Original Article

Effect of Nicotine on the Structure of Gastric Mucosa of Adult Male Albino Rats and the Possible Protective Effect of Royal Jelly (Light and Scanning Electron Microscopic Study)

Abeer Fareed Abd El-Naeem

Department of Anatomy and Embryology, Faculty of Medicine, Sohag University, Egypt

ABSTRACT

Background: The smoking effects on human health are well known and its impact on vital organs can leads to death in many cases. There is a strong association between cigarette smoking and gastric lesion especially gastric erosions .Nicotine is the main component of cigarette, and it is the cause of most of gastrointestinal hazards from erosions to cancers. Royal jelly is one of the attractive ingredients of healthy food. It has also antioxidant effect against many toxins. Its anti-inflammatory role and its role in protection from erosions and ulcers is due to its components.

Aim of the Work: To examine the role of royal jelly in protection against lesions on gastric mucosa caused by nicotine intake.

Materials and Methods: Thirty rats were used for the study equally divided into 3groups: 1st group (10 rats) were used as a control, 2nd group (10 rats) were administrated nicotine I.P in a dose of 0.5 mg/kg body weight for 30 days , and 3rd group (10 rats) were administrated nicotine as previously +royal jelly with a dose of 300 mg/kg/ body weight every day orally for 30 days. Then they were dissected 24 hours after last dose, the stomach was prepared for examination with Haematoxylin and Eosin, Masson Trichome, Periodic Acid Schiff (PAS) stain and scanning electron microscope.

Results: The rats that were injected with nicotine showed multiple histolopathological changes: erosions on the surface epithelium, degeneration of cells and distortion of the shape of the gland. Collagen fibers are more in the nicotine group, and the thickness of mucosa decreased in this group. The covering mucous layer is defective in the nicotine group, rats treated with nicotine with royal jelly the stomach appeared nearly normal.

Conclusion: Nicotine is a toxic substance to the stomach causing histopathological changes, and the royal jelly ameliorates these changes.

Received: 29 October 2020, **Accepted:** 09 April 2021

Key Words: Nicotine, royal jelly, scanning, stomach, ulcer.

Corresponding Author: Abeer Fareed Abd El-Naeem, MD, Department of Anatomy and Embryology, Faculty of Medicine, Sohag University, Egypt, **Tel.**: +20 10157 70097, **E-mail:** abeerfareed_2013@yahoo.com

ISSN: 1110-0559, Vol. 45, No.2

INTRODUCTION

Although awareness of the hazards of smoking increased and the prevalence of smoking has decreased since the US Surgeon General's report on smoking in 1964, smoking continues to be a big public health problem[1].

Chronic nicotine intake increased the levels of ghrelin, gastrin and histamine but decreased prostaglandin E_2[2]. The toxic effect of nicotine inhalation on stomach is due to the previous factors, and heavy smokers are more liable to gastric/peptic ulcers[2].

Cigarette smoking continues to be the leading cause of death and it is the main risk factor for major diseases, such as chronic obstructive pulmonary disease[3]. Smokers who continue smoking may lose at least one decade of life expectancy[4]. Nicotine has been shown to delay ulcer healing in stress-induced, ethanol-induced or Helicobacter pylori-induced gastric injury in rats, as well as increased recurrence of duodenal ulcer[5].

It has been suggested that cigarette smoke induces ulcer formation and delays their healing through enhancement of secretion of acid and pepsin, production of free radicals and infiltration of neutrophils[6]. A reduction of the gastric blood flow and secretion of mucus, prostaglandin (PG)[7], as well as the reduction of ornithine decarboxylase activity and polyamine synthesis[8], are other reported causes.

Natural products are used recently as therapeutic agents for the treatment of various diseases due to their benefits and their minimal side effects[9].

Royal Jelly is honeybee secreted by hypo pharyngeal gland of young worker bee (Apis mellifera Linne)[10], and it is the only nourishment for the bee Queen. Recently, royal Jelly and its components have been subjected to extensive usage for several diseases. Also, it was reported that the crude royal jelly or its major proteins such as royalisin, 10-hydroxy-2- decenoic acid, and jelleines show a great effect on different types of bacteria, especially on Gram positive bacteria[11].

Royal jelly has a complex composition of fats, proteins, fatty acids, free amino acids, sterols, organic acids, phenols, mineral salts, sugars, vitamins, and other unknown substances[12,13].

 DOI: 10.21608/ejh.2021.48094.1382

RJ has been shown to exhibit promising anti-oxidant[14], anti-inflammatory[15], immunomodulatory[16], anti-tumor[17], anti-hypercholesterolemic[18], anti-microbial[19]and anti-allergic properties[20].

MATERIAL AND METHODS

Animals

Thirty adult male albino rats, aging 4 - 6 months and weighing 200 - 250 gms, were used for the study, they were obtained from the Animal House of Assiut Faculty of Medicine. Housed in Animal Facility at Sohag Faculty of Medicine, Egypt. All rats were given access for rodent chow diet and water. The experiment was performed following the recommendations of the "Guide for the Care and Use of Laboratory Animals" Institutes of laboratory Animal Research[21] and in accordance with the Guidelines of the University Animal Ethics.

Experimental design

The animals were randomly equally divided into three groups as follows.

Group I (Control Group): It was composed of 10 adult male albino rats. They were subdivided into 2 equal subgroups: Ia (Negative control): included five rats. They were kept without treatment and served as control for all the experimental groups and Ib (positive control): included five rats. Each ingested saline with the normal diet .

Group II (Nicotine exposure group): It included 10 adult male rats subjected to nicotine intraperitoneal (I.P). The rats were injected I.P. with nicotine tartrate at a dose of 0.5 mg, dissolved in 0.9% normal saline once daily /kg body weight for 30 consecutive days[22].

Group III (Nicotine and royal jelly): It included 10 adult male rats subjected to nicotine intraperitoneal as before + Royal Jelly 300 mg/kg/day orally for 30 days (available in the form of tablets (Royal Vit) 1000 mg soft gelatin capsules from SEDICO pharmaceutical industries, Egypt)[23].

24 hours after the last dose, the rats were anesthetized, sacrificed, carefully dissected and specimens from the stomach were taken.

Preparation of the specimens for light microscopic examination: The specimens were fixed using perfusion fixation. They were fixed in 10% neutral buffered formalin and prepared for light microscopic study[24]. Paraffin sections of 6µm thickness were obtained for Haematoxyline and Eosin (H&E), Periodic Acid Schiff (PAS) and Masson trichrome stains.

Scanning electron microscope examination

The mucosal surface of the stomach were washed with normal saline, rinsed with cocodylate buffer and placed in 2.5%glutaraldehyde.Then they were prepared for scanning electron microscope[25]. The specimens were examined with a Jeol-JSM-3400 scanning microscope in the Electron microscope Unit of Assiut University.

Ulcer index

The number and severity of ulcers were counted by using the magnifying glass.

Score of severity[26,27]:

- normal color : 0.
- Reddish color: 0.5.
- spot ulcer 1.0.
- small ulcer with hemorrhage 1.5.
- deep ulcer 2.0 .
- perforations 3.0.

Ulcer index[28] = (UN + US+ UP) x 1/10

- UN = Average of number of ulcer per animal
- US = Average of severity score
- UP = Percentage of animal with ulcer.

Morphometric study

Collagen quantification: Semi-automated image analysis was applied[29] from each Masson's trichrome stained section, 5 random fields for each group were selected and imaged using an objective lens magnification of 10x. Image J software (version 1.5, Wayne Rasband, National Institutes of Health, USA) was used for the analysis.

Mucosal thickness: The thickness of the gastric mucosa was done using (Image Analyzer Computer System) digitizer version 4.3.They were measured on H&E stained sections using an objective lens magnification of 10[30].

