

*Full Length Research Paper*

## **Gastroprotective effects of amygdalin on experimental gastric ulcer: Role of NO and TNF- $\alpha$**

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Natural plant origin products like amygdalin with anti-inflammatory effects are still a major part of traditional medicine. We conducted the present investigation to elucidate the amygdalin effects on alcohol-induced gastric ulcer together with the possible role of nitric oxide (NO) and tumor necrosis factor-alpha (TNF- $\alpha$ ). Thirty-two male Wistar rats were used to study therapeutic and preventive effects of amygdalin on absolute alcohol-induced gastric ulcer. Animals were equally divided into 4 groups (n=8): (I) control, (II) Alcohol; absolute alcohol (1 ml/200 g b.w) used to induce gastric ulcer, (III) Alcohol/Amygda; amygdalin (2 mg/kg/day intramuscular) administrated for three weeks after gastric ulcer induction and (IV) Amygda/Alcohol; amygdalin was given for three weeks before gastric ulcer induction. Ulcer index, gastric acid secretion, gastric tissue's NO metabolites and TNF- $\alpha$  level measured. Ulcer index was significantly decreased in Alcohol/Amygda than Alcohol group (p<0.05). Gastric tissue's NO metabolites level and gastric acid secretion decreased in Alcohol and Amygda/Alcohol groups than control (p<0.05). Gastric tissue TNF- $\alpha$  level increased in Alcohol and Amygda/Alcohol rats than control group (p<0.05). The results of this study show that amygdalin protected gastric mucosa from alcohol-induced gastric ulcer. This gastroprotection may mediate via gastric mucosal nitric oxide production and TNF- $\alpha$  suppression.

**Key words:** Amygdalin, gastric ulcer, nitric oxide, TNF- $\alpha$ .

### **INTRODUCTION**

Natural plant origin products like amygdalin, a plant glycoside isolated from the stones of peaches, bitter almond and plums that has anti-inflammatory effects (Moertel et al., 1981; Yang et al., 2007) are still a major part of traditional medicine (Hwang et al., 2008). Many factors are involved in gastric and duodenal ulcer pathology such as stress, smoking, nutritional deficiencies, alcohol and nonsteroidal anti-inflammatory drugs (Basil and Howard, 1995). Protective measures should focus on gastric mucosa integrity and acid secretion, inflammation and atrophy of parietal cells,

blood flow, and endogenous agents especially nitric oxide (NO) and tumor necrosis factor-alpha (TNF- $\alpha$ ) (Singha et al., 2008).

Amygdalin is a cyanogenic glycoside claimed to show anti-inflammatory effects through COX-2 suppression and inducible nitric oxide synthase (iNOS) expression (Chang et al., 2005). Nitric oxide has been expressed in rat gastric mucosa and showed to reduce neutrophil adhesion, increase gastric blood flow and mucus secretion (Souza et al., 2004). It was also showed that amygdalin could suppress TNF- $\alpha$  expression in some tissues (Chang et al., 2005; Chang et al., 2006; Yang et al., 2007). An increase of pro-inflammatory cytokine level such as TNF- $\alpha$  has been shown in gastric and duodenal ulcers. TNF- $\alpha$  is involved in several physiological steps of inflammation including cell migration, edema and fever

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(Boraschi et al., 1998; Hwang et al., 2008; Lychkova et al., 2010). Since, estimates of the annual incidence of peptic ulcer disease range from 0.1 to 0.3% is in the world (Sung et al., 2009), we conducted the present study for the first time about amygdalin effects on gastric acid secretion, gastric tissue's NO metabolites and TNF- $\alpha$  level.

## MATERIALS AND METHODS

### Animals

Male Wistar rats (250 to 300 g) were obtained from the physiology department of Tehran Medical Sciences University animal room and kept in a temperature controlled environment on a 12:12 h light/dark cycle with free access to food and water.

The procedures were in accordance with the guidelines for the care and use of laboratory animal of Tehran University Medical Science.

