Gastroprotective Effects of *Ziziphus nummularifolia* on Hcl/Ethanol-Induced Gastric Damage in Rats.

Mirzaei A[1], Shariati A[2*], Ghavamizadeh M[2]

[1]Medicinal Plant Research Center, Yasuj University of Medical Sciences, Yasuj, Iran.
[2]Student Research Committee, Yasuj University of Medical Sciences, Yasuj, Iran.
*Corresponding author: alishariati66@gmail.com

Abstract: Plants are the riche stresources of new drugs for traditional and modern systems of medicine and play an important role in human health. Over three-quarters of the world population relies mainly on traditional medicine for health care. *Ziziphus*is a genus (family: *Rhamnaceae*) of plants found in tropical regions. *Z. nummularifolia* is a medicinal plant and has been used in different diseases in traditional medicine in Iran. The aim of this study was to evaluate the gastroprotective effect of hydro-alcoholic extract of *Z. nummularifolia (HEZN)* on Hcl/Ethanol-induced gastric damage in rats. **Material and Methods:** The *HEZN* at doses of 250 and 500 mg/kg was orally administered once daily for 8 days. The gasteroprotectivie activity was evaluated by assessment of various biochemichal antioxidant enzymes such as superoxide dismutase (SOD) and catalase (CAT). Also, malondialdehyde (MDA) as a product of lipid peroxidation, nitric oxide radical (NO°) and reduced glutathione (GSH) were measured. **Results:** Levels of antioxidant enzymes (e.g. SOD and CAT) and GSH decreased as compared to normal control group. MDA and NO radical were increased in positive control group. Although SOD, CAT, GSH, MDA and NO radical have been restored toward normal state by the *HEZN*, significantly ($p<0.05$). The *HEZN* proved to display a dose-dependent gastroprotective effect. **Conclusion:** The *HEZN* displayed significant protective effect on Hcl/Ethanol-induced gastric damage in rats which may be related to the antioxidant activity of the *HEZN*.
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Introduction

The stomach is a muscular organ located on the left side of the upper abdomen. The stomach secretes acid and enzymes that digest food. The peptic ulcers are open sores that develop on the inside lining of stomach and other organs e.g. esophagus and upper part of small intestine. It is considered a disease of modern times. Treatment with natural products presents a safe with less side effects of a cure. Plants have been raw material for the synthesis of many drugs and they remain an important source of new therapeutic agents. Many studies (Borrelli and Izzo, 2000) demonstrated the enormous variety of chemical substances isolated from plants that present gasteroprotective activity, indicating their great potential in the detection of new therapies for gastric ulcers. Plants are free gifted natural source and plant constituents like flavonoids, poly phenols are broadly distributed in them and have been reported to display marked *in vitro* and *in vivo* anti-inflammatory properties (Havsteen (2002), Middleton et al., 2000). In the last years, some reports on the gastroprotective activity of diterpenes from different medicinal plants with various types of structures have been published. Gasteroprotectiveterpenoids have been isolated from several plants (Giordano et al., 1990). Many species have been used since ancient times as folk remedies because they have chemical constituent (e.g. flavonoid, triterpenoids and tannins) which protect the stomach mucusa through the induction of gasteroprotective mechanisms of acting as natural antioxidant(Gonzalez et al., 2001). Many medicinal plants contain amounts of antioxidants, which play an important role in adsorbing and neutralizing free radicals, the medicinal values of plants has assumed a more important dimension in the past few decades owning largely to the discovery that extracts from plants contain a diverse array of secondary metabolites with antioxidant potential (Akinmoladun et al., 2007). The therapeutic effect of plants which are used in traditional medicine, are usually ascribed to their antioxidant compounds. They prevent oxidative deterioration of lipids. Plant derived antioxidants are now preferred to the synthetic ones because of safety concerns (David and Timothy, 2009). These factors have inspired the widespread screening of plants for possible medicinal and antioxidant properties, the isolation and characterization of different phytochemicals and the development and utilization of antioxidants of natural origins (Jayaprakasha et al., 2001). *Ziziphus nummularifolia* (family: *Rhamnaceae*) is an herbaceous plant found in tropical regions such as India, Saudi Arabia and Iran. Different extract from *Z.nummularifolia* showed anti-ulcer and anti-inflammatory activities as well as the wound healing