For each measured parameter, five non overlapped fields from each slide were used. Five slides were selected from each animal. The mean for each animal was calculated then the mean for each group was estimated

Statistical Analysis

Statistical analysis in each group, the numerical data in the form of gastric mucosal thickness, and area % of collagen were estimated and expressed as mean ± SD. One way ANOVA test was used to calculate the statistical differences between the groups using Statistical Package for the Social Science Software, version 16 (SPSS, Inc., Chicago, USA). Followed by LSD as a post-hoc test. In all statistical analysis, *P value* ≤0.05 was considered statistically significant[31].

RESULTS

Light microscopic results

No difference was seen between group Ia and Ib so they both considered as control group.

Control group (group I): H&E stained sections on the mucosa showing a normal appearance of the gastric mucosa, fundic glands are seen perpendicular on the

surface. The gland consists of an isthmus, a neck and a base. Gastric pits are seen invaginating the glands and open into the lumen (Figure 1).

By high magnification

Surface mucous secreting cells which appeared as columnar cells with pale cytoplasm and basal oval nuclei, Each fundic gland is seen consisting of: Parietal cells appeared large polyhedral with acidophilic cytoplasm and central rounded nuclei with prominent nucleoli. Mucous neck cells (columnar cells with pale cytoplasm and round nuclei) appeared between parietal cells in the neck of the gland. The chief cells (peptic) basally located and appeared low columnar with basophilic cytoplasm and basally located nuclei, giving the basal part of the gland its dark appearance (Figures 2,3).

Nicotine group (group II): By H&E there were multiple areas of ulceration in the mucosa ranging from small erosion due to loss of surface mucous cells to a deep ulcer reaching to lamina propria (Figures 4a,4b). With high magnification, most of surface mucous cells appeared degenerated with pyknotic nuclei ,at the base of the gland parietal and chief cells appeared degenerated and a detached from the basement membrane, there was also a distortion to the shape of the gland (Figures 5,6).

Nicotine+royal (group III): By H&E The glands appeared nearly normal (Figure 7). With high magnification the cells appeared nearly like the control group (Figures 8,9) .

With Masson Trichome stain

Control group appeared with few amount of collagen (Figure10)

Collagen fibers in group II increased in lamina propria (Figure 11) more than any other group. Royal jelly intake in group III preserved its normal appearance (Figure 12).

With PAS stain: In the control group there is a strong positive reaction on the surface of the gland and filling pits, a positive reaction is seen also on the neck of the gland (Figure 13). In group II there is interrupted week reaction on the surface of the gland in comparison with the control (Figure 14). In group III the gland preserved its normal shape with a strong positive reaction (Figure 15).

Scanning electron microscopic examination

In the control group (group Ia and Ib as there was no difference found between them): the mucosa shows a normal velvety appearance with numerous longitudinal folds or rugae with normal gastric pits (Figure 16). With high magnification, a healthy epithelium appears surrounding the normal pits (Figure 17). In group II (nicotine I.P): ulcers appeared extending deep in the mucosal layer. Complete shedding of the surface mucosal cells leaving the walls and also the opening of gastric pits giving the picture of a honeycomb (Figure 18) . The epithelium appeared destructed with high magnification (Figure 19). In group III (nicotine +royal jelly) the mucosa appeared normal like control group (Figures 20,21).

Ulcer Index

In group II (nicotine) the index was (110.217) which was higher than group 3 (nicotine+royal) (20.06).

24% of ulcers in the nicotine group were of grade 2 (deep ulcer) while 76% were of grade1 (spot ulcer).

Morphometric study

Mucosal thickness: (Graph 1) the mean thickness of control group I was 6.0312±70.68582 pixel, while the mean thickness of group II (nicotine) was 3.6962E2±130.56113 pixel, there is a very highly significant decrease in thickness from the control to the nicotine group ($p \leq .000$), the thickness in group III (nicotine I.P +royal jelly) was 6.7440E2 ± 55.37147 pixel, with a very high significant increase from group II ($p \leq .000$), but there is a highly significant increase of group III more than the control ($p \leq .003$) (Chart 1).

With ANOVA, test there is a very highly significant change between groups ($p=0.000$).

Collagen percent: (Graph 2): The mean collagen percent of control group is 3.8200± 1.27118%, there is very highly significant increase in the percent in group II 8.0200±.60444 % than control $p \leq .003$.There is a non-significant decrease in collagen percent in group III 7.7220±.50791 than group II: $p \leq .4$. But, there is a highly significant increase in the royal group than the control. $p \leq .000$ (Chart 2).

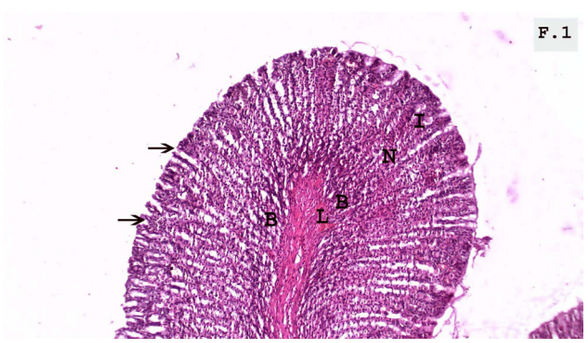


Fig. 1: A photomicrograph of a longitudinal section of the gastric mucosa showing the normal appearance of long tubular glands formed of isthmus (I), neck (N) and base (B).Gastric pits opening in the lumen appears as (arrows). Lamina propria is also seen (L) (H&E ×100).

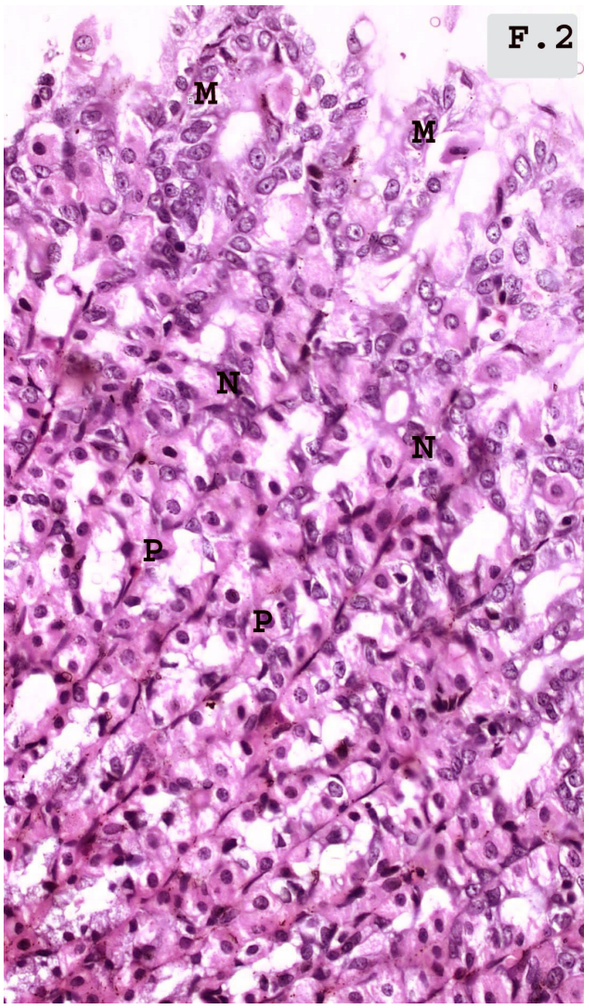


Fig. 2: High magnification of a section in the fundic gland of the stomach of the control group showing normal appearance mucous surface columnar epithelium (M).mucous neck cells(N) and Parietal cells (P) (H &E x 400).

