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Original article

To What Extent Garlic and Royal Honey Mitigating the Gastric Toxicity Induced by Bisphenol A in Adult Albino Rats: Histological, Immunohistochemical and Scanning Electron Microscopic Study

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Abstract:

Background: Bisphenol A (BPA) is among the most industrial compounds worldwide. BPA belongs to a group of compounds that are categorized as endocrine disruptive chemicals resulting in multi organ toxicities. Garlic, a natural plant, has a recorded history in ancient medicine. Royal Honey possesses antioxidant and anti-inflammatory properties against many contaminants. **Aim:** to compare effect of aged garlic extract (AGE) and royal honey (RH) on protection against gastric mucosal damage by Bisphenol A consumption. **Materials and Methods**: For the study, four equal groups of forty rats were established. The control group had ten rats. For 28 days, the remaining thirty rats were given an oral dosage of 50 mg/kg of bisphenol A; ten rats of whom were assigned to group II. Other ten rats (group III) were pretreated with AGE (200 mg/kg) daily orally one hour before Bisphenol A. whereas the remaining ten rats (group IV) were pretreated with RH (250 mg/kg) daily orally one hour before Bisphenol A. The stomach was investigated using Haematoxylin and Eosin, a scanning electron microscope, Periodic Acid Schiff stain, caspase 3 and TNF- α immunostaining. **Results:** Bisphenol A treated group showed cell degeneration and erosions on the epithelial surface. The cytoplasmic reactivity of TNF- α and caspase 3 immunostaining is elevated, indicating a malfunction in the gastric mucosa. The pretreatment of AGE and RH mitigates the histopathological alterations with a significant decrease in TNF- α and caspase 3 immunreactivity. **Conclusion:** This study highlights the protective effects of natural compounds (AGE and RH) against BPA-induced gastric mucosal damage by reducing inflammation and apoptosis markers.

Keywords: gastric toxicity, Bisphenol A, Garlic and Royal honey

Introduction

A chemical used all over the world is bisphenol A; 2,2-bis 4-hydroxyphenyl propane. The polycarbonate plastic industry and everyday items including toys, food packaging, baby bottles, medical equipment, and medications heavily rely on bisphenol A (BPA) [(1)]. Due to its association with a number of health issues that arise following direct or indirect human exposure, BPA has been receiving increased attention [(2)]. The detection of BPA in plasma and urine has confirmed human exposure to the chemical, which has been linked to a number of illnesses [(3)]. These conditions include cardiovascular diseases [(4)], reproductive abnormalities [(5)], gastrointestinal barrier damage [(6)], and nephrotoxicity [(7)].

Because BPA can cause oxidative stress, there have been a lot of contradictory findings on it. These findings suggest that BPA depletes antioxidants [(8)], causes apoptosis [(9)], and has pro-oxidant/antioxidant behavior [(10)]. BPA may be harmful and carcinogenic due to its generation of reactive oxygen species [(11)]. Since an oestrogen receptor antagonists have been shown to reverse production of cytokine, BPA may influence cytokine via ER dependent mechanism [(12)].

Garlic is a popular spice and medicinal herb (Allium sativum, Liliaceae). Its chemical components, all of which are found in significant quantities, include diallyl-disulfide, diallyl sulphide, thiacremonone, alliin, S-allyl cysteines, and allicin [(13)]. This plant and its constituents have anti-inflammatory, anti-cholesterolemic, anti-gastric ulcer, anti-microbial, anti-cancer, antioxidant, and free radical scavenging properties [(14)]. Because it inhibits lipid-forming enzymes, stops lipid peroxidation, lowers LDL oxidation by scavenging superoxide, and increases antioxidant levels, garlic has promise [(15)].

Honey is a natural substance with long-standing medicinal uses [(16)]. Among the many nutrients that are abundant in this food are enzymes, water, fructose (38.2%), glucose (31.2%), sucrose (0.7%–1%), phenolic acids, toco pherols, ascorbic acid, which can reduce glutathione, catalase, superoxide dismutase and flavonoids [(17)]. It was used to cure wounds and digestive diseases through the ancient Greeks and Egyptians [(18)]. It has also been used to treat earaches, cough, and sore throat [(19)]. Honey has recently been used for its anti-inflammatory, antibacterial, and antioxidant qualities as well as to strengthen the immune system [(20)].

This study aimed to evaluate the cytoprotective effect of royal honey and aged garlic extract against gastric toxicity induced by bisphenol A in rat models.

Materials and Methods:

Chemicals:

Bisphenol A powder from Sigma Company in Egypt (CAS number: 80-05-7), white powder liquified in corn oil (10 mg BPA: 1 ml corn oil).

Aged garlic extract was obtained from Wakunaga of America (Mission Viejo, CA) as kyolic supplement. It contains 100 capsules and Aged garlic extract (AG) was present at 300 mg per one.

Royal Honey (RH): was bought from a nearby apiculture store (Benha, Qalyubia, Egypt) dissolved in 5 ml distilled water.

Animals:

Male albino rats (eight weeks old) weighing between 220 and 255 g were acquired from the Helwan breeding farm in Helwan, Egypt. The rats were given water, regular rat food, and libitum while being exposed to room temperature (between 25°C and 27°C), normal atmospheric conditions, and alternate day and night cycles. Before beginning the experiment, the animals were given two weeks to get used to the lab environment at anatomy department Faculty of Medicine Benha University. The experimental protocol was conducted in compliance with the rules established by Benha University's Animal Care and Use

Research Ethics Committee (Ethical Approval No. RC 7-12-2024)

Selection of doses and induction of gastric lesion:

Forty rats were divided into four groups at random, with ten rats in each group:

Control group (Group I): rats were divided into three sub groups:

Ten rats were equally subdivided into:

Subgroup (IA): rats were fed on basal diet and water

Subgroup (IB): rats administered orally corn oil 5 ml/kg which used as BPA vehicle

Subgroup (IC): rats received distilled water 5ml/kg which used as AG and RH vehicle

Subgroup (ID): rats administered orally AGE (200 mg/kg BW) [21].

Subgroup (IE): rats administered orally RH (250 mg/kg BW) [22].

