity and role of central catecholamine. *Eur J Pharmacol* 1985; **107**(2): 149-155.

[33] **Goto Y, Tache Y.** Gastric erosions induced by intracisternal thyrotropin-releasing hormone (TRH) in rats. *Peptides* 1985; **6**(1): 153-156.

[34] **Kwiecien S, Brzozowski T, Konturek PC, Pawlik MW, Pawlik WW, Kwiecien N, Konturek SJ.** Gastroprotection by pentoxyfilline against stress-induced gastric damage. Role of lipid peroxidation, antioxidizing enzymes and proinflammatory cytokines. *J Physiol Pharmacol* 2004; **55**(2): 337-355.

تأثير الإجهاد نتيجة التعرض للبرد على الغشاء المخاطى للمعدة فى ذكور الفئران البيضاء البالغة باستخدام المجهر الضوئى

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جدة – المملكة العربية السعودية

المستخلص. الإجهاد الناتج عن البرودة يؤثر على الجهاز التنفسي والهضمي والقلب والأوعية الدموية فى جسم الإنسان. تم دراسة التغيرات التي حدثت في الغشاء المخاطي للمعدة فى الفئران لتقييم تأثير التعرض للبرد في المختبر. استخدم في الدراسة الحالية عشرون من ذكور الفئران البيضاء البالغة. تم تقسيمهم إلى مجموعتين: المجموعة الضابطة التي وضعت في درجة حرارة الغرفة، والمجموعة التجريبية التي تعرضت للطقس البارد (٨ درجات مئوية) لمدة ساعتين. وكشفت النتائج أن التعرض للبرد يؤدى إلى انكماش الغشاء المخاطي للمعدة وزيادة ثناياه المخاطية، بالإضافة إلى تدمير الخلايا الطلائية التي تبطن تلك الثنايا. علاوة على ذلك، نتج عن التعرض للبرد زيادة في إفرازات الغدد المخاطية في المعدة مع فقدانها الشكل الطبيعي. وقد لوحظ زيادة الاحتقان وتسرب الخلايا من الغشاء المخاطي. ومن ثم نجد أن التعرض للبرد أثر على الغشاء المخاطي للمعدة مما قد يؤدي إلى قرحة المعدة مع التعرض للبرد لفترات طويلة.