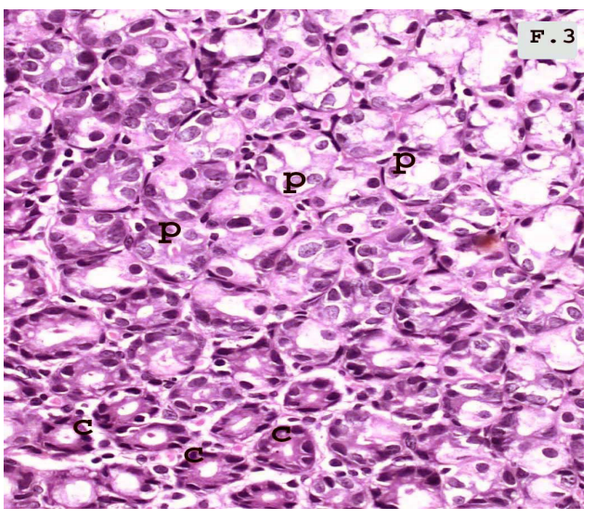


Fig.3: A photomicrograph of the base of fundic glands of control group showing more chief cells(c),and less parietal cells. (H&E ×400).

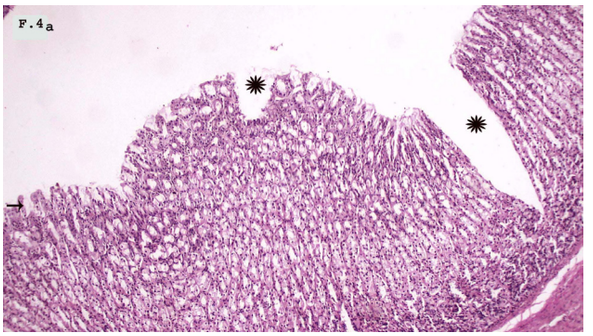


Fig. 4a: A photomicrograph of a longitudinal section of gastric mucosa of group 2 (Nicotine) showing erosion on the surface (arrow) superficial ulcer reaching the neck and deep ulcer down to the base of the gland(stars) (H&E ×100).

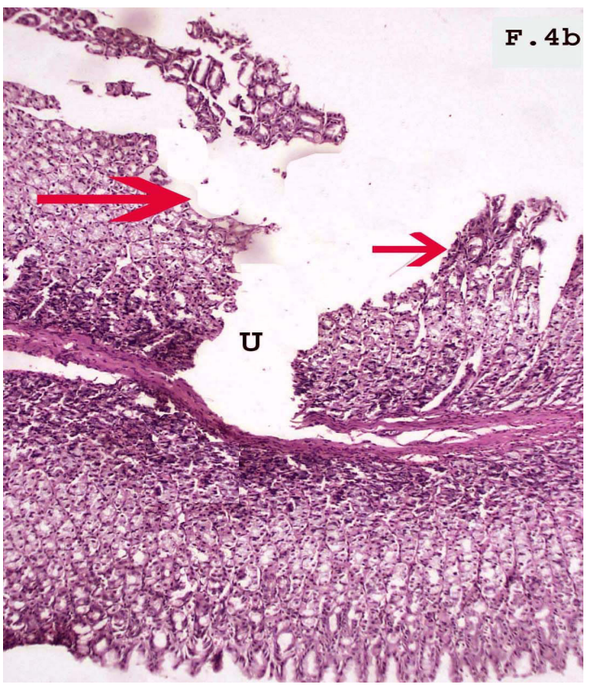


Fig. 4b: A photomicrograph of a longitudinal section of gastric mucosa of group II (Nicotine) showing deep ulcer reaching the lamina propria(U),the tissue around the ulcer appeared destructed (arrows) (H&E ×100).

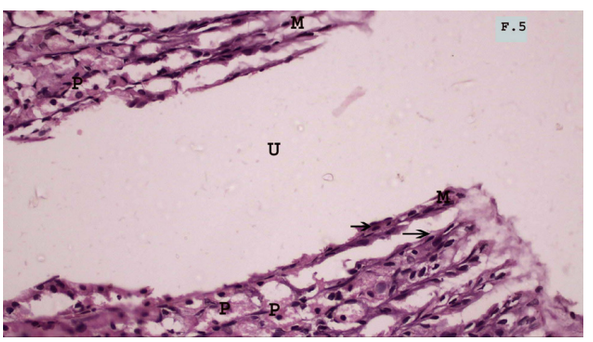


Fig. 5: High magnification of gastric gland of the nicotine group showing a deep ulcer (U)., degeneration of mucous cells (M) with pyknotic nuclei(arrows)appeared.Parietal cells(p) on the edges of the ulcer appeared degenerated and detached from basement membrane. (H&E×400).

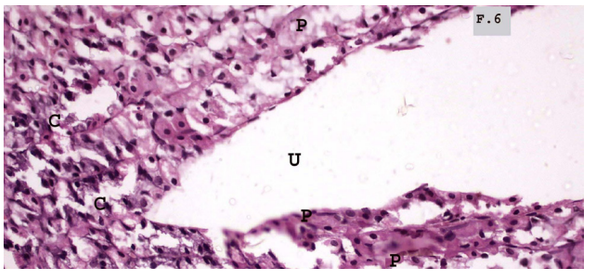


Fig. 6: A photomicrograph of a section of the base of the gland showing the ulcer reaching base of the gland (U), degeneration of parietal(p) and chief cells(c),with distortion of the shape of the gland are seen. (H&E×400).

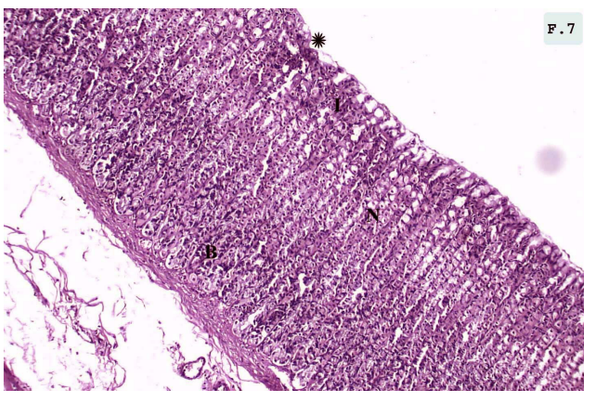


Fig. 7: A photomicrograph of a longitudinal section of gastric glands of group III treated with nicotine and royal showing regularly arranged fundic gland with normal isthmus (I) ,neck (N)and base(B). Small superficial erosion is seen (star) (H & E x 100).

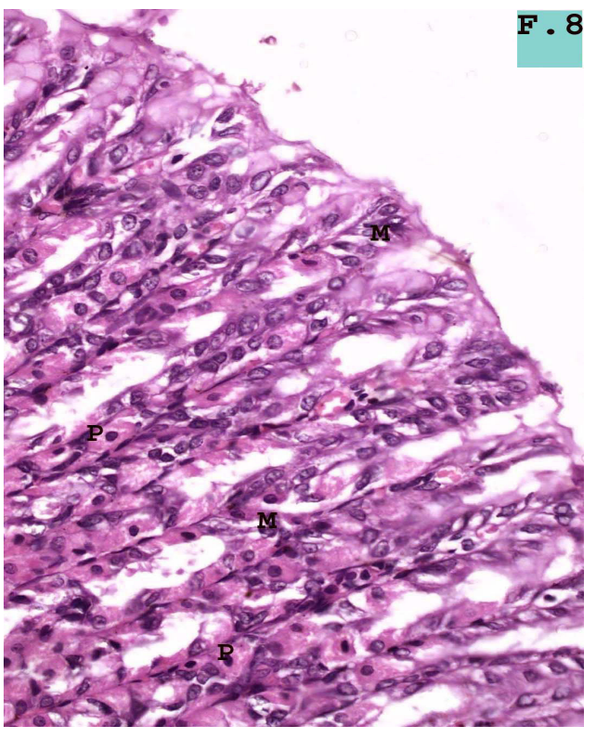


Fig. 8: High magnification of group III showing nearly like normal appearance of mucous cells (M) and parietal cells (p). (H&E×400).