### Study design

Thirty-two male Wistar rats were used to study therapeutic and preventive effects of amygdalin on absolute alcohol-induced gastric ulcer. Animals equally divided into 4 groups (n=8): (I) control, (II) Alcohol; absolute alcohol (1 ml/200 g b.w) administrated to induce rat gastric ulcer one hour before each experiment (Ozdil et al., 2004), (III) Alcohol/Amygda; amygdalin (2 mg/kg/day intramuscular) administrated for three weeks after gastric ulcer induction and (IV) Amygda/Alcohol; amygdalin was given for three weeks before gastric ulcer induction. The stock solution of amygdalin powder was dissolved in distilled water. Animals were deprived of food but free to drink water 24 h before each experiment (Nabavizadeh and Vahedian, 2004).

### Surgical procedure

Animals were first anesthetized with sodium thiopental (50 mg/kg i.p). Then, tracheostomy performed and cervical esophagus tied to prevent gastric reflux into the oral cavity (Nabavizadeh et al., 2009). Laparotomy was performed and a polyethylene cannula (3 mm diameter) placed in the stomach via a duodenal incision. Residual gastric secretions were lavaged several times with 1 to 2 ml normal saline (37°C). A recovery time of 30 min was given to reach a steady state (Nabavizadeh et al., 2009).

### Measurement of gastric acid and nitric oxide metabolites

In all groups, normal saline (1 ml) was introduced into the stomach. Normal saline injected again at the same dose 15 min later. Gastric contents were then collected using washout technique (Nabavizadeh et al., 2009; Salim, 1988). Basal acid secretion was measured with a digital titrator system (Basic Titrimo, Metrohm, 794). Stimulated acid secretion was measured 15 min post pentagastrin (25  $\mu$ g/kg i.p) injection (Nabavizadeh et al., 2009; Kato et al., 1998).

Gastric tissue's NO metabolites level was measured using Griess micro assay method (Nahrevarian et al., 2006). Also, number of ulcers was counted. Ulcer scoring was done according to the method by Vogel et al. (1997) as follows; the scores were: 0= no ulcer, 1= superficial ulcer, 2= deep ulcer, 3= perforation. Ulcer

index was measured by using the following formula (Vogel et al., 1997):

$$UI = UN + US + UP \times 10 - 1$$

UI = Ulcer Index

UN = Average number of ulcers per animal

US = Average number of severity score

UP = percentage of animals with ulcers

Percentage inhibition of ulceration was calculated as follows:

$$\% \text{ inhibition of ulceration} = \frac{(\text{Ulcer index Alcohol} - \text{Ulcer index Test})}{\text{Ulcer index Alcohol}} \times 100$$

### Histopathology study

Total stomach and proximal duodenum were removed and kept in fixative solution (10% formaldehyde). Then, they passaged and embedded in paraffin.

Paraffin blocks were sectioned by 3 to 5  $\mu$ m thickness for hematoxylin and eosin (H and E) staining to assay gastric or duodenal ulcers, inflammation and hemorrhages.

### Preparation of gastric tissue homogenate

Some samples of stomach were kept at -70°C to measure TNF- $\alpha$  (ELISA technique). Weighed samples of gastric tissue (approximately 0.2 g) were placed in 1.5 ml microfuge tubes and homogenized using an electrical homogenizer (Model RS541-242, RS Components, Corby, UK). Then homogenates tested for TNF- $\alpha$  level using a TiterZyme  $\text{EIA}$  rat TNF- $\alpha$  Enzyme Immunometric Assay (EIA) Kit (Catalog No. 900-042, 96 Well Kit, Germany) (Golestan et al., 2010).

### Materials

Amygdalin (linear formula;  $\text{C}_{20}\text{H}_{27}\text{NO}_{11}$ , molecular weight; 457.43, CAS number; 29883-15-6) and pentagastrin obtained from Sigma Chemical Co. (St. Louis, MO, USA).

### Statistical analysis

Results were expressed as mean  $\pm$  SE. Analysis of variance (ANOVA) and post hoc Tukey test used for comparison among groups by using SPSS software.  $P < 0.05$  was considered to be statistically significant.

