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EFFECT OF HYOSCIN N-BUTYL BROMIDE ON GASTRIC ULCERATION INDUCED BY INDOMETHACIN AND RESTRAINT STRESS, AND ON GASTRIC SECRETION IN ADULT MALE ALBINO RATS

By

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ABSTRACT

Background: Hyoscine N-butylbromide (Buscopan) is clinically used as an anticholinergic antispasmodic for the treatment of abdominal cramping or visceral pain associated with cramps.

Objectives: we aimed to study the effect of pretreatment by a single injection of various doses of Hyoscin N-butylbromide (HBB) (1mg/Kg, 2.5mg/Kg and 5mg/Kg), on changes in gastric contents in pyloric ligated rats receiving indomethacin in a dose of 100mg/Kg i.p, and on gastric ulcer formation induced in rats by the same dose of indomethacin and subjected to restraint stress.

Materials and Methods: This study was conducted on 60 adult male albino rats, divided into two equal categories, which were further divided into five equal subgroups.

Results: Administration of HBB in doses of 1mg/Kg (i.p) to rats subjected to restraint stress and indomethacin administration in a dose of 100mg/kg led to insignificant decrease in the gastric ulcer index relative to the group received indomethacin and subjected to restraint stress with a protection ratio of (3%). Administration of HBB in doses of 2.5mg/Kg and 5mg/Kg (i.p) to rats subjected to restraint stress and indomethacin administration in a dose of 100mg/kg led to a significant decrease in the gastric ulcer index relative the group received indomethacin and subjected to restraint stress with a protection ratio of 60.46% and 66.41% respectively. Administration of HBB in doses of 1mg/Kg, 2.5mg/Kg and 5mg/Kg (i.p) to pyloric-ligated rats led to a significant decrease in gastric juice volume, acid concentration and the total acid output compared to the group receiving indomethacin.

Conclusion: Intraperitoneal administration of HBB in doses of 1mg/Kg, 2.5mg/Kg and 5mg/Kg to rats in a dose-dependent manner led to a prophylactic effect against stress induced gastric ulcers, with a protection ratio of 3%, 60.46% and 66.41% respectively.

Key words: HBB, gastric secretion, gastric ulcer, restraint stress, Indomethacin.

INTRODUCTION

Hyoscine N-butylbromide (HBB) is a quaternary ammonium compound, which blocks the action of acetylcholine at parasympathetic sites (both muscarinic and nicotinic receptors) in smooth muscle, and in secretory glands. It causes decreased motility of the gastrointestinal tract. So, it is useful in the treatment of abdominal cramping or visceral pain associated with cramps *(Zhang et al., 2016)*, allowing the system to reset normal peristaltic gut activity, i.e. sympatholytic effect in patients and healthy volunteers *(Krueger et al., 2013)*. It is unable to cross the blood brain barrier to exert central nervous system effects *(Lacy et al., 2013)*. It also retains its polar nature regardless of surrounding pH, and is, therefore, poorly absorbed (8%) after oral intake with a systemic bioavailability less than 1%. Although orally administered HBB is excreted in both the feces and in the urine, the metabolites excreted via the renal route bind poorly to the muscarinic receptors and are, therefore, not considered active there *(Samuels et al., 2009)*.

Successful widespread use as well as accumulation of scientific evidence of oral HBB has paved the way for pharmaceutical reformulation, indication expansion, and generic availability as well as rescheduling from prescription only to over-the-counter status in many countries *(Whittaker, 2010)*. Oral HBB is indicated for the relief of smooth muscle spasm of both the gastrointestinal and genitourinary systems *(Ruepert et al., 2011)*, while the parenteral ampoules for injection are indicated for the relief of acute genitourinary or gastrointestinal spasm e.g., renal or biliary colic *(Tytgat, 2008)*. From a pharmacological perspective, it is gratifying that hyoscine, under the wings of N-butylbromide, has emerged from its deadly shadow to become a useful therapy for functional cramping abdominal pain, a condition estimated to affect 30% of the Western adult population *(Lacy et al., 2013)*.

Indomethacin is an indol derivative, non-steroidal, anti-inflammatory drug with anti-inflammatory, analgesic, and antipyretic effects. Indomethacin is the first-choice drug to produce an experimental ulcer model as a result of having a higher ulcerogenic potential than other non-steroidal anti-inflammatory drugs (NSAIDs). Some antiulcer drugs have been shown to inhibit indomethacin-induced ulcers without affecting acid and mucus secretion or oxidant parameters, as well as to inhibit the production of protective factors like COX-1, PGE2, and bicarbonate, and to reduce antioxidant parameters *(Suleyman et al., 2010)*.

The aim of the present work was to study the effect of single injection of various doses of Hyoscin N-butylbromide (HBB) (1mg/Kg, 2.5mg/Kg and 5mg/Kg) on gastric contents (volume and acidity), as well as on gastric ulcer formation induced in rats by indomethacin and subjected to restraint stress.

MATERIALS AND METHODS

Animals:

Sixty adult male Sprague – Dawley rat weighing 120-170 g were purchased form Abu-Rawash Animal House (Giza, Egypt). Animals were given fed with standard commercial rat chow and water. They were left to accommodate for one

week and kept on normal dark/light cycle in partially humid and well – aerated room, at room temperature (22 ± 2oC). Rats were kept in cages (20x32x20 cm for every four rats).

The rats were fasted for 24 hr prior to the induction of the gastric ulcers in wide meshed cages to minimize coprophagia. The animals had free access to water except for the last hour before the experiment. All the experiments were performed during the same time of the day to avoid variations due to diurnal rhythms of putative regulators of gastric functions.

Immediately after indomethacin injection (100mg/kg) (i.p) according to the method of Berenguer et al. (2006), rats were restrained for 24 hr. according to the method of Anchkov and Zovodyskaya (1968) to increase the vulnerability of the rat gastric mucosa to the ulcerogenic effect of indomethacin. Rats were divided into two main equal groups:

A-The first group (group A) was used for the study of the effect of HBB on gastric ulcers induced by immobilization for 24 hr and indomethacin administration in a dose of 100mg/Kg. This group was further subdivided into 5 equal groups:

Group AI received saline i.p. in a dose of 1mL/rat.