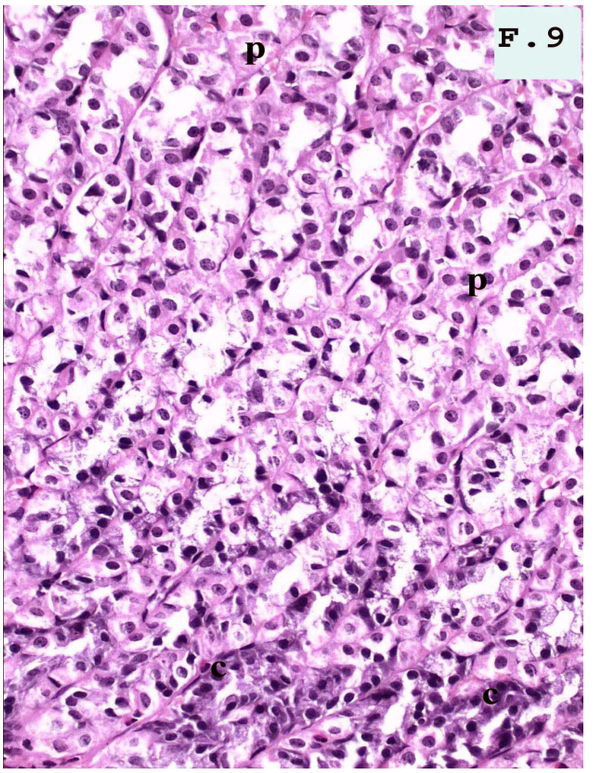


Fig. 9: A photomicrograph of a longitudinal section of bases of gastric glands. Parietal (p) and chief cells(c) appeared nearly like the control group. (H&E×400).

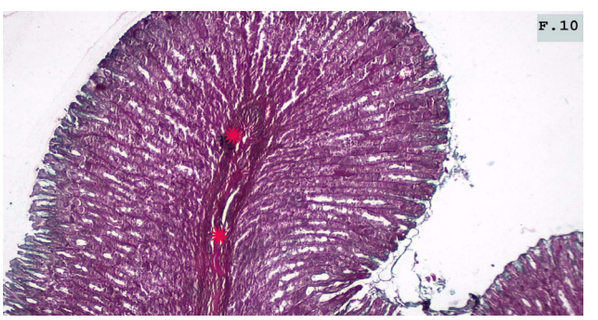


Fig. 10: A photomicrograph of a longitudinal section of the gastric mucosa of the control group showing lamina propria with few collagen fibers (stars) (Masson ×100).

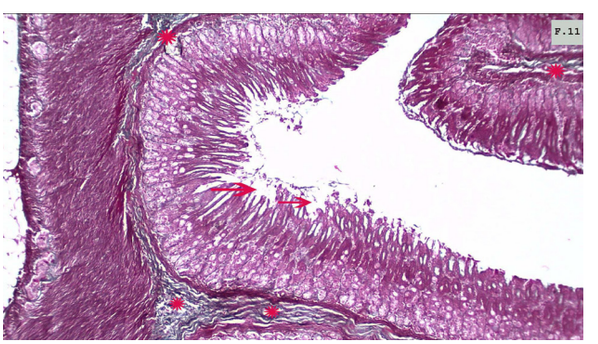


Fig. 11: A photomicrograph of a longitudinal section in the gastric mucosa of the nicotine group showing an increase in the collagen fibers in the lamina propria (stars), notice erosions on the surface (arrows) (Masson×100).

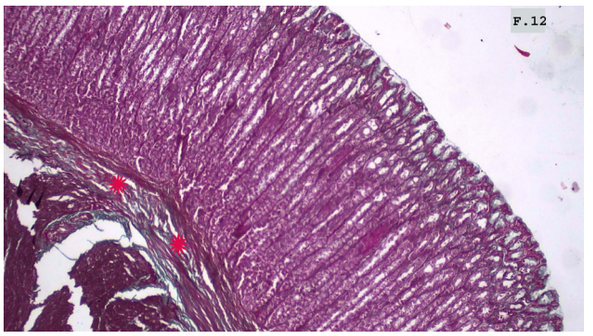


Fig. 12: A photomicrograph of a longitudinal section in the gastric mucosa of the Royal group showing less collagen fibers than the nicotine group (stars) (Masson ×100).

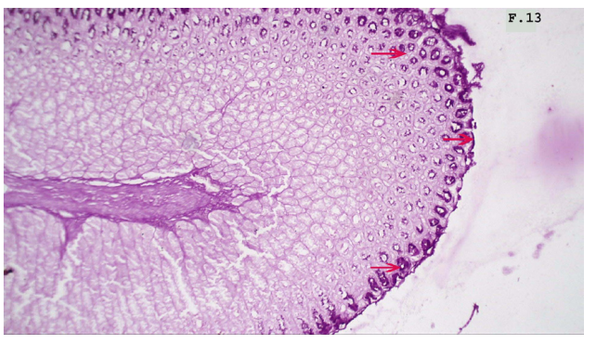


Fig. 13: A photomicrograph of a longitudinal section of the gastric mucosa in the control group showing: a strong positive reaction on the surface of the gastric glands and filling the pits, a moderate reaction on the neck is also noticed (arrows). (PAS×100).

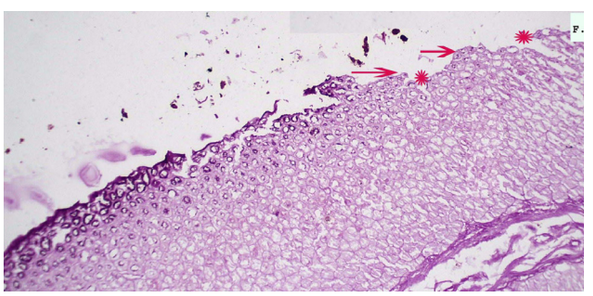


Fig. 14: A photomicrograph of a longitudinal section of the gastric mucosa in the nicotine group showing a thin interrupted mucous film on the surface and neck (arrows). Notice erosions on the surface (stars). (PAS×100).

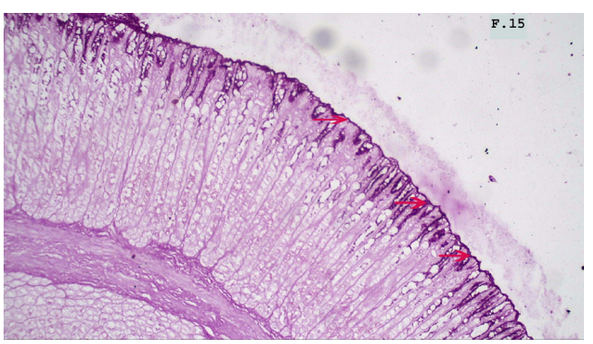


Fig. 15: A photomicrograph of a longitudinal section of the gastric mucosa in the Royal group showing near normal appearance of mucous film on the surface (positive reaction) extending to the neck (arrows) (PAS×100).

