INTRODUCTION

The normal stomach mucosa maintains a balance between protective and aggressive factors. Some of the main aggressive factors are gastric acid, abnormal motility, pepsin, bile salts, use of alcohol and nonsteroidal anti-inflammatory drugs (NSAID), as well as infection with microorganisms (*Helicobacter pylori* and others). Although in most cases the etiology of ulcer is unknown yet, it is generally accepted that gastric ulcers are multifactorial and develop when aggressive factors (endogenous, exogenous and/or infectious agents) overcome mucosal defense mechanisms **(Tulassay and Herszényi, 2010).** Resveratrol has many biological functions and activities, including antioxidant and anti-inflammatory effect **(Xiaolin *et al.*, 2015).** Resveratrol (3,5,4'-trihydroxy-trans-stilbene), a natural phytoalexin from the stilbenes subgroup, is isolated from berries, grape skin, and peanuts (**Yadav *et al*., 2009).** The potential of resveratrol in the management of gastric and intestinal injury and inflammation, due to its strong antioxidant, anti-oxidative stress, free radical scavenging, and anti-inflammatory activities, is widely evaluated**.** The protective effect of resveratrol in the models of peptic ulcer is reasonably well-reported, with most investigations being based on its potential to suppress the production of key inflammatory mediators. It is now well-recognized that resveratrol has an excellent antibacterial effect against **H. pylori** infection. Resveratrol inhibits the virulence factor of **H. pylori** and urease enzyme activity significantly **(Mohammad *et al*., 2015).** Resveratrol is another poly-phenolic compound present in red grapes, wine, nuts and common garden plants, and is one of the most intensively investigated of all the phytochemicals with putative

beneficial effects on human health. The effects of resveratrol include anti-oxidant and anti-aging properties, improvement of insulin sensitivity and reduction of cardiovascular disease risk. Previous studies have also indicated that resveratrol is able to function as a chemo-preventive and chemotherapeutic agent in certain types of human carcinomas **(Zixuan *et al*., 2017).**

MATERIALS AND METHODS

1. Experimental rats:

Twenty two (24) Sprague – Dawley male albino rats weighting 150±5g b.wt., each, used in this study. Each rat was housed in an individual stainless steel cage with food pellets and water under controlled condition of temperature (22 °C) in the Animal House of Ophthalmology Hospital, Giza, Egypt. All rats fed the control (casein) diet for four consecutive days. The basal diet which called (casein - basal diet) was composed of 12.3 g casein (10% protein), 10g corn oil (10% fat), 4 g minerals (4% minerals), 1g vitamin mixture (1% vitamin) 5 g cellulose (5% fiber) and corn starch up to 100g according to (**Campbell, 1963)**. Diets introduced to rats in special non- scattering feeding cups to avoid loss of feed and contamination. Tap water provided to rats by mean of glass tubes projecting through wire cages from inverted bottles supported to one side of the cage. They kept on a 12-h darkness:12-h light cycle with light at 24:00 h. Meanwhile, resveratrol which used as a treatment of aspirin induced acute gastric ulcer was used as a dry substance purchased from Al-Gomhoria Company for Chemicals then dissolved in water with the required concentration to make a dose of 25 and 50 mg/kg b.wt. of rats.

2. Experimental design:

Rats were fed for one week on basal diet before starting the experiment for acclimatization. After one week, rats were divided into two main groups. The first group (n= 6 rats) was fed on basal diet only as a control negative (healthy rats). The second main group (n= 18 rats) were given orally aspirin at a dose of 200mg/kg b.wt., for induction of acute gastric ulcer according to **Agrawal *et al.*, (2000),** then divided as the following:

Group (2): Was fed on basal only as a control positive group.

Group (3): Was fed on a basal diet with oral injection of (resveratrol (3,5,4′-trihydroxylstilbene), at a dose of 25 mg/kg b.wt., for 7 days.

Group (4): Was fed on a basal diet with oral injection of (resveratrol (3,5,4′-trihydroxylstilbene), at a dose of 50 mg/kg b.wt., for 7 days.