Group AII received indomethacin in a dose of 100mg/Kg i.p immediately before the immobilization stress.

Group AIII received HBB i.p. in a dose of 1mg/Kg, ½ h before immobilization stress and indomethacin administration (i.p).

Group AIV received HBB i.p. in a dose of 2.5mg/Kg, ½ h before the immobilization stress and indomethacin administration (i.p).

Group AV received HBB i.p. in a dose of 5mg/Kg, ½ h before the immobilization stress and indomethacin administration (i.p).

B-The Second group (group B) was fasted for 48 hr in a wide meshed bottom cages used for the study of the effect of HBB on gastric secretion in pyloric ligated rats received indomethacin i.p in a dose (100 mg/Kg), which was further subdivided into 5 equal groups:

Group BI received saline i.p. in a dose of 1mL/rat immediately before pyloric ligation.

Group BII received indomethacin in a dose of 100mg/Kg (i.p) immediately before pyloric ligation.

Group BIII received HBB i.p. in a dose of 1mg/Kg, ½ h before indomethacin administration (i.p) and pyloric ligation.

Group BIV received HBB i.p. in a dose of 2.5mg/Kg, ½ h before indomethacin administration (i.p) and pyloric ligation.

Group BV received HBB i.p. in a dose of 5mg/Kg, ½ h before indomethacin administration (i.p) and pyloric ligation.

Drugs and chemicals:

- Saline: Physiological saline (0.9% NaCl) was obtained from a local pharmacy.
- Indomethacin ampoules (50mg/2mL) were obtained from El-Nile Company, Egypt.
- Hyoscine N-butylbromide (HBB) ampoules (20mg/mL): were obtained from Chemical Industries Development (CID) Giza, Egypt.

Method of induction of gastric ulcer by restraint stress: The animals were immobilized in supine position for 24 hr, after 24 hr fasting, by fixing the four limbs at the four corners of the wooden board (35cm x 45cm). Immobilization was sufficient to prevent the animal from turning and wedging itself, without hindrance of respiration. The mechanism of induction of gastric lesion by the use of the immobilization technique was according to *(Anchkov and Zovodskoya, 1968)*. After 24 hr, animals were sacrificed, and abdominal cavity was opened, and the stomach was quickly removed. It was opened along the greater curvature. The mucosa was washed with normal saline, pinned out on cork, and was inspected for the presence of ulceration and hemorrhage by the naked eye, and with the help of a binocular magnifying lens (2X). Lesions were defined as erosions of the gastric mucosa, which may be linear along the regular folds or punched out, and their bases were red or black.

Method of collection and analysis of gastric secretion: Gastric juice was collected according to the technique of *(Shay et al., 1954)*. Rats were kept individually in separate wide meshed cages to insure immediate passage of feces from the cage and to prevent coprophagia.

The animals were starved for 48 hours before the experiment to ensure emptying of the stomach, and water was permitted ad libitum. Fasting of animals was started in early morning. The animals were weighed immediately before fasting and at the end of 48 hrs of starvation. One hour before the experiment, water was removed from the cages.

Operative procedures: Under light isoflurine anesthesia, a midline incision was made extending from the xiphoid process downwards for 2 cm. The duodenum was exposed and the pylori-duodenal junction was picked up gently by a curved probe. A pyloric ligature was made by silk suture. The abdominal wound was then closed with interrupted sutures. The abdominal wound was cleaned thoroughly with physiological saline, dried and covered with collodion solution.

Dehydration before operation was avoided as it affects the rate of gastric secretion. Three hours later, rats were anesthetized again with isoflurine, abdomen was opened, the esophagus was ligated, and the stomach was removed and washed with saline. The stomach was then opened at the greater curvature, and gastric juice was drained into a graduated centrifuge test tube through a funnel.

Analysis of the gastric juice contents: Gastric juice of each stomach was analyzed individually, drained into a graduated centrifuge test tube, and centrifuged at 3000 (rpm) for 15 minutes. The clear supernatant fluid was estimated and analyzed for:

1. The volume of gastric juice by using graduated test tube.
2. Titritable acidity was measured colorimetrically by determining the number of millimeters of 0.01 N NaOH required for neutralizing 100 mL of gastric juice. A given volume of gastric juice (0.2 mL) was titrated to pH 7.0 against 0.01 N NaOH

using phenol red as an indicator (Grossman, 1963).

3. The total titritable acid output (microequvilant/3h) was calculated by multiplying the volume of gastric juice (in mL) by the titritable acidity (in mEq/L) divided by 1000 (Brodie & Hook, 1971 and Okabe et al., 1975), and it was expressed as µEq/3 hours.

Estimation of the ulcer index: The total lengths of gastric lesions per stomach (expressed in mm) were judged by two independent researchers blinded to the protocol of *(Jansson et al., 2007)*. The sum of the lengths of gastric ulcers (in mm) in the group was used as an ulcer index according to the method of *(Kasuya et al., 1978)*.

The preventive index (%) was estimated according to the method of *(Cuparencu and Sandor, 1977)*.

Preventive index (%) =

$$\frac{\text{Mean ulcer index (control)} - \text{Mean ulcer index (treated)}}{\text{Mean ulcer index (control)}} \times 100$$

The preventive index (the protection ratio) was considered to be significant if its percentage exceeded 33%.

Histopathological examination: The specimens were stained with hematoxylin and eosin for examination of the stomach by light microscope according to the method of *(Drury and Wallington, 1980)*.

Statistical analysis: One way ANOVA (Analysis Of Variance) test was used to do the following: Calculation of the descriptive statistics in studied groups (means ± standard deviations). Detection of any significant difference between different groups and between different samples. Performing multiple comparisons between each group and another and each sample and another by using the "Post Hoc LSD" multiple comparison tests. The computer program SPSS version "17" was used to perform ANOVA test. P value < 0.05 was considered significant.