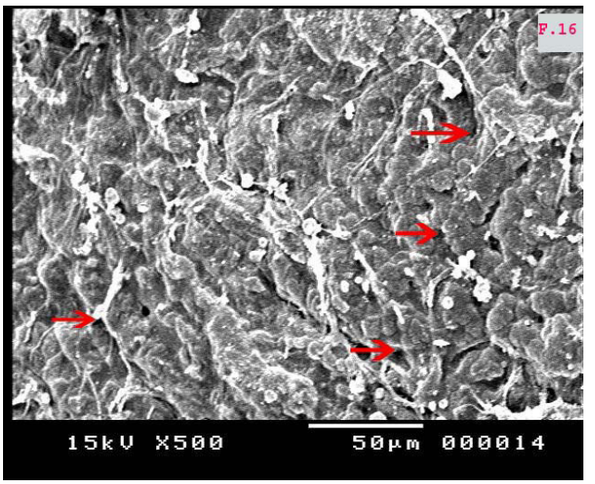


Fig. 16: A scanning electron photomicrograph of the gastric mucosa of the control group: showing a normal velvety appearance of the surface with pits which invaginated into it (arrows). (×500).

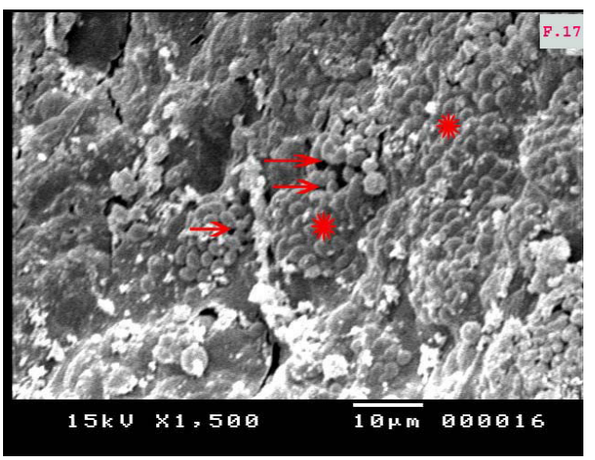


Fig. 17: A scanning electron micrograph of the control group showing intact cells (stars) with an intact pit (arrows) (×1.500).

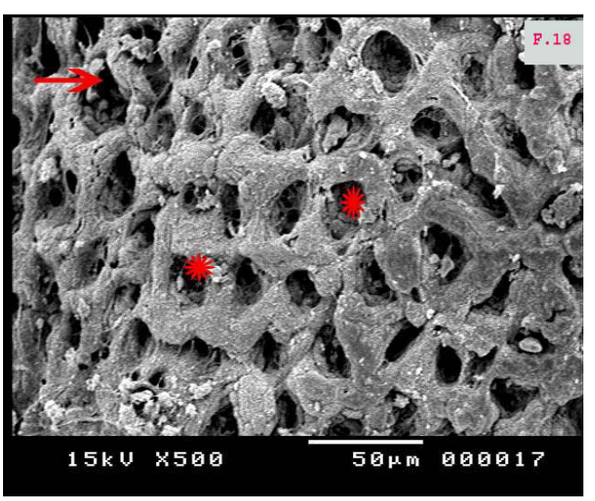


Fig. 18: A scanning electron micrograph of the nicotine group showing wide pits (stars) and a shedding of epithelium (arrows) giving a honeycomb appearance (×500).

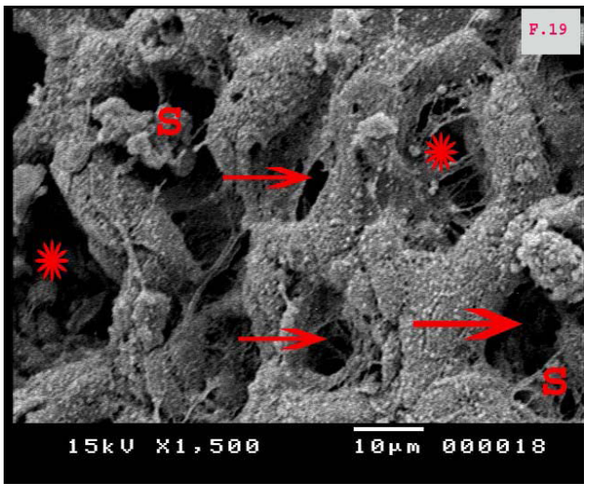


Fig. 19: High magnification of mucosa showing destruction of the mucosa with wide pits (arrows),areas of ulcers (stars) ,and the surface epithelium appears destructed (S) (×1.500).

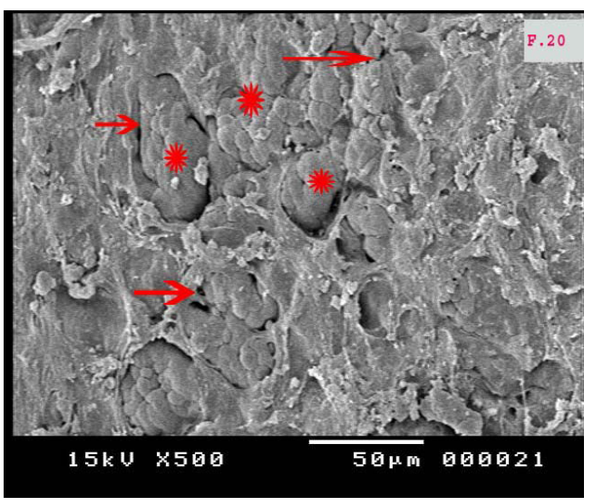


Fig. 20: A scanning electron microscope of gastric mucosa after royal intake showing a healthy surface (stars) with normal pits (arrows)(×500).

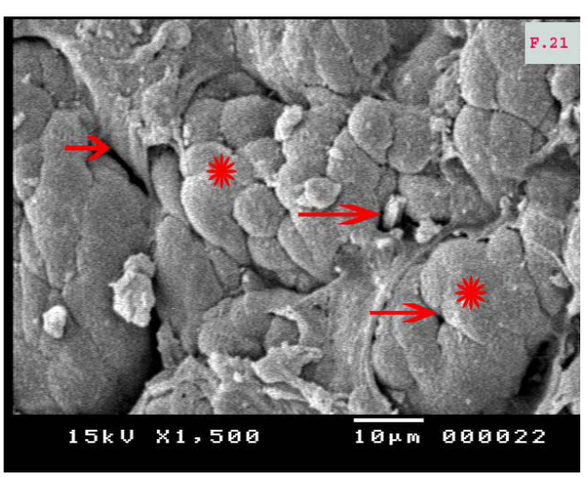
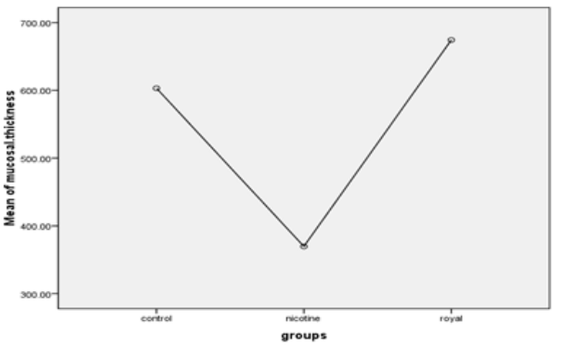
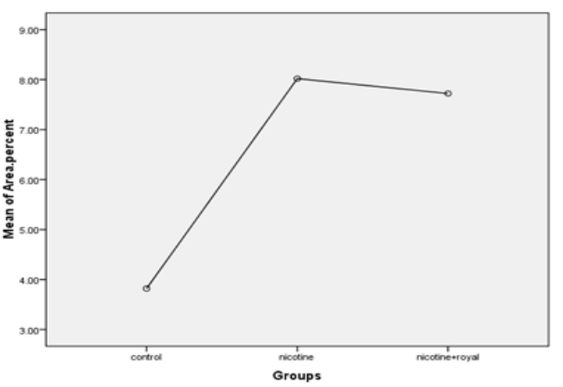


Fig. 21: High magnification of the gastric mucosa showing healthy epithelial cells (stars) with intact pits (arrows). (×1.500).