3. Measurement the gastric juice volume and the length of gastric ulcer:

At the last day of the experiment, rats were fasted for 12 hrs and only allowed for drinking water. Next day, all rats were sacrificed and their stomachs were tied around both openings (cardiac & pyloric sphincters) then injected with 3ml of distilled water. After that gastric juice was collected in sterilized tubes and centrifuged at 500 r p m, for 5 minutes. The volume of gastric juice was measured by graduated cylinder and expressed as ml. The stomachs were opened longitudinally, then washed with saline followed by examination under dissecting microscope for measuring ulcer. The length of gastric ulcer was measured and

expressed as mean±SE for each group according to the method described by **Akhtar and Ahmad (1995)**

4. Determination the total acidity and pH of gastric juice:

Total acidity was determined by titration of 1ml gastric juice in 10ml of distilled water with 0.01N NaOH using two drops of phenolphthalein as an indicator. Data were expressed as percentage. The pH degree was determined by pH meter.

5. Histopathological study:

Specimens from stomachs were collected from rats of all experimental groups at the end of the experimental period, fixed in 10% neutral buffered formalin (pH=7.0), dehydrated in ethyl alcohol, then cleared in xylol and embedded in paraffin; 4-6 microns thickness, sections prepared and stained with haematoxylin and eosin for examining both fore and glandular parts of the stomach **(Carleton, 1976).**

6. Statistical analysis of data:

The obtained data were statistically analyzed using computerized SPSS (Statistic Program Sigma stat, statistical soft-ware, SAS Institute, Cary, NC). Effects of different treatments were analyzed by one way ANOVA (Analysis of variance) test using Duncan's multiple range test and $p<0.05$ was used to indicate significance between different groups **(Snedecor and Cochran, 1967)**.

RESULTS AND DISCUSSION

The present investigation aimed to study the potential effect of resveratrol on aspirin induced acute gastric ulcer in rats.

1. Effect of resveratrol on the length of gastric ulcer in rats:

Data listed in table (1) show the effect of resveratrol at two doses on aspirin induced gastric ulcer in rats.

Table (1): Effect of resveratrol at two doses on the length of gastric ulcer in rats

Groups		Aspirin and resveratrol	Doses (mg/kg b.wt.)	Gastric ulcer length (mm.)
				Mean ± SE
Control –ve	1	-	-	0.00
Control +ve	2	Aspirin(Asp)	200	6.38 ± 0.04 [a]
Treated Groups	3	(Asp) + Resveratrol	25	3.94 ± 0.03 [b]
	4	(Asp) + Resveratrol	50	2.19 ± 0.02 [c]

- Values denote arithmetic means ± standard error of the means
- Means with different letters (a, b, c, d) in the same column differ significantly at $p \leq 0.05$ using one way ANOVA test, while those with similar letters are non-significant.

It could be observed that the length of gastric ulcer in control +ve group was 6.38 ± 0.04 mm., compared to zero in control -ve group (normal rats). This mean that there were significant increase in gastric ulcer length in control positive group when compared to control negative. All treated groups including high and low doses of resveratrol (25 and 50 mg/kg b.wt) showed significant decrease in gastric ulcer length as compared to control positive group which were 3.94 ± 0.03 and 2.19 ± 0.02mm, respectively. Rats administered with resveratrol at high dose (50 mg/kg b.wt) showed the highest significant decrease in the length of gastric ulcer compared to low dose group. These results were in agreement with **(Peng *et al.*, 2016)** who demonstrated that resveratrol, a naturally dietary polyphenol, exhibited anti-inflammatory activity and a protective effect against gastric mucosa damage induced by NSAIDs. In this regard, it had been synthesized a series of resveratrol-based NSAIDs derivatives and evaluated their

anti-inflammatory activity against NO overproduction in LPS-stimulated RAW 264.7 macrophages. They identified mono-substituted resveratrol–ibuprofen combination 21 as the most potent anti-inflammatory agent, which is more active than a physical mixture of ibuprofen and resveratrol, individual ibuprofen, or individual resveratrol. In addition, compound 21 exerted potent inhibitory effects on the LPS-induced expression of TNF-α and IL-1β. Furthermore, compound 21 significantly increased the survival rate in an LPS-induced acute inflammatory model and produced markedly less gastric damage than ibuprofen. It was found that compound 21 may be a potent anti-inflammatory agent for the treatment of inflammation-related diseases.