BPA group (Group II): the rats administered a dose of BPA (50 mg/kg BW) daily [23]

AGE group (Group III): rats were treated with AGE (200 mg/kg BW) [21] one hour prior to BPA (50 mg/kg BW) [23] treatment daily.

RH group (Group IV): rats were treated with RH (250 mg/kg BW) [22] one hour prior to BPA (50 mg/kg BW) [23] treatment daily.

All treatments were administered via oral rout using oral cannula daily for 28 consecutive days in each group.

Rats were sacrificed under anesthesia (inhaling 1.9% diethyl ether) at the end of the experiment. Each fundic stomach of rats was removed after the front abdominal wall was opened and carefully cleaned with saline. Each rat's stomach was split along the length into two sections, one of which was fixed in 2.5% glutaraldehyde and the other in neutral-buffered 10% formalin.

Light microscopy.

After the specimens were embedded in paraffin blocks, slices (5 µm) were cut out and stained by Hematoxylin and Eosin (Hx.&E.) confirming histological features and Periodic Acid–Schiff stain (PAS) to detect mucins [24].

Monoclonal anti-TNF- α (Cat. No. GTX54419, GeneTex, Inc., North America) (1:300) and a polyclonal antibody to cleaved caspase (Cat. No. GTX110543, GeneTex, Inc., North America) (1:200) as primary antibodies, immunohistochemistry was used to identify tumor necrosis factor (an indicator of gastric inflammation) and cleaved caspase-3 (an indicator of apoptosis) in the cells of the gastric mucosa. After being put on positively charged glass slides, the specimens were completely deparaffinized in xylene and then rehydrated in decreasing alcohol grades. The antigens were then recovered by inhibiting the endogenous hydrogen peroxidase activity and then exposing the sample to 500 W of microwave radiation for 10 minutes in a 10 mM citrate buffer with a pH of 6.0. By diaminobenzidine (DAB), sections containing primary antibodies of TNF- α - and cleaved caspase 3 were subsequently kept in a whole night at 4C. Hematoxylin was used as a counterstain after the slides had been cleaned with distilled water. The cytoplasm became brown as a result of the positive cellular response [25] In five fields of view, positive reaction of TNF- α - and cleaved caspase-3 cells were counted count method in blind manner.

A light microscope (Olympus CX 41, Japan) and an attached camera (Olympus E 330, Japan) were used to analyze and record Hx&E and immunohistochemical sections at Anatomy Department, Faculty of Medicine, Benha University.

Electron microscopy

Each group had one stomach tissue block removed for scanning electron microscopy. For a whole night at 4 C, the blocks were preserved in 2.5% glutaraldehyde. Following three rounds of washing in phosphate buffer, the samples were osmicated for two hours in 1% osmium tetra oxide. Following dehydration in a series of graded ethanol solutions and washing in buffer. The specimens were placed on aluminum stubs, vacuum-coated with palladium gold, and dried using liquid carbon dioxide [26]. Following that, the coded specimens were examined using a scanning electron microscope (Jeol-JSM-3400) at Mansoura University's Electron Microscopy Unit.

Morphometric study

Version 6.0 of the Image-Pro Plus software (Media Cybernetics Inc., Bethesda, Maryland, USA) was utilized to compute the mean area percentage of TNF- α and caspase 3 positive expression in the immunohistochemical gastric sections at 200× magnification, as well as the percentage area of mucin stain (on PAS-stained sections).

Statistical Analysis

All data, including the mean area (%) of positive immunohistochemistry expression levels and the percentage area of mucin stain (on PAS-stained sections), were displayed as mean±SD for each group using IBM SPSS Statistics software for Windows, Version 20 (IBM Corp., Armonk, NY, USA). The differences between the groups of morphometric findings were compared by one-way analysis of variance (ANOVA) with the Post Hoc LSD test. Standard deviation (SD) and mean (M) values were represented, and any variation was considered significant at $P < 0.01$.

Results

Evaluation of gastric samples by Hx. & E.

Examinations of gastric sections in control group showed that no differences were seen between subgroups. Sections from all rats in the control group demonstrated that the fundic mucosa was showed simple, tubular glands in a little lamina propria. The glands were densely packed over the whole thickness of the mucosa and perpendicular on the surface. Short, small pits allowed the glands to open into the lumen. The body, neck, and isthmus had formed the fundic glands. Simple columnar epithelium with pale cytoplasm and basal oval nuclei comprised the surface epithelium. The isthmus and fundic pits were coated with surface columnar cells that secreted mucus. The neck was surrounded by polyhedral parietal cells scattered between mucous neck cells. Its cytoplasm was acidophilic and had central round nucleus, as well as mucous neck cells were low columnar with basal flat nuclei and pale vacuolated cytoplasm. Chief cells predominate near the base of the gland, which contain rounded basal nuclei and basophilic cytoplasm with a pale apical part, (Fig. 1a-c).

Sections of BPA group were examined, and it was found that there were mucosal ulcers with discontinuities in the surface epithelial cells and sloughing the epithelium in the lumen. The architecture of Fundic glands was disturbed with cellular infiltrations in lamina propria. The gastric pit was wide. The surface mucous cells have minimal cytoplasm and flattened nuclei. Most parietal cells showed pyknotic nuclei within vacuolated cytoplasm. Decreases in the density of the chief cells were detected. The chief cells showed decreased basophilia vacuolated cytoplasm and dark pyknotic nuclei (Fig. 2 a- c).

Sections of AGE group were examined, and the results showed that the mucosa fundic glandular tissue architecture was almost normal with wide spaces in the glands. The fundic glands had elongated gastric pits with most of surface mucous cells were normal and enlarged with mucus. Few of these cells had darkly stained nuclei. Also, extravasated blood was seen. The most of parietal cells seemed normal, despite a small number having vacuolated cytoplasm and pyknotic nuclei. Some of cheif cells were normal while few of them showed darkly stained nuclei (Fig. 3 a- c).