RESULTS

Studies on gastric ulcer:

A- Effect of i.p administration of various doses of Hyoscine N-butylbromide (HBB) (1mg/kg, 2.5mg/kg, and 5mg/kg) on gastric ulcers induced by the synergistic effect of i.p administration of 100mg/kg indomethacin and restraint stress for 24hr in adult male albino rats (Table 1).

1. Effect of saline administration in a dose 1mL (i.p) on the gastric mucosa of the rats (control group).

The total of lengths of ulcers in mms per group = 0 mms

The mean lengths of ulcers in mms per group = 0 mms

2. Effect of indomethacin administration (i.p) in a dose of 100 mg/Kg on the gastric mucosa of rats subjected to immobilization stress.

The total lengths of ulcers in mms per group = 134 mms.

The mean lengths of ulcers in mms per group = 134/6 = 22.33.

3. Effect of HBB administration (i.p) in a dose of 1mg/Kg, ½ h before immobilization stress and indomethacin administration (i.p) on the gastric mucosa of rats.

The total lengths of ulcers in mms per group = 130 mms.

The mean lengths of ulcers in mms per group = 130/6 = 21.67.

The preventive index = $\frac{22.33 - 21.67}{22}.33 = 3\%$.

4. Effect of HBB administration (i.p) in a dose of 2.5mg/Kg, ½ h before immobilization stress and indomethacin administration (i.p) on the gastric mucosa of rats.

The total lengths of ulcers in mms per group = 53 mms.

The mean lengths of ulcers in mms per group = 8.83mms

The preventive index = $\frac{22.33 - 8.83}{22}.33 = 60.46\%$

5. Effect of HBB administration (i.p) in a dose of 5mg/Kg, ½ h before immobilization stress and indomethacin administration (i.p) on the gastric mucosa of rats.

The total lengths of ulcers in mms per group = 45 mms.

The mean lengths of ulcers in mms per group = 7.5 mms

The preventive index = $\frac{22.33 - 7.50}{22}.33 = 66.41\%$

Table (1): Effect of i.p administration of various doses of Hyoscine N-butylbromide (HBB) (1mg/kg, 2.5mg/kg, and 5mg/kg) on gastric ulcers induced by the synergistic effect of i.p administration of 100mg/kg indomethacin and restraint stress for 24hr in male albino rats

Parameters / Groups (n=6)	Ulcer index		Preventive index (%)
	The sum of the lengths of ulcers in mms / group (Total)	The Mean lengths of ulcers in mms / group (Mean)	
Group (AI)	0	0	-
Group (AII)	134	22.33	-
Group (AIII)	130	21.67	3%
Group (AVI)	53	8.83	60.46%
Group (AV)	45	7.50	66.41%

-n = Number of rats in each group.
-Group AII, was compared to the control group AI.
-Groups AIII, AIV and AV were compared to groups AII.

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Group AI received neither Indomethacin (Indo) nor subjected to Restraint Stress (R.S).

Group AII received i.p Indomethacin (Indo) in a dose of 100mg/Kg and subjected to Restraint Stress for 24 hrs.

Group AIII received i.p (HBB) in a dose of 1mg/Kg before i.p (Indo) in a dose of 100mg/Kg + R.S for 24 hrs.

Group AVI received i.p (HBB) in a dose of 2.5mg/Kg before i.p (Indo) in a dose of 100mg/Kg + R.S for 24 hrs.

Group AV received i.p (HBB) in a dose of 5mg/Kg before i.p (Indo) in a dose of 100mg/Kg + R.S for 24 hrs.

B- Histopathological examination: Examination of the rat gastric mucosa :

Effect of i.p administration of various doses of Hyoscine N-butylbromide (HBB) (1mg/kg, 2.5mg/kg, and 5mg/kg) on the gastric mucosa of rats subjected to restraint stress and immediately received indomethacin in a dose of 100mg/kg (i.p).

The gastric mucosa in rats subjected to restraint stress and immediately received indomethacin in a dose of 100mg/kg (i.p) showed by naked eye examination multiple ulcers in each corpus of the stomach, and by light microscope showed deep ulcers reaching the submucosal layer, complete loss of superficial mucus layer, marked submucosal oedema, loss of architecture of the oxyntic cells and marked congested mucosal blood vessels (Figures 1, 2& 3).

The gastric mucosa in rats received Hyoscine N-butylbromide in a dose of 1mg/kg (i.p) 1/2hr before restraint stress and immediate indomethacin administration in a dose of 100mg/kg (i.p) showed by naked eye examination multiple ulcers in each corpus of the stomach, and by light microscope showed partial preservation of the mucus layer, deep mucosal ulcer not penetrating the submucosa, moderate submucosal oedema, scattered parietal cells and marked dilated congested submucosal blood vessels (Figures 4, 5& 6).

The gastric mucosa in rats received Hyoscine N-butylbromide in a dose of 2.5mg/kg (i.p) 1/2hr before restraint stress and immediate indomethacin administration in a dose of 100mg/kg (i.p) showed by naked eye examination multiple ulcers in each corpus of the stomach, and by light microscope showed deep ulcers involving the whole mucosal thickness and lined by granulation tissue, mild submucosal oedema and partial loss of superficial mucus layer (Figures 7, 8& 9).

The gastric mucosa in rats received Hyoscine N-butylbromide in a dose of 5mg/kg (i.p) 1/2hr before restraint stress and immediate indomethacin administration in a dose of 100mg/kg (i.p) showed by naked eye examination very few superficial gastric mucosal ulcers, and by light microscope showed gastric mucosal ulcers not penetrating the deep epithelial layer, preservation of the mucus layer and very mild submucosal edema (Figures 10, 11& 12).