In addition, a wide range of investigations has been executed to enhance resveratrol bioavailability to overcome its normally poor bioavailability. These strategies are generally based on encapsulations, inhibition of Cytochromes P450 (CYPs) by means of specific inhibitors such as piperine, and modifications of resveratrol's structure. **(Santos *et al* ., 2011).** The potential of resveratrol in the management of gastric and intestinal injury and inflammation, due to its strong antioxidant, anti-oxidative stress, free radical scavenging, and anti-inflammatory activities, is widely evaluated . The protective effect of resveratrol in the models of peptic ulcer is reasonably well-reported, with most investigations being based on its potential to suppress the production of key inflammatory mediators. In vitro studies demonstrated that resveratrol inhibits the expression and activation of nuclear factors (NF-kB) and intracellular transcriptional enzymes (MAPKs) **(Panaro *et al*., 2012**).

2. Effect of resveratrol on volume of gastric juice in rats:

Table (2) show the effect of aspirin with and without resveratrol on the volume of gastric juice in rats.

Table (2): Effect of resveratrol at two doses on the volume of gastric juice in rats

Groups		Aspirin And Resveratrol	Doses (mg/kg B.Wt.)	Volume of gastric juice (mL.)
				Mean ± SE
Control –ve	1	-	-	1.30 ± 0.003 [d]
Control +ve	2	Aspirin (Asp)	200	3.99 ± 0.008 [a]
	3	(Asp) + Resveratrol	25	2.78 ± 0.005 [b]
	4	(Asp) + Resveratrol	50	2.11 ± 0.004 [c]

- Values denote arithmetic means ± standard error of the means
- Means with different letters (a, b, c, d) in the same column differ significantly at $p \leq 0.05$ using one way ANOVA test, while those with similar letters are non-significant.

It is clear from the table that ulcerated rats (control positive group) showed significant increase ($p \leq 0.05$) in the volume of gastric juice in rats when compared to normal rats which were 3.99 ± 0.008 and1.30± 0.003 ml, respectively. All experimental groups that treated with resveratrol at two doses showed significant decrease in the volume of gastric juice as compared to control positive group. Oral administration with resveratrol at a dose of 50mg/kg b.wt showed the highest significant decrease in the volume of gastric juice which was 2.11 ± 0.004 ml., when compared to control positive group which revealed 3.99 ± 0.008 ml. These data agreed with that of **Solmaz *et al*.,(2009)** who demonstrated that resveratrol has both protective and therapeutic effects on oxidative gastric damage by suppressing pro-inflammatory cascades, including the activation of pro-inflammatory cytokines, accumulation of neutrophils and release of oxygen-derived free radicals.

3. Effect of resveratrol on pH of gastric juice in rats:

The effect of resveratrol at two doses (25 and 50 mg.kg b.wt.) on the pH of gastric juice is listed in table(3).

Table (3): Effect of resveratrol at two doses on the pH of gastric juice in rats

Groups		Aspirin and Extracts	Doses (mg/kg B.Wt.)	pH of gastric juice
				Mean ± SE
Control –ve	1	-	-	5.13 ± 0.024 [a]
Control +ve	2	Aspirin (Asp)	200	2.39 ± 0.080 [d]
	3	(Asp) + Resveratrol	25	3.73 ± 0.062 [c]
	4	(Asp) + Resveratrol	50	4.63 ± 0.049 [b]

- Values denote arithmetic means ± standard error of the means
- Means with different letters (a, b, c, d) in the same column differ significantly at $p \leq 0.05$ using one way ANOVA test, while those with similar letters are non-significant.