Sections of RH group showed a picture that was much the same as the control group, regular arrangement of the fundic gastric glands which appeared straight, crowded. Oval basal nuclei are seen in the majority of surface epithelial cells. The majority of parietal cells seemed normal. Chief cells were found to be closely packed, having basal spherical nuclei and basal basophilic cytoplasm (Fig. 4 a- c).

Scanning electron microscopic examination

Examination of the control group revealed that the mucosa has a typical velvety texture with dome-shaped surface mucous epithelial cells and intact border gastric pits. Mucous over the epithelial surface was also detected (Fig. 5a). In BPA group, the surface epithelial cells appeared with rough eroded surfaces, as well as loss of its dome shaped appearance and the boundaries between cells with disturbed wide gastric pits creating a honeycomb-like appearance (Fig. 5b). The mucosa in AGE group contained surface epithelial cells with slightly rough eroded borders around wide gastric pits. Red blood cells were observed over the surface (Fig. 5c). The mucosa in RH group resembled that of the control group; dome shaped surface and intact cellular boundaries covered by mucous, surrounding the normal gastric pits was observed (Fig. 5d).

Periodic acid Schiff (PAS) stained

The gastric mucosal surface, extending to the pits, and neck regions, was continuously coated with strong PAS-positive magenta red mucous coating in the control group. Even though there was only a slight PAS-positive reaction in the gastric pits in BPA group across the eroded surface epithelium. AGE group showed a PAS-positive response at the surface epithelium and gastric pits. In otherwise, RH group displayed a positive PAS reaction in the gastric mucosa near control group at the epithelial surface and gastric pits with mild reaction at neck areas (Fig.6 a-d). Morphometric and statistical evaluation revealed BPA, AGE and RH groups had a significant reduced comparing to the control group, while AGE and RH groups had a significant increase comparing with the BPA group (table 1).

TNF- α immunohistochemical expression

The brown cytoplasmic response, which is an indicator of inflammation, was a sign of positive TNF- α immunohistochemical staining. TNF- α expression in the control group had a weak response. BPA group exhibited a high positive immunoreactivity cytoplasmic response in gastric mucosa. AGE group had moderate TNF- α -a immunoreactivity, while RH group showed mild TNF- α -a immunoreactivity (Fig. 6 e-h). Morphometric and statistical evaluation revealed BPA, AGE and RH groups had a significant increase as compared to the control group, while AGE and RH groups had a significant decrease as compared to the BPA group (table 1).

Caspase-3 immunohistochemical stain

Gastric sections of control group showed a weak cytoplasmic response in the gland cells. BPA group rats showed widespread positive cytoplasmic reaction to caspase-3. However, gastric mucosa in AGE and RH groups revealed moderate cytoplasmic immunoreactivity expression for caspase-3 in the gland cells (Fig. 6 I-L). Morphometric and statistical evaluation revealed BPA, AGE and RH groups had a significant elevation comparing with the control group, while AGE and RH groups had a significant decrease comparing with the BPA group (table 1).

Table (1): Demonstrating the morphometric comparison between the experimental groups regarding the mean area% of PAS-positive reaction, TNF-α immunoreaction and caspase-3 positive immunoreaction by Post Hoc LSD test.

	PAS-positive reaction	TNF positive immunoreaction	Caspase-3 positive immunoreaction
	Area% (Mean ± Standard deviation)		
Control group	15.99 ±0.38^{bcd}	0.46 ±0.05^{bcd}	0.31 ±0.27^{bcd}
BPA group	1.73 ±0.16^{acd}	3.48 ±0.54^{acd}	6.88 ±0.94^{acd}
AGE group	6.87 ±0.78^{abd}	1.81 ±0.74^{abd}	4.26± 1.3^{abd}
RH group	13.72 ±0.58^{abc}	1.02 ±0.04^{abc}	2.32 ±0.6392^{abc}

Data are represented as mean ±SD
a Significantly different from control group	b Significantly different from BPA group
c Significantly different from AGE group	d Significantly different from RH group