Graph 1: Mucosal thickness between the different groups



Graph 2: Collagen percent between the different groups

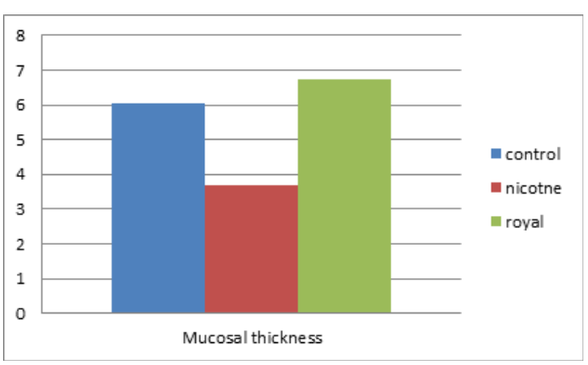


Chart 1: Mucosal thickness between the different groups

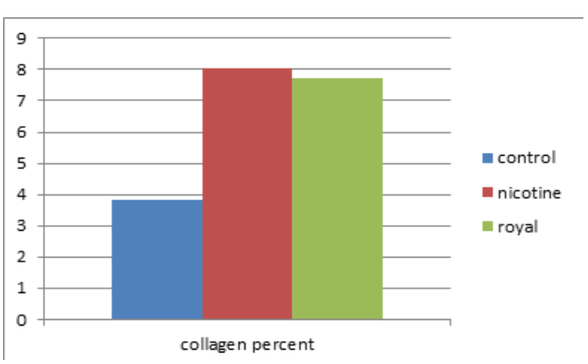


Chart 2: Collagen percent between the different groups

DISCUSSION

The main factor in gastric ulcer formation is s defect in the balance between the defensive factors such as mucus-bicarbonate phospholipid barrier which constitutes the first line of mucosal defense[32]. This barrier is formed of phospholipids and bicarbonate which protect the gastric mucosa against penetration of gastric acid , pepsin and any other proteolytic agent[33,34,35].

Injection of I.P nicotine to the experimental rats in the current study resulted in gastric ulceration, and shedding of the superficial epithelial cells. The cells became degenerated.

Nicotine causes ulcerogenesis which are difficult to heal[36] ndue to many factors such as inhibiting prostaglandins production, and increasing the production of free radicals reactive oxygen species but gastric acid secretion is regarded as one of the major aggressive factors in ulcer formation[37].

The nicotine-induced free radicals react with bio membranes causing oxidative destruction of polyunsaturated fatty acids .This resulted in pathogenesis of a number of diseases[38,39]. Gastric and intestinal metaplasia is one of the major hazards of nicotine as reported by Morais *et al*.,[40].

I.P. nicotine injection could also be attributed to stimulation of gastrin-secreting cells in and outside the stomach compared with the control group. Nicotine cause elevations in circulating adrenal catecholamine concentrations, as it was reported by other researchers[2].

Wong *et al*.,[41] found that 10 days of nicotine administration decreased the mucous in mucosal layer in a dose-dependent manner in rats, suggesting that nicotine in cigarette smokers could decrease the synthesis of mucous ,and so affect the defensive mechanism of the stomach.

Other studies revealed that cigarette smoke and nicotine reduced the levels of epidermal growth factor and prostaglandins, which they are the factors essential for the repair of gastric ulcers[42]. Furthermore, nicotine causes a reduction in the gastric mucosal blood flow and the mucus volume[43,44].

Royal jelly administration showed a protective effect against most of ulcers, this was agreed with[13] who proved that royal jelly (dose dependent) improved gastric ulcers done by omeprazole. Also Belostotskiĭ *et al*.[45] revealed that royal jelly has a healing effect on different ulcers. Mostafa *et al*., showed that RJ decreased ulcers in GIT caused by diclofenac[23]. Kaynar *et al*., described the efficacy of RJ against methotrexate-induced small intestine damage in rats[46].

In this study collagen amount which appeared in inflammation increased in nicotine group as seen by masson stain. This means more inflammation in this group. This was agreed by Ali *et al*., who studied effect of nicotine on coronaries and said that inflammation which appeared by masson stain increased with nicotine[47]. Nada *et al*., studied the effect of aspirin on gastric mucosa revealed that collagen fibers increased as a response of the cells to inflammatory effect of aspirin , while it showed significant decrease after using Platelet Rich Plasma which mean less fibrosis and more regeneration[48].

Ghonimi *et al*., who studied the protective effect of RJ against tartrazine induced ulceration and found that the groups treated with RJ its results were nearly normal in Masson stain[49].

On the other hand, Liu *et al*., who studied the gastroprotective effects of chebulagic acid against ethanol-induced gastric injury revealed that with Masson stain, the collagen fibers decreased sharply as an inflammatory response in mucosa to ethanol, while the fibers increased as a reverse to damage of mucosa and its micro vessles after using of chebulagic acid[50].

The periodic acid-Schiff (PAS) staining results showed a week reaction (a decrease of the mucous film) in the gastric glands of the rats injected I.P nicotine approving its necrotic effect on the mucous secreting cells, while the group treated with the royal jelly has a positive reaction (a well mucosal film) which suggests the gastro-protective activity of it.

These results are in agreement with several other studies that showed a significant increase of gastric mucus in rats which were treated with the various natural compounds against agents the induced gastric mucosal injury[51].

These results were also in line with those of[52] who demonstrated that RJ protected the mucosa of the colon against acetic acid-induced injury .

Scanning electron microscope showed that rats received nicotine showed ulcers ,this is agreed with Ali *et al*., who showed that erosions and loss of epithelium appeared with oral or I.P injection of nicotine[2].

The ulcer index was used to evaluate the severity of the ulcer. The ulcer index was higher in nicotine group in comparison with royal group, which indicate the ulcerative effect of nicotine, and the protective effect of royal against ulcers.

In agreement with this Mostafa *et al*., who count ulcers induced by diclofenac and explained that the number of ulcers decreased in rats treated by royal jelly[23].

RJ is a natural product which has protective components against gastric lesions especially ulcers. The phenolic compounds of RJ induce synthesis of prostaglandin E_2 (PGE2), which is in contrary declined mucin liked glycoproteins, via mucus secretion activities[53].

It has been reported that, the RJ appears to have a stimulant effect on gastric mucosa .One of the unique compounds of royal jelly is a hydroxydicanoic unsaturated trans fatty acids, which may act as a neurotrophic factor. This compound may possibly have this proliferative effect on the gastric mucosa[54].

CONCLUSION

Nicotine has a toxic effect on the gastric mucosa, and the royal jelly has a protective role against it . More studies are needed on effect of royal jelly against different toxins on different organs.

CONFLICT OF INTERESTS

There are no conflicts of interest.