## RESULTS

### Effects of amygdalin on ulcer index and gastric acid secretion

Gastric ulcer measurements showed that amygdalin significantly decreased the number of ulcer, ulcer score and index at Alcohol/Amygda than Alcohol group ( $p < 0.05$ ). But, the changes have not been noticeable in Amygda/Alcohol group (Table 1).

**Table 1.** Effects of amygdalin on alcohol-induced ulcer of gastric tissue.

Parameters Groups	Number of ulcers	Ulcer score	Ulcer index	Ulcer inhibition (%)
Control	-	-	-	-
Alcohol	4.9 ± 1.1	2.7 ± 0.9	23.6 ± 2.5	-
Alcohol/Amygda	1.9 ± 0.7*	1.1 ± 0.6*	9.0 ± 2.2*	48.5*
Amygda/Alcohol	5.1 ± 1.1	2.5 ± 0.7	20.5 ± 2.4	4.5

Data were expressed as mean ± SE, n=8, Amygda; Amygdalin, \*p<0.05 compared to Alcohol group.

**Table 2.** Gastric acid and NO metabolites levels due to amygdalin administration.

Parameters Groups	Basal acid (mmol/ml/15 min)	Stimulated acid (mmol/ml/15 min)	NO metabolites (µmol/g.wet weight)
Control	0.45 ± 0.05	1.55 ± 0.07	15.2 ± 0.3
Alcohol	0.06 ± 0.02*	0.12 ± 0.04 *	4.3 ± 0.22 *
Amygda/Alcohol	0.07 ± 0.03*	0.14 ± 0.03 *	5.2 ± 0.24 *
Alcohol/Amygda	0.6 ± 0.04 #	1.9 ± 0.06#	14.2 ± 0.4 #

Data were expressed as mean ± SE, n=8, Amygda; Amygdalin, NO; nitric oxide, \*p<0.05 compared to control, # p<0.05 compared to Alcohol group.

### Effects of amygdalin on gastric tissue's NO metabolites level

Gastric tissue's NO metabolites level, basal and stimulated acid secretion were less in Alcohol and Amygda/Alcohol groups than control (p<0.05). There was no difference in the level of these items between control and Alcohol/Amygda animals (Table 2).

### Effects of amygdalin on gastric tissue's TNF-α level

Gastric tissue's TNF-α level was more in Alcohol and Amygda/Alcohol rats than control (p<0.05). But, gastric tissue's TNF-α level was the same in Alcohol/Amygda and control groups (p<0.05) (Figure 1).

### Effects of amygdalin on histopathological of gastric tissue

Histological study showed gastritis and ulcer formation in Alcohol and Amygda/Alcohol groups unlike control and Alcohol/Amygda animals (Figures 2A to C).