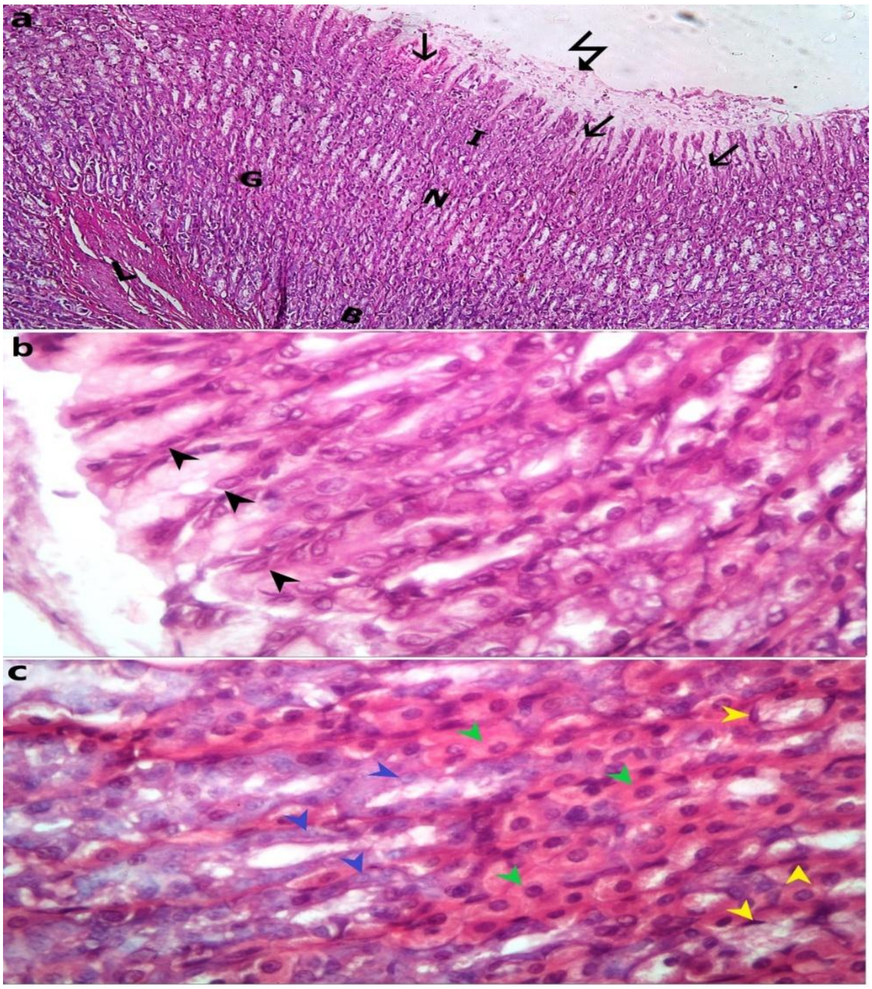

Fig. 1: Photomicrograph of fundic mucosa of a control group rat showing:
a) thick layer of mucus covering the epithelium (zigzag arrow). There are several closely spaced tubular fundic glands (G) that run parallel to one another and have small pits (arrows). The glands have isthmus (I), neck (N), and base (B) regions and are located inside a little lamina propria (L). **(Hx&E x 100**).
b) Surface mucous cells (black head arrow) with basal oval nuclei and acidophilic cytoplasm are seen at the upper portion.
c) The bottom of the gland has a large number of cheif cells (blue head arrow), each of which has a pale apical area, basal basophilic cytoplasm, and basal nuclei. There are polyhedral parietal cells

(green head arrow), which have rounded center nuclei and acidophilic cytoplasm. Also, there are mucous neck cells (yellow arrow) with flat basal nuclei and pale cytoplasm. **(Hx&E b & c x 400)**

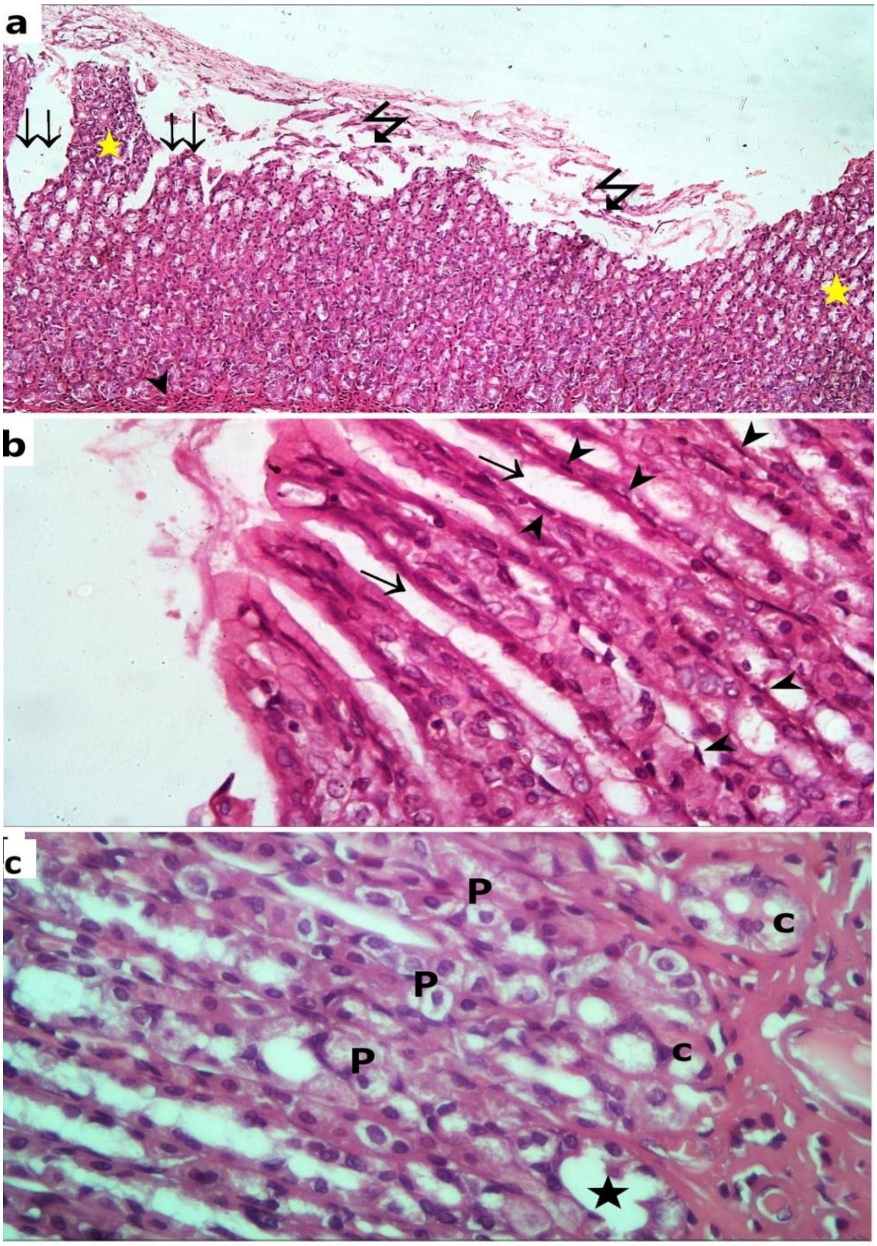

Fig. 2: Photomicrograph of fundic mucosa of BPA group rat showing:
a) ulceration (double arrow) with sloughing the surface epithelial cells into the lumen (zigzag arrow). The architecture of some gastric glands (star) is deformed. Notice inflammatory infiltrations (head arrow) **(Hx&E, x 100)**.
b) The pits of fundic glands are broad (arrow). The surface mucous cells have thin, nuclei and little cytoplasm (black head arrow).
C) the parietal cells (P) have deeply stained nuclei and cytoplasmic vacuolization. Fundic gland cystic dilatations are accompanied with chief cell flattening (star). Apparent decrease in the density of the cheif cells (C) which appear with dark stained nuclei and decreased cytoplasmic basophilia **(Hx&E b & c x 400)**