REFERENCES

1. Smith TT, Rupprecht LE, Denlinger-Apte RL, Weeks JJ, Panas RS, Donny EC, Sved AF. Animal .Research on Nicotine Reduction: Current Evidence and Research Gaps. Nicotine & Tobacco Research, 2017; 19(9):1005–1015.
2. Ali SS, Hamed EA, Ayuob NN, Ali AS and Suliman MI. Effects of different routes of nicotine administration on gastric morphology and hormonal secretion in rats. Experimental Physiology, 2015; 100 (8): 881–895.
3. Kotz D, Simpson C, Viechtbauer W, vanSchayck OC, West R and Sheikh A. Cardiovascular and neuropsychiatric safety of varenicline and bupropion compared with nicotine replacement therapy for smoking cessation: study protocol of a retrospective cohort study using the Q Research general practice database. BMJ, 2014; 4(8):28.
4. Jha P, Ramasundarahettige C, Landsman V, Rostron B, Thun M, Anderson RN, McAfee T and Peto R. 21st-century hazards of smoking and benefits of cessation in the United States. The New England Journal of Medicine, 2013; 368: 341–350.
5. Zhang Z, Zou YY, Zhou Y, Zhou H and Li YJ. The aggravatory effect of nicotine on Helicobacter pylori-induced gastric mucosa injury: role of asymmetric dimethylarginine. Journal of Clinical Gastroenterology, 2009;43: 261–266.
6. Chow JY, Ma L and Cho CH. Involvement of free radicals and histamine in the potentiating action of cigarette smoke exposure on ethanol-induced gastric mucosal damage in rats. Free Radical Biology & Medicine – Journal, 1998; 24: 1285–1293.
7. Ma L, Wang WP, Chow JY, Yuen ST and Cho CH. Reduction of EGF is associated with the delay of acetic acid-induced gastric ulcer healing by cigarette smoking. American Journal of Physiology-Gastrointestinal and Liver Physiology 2000; 278: 10–17.
8. Shin VY, Liu ES, Koo MW, Wang JY, Matsui H and Cho CH. Cigarette smoke extracts delay wound healing in the stomach: involvement of polyamine synthesis. Experimental Biology and Medicine, 2002; 227: 114–124.
9. Khan, RA. Natural products chemistry: the emerging trends and prospective goals. Saudi Pharmaceutical journal, 2018; 26(5): 739-753.
10. Ahmed WM, Khalaf AA, Moselhy WA, Safwat GM: Royal jelly attenuates azathioprine induced toxicity in rats. Environmental Toxicology and Pharmacology, 2014; 37 (1): 431–437.
11. Fratini F, Cilia G, Mancini S, and Felicioli A. Royal Jelly: An ancient remedy with remarkable antibacterial properties. Journal of Food Science and Technology, 2016;192:130-141.
12. Viuda-Martos M, Ruiz-Navajas Y, Fernández-López J, and Pérez-Alvarez JA. Functional properties of honey, propolis, and royal jelly. Journal of Food Science and Technology, 2008; 73:117-124.
13. Sofiabadi M and Samiee-Rad F. Royal jelly accelerates healing of acetate induced gastric ulcers in male rats. Research Institute for Gastroenterology and Liver Diseases, 2020; 13(1):14-22.
14. Abdel-Hafez SMN, Rifaai RA, and Abdelzaher WY. Possible protective effect of royal jelly against cyclophosphamide induced prostatic damage in male albino rats; a biochemical, histological and immuno-histo-chemical study. Biomedecine and pharmacotherapie, 2017; 90: 15–23.
15. Karaca, T., Simsek, N., Uslu, S., Kalkan, Y., Can, I., Kara, A., *et al*. The effect of royal jelly on CD3(þ), CD5(þ), CD45(þ) T-cell and CD68(þ) cell distribution in the colon of rats with acetic acid-induced colitis. Allergologia et Immunopathologia, 2012; 40 (6): 357–361.
16. El-Nekeety AA, El-Kholy W, Abbas NF, Ebaid A, Amra HA, and Abdel-Wahhab MA. Efficacy of royal jelly against the oxidative stress of fumonisin in rats. Toxicon – The International Society on Toxinology, 2007; 50 (2): 256–269.
17. Zhang S, Nie H, Shao Q, Hassanyar A, and Su S. RNA-Seq analysis on effects of royal jelly on tumour growth in 4T1-bearing mice. Journal of Functional Foods, 2017; 36: 459–466.
18. Ibrahim A. Immunomodulatory effects of royal jelly on aorta CD3, CD68 and eNOS expression in hypercholesterolaemic rats The Journal of Basic and Applied Zoology, 2014; 67: 140–148.
19. Chan Q, Melathopoulos A, Pernal S, and Foster L.Theinnate immune and systemic response in honey bees to abacterial pathogen, Paenibacillus larvae. (2009): BMC Genomics, 2009; 10: 387.
20. Oka H, Emori Y, Kobayashi N, Hayashi Y, and Nomoto K. Suppression of allergic reactions by royal jelly in association with the restoration of macrophage function and the improvement of Th1/Th2 cell responses. International Immunopharmacology, 2001; 1 (3): 521–532.

21. Institute Of Laboratory Animal Resources: Commission on Life Science, National Research Council of national academies. Guide for the Care and Use of Laboratory Animals. 18th ed. National Academy press, Washington, D.C; 2011 Pp, 11-55.
22. Azad FA, Nejati V, Shalizar-Jalali A, Najafi G, and Rahmani F. Antioxidant and anti-apoptotic effects of royal jelly against nicotine-induced testicular injury in mice. Environmental Toxicology and Chemistry, 2019; 34:708–718
23. Mostafa RE, El-Marasy SA , Abdel Jaleel GA, and Bakeer RM. Protective effect of royal jelly against diclofenac-induced hepato-renal damage and gastrointestinal ulcerations in rats Heliyon, 2020; 6:1-9.
24. Bancroft J, Stevens A, Turner D. Theory and Practice of Histological Techniques. Churchill Livingstone, New York, 1996; pp: 766.
25. Dykstra MJ and Reuss LE. Biological electron microscopy: theory, techniques, and troubleshooting. 2nd ed. New York: Library of congress, 2003; Pp, 74-100.
26. Sabiua S, Garuba T, Sunmonuc T, Ajani E, Sulymana A, Nuraina I, Balogun A. Indomethacin-induced gastric ulceration in rats: Protective roles of Spondias mombin and Ficus exasperate. Toxicology Reports, 2015; 2:261–267.
27. Dashputre N and Naikwade N. Evaluation of anti-ulcer activity of methanolic extract of Abutilon indicum Linn leaves in experimental rats. International Journal of Pharmaceutical Sciences and Research, 2011; 3:97-100.
28. Ozbakis GD and Gursan N. Effects of Momordica charantia L. (Cucurbitaceae) on indomethacin-induced ulcer model in rats. Turkish Journal of Gastroenterology, 2005; l16: 85-88.
29. Schipke J, Brandenberger C, Rajces A, Manninger M, Alogna A, Post H *et al*. Assessment of cardiac fibrosis: a morphometric method comparison for collagen quantification. Journal of Applied Physiology, 2017; 122 (4):1019-1030.
30. Acipayam C, Bayram I, Daglioglu K, Doran F, Yilmaz S, Sezgin G, Ateş BT, Ozkan A, and Tanyeli A. The protective effect of hesperidin on methotrexate-induced intestinal epithelial damage in rats: an experimental study. Medical Principles and Practice, 2013; 23:45–52
31. Peat J and Barton B. Medical Statistics. A Guide to data analysis and critical appraisal. First Edition. Wiley-Blackwell. 2005.
32. Laine, L, Takeuchi K, and Tarnawski A. Gastric mucosal defense and cyto-protection: bench to bedside. Gastro-enterology, 2008; 135(1):41-60.
33. Arab H, . Salama S, Omar H, Arafa E ,and Maghrabi I. Diosmin protects against ethanol-induced gastric injury in rats: novel anti-ulcer actions. PLos One, 2015; 10 (3):1-21.
34. Thomas D, Govindhan S, Baiju E, Padmavathi G, Kunnumakkara A, and Padikkala J. Cyperus rotundus L. prevents non-steroidal anti-inflammatory drug-induced gastric mucosal damage by inhibiting oxidative stress. Journal of Basic and Clinical Physiology and Pharmacology, 2015; 26: 485–490.
35. Allen A, and Flemstrom G. Gastroduodenal mucus bicarbonate barrier: protection against acid pepsin. American Journal of Physiology,2005; 288: 1-19.
36. Li LF, Chan RLY, Lu L, Shen J, Zhang L. Wu W, Wang L, Hu T, Li MX,and Cho CH. Cigarette smoking and gastrointestinal diseases: The causal relationship and underlying molecular mechanisms (Review). International Journal of Molecular Medicine – Spandidos, 2014; 34 (2): 372-380.
37. Wu WK, and Cho CH. The Pharmacological Actions of Nicotine on the Gastrointestinal Tract. Journal of Pharmacological Sciences, 2004; 94: 348 – 358.
38. Niki, E. Lipid peroxidation: Physiological levels and dual biological effects. Experimental and Molecular Pathology, 2009; 47: 469-484.
39. Al Anany MGE, Kamal AM, and El Saied K. Effects Of Curcumin And/Or Quercetin On Nicotine-Induced Lung And Liver Toxicity In Adult Male Albino Rat. AL-Azhar Assuit Medical Journal, 2015; 13(2):92-102.
40. Morais S, Rodrigues S, Amorim L, Peleteiro B and Lunet N. Tobacco smoking and intestinal metaplasia: systematic review and meta-analysis. Digestive and Liver Disease, 2014; 46:1031–1037.
41. Wong D, Koo MW, Shin VY, Liu ES, and Cho CH. Pathogenesis of nicotine treatment and its withdrawal on stress-induced gastric ulceration in rats. European Journal of Pharmacology, 2002; 434: 81–86.
42. Maity P, Biswas K, Roy S, Banerjee RK, and Bandyopadhyay U. Smoking and the pathogenesis of gastroduodenal ulcer—recent mechanistic update. Molecular and Cellular Biochemistry, 2003; 253: 329–338.
43. Thomas GA, Rhodes J, and Ingram JR .Mechanisms of disease: nicotine—a review of its actions in the context of gastrointestinal disease. Clinical Practice Gastroenterology and Hepatology, 2005; l2: 536–544.
44. Chu K, Cho CH, and Shin VY. Nicotine and gastrointestinal disorders: its role in ulceration and cancer development. Current Pharmaceutical Design, 2013;19: 5–10.