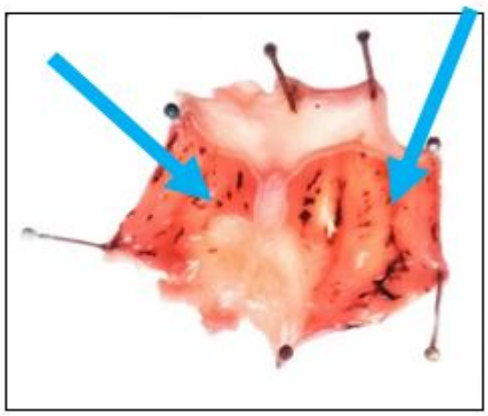


Fig. (1)

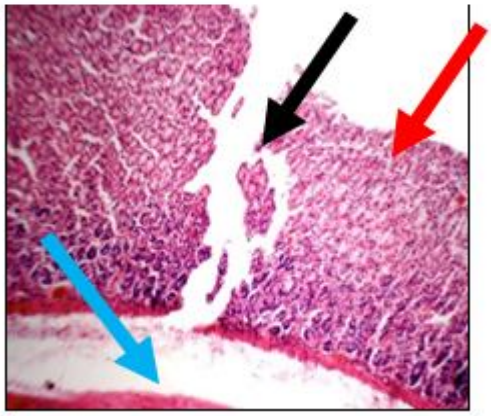
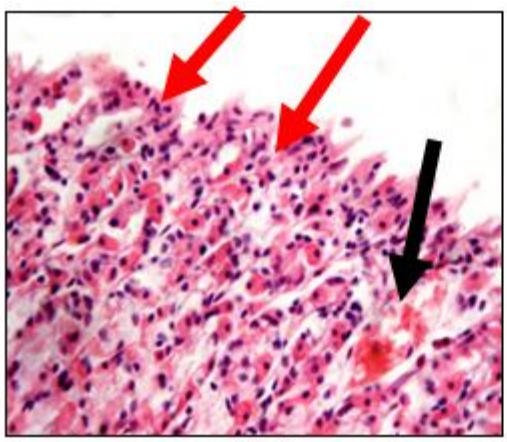


Fig. (2)

Figure (1): Rats subjected to restraint stress and immediately received indomethacin in a dose of 100mg/kg (i.p). Naked eye examination showed multiple ulcers in each corpus of the stomach (blue arrows).

Figure (2): Rats subjected to restraint stress and immediately received indomethacin in a dose of 100mg/kg (i.p). Histopathological examination showed (i) Deep ulcers reaching the submucosal layer (black arrow), (ii) Complete loss of superficial mucus layer (red arrow),(iii) Marked submucosal oedema (blue arrow) (H&E x235).



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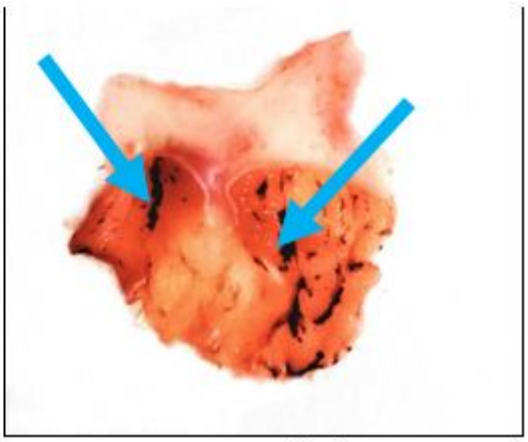
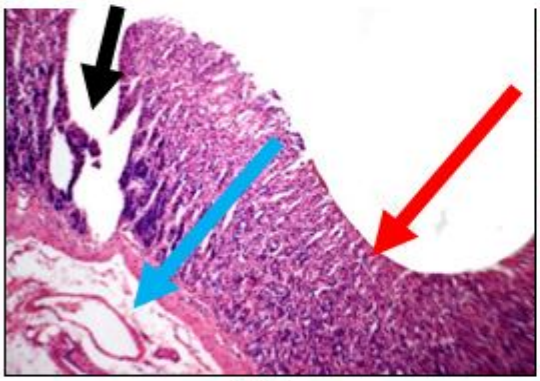


Fig. (4)

Figure (3): Rats subjected to restraint stress and immediately received indomethacin in a dose of 100mg/kg (i.p). Histopathological examination showed: (i) Loss of architecture of the oxyntic cells (red arrows), (ii) Marked congested mucosal blood vessels (black arrow) (H&E x360).

Figure (4): Rats received Hyoscine N-butylbromide in a dose of 1mg/kg (i.p) ½ hr before restraint stress and immediate indomethacin administration in a dose of 100mg/kg (i.p). Naked eye examination showed multiple ulcers in each corpus of the stomach (blue arrows).



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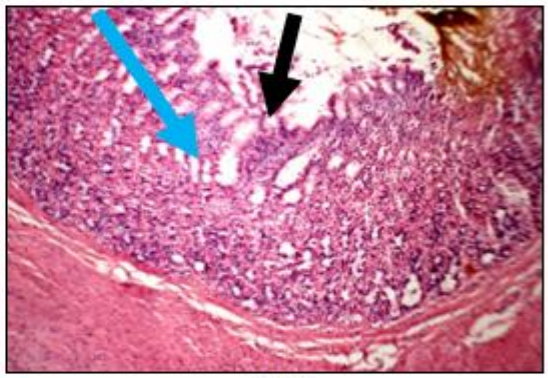
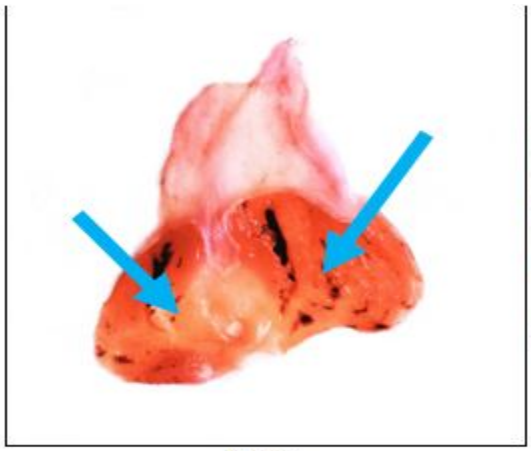


Fig. (6)

Figure (5): Rats received Hyoscine N-butylbromide in a dose of 1mg/kg (i.p) ½ hr before restraint stress and immediate indomethacin administration in a dose of 100mg/kg (i.p). Histopathological examination showed (i) Partial preservation of the mucus layer (red arrow), (ii) Deep mucosal ulcer not penetrating the submucosa (black arrow), (iii) Moderate submucosal oedema (blue arrow) (H&E x235).