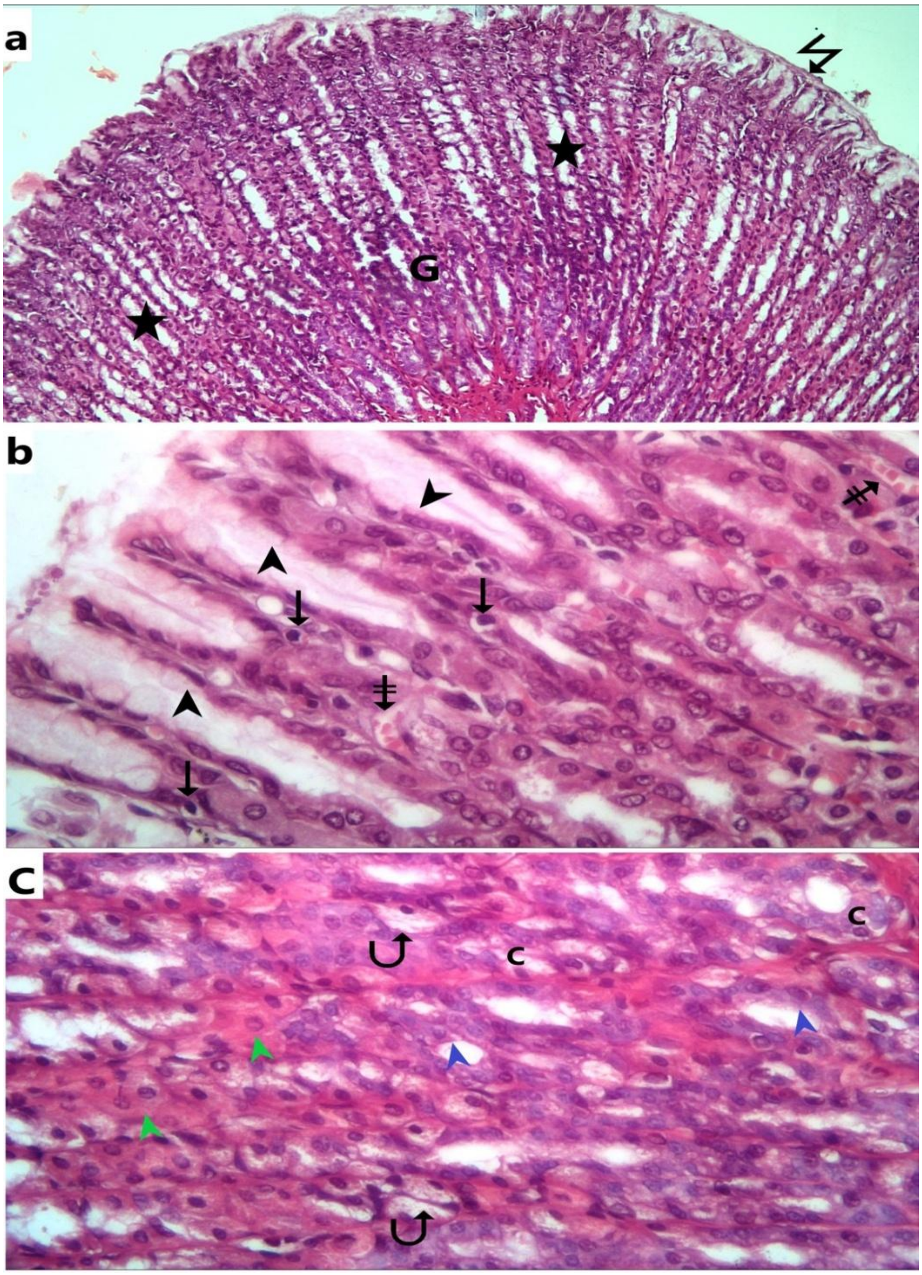

Fig. 3: Photomicrograph of fundic mucosa of AGE group rat showing:
a) nearly normal appearance of gastric glands (G) and wide fundic glands (star). The surface is covered by thin film of mucous (zigzag) **(Hx&E x 100).**
b) Surface mucous cells seem normal and filled with mucus (head arrow), but some of them have darkly stained nuclei (arrow). Notice extravasated blood (feathery arrow).
c) The parietal cells show round nuclei and acidophilic cytoplasm (green head arrow); a few cells have pyknotic nuclei and cytoplasmic vacuolization (curved arrow). Some of cheif cells are normal (blue head arrow) while few cheif cells are observed with dark stained nuclei and vacuolated cytoplasm (C) **(Hx&E b & c x 400)**.