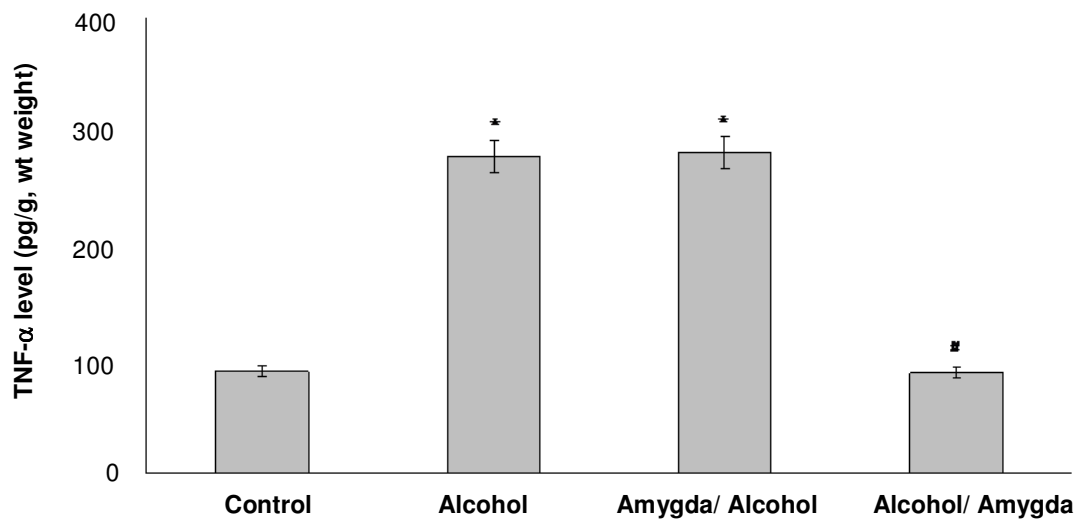
## DISCUSSION

The aim of our study was to survey protective and treating effects of amygdalin on alcohol-induced gastric ulcer in rats. We showed that there is a significant

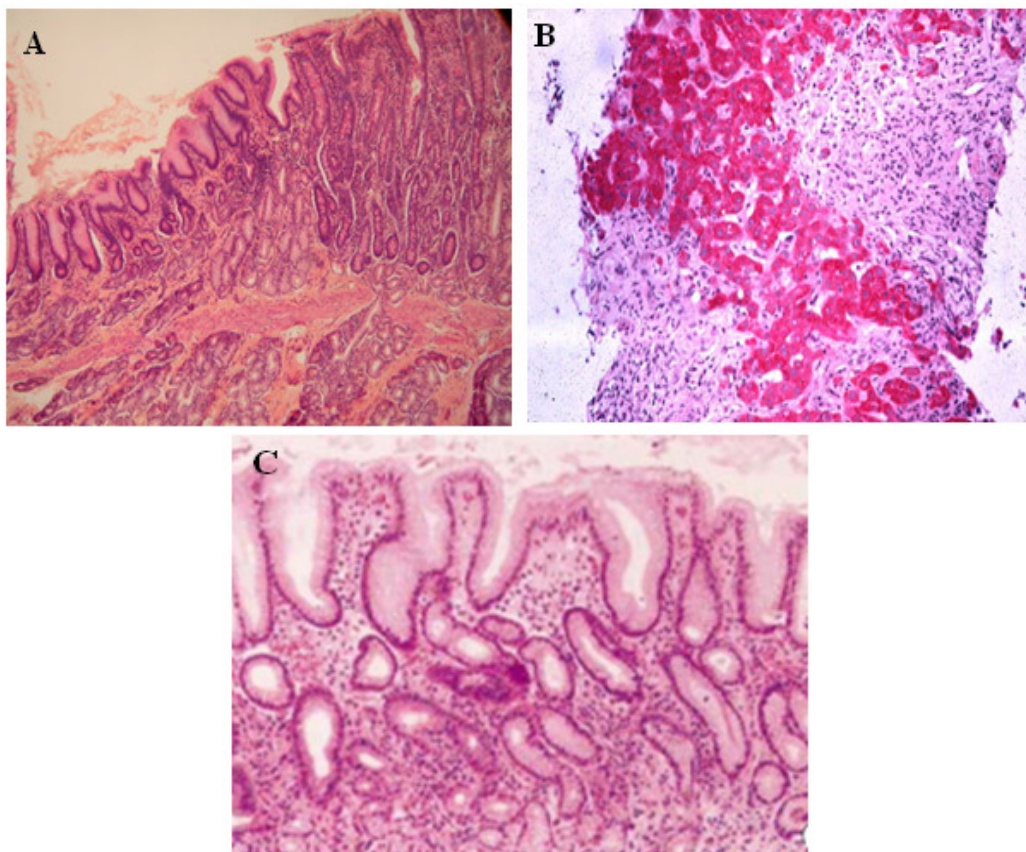
difference in gastric ulcer index, gastric tissue's NO metabolites and TNF-α level in Alcohol and Amygda/Alcohol groups than control. These therapeutic effects may be due to gastric tissue's NO and TNF-α level changes followed by amygdalin administration. Absolute alcohol gavage created remarkable gastric macroscopic and microscopic mucosal injury. The lesions were long, hemorrhagic and glandular confined (Figure 2B). Here, alcohol impairs gastric mucus natural defensive mechanisms and circulation (Kushima et al., 2005). Treatment with amygdalin could reduce the ulcer index and promote gastric healing in Alcohol/Amygda group (Table 1) (Figure 2), but amygdalin prescription before ulcer induction for preventive approach in Amygda/Alcohol group was not successful in the healing process of gastric lesions.