Figure (6): Rats received Hyoscine N-butylbromide in a dose of 1mg/kg (i.p) ½ hr before restraint stress and immediate indomethacin administration in a dose of 100mg/kg (i.p). Histopathological examination showed: (i) Scattered parietal cells (blue arrow), and (ii) Marked dilated congested submucosal blood vessels (black arrow) (H&E x360).



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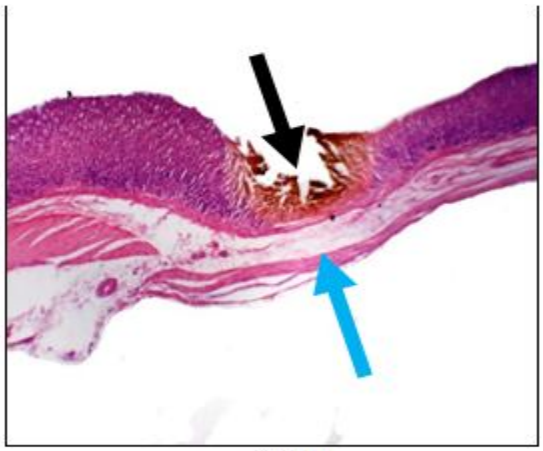
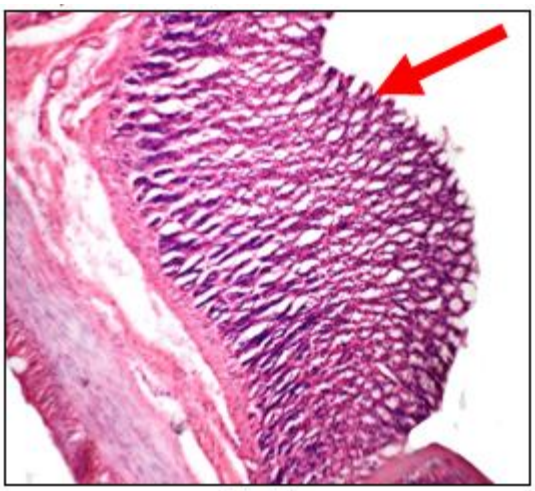


Fig. (8)

Figure (7): Rats received Hyoscine N-butylbromide in a dose of 2.5mg/kg (i.p) ½ hr before restraint stress and immediate indomethacin administration in a dose of 100mg/kg (i.p). Naked eye examination showed multiple ulcers in each corpus of the stomach (blue arrow).

Figure (8): Rats received Hyoscine N-butylbromide in a dose of 2.5mg/kg (i.p) ½ hr before restraint stress and immediate indomethacin administration in a dose of 100mg/kg (i.p). Histopathological examination showed: (i) Deep ulcers involving the whole mucosal thickness and lined by granulation tissue (black arrow), (ii) Mild submucosal oedema (blue arrow) (H&E& x150).



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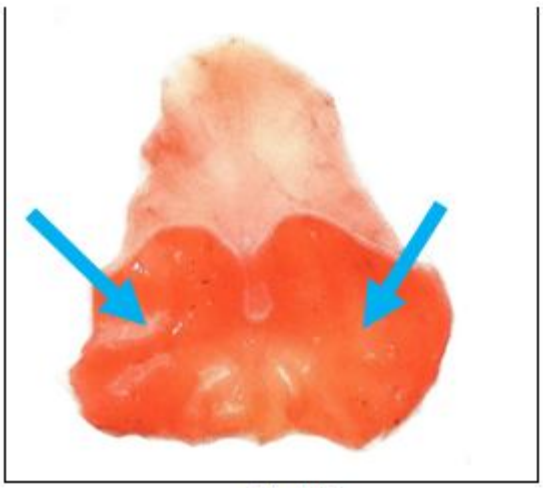
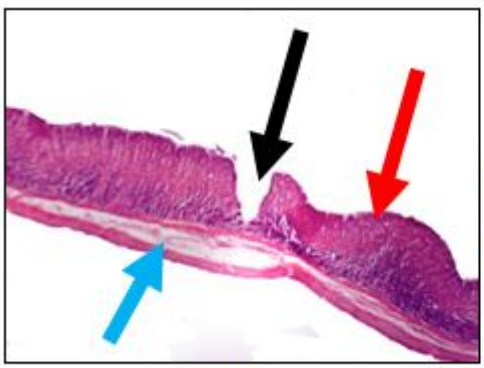


Fig. (10)

Figure (9): Rats received Hyoscine N-butylbromide in a dose of 2.5mg/kg (i.p) ½ hr before restraint stress and immediate indomethacin administration in a dose of 100mg/kg (i.p). Histopathological examination showed partial loss of superficial mucus layer (red arrow) (H&E x235).

Figure (10): Rats received Hyoscine N-butylbromide in a dose of 5mg/kg (i.p) ½ hr before restraint stress and immediate indomethacin administration in a dose of 100mg/kg (i.p). Naked eye examination showed very few superficial gastric mucosal ulcers (blue arrow).



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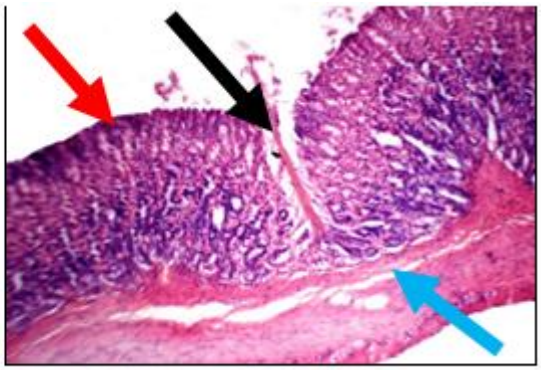


Fig. (12)

Figure (11): Rats received Hyoscine N-butylbromide in a dose of 5mg/kg (i.p) 1/2hr before restraint stress and immediate indomethacin administration in a dose of 100mg/kg (i.p). Histopathological examination showed: (i) Gastric mucosal ulcers not penetrating the deep epithelial layer (black arrow), (ii) Preservation of the mucus layer (red arrow), (iii) Very mild submucosal oedema (blue arrow) (H&E x150).