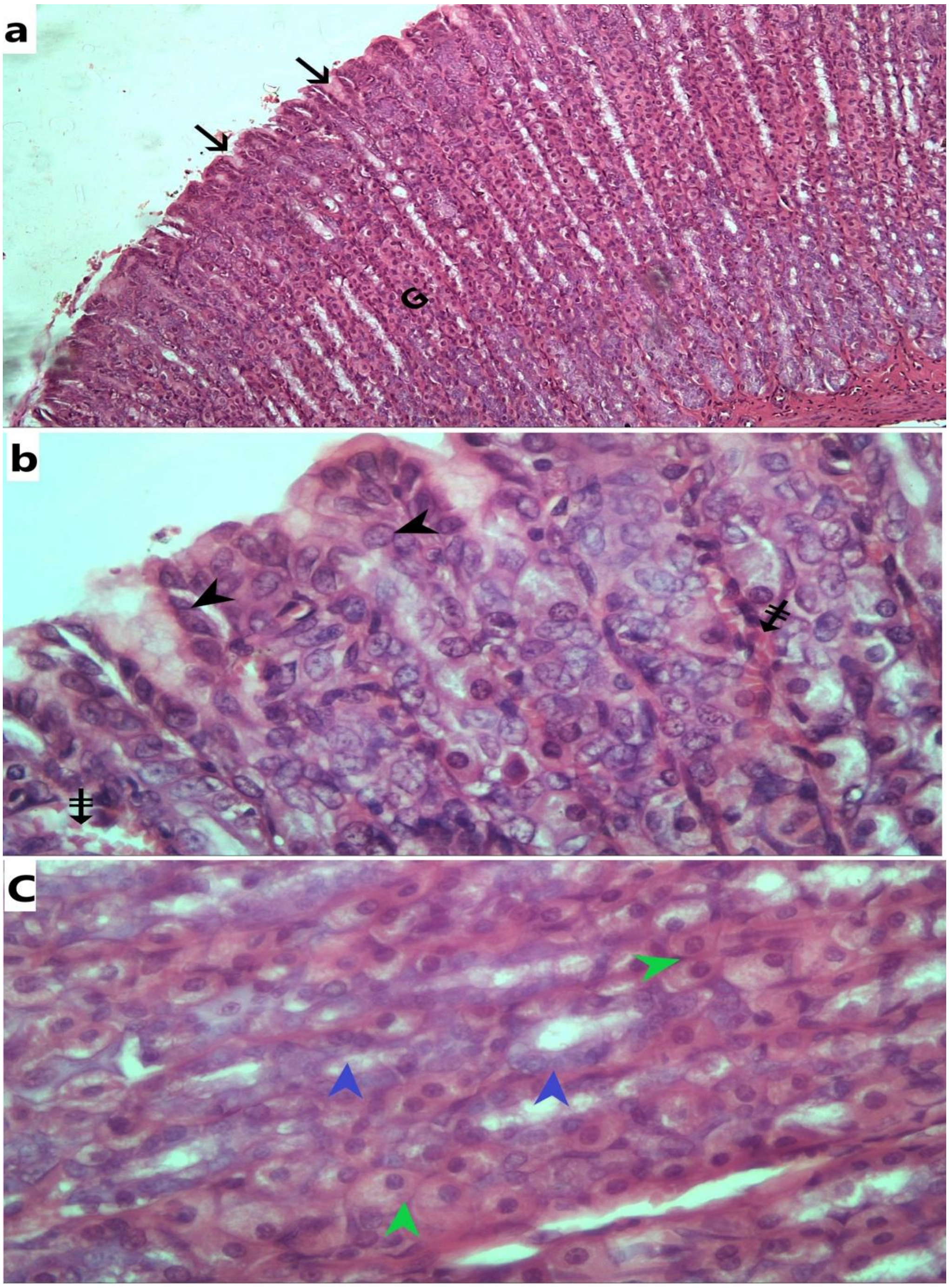

Fig. 4: Photomicrograph of fundic mucosa of RH group rat showing:
a) the fundic mucosa has a normal architecture of overcrowded gastric glands (G) that have narrow pits (arrow) **(Hx&E x 100).**
b) Surface epithelial cells have oval nuclei and acidophilic cytoplasm (black head arrow) can be observed. Notice extravasated blood (feathery arrow)
c) the parietal cells have normal round nuclei and acidophilic cytoplasm (green head arrow). The cheif cells are normal (blue head arrow). **(Hx&E b & c x 400)**

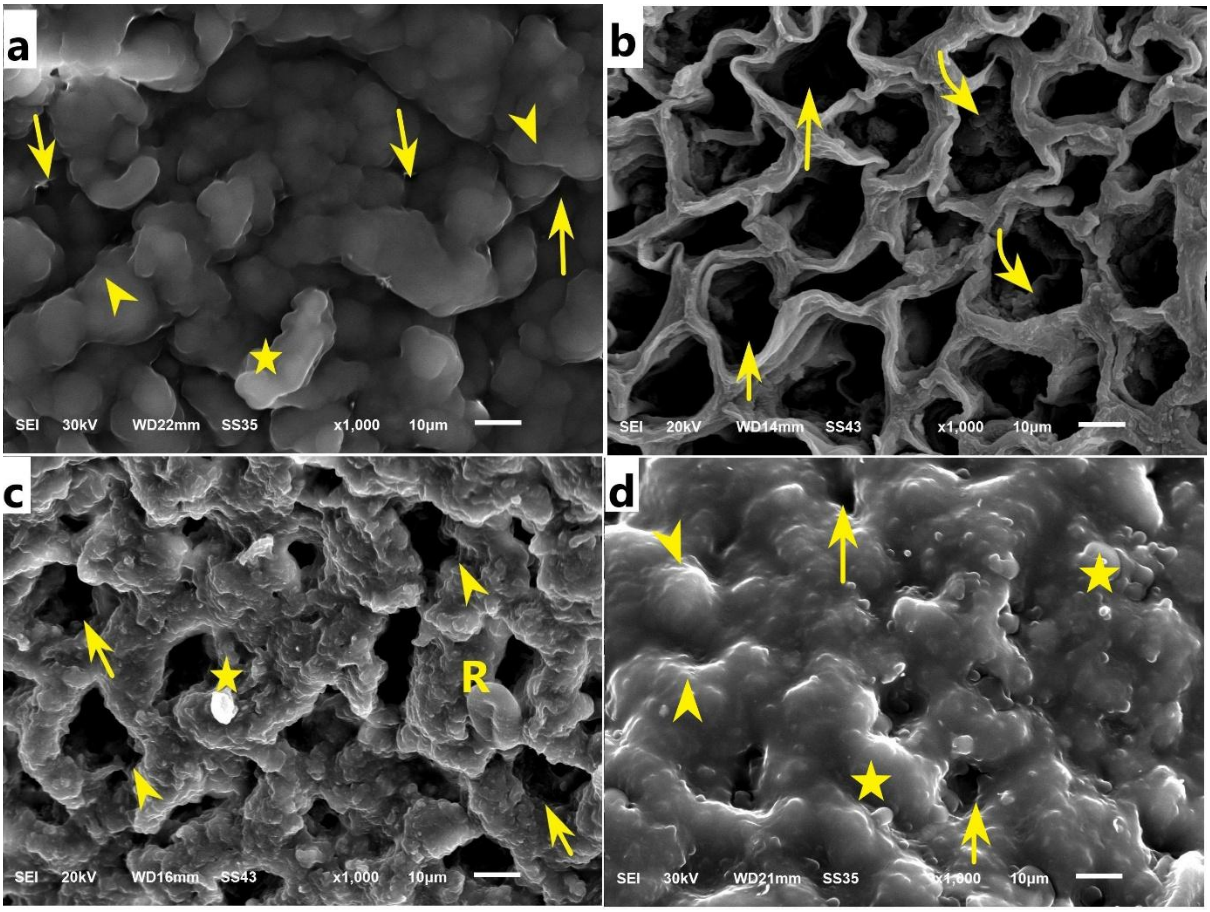

Fig. 5: A Scanning electron microscopy (SEM) of rat gastric mucosa:

a) control group demonstrates the dome-shaped surface mucous epithelial cells (head arrow) that encircle the intact pits (arrows) that have well defined borders. There is mucus (star) covering the surface epithelium.

b) BPA group shows broad pits (arrow) and epithelium shedding (curved arrows) that resemble honeycombs.