activity (Hasan Soliman, 2011). It is widely used as a medicinal plant for the treatment of digestive diseases in Iran. Some studies have shown that other species of this family, *Zyzyphus jujube*, *Zizyphusspina-christi* and *Ziziphus sativa* have useful in inflammatory reaction, mental retardation, cold, piles, mouth ulcers, sedative, antidote, astringent, pectoral, fragrance, hepatic disorder, anxiety and anti-diabetic activities (Anand et al., 1989-12; Glombitza et al., 1994; Ignacimuthu et al., 1998). Therefore, considering the therapeutic properties attributed to this plant and the anti-inflammatory effects demonstrated by previous studies, we investigated the gastro protective action of the hydro-alcoholic extract of aerial parts *Z.nummularifolia*on Hcl/ ethanol-induced gastric damage in male wistar rats.

2. Material and Methods

2.1. Plant materials

Z. nummularifolia was collected from Yasuj and identified by a taxonomist. The aerial parts were shade dried at 25–30 °C. A voucher specimen was deposited in the herbarium of medicinal plant research center, Yasuj, Iran.

2.2. Preparation of plant extract

Aerial parts of *Z. nummularifolia* cut into small pieces by a grinding machine. Plant extraction carried out by maceration method (Sukhdev et al., 2008).In this process, 100 gram of the coarsely powdered crude plant was placed in a beaker with ethanol-water 70% (V/V) and allowed standing at room temperature for 48 hours with frequent agitation. The mixture then clarified by filtration with a Wathman filter paper No.1. The solvent was evaporated using rotary evaporator (Hyedolph, type:HeizbadHei-VAP, Germany).

2.3. Antioxidant Assessment

2.4. Toxicity study

An approximate LD_{50} of *HEZN*was initially determined in a pilot study by a so-called staircase method using a small number of animals (Miller and Tainter, 1944).

2.5. Animals

Male wistar rats weighting 200–250g from the Yasuj Laboratory Animal House (YLAH) were fasted for 24 hr before the experiments but allowed free access to water. They maintained under conditions of controlled temperature (25±2°C) and illumination (12 h light cycle starting at 06:00 am) (Robert et al., 1979).

2.6. Experimental design

2.6.1 Treatment groups

The rats were divided into five groups of six each as shown in the below.

Group I – Negative Control received distilled water 1ml/kg, p.o.

Group II – Positive Control received Hcl/Ethanol, 1ml/kg, p.o)

Group III – Standard received Hcl/Ethanol (1ml/kg, p.o) + *Ranitidine* (500mg/kg, p.o).

Group IV – treatment 1receivedHcl/Ethanol (1ml/kg, p.o) + *HEZN* (250mg/kg, p.o).

Group V – treatment 2received Hcl/Ethanol (1ml/kg, p.o) + *HEZN* (500mg/kg, p.o).

2.6.2 Procedure for producing gastric lesions

In groups IV and V the *HEZN* administered orally 4 days prior to induction of gastric damage. Then prescription continued by the *HEZN* (250, 500mg/kg, p.o) and Hcl/Ethanol (1ml/kg, p.o) simultaneously for 4 days later, except for standard group (group III) which received ranitidine (500mg/kg, p.o) and Hcl/Ethanol (1ml/kg, p.o) simultaneously. Animals were sacrificed under light ether anesthesia After 8 days. Blood was collected by heart puncture for MDA and NO assays in serum. The stomach was removed; cut along the greater curvature and washed with normal saline and the mucosa was rinsed with normal saline to remove any contamination. A small part for stomach GSH, SOD and Cat assay, kept in a micro tube for gastric mucosa homogenate.

2.7. Biochemical Assessments

2.7.1. Malondialdehyde (MDA) assay

MDA was assayed in serum by the method of Hoyland (Hoyland and Taylor, 1991). In this assay, 500 µl serums were shaken with 2 ml of MDA reagent (15gr, TCA+375 mg, TBA dissolved completely in 0.25 M Hcl) in a 10 ml centrifuge tube and warmed for 15 min in a boiling water bath followed by rapid cooling in ice. Then it was centrifuged at 2000g for 5min. Supernatant decant into a cuvette and MDA content in the serum was determined from the absorbance at 535 nm by spectrophotometer (Pharmacia LKB·Novaspec II, Germany). The standards of 0, 2.5, 5, 10, 20, 40 µmol/ml were used. The results were expressed as µmol/ml serum.