Figure (12): Rats received Hyoscine N-butylbromide in a dose of 5mg/kg (i.p) 1/2hr before restraint stress and immediate indomethacin administration in a dose of 100mg/kg (i.p). Histopathological examination showed: (i) Narrow gastric mucosal ulcers not penetrating the deep epithelial layer (black arrow), (ii) Preservation of the mucus layer (red arrow), (iii) No submucosal oedema (blue arrow) (H&E x235).

EFFECT OF HYOSCIN N-BUTYL BROMIDE

Studies on Gastric secretions:

A- Effect of different doses of HBB (1mg/kg, 2.5mg/kg, and 5mg/kg) on gastric secretion in pyloric ligated rats received indomethacin i.p in a dose of 100 mg/Kg. (Figures 13, 14, 15).

-Effect of saline administration in a dose 1mL (i.p)/rat on gastric secretion in pyloric ligated rats (control group).

- The mean volume of the gastric juice (2.4000 ± 0.05164 mL).
- The mean titritable acidity (14.0000 ± 0.25820 mEq/L).
- The mean total acid output (0.0336 ± 0.00082 micro equivalent/3 hrs).

B- Effect of indomethacin administration (i.p) in a dose of 100 mg/Kg on gastric secretion in pyloric ligated rats.

- A non-significant decrease in the mean volume of the gastric juice (2.2833 ± 0.04014 mL) as compared to the control group received saline (2.4000 ± 0.05164 mL).
- A significant decrease in the mean titritable acidity (7.0000 ± 0.44721 mEq/L) as compared to the control group received saline (14.0000±0.25820 mEq/L).
- A significant decrease in the mean total acid output (0.0159 ± 0.00077 micro equivalent/3hrs) as compared to the control group received saline (0.0336 ± 0.00082 micro equivalent/3 hrs).

C- Effect of (i.p) administration of HBB (i.p) in a dose of 1mg/Kg ½ h before pyloric ligation and indomethacin administration :

- A significant decrease in the mean volume of the gastric juice (1.4000 ± 0.02582 mL) as compared to the group received indomethacin only (2.2833 ± 0.04014 mL).
- A significant decrease in the mean titritable acidity (4.0000 ±0.00000 mEq/L) as compared to the group received indomethacin only (7.0000 ± 0.44721 mEq/L).
- A significant decrease in the mean total acid output (0.0056 ± 0.00010 micro equivalent/3hrs) as compared to the group received indomethacin only (0.0159 ± 0.00077 microequivalent/3 hrs).

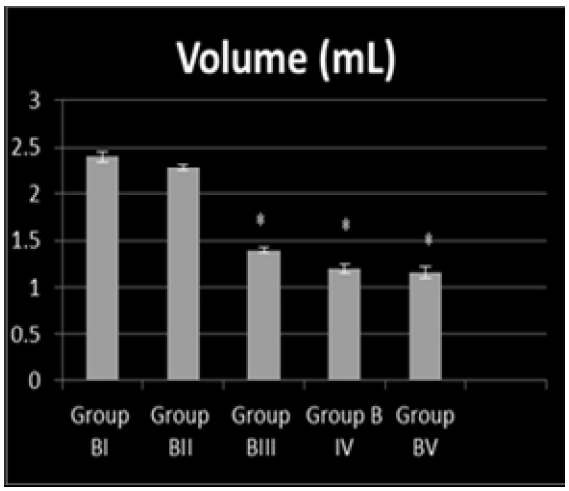
D- Effect of (i.p) administration of HBB (i.p) in a dose of 2.5mg/Kg ½ h before pyloric ligation and indomethacin administration :

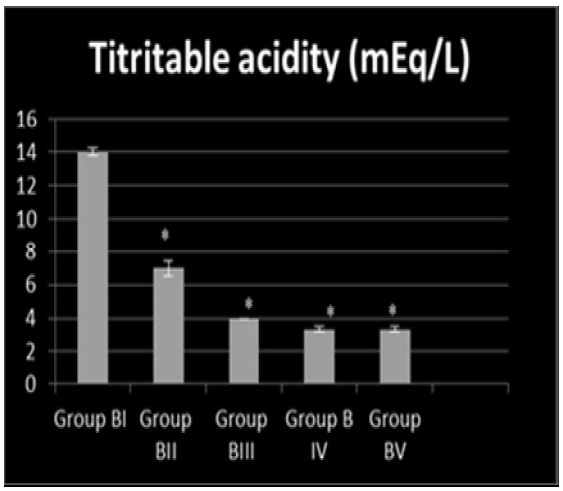
- A significant decrease in the mean volume of the gastric juice (1.2000 ± 0.04472 mL) as compared to the group received indomethacin (2.2833 ± 0.04014 mL).
- A significant decrease in the mean titritable acidity (3.3333 ± 0.14926 mEq/L) as compared to the group received indomethacin (7.0000 ± 0.44721 mEq/L).
- A significant decrease in the mean total acid output (0.0040 ± 0.00026 micro equivalent/3hrs) as compared to the group received indomethacin (0.0159 ± 0.00077 micro equivalent/3 hrs).

Effect of (i.p) administration of HBB (i.p) in a dose of 5mg/Kg ½ h before pyloric ligation and indomethacin administration :

- A significant decrease in the mean volume of the gastric juice (1.1667 ± 0.06146 mL) as compared to the group received indomethacin (2.2833 ± 0.04014 mL).
- A significant decrease in the mean titritable acidity (3.3333 ±0.16667 mEq/L) as compared to the group received indomethacin (7.0000 ± 0.44721 mEq/L).

- A significant decrease in the mean total acid output (0.0038 ±0.00009 micro equivalent/3hrs) as compared to the group received indomethacin (0.0159 ± 0.00077 micro equivalent/3 hrs).