C) AGE group shows broad gastric pits (arrow) surrounded by slight rough, degraded surface (head arrow). Notice mucous (star) and escaped red blood cell (R) over cells.

d) RH group relieves normal pits (arrows) are surrounded by dome-shaped surface epithelial (head arrow). Observe mucous over cells (star) **(SEM, x1000, scale bar 10µ).**

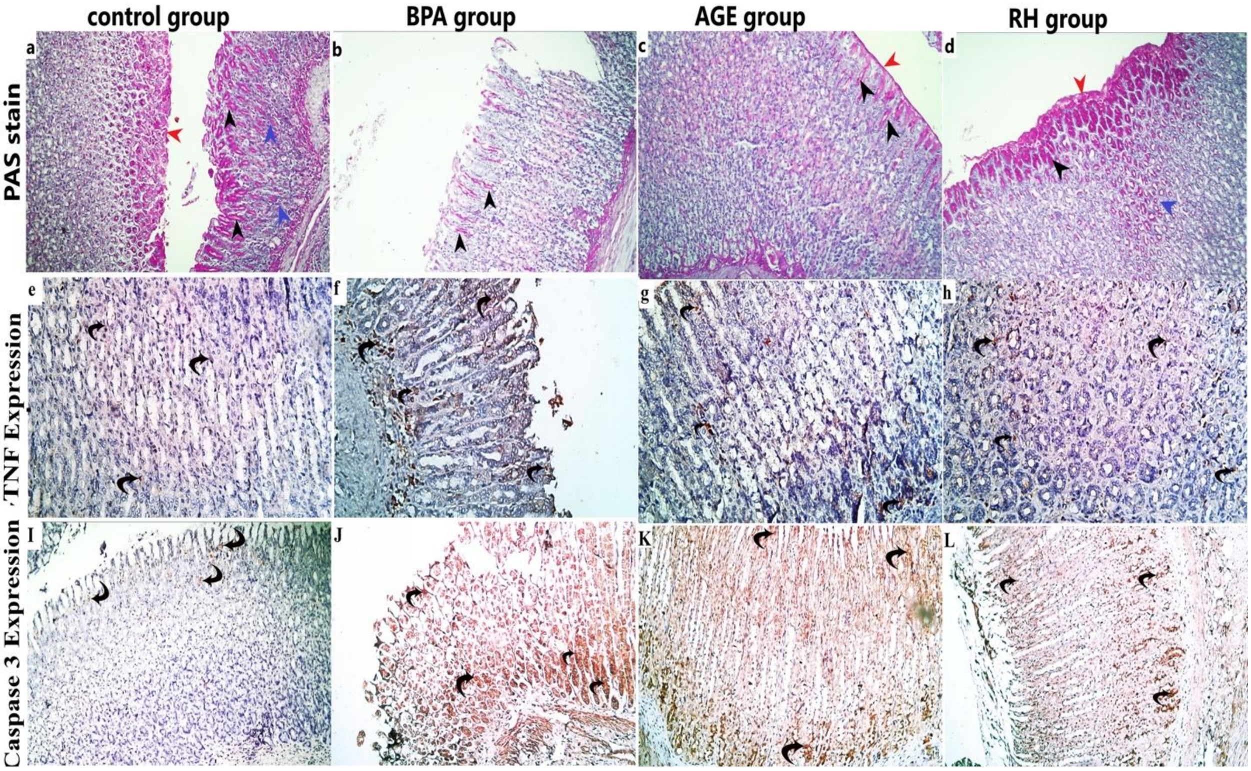

Fig. 6: photomicrographs of PAS and immunohistochemical expression to TNF-α and Caspase 3 in gastric mucosa of all experimental groups.

PAS staining (a-d) showing (a): The control group has positive PAS over the surface epithelium (red head arrow), filling fundic pits (black head arrow) and neck of the gland (blue head arrow). **(b) BPA group** exhibit interrupted PAS response in fundic pits (black head arrow). **(c): The AGE group** has mild positive PAS response across surface epithelium (red head arrow) and gastric pits (black head arrow). **(d): RH group** has positive PAS reaction throughout the surface epithelium (red head arrow), fundic pits (black head arrow) and mild in the neck of the fundic glands (blue head arrow **(X 200).**

Immunohistochemical expression to TNF-α (e-h). e) control group has a weak TNF-α immune expression in gastric mucosa (curved arrow). **f) BPA group** shows a strong TNF-α immune expression in gastric mucosa (curved arrow). **g) The TNF-α immunoreaction in the AGE group** is mild positive in gastric mucosa (curved arrow). **h) RH group** shows weak positive TNF-α immune expression in in gastric mucosa (curved arrow). **(X 200).**

Immunohistochemical expression to caspase 3 (I-L). I) control group exhibit a weak cytoplasmic response to caspase-3 in gastric mucosa (curved arrow). **J)** The gland cells in the BPA group have a high positive cytoplasmic reactivity for caspase-3 (curved arrow). **K)** AGE group is shown moderate immunoreaction in the glands' cells (curved arrow). **L)** RH group, it has moderate positive caspase-3 immunoreaction. **(X 200).**

Discussion

One of the most common gastrointestinal conditions impacting people globally is gastric ulcers. The widespread usage of BPA as the main component in producing plastics raises several concerns regarding its potential effects on human health. Similar in size and structure to estradiol, BPA disrupts endocrine function by acting on estrogen receptors [(27)].