2.7.2 Reduced Glutathione (GSH) assay in gastric mucosa

Reduced Glutathione (GSH) levels of gastric tissue of animals were determined by Ellman's reagent by the method of *Moron* (Moron et al., 1979). In this assay, briefly, the homogenate was precipitated with 25% tri-chloroacetic acid (TCA) and centrifuged. The supernatant used for GSH

estimation. The intensityof the yellow color was read at 412 nm in spectrophotometer.

2.8. Antioxidant Enzymes Assessment

2.8.1. Superoxide dismutase (SOD) assayin gastric mucosa

The method of Misra and Fridovish was used for the determination of SOD activity (Misraand Fridovich, 1972).

2.8.2. Catalase (CAT) assayin gastric mucosa

The method of Beers and Sizer was used for the determination of CAT activity (Beers and,1952).

2.8.3. Nitric oxide radical (NO°) assay:

The method of Garrat was used for the determination of Nitric oxide radical on serums (Garrat, 1964).

2.9. Statistical analysis

Statistical analyzes were performed using the SPSS statistics software ver.20.Values are expressed as Mean±S.D. p <0.05 were considered significant.

3. Results

Toxicity

Median lethal dose (LD_{50}) values of the *HEZN* in rats was found to be >5000 mg/kg after oral gavages.

3.4. Biochemical assays

3.4.1Antioxidant enzyme assessment

Oral administration of Hcl/Ethanol (1ml/kg) led decrease levels of gastric SOD and CAT in all groups compared to normal control .Although Co-administration of Hcl/Ethanol (1ml/kg, p.o) and *HEZN* (500mg/kg, p.o) restored those serum enzymes toward normal status significantly p<0.05 (Table 1).

Table 1.The effect of *HEZN* on gastric SOD and CAT activity in Hcl/ethanol-induced gastric damage in rats

Group	SOD (U/mg protein)	CAT(U/mg protein)
NC	517.61±30.3	1.61±0.33
PC	332.83±32.21	0.83±0.01
ST	604.25±51.43[a]	1.36±0.13[b]
T 1	702.38±27.09[a]	2.38±0.09[b]
T 2	689.50±45.22[a]	2.5±0.22[b]

(NC): Norml Control (distilled water,1ml/kg, p.o), (PC): Positive Control (Hcl/Ethanol,1ml/kg, p.o), (ST): Standard, Hcl/Ethanol (1ml/kg, p.o) + *Ranitidine* (500mg/kg, p.o), (T_1): Treatment 1: Hcl/Ethanol (1ml/kg, p.o) +*HEZN* (250mg/kg, p.o), (T_2): Treatment 2: Hcl/Ethanol (1ml/kg, p.o) + *HEZN* (500mg/kg, p.o). [a]Significant differences compared to positive control group (p<0.05). [b]Significant differences compared to positive control group (p<0.05).

3.4.2 MDA, GSH and NO assessment

Orally administration of Hcl/Ethanol (1ml/kg) caused to an increase in serum levels of serum MDA and NO in all groups except normal control group. However, Co-administrationof *HEZN* (500mg/kg, p.o) and Hcl/Ethanol (1ml/kg, p.o) restored serum levels of MDA and NO toward normal status significantly p<0.05 (Table 1). *HEZN* consumption at highest dose (500mg/kg, p.o) caused a significant increase in GSH level p<0.05 (Table 2).