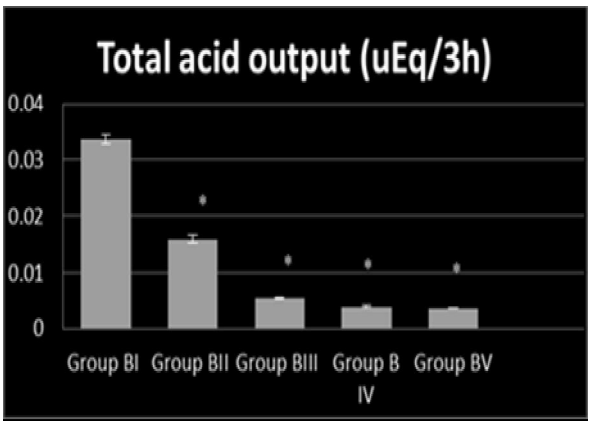


Fig (13)

Fig (14)

Fig (15)

Figure (13): Effects of (i.p) administration of various doses of HBB (1, 2.5 and 5 mg/kg) on the mean volume of gastric juice in pyloric ligated rats received indomethacin in a dose of 100 mg/kg.

Figure (14): Effects of (i.p) administration of various doses of HBB (1, 2.5 and 5 mg/kg) on titritable acidity in pyloric ligated rats received indomethacin in a dose of 100 mg/kg.

Figure (15): Effects of (i.p) administration of various doses of HBB (1, 2.5 and 5 mg/kg) on total acid output in pyloric ligated rats received indomethacin in a dose of 100 mg/kg.

Group BI: Rats received saline i.p. in a dose of 1mL/rat immediately before pyloric ligation.

Group BII: Rats received indomethacin in a dose of 100mg/Kg (i.p) immediately before pyloric ligation.

Group BIII: Rats received HBB i.p. in a dose of 1mg/Kg, ½ h before indomethacin administration (i.p) and pyloric ligation.

Group BIV: Rats received HBB i.p. in a dose of 2.5mg/Kg, ½ h before indomethacin administration (i.p) and pyloric ligation.

Group BV: Rats received HBB i.p. in a dose of 5mg/Kg, ½ h before indomethacin administration (i.p) and pyloric ligation.

Group BII was compared to the control **group BI. Groups BIII, BIV, and Group BV** were compared to **Groups BII.**

DISCUSSION

Our results showed that indomethacin administration in a dose of 100mg/kg (i.p) to pyloric ligated rats led to a nonsignificant decrease in gastric juice volume as compared to the control group. Our results are in contradictory to the result of *(Naito et al., 2008)*, who reported a marked reduction in gastric blood flow produced by drugs was accompanied by reduction of the volume of gastric secretion, but *(Biplab et al., 2011)* and *(Muhammed et al., 2012)*, reported that indomethacin have caused alternation in gastric secretions of rats.

Our results showed that indomethacin administration in a dose of 100mg/kg (i.p) to pyloric ligated rats led to a significant decrease in gastric acid concentration and gastric acid output as compared to the control group received saline (i.p). Our results are in controversy to the results of *(Filaretova et al., 2002)*, whom reported an increased gastric acid secretion in the occurrence of sever indomethacin induced stomach damage.

In our results, the decrease in gastric acid concentration, and gastric acid output induced by indomethacin administration was most probably due to a decrease in gastric blood flow *(Muhammed et al., 2012)*, the direct toxic effect on the oxyntic cells, induction of apoptosis and inhibition in epithelial cell proliferation in the ulcer margin *(Beck et al., 2000)*.

Our results showed that pretreatment by Hyoscin N-butylbromide in doses of 1mg/kg, 2.5mg/kg and 5mg/kg (i.p) to pyloric ligated rats received indomethacin

in a dose of 100mg/kg (i.p) led to a significant decrease in gastric acid concentration and gastric acid output as compared to the group-received indomethacin only.

Our results showed that pretreatment by Hyoscin N-butylbromide in doses of (1mg/kg, 2.5mg/kg and 5mg/kg) (i.p) to groups of rats subjected to restraint stress and indomethacin administration in a dose of 100mg/kg (i.p) led to a decrease in the ulcer index as compared to group of rats subjected to restraint stress and received indomethacin only with a protection ratio of 3%, 60.5% and 66.4% respectively .

Our results support the concept that most of the anti-ulcer drugs presently used as anticholinergic and anti-acid drugs are produced with the aim of reducing gastric acid secretion, and treatment of gastric ulcer by anticholinergic drugs as a result of gastric acid inhibition can be exemplified *(Zanatta et al., 2009;* and *Suleyman et al., 2010)*.

The parasympathetic blocker pirenzepine are used for the treatment of gastric ulcer and as a gastroprotective drug acting on offensive factors interfere with acid secretion *(Mahmood et al., 2010* and *Saravanan et al., 2011)*. Propanthocyanidine extracts of the black grape seeds (vitis vinifera), has a prophylactic effects against indomethacin – induced gastric ulceration in rabbits due to its anti-secretory activity (significant increase in gastric pH, a decrease in gastric juice volume, and total gastric acidity *(Anamad et al., 2014; Ingale et al., 2014* and *Abdul-Razek et al., 2015)*.

Our results are also in concomitant with the results of *(Abdul-Razek et al., 2015)*, whom reported that propanthocyanidine extracts of the black grape seeds (vitis vinifera), has a prophylactic effects against indomethacin – induced gastric ulceration due to its anti-secretory activity.

Hyoscin N-butylbromide clearly antagonized muscarinic receptor induced activation of the muscle, the epithelium and enteric neurones, and significantly reduced bethanecol-induced action potential in enteric neurones *(Krueger et al., 2013)*. Hyoscin N-butylbromide obviously reaches its target structures, i.e. muscarinic receptors of smooth muscle cells, parasympathetic ganglia, and / or possibly nicotinic receptors on enteric neurones *(Weiser and Just, 2009)*. Peripheral anticholinergic effects of Hyoscin N-butylbromide results from a ganglion – blocking action within the visceral wall, as well as from antimuscarinic activity *(Samuels, 2009;* and *Krueger et al., 2013)*.