Additionally, BPA treatment damaged the gastric glands, as seen by light microscopy of the stomach sections. The parietal cells and Chief cells displayed cytoplasmic vacuolation and pyknotic nuclei. Rats given BPA developed ulcers with shedding of surface epithelium. There were cellular infiltrations. According to SEM, it supports earlier findings. It is consistent with earlier findings by Ismail and El-Meligy [(27)], Abo-Elsoud, et al., [(28)], and Ige, et al., [(29)]. According to a prior study [(23)], the gastric tissues of the BPA-only group had higher levels of Myeloperoxidase (MPO), a marker of leucocyte infiltration that is thought to be an indicator of inflammation and leukocyte-mediated mucosal tissue damage. It demonstrated how the integrity of the stomach mucosa in this group may be affected by mechanisms associated with increased oxidative stress and inflammation.

In this work, mucin depletion and PAS-positive reactivity were significantly decreased in the BPA-administered group. These results are consistent with a prior study's findings that the BPA group was noticeably worse than the control group [(27)]. Additionally, this study's result is consistent with another that found that rats given BPA had a modest PAS-positive reaction in rat prostate [(30)]. A reduction in gastric mucus raises the risk of gastrointestinal mucosal injury as the mucus shields the gastric mucosa against oxidative damage [(31)].

The current study reveals a significant rise in the number of positive TNF- α gastric mucosal cells. This result aligned with the findings of Ige [(23)]. According to a prior research elevated TNF -α expression may be a sign of stomach inflammation [(32)]. Reactive oxygen species and the ensuing oxidative state may be mostly caused by invading inflammatory cells [(33)].

The majority of the gland cells exhibited a significant positive cytoplasmic response when the immunohistochemically stained sections for caspase-3 in the BPA-administered group were examined. BPA may cause apoptosis by increasing the amount of caspase-3 proteins in gastric tissue [(28)].

In this work Hx & E and SEM results demonstrated that using aged garlic extract helped to partially heal the disturbance in the stomach mucosa. However, few of parietal and chief cells showed degenerative changes. Also, extravasated blood was seen. This was in line with findings of Kuna et al. [(34)], who found that pretreatment with garlic oil had a gastroprotective effect in a peptic ulcer disease cell culture. In model of stomach ulcer caused by a nonsteroidal anti-inflammatory medication, it was discovered that using single dose (275 mg. rat) of garlic extract had partial gastroprotective benefits [(35)]. In a prior study, it was shown that giving rats 200 mg.kg-1 of garlic for 10 days prevented stomach ulcers as compared with the group given omeprazole [(21)]. But according to an earlier research, AGE improved the histological abnormalities in the stomach tissue and demonstrated a potential gastroprotective function in stomach ulcers caused by aspirin and indomethacin [(36, 37).]

In previous work, it was demonstrated that when the actin filaments became amorphous, the garlic extract's allicin has a potently positive impact on the actin cytoskeleton [(38)]. By raising glutathione and prostaglandin E2 levels, garlic extract (GE) has anti-ulcer preventive properties such cytoprotective actions and antioxidant activity. The GE gastroprotective benefits against sodium taurocholate-induced peptic ulcers are related to oxidative stress through the NFκB pathway-mediated suppression of pro-inflammatory signaling [34].

The study found that rats treated with garlic extract produced more mucus, as seen by a significant increase in PAS reaction. This might be explained by AGE ability to increase the production of mucus from the stomach glands as Prostaglandin E2 stimulates the production of gastric mucus and bicarbonate and is a potent inhibitor of gastric acid release [34].

Additionally, AGE therapy reduced positive expression of TNF- α. This was in line with Badr and Al-Mulhim[36] and Choi, Y. J [37]. Garlic allicin reduced the release of TNF-α when tested for its anti-inflammatory effects on intestinal epithelial cells [39]. Additionally, it inhibited the activation of NF-kappa B in human T lymphocytes that was triggered by both TNF-α and H2O2 [40].

In the present work, Garlic extract has antiapoptotic properties that were investigated. Garlic extract treatment significantly reduced the expression of caspase-3 comparing to the BPA group, according to immunohistochemical analysis of the gastric mucosa employing apoptotic protein. The treatment of garlic decreased caspase-3 positive expression on the rats' brain tissue exposed to acrylamide [41]. According to Hossain, et al. [42], this was explained by the fact that garlic contains over 200 chemical components, including sulfur-containing allicin, alliin, and peroxidase. Due to its strong antioxidant activity, it was found that when given with gentamicin, Garlic extract helps oxidants and antioxidants of renal tissue return to their normal balance [43].

In contrast to the BPA group, the royal honey-treated rats in this study showed no histopathological alterations and the typical histological structure of the stomach mucosa. According to earlier research, royal honey protected against damage to the stomach mucosa. Royal honey therapy was found to promote healing from gastric ulcers [44, 45], and gastro-protective and anti-ulcerogenic properties on ethanol-induced and nicotine induced ulcers [23, 46].

Rats had royal honey treatment, restored positive PAS reactions on their stomach mucosa. Our findings are consistent with a previous study discovered that honey significantly increased PAS reaction considerable continuous mucous layer lining the stomach mucosal and the mucus production of the gastric mucosa [45, 46]. A prior study examined honey's antiulcer effects in rats and found that it boosted the synthesis of mucosal glycoproteins, which in turn restored mucus production. Honey has ability to prevent mucosal injury was attributed to its phenol concentration, which promotes the creation of PGE2 [47].The observation of this study revealed significant low expression of TNF- α in the mucosa. This finding was consistent with a study that demonstrates the gastroprotective benefits of Wadi and Talh honey that are mediated by TNF-α suppression, and IL-10 [48-51].

According to the current study, honey significantly reduced the immunological expression of caspase-3 in the gastric mucosa. A previous researcher discovered honey's anti-apoptotic properties, demonstrating a significant reduction in the percentage immune expression of Caspase 3 in nephrotoxicity caused by doxorubicin [52].

Conclusion

Aged garlic extract and royal honey treatment shown therapeutic promise in reducing fundic stomach changes in this rat model of Bisphenol A gastric poisoning. However, more studies are needed to determine the defined preventative mechanism on stomach ulcers.

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Author contribution

The authors contributed equally in the study.

Conflict of interest

The authors declare no conflicts of interest

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