It is clear from data that oral administration with aspirin at a dose of 200mg/kg B.Wt., (control +ve) decreased the pH value of gastric juice which was 2.39 ± 0.080 compared with 5.13 ± 0.024 for normal rats (control – ve). Oral administration with resveratrol at doses of 25 & 50 mg/kg b,wt., showed significant increase in the pH of gastric juice as compared to control positive group which were 3.73 ± 0.062, 4.63 ± 0.049 and 2.39 ± 0.080, respectively. Oral administration with high dose of resveratrol (50 mg/kg b,wt.) showed the highest significant increase in the pH of gastric juice when compared low dose.

In an experimental study by **Solmaz *et al,.(*2009)** showed that resveratrol exhibited both protective and therapeutic effects in an animal model of gastric ulcer induced by acetic acid, which was due to attenuating gastric MPO activity (inhibition of leukocyte infiltration), MDA (suppressing tissue lipid peroxidation), elevating collagen content, and restoring depleted GSH. Suppressing the key pro inflammatory agent TNF-α has an essential role in its therapeutic function in gastric tissue. This polyphenol also

has significant suppression on intracellular and extracellular oxidative events in gastric mucosal tissue. In another experimental study executed by Mohammad *et al.*,(2015) this polyphenol protects against gastric injury induced by acidified ethanol, which is attributed to the enhancement of dimethylarginine dimethylaminohydrolase (DDAH) activity and the subsequent reduction of asymmetric dimethylarginine (ADMA) content, resulting in blocked methylarginines accumulation, the induction of NO synthesis, and vasodilation in mucosal tissue. Thus, augmentation of gastric NO production is among the most important abilities of resveratrol in the protection of gastric mucosa.

4. Effect of resveratrol on the total acidity of gastric juice in rats:

Data listed in table (4) show the effect of aspirin with and without resveratrol at two doses on the total acidity of gastric juice in rats.

Table (4): Effect of resveratrol at two doses on the total acidity of gastric juice in rats

Groups		Aspirin And resveratrol	Doses (mg/kg B.Wt.)	Total acidity (%)
				Mean ± SE
Control –ve	1	-	-	0.029± 0.003 [d]
Control +ve	2	Aspirin (Asp)	200	0.083± 0.008 [a]
	3	(Asp) + Resveratrol	25	0.065± 0.002 [b]
	4	(Asp) + Resveratrol	50	0.049± 0.005 [c]

- Values denote arithmetic means ± standard error of the means
- Means with different letters (a, b, c, d) in the same column differ significantly at $p \leq 0.05$ using one way ANOVA test, while those with similar letters are non-significant.

It is clear that in (C +ve) group there were significant increase in total acidity of gastric juice in rats compared to (C –ve) group which were 0.083 ± 0.008 and 0.029±

0.003%, respectively. Ulcerated rats and orally administered with low and high doses of resveratrol (25 & 50 mg/kg b,wt.) showed significant decrease in the percentage of the total acidity compared to control positive group. Oral administration with resveratrol at a dose of 50 mg/kg body weight showed the highest significant decrease in the percentage of total acidity in rats when compared to control positive group which were 0.049 ± 0.005 and 0.083± 0.008%, respectively. These results were in agreement with **(Dey *et al*., 2009)** who reported that resveratrol has a biphasic protective effect. It is now well-recognized that resveratrol has an excellent antibacterial effect against *H. pylori* infection. Resveratrol inhibits the virulence factor of *H. pylori* and urease enzyme activity significantly. The inhibitory property of resveratrol on this enzyme is non-competitive and dose dependent . *H. pylori* infection stimulates pro-inflammatory mediators, causing disturbance of surface epithelial cell integrity, irregularity of the luminal border, damage to the gastric microvilli, and inducing mucosal vacuolation Among the pro-inflammatory cytokines, IL-8 possesses an obvious effect in the pathogenesis of *H. pylori* diseases by activating neutrophils and inducing cellular chemoattraction.

However, **Paulo *et al*., (2011**) noted that resveratrol prevents *H. pylori*-induced gastric mucosal damage and gastritis by suppressing the secretion of IL-8 from *H. pylori* infected cells. *H. pylori* infection associates with ROS generation, oxidative DNA damage, and epithelial proliferation. Pretreatment with this polyphenol mitigates intracellular ROS generation and oxidative DNA fragmentation induced by *H. pylori* infection dose dependent l in gastric epithelial cell. The interaction of *H. pylori* with gastric epithelial cells is associated with

morphological changes that result in the dysregulation of host cell functions, cell motility, and the "hummingbird phenomenon" (altered migration, motility, and adhesion of the gastric endothelial cells), leading to gastropathy. Cellular studies showed that *H. pylori*-initiated gastric morphological changes were noticeably suppressed by resveratrol.