Table 2. The effect of *HEZN* on serum MDA and NO and gastricGSH in Hcl/ethanol-induced gastric damage in rats

Group	GSH (µg/mg protein)	MDA (µg/ml)	NO (µmol/ml)
NC	2.38±0.33	36.61±2.25	2.78±0.14
PC	0.83±0.10	43.49±12.01	9.82±0.52
ST	1.28±0.13[a]	37.90±11.11[*]	3.52±0.09[c]
T 1	1.38±0.09[a]	32.38±0.09[b]	6.82±0.09[c]
T 2	1.14±0.22[a]	33.41±0.22[b]	4.21±0.09[c]

(NC): Norml Control (distilled water,1ml/kg, p.o), (PC): Positive Control(Hcl/Ethanol,1ml/kg, p.o), (ST): Standard, Hcl/Ethanol (1ml/kg, p.o) + *Ranitidine* (500mg/kg, p.o), (T_1): Treatment 1: Hcl/Ethanol (1ml/kg, p.o) +*HEZN* (250mg/kg, p.o), (T_2): Treatment 2: Hcl/Ethanol (1ml/kg, p.o) + *HEZN* (500mg/kg, p.o).

[a]Significant differences compared to positive control group(p<0.05). [b]Significant differences compared to positive control group (p<0.05). [c]Significant differences compared to positive control group (p<0.05).

4. Discussion

The plants of genus *Ziziphus* (family: *Rhamnaceae*) are used in traditional medicine for the treatment of several conditions, including anti-inflammatory, mental retardation, cold, piles , hair growth, mouth ulcers, sedative, anodyne, antidote, astringent, amollient, hypnotic, pectoral, fragrance, hepatic disorder, anxiety and anti-diabetic properties (Hasan Soliman (2011); Anand et al., 1989; Glombitza et al, 1994; Ignacimuthuand Amalraj, 1998). Also herbal medicine cures many diseases related to inflammation such as cardiovascular diseases, diabetes, cancer and etc. In recent years, natural compounds present in plants e.g. tannins, flavonoids, triterpenes and alkaloids act as antioxidant agents. Thus using natural products as a source for treatment of disease is a good choice and new alternate. This study reveals that hydro alcoholic extract of *Ziziphus nummularifolia* have gasteroprotective potential along with their other medicinal activities of its family. According to chemical analysis on some species belong ingto this family many constituents, such as flavonoid,

triterpenoids and tannins was reported. Some flavonoids have been shown gastroprotective property (Alarcon etal., 1993). It is also several biological effects in different extracts from other species of this family were reported. Protective potential of present extract has been attributed to the inhibition of ROS, RNS. The role of free radical in the pathophysiological processes of gastrointestinal injury recently reported in literature. In the case of Hcl/Ethanol-induced ulcers, damage is caused by a direct necrotizing effect over the gastric mucosa, which produces a hypoxic state that triggers the generation of free radicals in the tissue. Oxygen reactive species cause the destruction of the gastric mucus barrier, allowing acidic gastric juices to reach the stomach tissue causing the ulceration (Calvo et al., 2007: Mohan et al., 2006). The present study revealed that protection against gastric injury is offered by HEZN as a free radical scavenger. Some studies same to present research have reported that pretreatment with plant extracts can prevent lesion formation by irritants through the biosynthesis of PGs (Robert et al., 1983). It is believed that HEZN may prevent the gastric damage produced by Hcl/Ethanol same as quercetin and quinacrine. They inhibit phospholipase A_2 which catalyzes the rate-limiting step in the biosynthesis of PGs (Lee et al., 1982; Flower and Blackwell, 1976). Previous studies have shown that gastric damages were accompanied by an increase in the lipid peroxide level of gastric mucosa (Mizuiand Doteuchi 1986). On the other hand, high concentrations of reduced glutathione (GSH) as an antioxidant, is found in the gastric mucosa of rats as well as prostaglandins (PGs) can prevent the gastric damage induced by Hcl/Ethanol (Boyd et al., 1981; Robert et al., 1979). According to present results reactive oxygen species (ROS) such as superoxide anion radicals, hydroxyl radicals and nitrogen reactive species (RNS) (e.g. NO radicals) contributes to lipid peroxidation (Perry et al., 1986) and cause damage formation in the gastric mucosa after Hcl/Ethanol-administration. It concluded that gastric damages were induced by oral administration of Hcl/Ethanol could be prevented by pretreatment and then co-administration with *HEZN* as an antiperoxidative crude extract.

Conclusion

This research demonstrated the gastero protective effects of *Z. nummularifolia* which monitor by determination of antioxidant enzyme markers. This protection may relate to the antioxidant property of the *HEZN*.

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