45. Belostotskiĭ NI, Kas'ianenko VI, Dubtsova EA,and Lazebnik LB:Influence of honey, royal jelly and propolis on accelerating acetate healing of experimental gastric ulcers in rats. Eksperimental'naija i Klinicheskaija gastroenterologiija , 2009;6:46-50.

46. Kaynar, L., Cetin, A., Hacioglu, S.K., Eser, B., Kocyigit, I., Canoz, O., *et al*., Efficacy of royal jelly on methotrexate-induced systemic oxidative stress and damage to small intestine in rats. The African Journal of Traditional, Complementary and Alternative Medicines, 2012;9 (3): 412–417.

47. Ali SS , Alama MN , HuwaitEA , Abed AS , and Karrouf G. Effect of nicotine-caffeine interaction on rats coronaries and cardiac muscle. Journal of Integrative Cardiology, 2017; 3(2): 1-4.

48. Nada FF, El-Safty F, El –mehi A, and Issa N. The Possible Protective Effect of Platelet Rich Plasma on Aspirin Induced Gastric Ulcer in Adult Male Albino Rat. The Egyptian Journal of Hospital Medicine, 2020; 81 (1):1240-1250.

49. Ghonimi WA and Elbaz A. Histological Changes of Selected Westar Rat Tissues Following the Ingestion of Tartrazine With Special Emphasis on the Protective Effect of Royal Jelly and Cod Liveroil Journal of Cytology & Histology, 2015; 6 :4.

50. Liu W, Shang P, Liu T, Xu H, Ren D , Zhou W , Wen A, and Ding Y. Gastroprotective effects of chebulagic acid against ethanol-induced gastric injury in rats. Chemico-Biological Interactions, 2017; 278(25): 1-8.

51. Tarnawski A, Ahluwalia A and Jones K. M. Gastric cytoprotection beyond prostaglandins: cellular and molecular mechanisms of gastroprotective and ulcer healing actions of antacids Current Pharmaceutical Biotechnology, 2013; 19(1):126–132.

52. Karaca, T., Bayiroglu, F., Yoruk, M., Kaya, M., Uslu, S., Comba, B., *et al*., Effect of royal jelly on experimental colitis induced by acetic acid and alteration of mast cell distribution in the colon of rats. European Journal of Histochemistry, 2010; 54.

53. Alanko J, Riutta A, Holm P, Mucha I, Vapaatalo H, and Metsä-Ketelä T:Modulation of arachidonic acid metabolism by phenols: relation to their structure and antioxidant/prooxidant properties. Free Radical Biology & Medicine, 1999; 26:193-201.

54. Chai J, Baatar D, and Tarnawski A. Serum response factor promotes re-epithelialization and muscular structure restoration during gastric ulcer healing. Gastroenterology, 2004; 126: 1809-1818.

الملخص العربى

تأثير مادة النيكوتين على تركيب الغشاء المخاطى لمعدة الفئران البيضاء البالغة والتأثير الوقائى المحتمل لمادة رويال جيللى. دراسة بالميكروسكوب الضوئى والميكروسكوب الالكترونى الماسح

عبير فريد عبد النعيم

قسم التشريح الادمى وعلم الاجنة، كلية الطب، جامعة سوهاج

المقدمة: لا شك أن هناك علاقة وثيقة بين تناول السجائر والقرح التى تصيب المعدة.وتعتبر مادة النيكوتين هى المكون الأساسى للسجائر.وهى السبب الرئيسى للأمراض المختلفة التى تصيب المعدة من القرح وحتى تصل الى الأورام الخبيثة). ولكن هناك أيضا مواد نافعة مثل رويال جيللى والذى يعد واحدا من المواد الصحية الطبيعية والجاذبة للانتباه. والتى لها تأثير جيد فى علاج السموم لأنها من مضادات الأكسدة. وبسبب ذلك فهى مفيدة فى علاج الالتهابات المختلفة و الأورام الخبيثة.

الهدف من البحث: دراسة تأثير مادة النيكوتين على تركيب معدة الفئران البيضاء البالغة والتأثير الوقائى المحتمل لمادة رويال جيللى.

مجموعات: المجموعة الأولى هى الضابطة. المجموعة الثانية تم حقنها بمادة النيكوتين فى الغشاء البريتونى بجرعة ٥.مجم/كجم وزن الجسم لمدة ٣ اسابيع.والمجموعة الثالثة تم حقنها بالنيكوتين كما سبق بالاضافة الى مادة رويال جيللى بجرعة ٣٠٠مجم/كجم وزن الجسم عن طريق الفم لمدة ٤ اسابيع. تم تشريحهم بعد يوم كامل من اخر جرعة. ثم تم تحضيرهم للفحص الميكروسكوبى الضوئي بصبغة هيماتوكسيلين وايوسين وصبغة ماسون, وصبغة التلوين الحمضى .وايضا تم فحصهم بالميكروسكوب الالكترونى الماسح.

النتائج: ظهر فى المجموعة التى تم حقنها بالنيكوتين العديد من التغيرات :حيث ظهرت قرح فى نسيج المعدة.كما ظهر ضمور فى بعض خلايا المعدة.وقد ظهرت الالتهابات على شكل زيادة فى انسجة كولاجين. أما بالنسبة لسمك المعدة فقد قل فى المجموعة التى حقنت بالنيكوتين.

وأما المجموعة الثالثة التى تناولت رويال فقد ظهر نسيج المعدة تقريبا بحالته الطبيعية.

الخلاصة: مادة النيكوتين هى مادة سامة للمعدة تسبب الكثير من التغيرات وتناول رويال جيلى يحميها من هذه التغيرات.