Moreover, **Mohammad *et al.*, (2015**) reported that **n**atural polyphenols have been to possess numerous beneficial roles in the gastrointestinal tract, including antispasmodic, anticolitis, anti-secretory, anti-diarrheal, anti-ulcer, and anti-oxidative stress properties . Additionally, it is possible that the therapeutic benefits of various traditional and complementary medicines in the treatment of peptic ulcer are related to the presence of polyphenol constituents .

5. Histopathological results:

Microscopically, the stomach of rat from control negative group revealed gastro-esophageal wall showing normal mucosal gastric gland's (Photo 1). Meanwhile, the stomach of rat from control positive group showed the mucosal layers of the stomach which was hyper-plastic and associated with focal superficial necrosis gastric (Photo 2). Stomach of rat from group 3 (resveratrol 25 mg/kg b.wt.) showing gastro-esophageal wall with marked gastric mucosal gland necrosis, and associated with lymphocyte cell infiltrate (Photo 3). Moreover, Stomach of rat from group 4 (resveratrol 50 mg/kg b.wt.) showing gastric wall with little focal superficial gastric mucosal gland necrosis and partial atrophy (Photo 4). These findings agreed with **Xiaolin *et al., (*2015)** who revealed that resveratrol treatment exerted significant

effects against oxidative stress and inflammation in *H. pylori*-infected mucosa through the suppression of IL-8, iNOS, and NF-κB, and moreover through the activation of the Nrf2/HO-1 pathway.

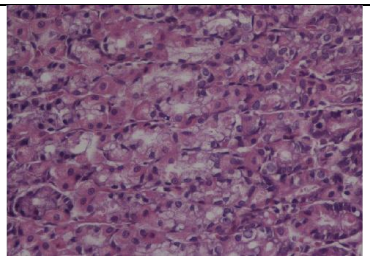
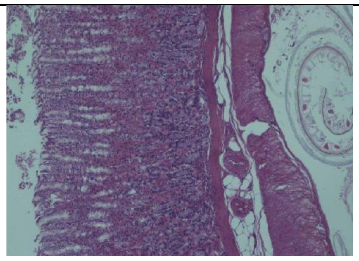
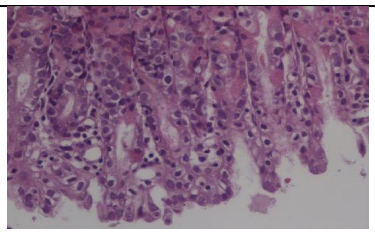
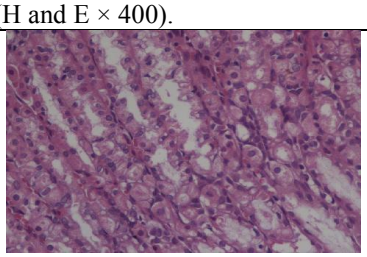
	

Photo (1): Stomach of rat from control negative (C -ve) group showing normal mucosal gastric gland's. (H and E × 400).	Photo (2): Stomach of rat from (C +ve) group showing the mucosal layers of the stomach which was hyper-plastic and associated with focal superficial necrosis gastric. (H and E × 400).
	
Photo(3): Stomach of rat from group 3 (resveratrol 25 mg/kg b.wt.) showing gastro-esophageal wall with marked gastric mucosal gland necrosis and associated with lymphocyte cell infiltrate. (H and E × 400).	Photo (4): Stomach of rat from group 4 (resveratrol 50 mg/kg b.wt.) showing gastric wall with little focal superficial gastric mucosal gland necrosis and partial atrophy. (H and E × 400).

CONCLUSION

Resveratrol could be used for healing aspirin induced acute gastric ulcer in rats especially at a dose of 50 mg/kg b.wt.

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