Stomach ulceration reduced the gastric mucin content *(Adhikary et al., 2011)*. This might reduce the ability of the mucosal membrane to protect the mucosa from physical damage and back diffusion of hydrogen ions, and hinder epithetical recovery *(Adhikary et al., 2011)*. Inhibition of PGs caused decrease in mucin secretion then allows hydrogen ions and pepsin to diffuse into the mucosa from the lumen. So, back diffusion of acid and pepsin into the tissue stimulate more acid and pepsin secretion to cause more damage *(Abdallah et al., 2011)*. It is difficult to attribute the gastro toxic effects of indomethacin to only one factor, specially the inhibition of COX – 1 *(Suleyman et al., 2010)*.

By histopathologicl examination, our results showed (Figures 1, 2, 3) deep ulcers reaching the submucosal layer with loss of the superficial mucus in rats subjected to restraint stress and indomethacin administration.

(Suleyman et al., 2010) reported that the relationship between the increase of mucus secretion and gastroprotection is not considerable. But treatment with Black Tea (BT) and Thea – flavins (Tf) significantly accelerated ulcer healing, which was associated with an increase in the mucin content of the gastric mucosa. The test samples BT and Tf, restored the mucin level to normalcy *(Adhikary et al., 2011)*.

Our results showed that pretreatment by Hyoscin N-butylbromide in doses of 1mg/kg, 2.5mg/kg (i.p) ½ hr before restraint stress and indomethacin administration led to a partial preservation of the mucus layer lining the stomach, and pretreatment by Hyoscin N-butylbromide in a dose of 5mg/kg (i.p) ½ hr before restraint stress and indomethacin administration led to complete preservation of the mucus layer lining the stomach.

Our results are in concomitant with the results of *(Adhikary et al., 2011)*, whom reported that preservation of the mucus content of the gastric mucosa has a prophylactic effect against both restraint and indomethacin – induced gastric ulcers .

Acetylcholine is one of the most important neurotransmitters in the gut, since the vagus nerve and the sacral parasympathetic nerve, as well as the cholinergic entire neurones, play a key role in regulation gastrointestinal motility *(Auli et al., 2008; O'Donnell and Puri, 2009)*. Muscarinic M2 and M3 receptor were the muscarinic cholinergic subtypes distributed in the gut wall *(Tobin et al., 2009)*, especially in the circular and longitudinal muscular layer, which contributed to the regulation of gastrointestinal motility *(Harrington et al., 2010)*. The inhibitory effects of Buscopan were mainly responsible for the antagonism of muscarinic M2 and M3 receptors *(Zhang et al., 2016)*. It is relatively less effective on the stomach than intestine *(Ge et al., 2011;and Papadopoulos et al., 2014)*. Hyoscine N-butylbromide (HBB) block the action of acetylcholine at parasympathetic sites (both muscarinic and nicotinic receptors) in smooth muscle, and in secretory glands, it causes decreased motility of the gastrointestinal tract *(Zhang et al., 2016)*.

CONCLUSION

Intraperitoneal administration of HBB in doses of 1mg/Kg, 2.5mg/Kg and 5mg/Kg, to rats in a dose-dependent manner led to a prophylactic effect against stress induced gastric ulcers with a protection ratio (3%, 60.46% and 66.41%) respectively.

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دراسة تأثير عقار الهيوسين ن-بيوتيل بروميد على قرحة المعدة المحدثة بطريقة التعرض للإجهاد بالشد وحقن عقار الإندوميثاسين وكذلك على إفرازات المعدة في ذكور الجرذان البيضاءالبالغة

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خلفية البحث : يستخدم عقار الهيوسين ن-بيوتيل بروميد كمضاد لإفراز الكولين ومضاد للتقلصات لعلاج التقلصات المعوية او آلام الأمعاء المصحوبة بالتقلصات.

الهدف من البحث: دراسة تأثير العلاج بجرعات مختلفة من عقار الهيوسين ن-بيوتيل بروميد (١ و٢,٥ و٥ ملجم / كجم) على قرحة المعدة المحدثة بطريقة التعرض للإجهاد بالشد وحقن عقار الاندوميثاسين وكذلك على إفرازات المعدة في ذكور الجرذان البيضاءالبالغة.

مواد وطرق البحث: إستخدم في هذا البحث ستون من ذكورالجرذان البيضاء وقد تم تقسيم الجرذان إلى قسمين متساويين. وقد تم تقسيم كلا من القسميين الكبيرين الي أقسام خمسة متساوية.

النتائج: إعطاء عقار االهيوسين ن-بيوتيل بروميد بجرعة (١ ملجم / كجم) عن طريق الحقن الصفاقي (البريتوني) قبل الاجهاد بالشد وحقن عقار الاندوميثاسين مباشرة له تأثير وقائي على الغشاء المخاطي المبطن للمعدة والذي ظهر على هيئة نقص في معامل التقرح مقارنة بالمجموعة التي تم فيها الإجهاد بالشد مباشرة بعد حقن عقار الاندوميثاسين. وإعطاء عقار االهيوسين ن-بيوتيل بروميد بجرعات (٢,٥ و٥ ملجم / كجم) عن طريق الحقن الصفاقي (البريتوني) قبل الاجهاد بالشد وحقن عقار الاندوميثاسين مباشرة له تأثير وقائي على الغشاء المخاطي المبطن للمعدة والذي ظهر على هيئة نقص في معامل التقرح مقارنة بالمجموعة التي تم فيها الإجهاد بالشد مباشرة بعد حقن عقار الاندوميثاسين.

الاستنتاج: إعطاء عقار االهيوسين ن-بيوتيل بروميد بجرعات (١ و٢,٥ و٥ ملجم / كجم) لذكورالجرذان البيضاء البالغة يعمل كوقاية ضد حدوث قرح المعدة وتزاد نسبة الوقاية مع زيادة الجرعة المعطاة.