Although, ulcer etiology is idiopathic in most cases, it is generally accepted that an imbalance between acid production and mucosal integrity would be a causative factor (Wallace and Granger, 1996). Focal hyperemia, submucosal hemorrhage and circulatory disturbances seen followed by absolute alcohol ingestion may be a clue of this imbalance manifestations acting via several gastric mechanisms. Conceivably, we may conclude that amygdalin, a known anti-inflammatory agent (Yang et al., 2007), has also a potential anti-ulcer effect. Still further studies should be designed to show the specific contributed mechanisms.

In our study, NO metabolites level of gastric tissue was significantly higher in Alcohol/Amygda than Alcohol group. Several studies have demonstrated the



**Figure 1.** The effect of amygdalin on gastric tissue's TNF- $\alpha$  level. Data were expressed as mean  $\pm$  SE, n=8, Amygda; Amygdalin. \*p<0.05 compared to control, # p<0.05 compared to Alcohol group.



**Figure 2.** Histological features of gastric tissue due to amygdalin administration. A: Normal gastric mucosal (100X), B: Gastric mucosal ulcer (100X) and C: Gastritis (100X).

importance of endogenous NO in the protection of gastric mucosa (Kim and Kim, 1998; Tanaka et al., 2001; Whittle et al., 1990). It is showed that about 50% of the nerves in the enteric nervous system contain neuronal NOS (nNOS) (Dijkstra et al., 2004) and rat parietal cells express nNOS. Also, endothelial NO plays an important role in the modulation of gastric mucosal integrity by interacting with sensory neuropeptides (Whittle et al., 1990; Tepperman and Whittle, 1992). These findings suggest that endogenous NO may participate in the regulation of gastric secretion via an intracellular signaling (Dijkstra et al., 2004). In the present study, alcohol significantly reduced gastric tissue's NO metabolites level together with increased mucosal injury compared to control group. These findings are in accordance with Tripp and Tepperman study in which they reported decreased NO biosynthesis along with mucosal damage (Tripp and Tepperman, 1995). Here, we may assume that NO release followed by amygdalin administration can increase basal and stimulated vagus nerve tone and augment acetylcholine effects on gastric muscular cells. It has also been showed that NO can presynaptically facilitate vagal neurotransmission which ultimately leads to presynaptic L-type  $Ca^{+2}$  channels phosphorylation. This pathway causes increased presynaptic calcium influx and vesicular acetylcholine release (Herring and Paterson, 2001). So, NO release in gastric tissue followed by amygdalin administration can cause healing process of gastric lesions. TNF- $\alpha$ , a known factor in gastric ulcer pathogenesis significantly decreased in gastric tissue of Alcohol/Amygda animals (Souza et al., 2004). It was also showed that amygdalin suppressed the expression of TNF- $\alpha$  mRNA in some tissues (Hwang et al., 2008).

Our results suggest that the inhibitory effect of amygdalin can be attributed to transcriptional mRNA suppression of pro-inflammatory cytokines such as TNF- $\alpha$  (Dorazil-Dudzic et al., 2004; Bianchi et al., 2004). It is also reported that TNF- $\alpha$  is capable of inhibiting gastric acid secretion and parietal cells apoptosis induction via NF-kB expression (Beales and Calam, 1998; Neu et al., 2003). This mechanism may contribute to gastric mucosal atrophy and ulcer. So, TNF- $\alpha$  suppress in gastric tissue followed by amygdalin administration can cause healing process of gastric lesions.

## Conclusion

The results of the present study show that amygdalin treated alcohol-induced gastric ulceration, but its preventive approach was not successful in the healing process of gastric lesions. This gastroprotection may be mediated via gastric mucosal nitric oxide production and TNF- $\alpha$  suppression.

## ACKNOWLEDGEMENT

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