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Gastroprotective Activity of *Artemisia Herba Alba* Aqueous Extract On Aspirin-induced Gastric Lesions in Albino Rats

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Abstract: In the recent decades, the use of traditional medicine worldwide has tremendously increased. The gastroprotective effects of aqueous leaves extract of *Artemisia herba alba* [Asso] were investigated against aspirin-induced gastric lesion in male albino rats. Aspirin [10mg/kg body weight] was orally administered to fasting male albino rats [n=6]. The acute mucosal damage was measured 30 min later. The oral administration of aqueous leaves extract of *Artemisia herba alba* before aspirin led to gastric protection detected by a decrease in both ulcers number and gastric fluid contents. The gastroprotective effects of *Artemisia herba alba* aqueous leaf extract at 2.5; 5 and 10 ml/kg body weight, as indicated by number of ulcer, were 16.7%, 56.4% and 74.8%, respectively. In conclusion, a concentration-dependent protective effect of the aqueous leaves extract of *Artemisia herba alba* against stomach injury induced by aspirin was achieved. This protective effect against ulcer formation may support the traditional use of *Artemisia herba alba* extract in treatment and protection.

Keywords: *Aspirin, Artemisia herba alba; Gastric ulcer; Albino rats.*

1. Introduction

The mucus layer that protects the gastroduodenal mucosa plays a protective role under normal physiological conditions. The thickness of this layer, made up of glycoprotein and water, and its physicochemical properties are thought to be important factors determining its efficacy as a barrier [24,20]. Stomach ulcers may develop from decreased resistance of the mucus lining the stomach to gastric acids, increased production of gastric acids or bacteria called *Helicobacter pylori* and it is more likely to occur in people who take aspirin or anti-inflammatory drugs, drink alcohol, smoke tobacco or feel stressed [2,12].

Akiba and collaborators had evaluated the mucus layer thickness in the stomach in response to exposure to acid, the effects of cyclogenase enzyme [COX] inhibition using indomethacin and the effects of exogenous prostaglandins [1]. The exposure to acid led to increase in the mucus layer thickness, while the indomethacin treatment with subsequent acid exposure led to opposite effects, which finally leads to stomach injuries that decreased in case of pretreatment with prostaglandins. Their study demonstrated that the COX inhibitors destabilize the mucosa barrier leading to an increased susceptibility to acid injury [1,30]. It was early found that there is a relation between the chronic ingestion of Non-steroidal anti-inflammatory drugs [NSAIDs] and the increased risk of gastric damage [26]. The gastric ulcers were found to occur by two independent mechanisms, either by topical injury to the mucosa or systemic depletion of cytoprotective prostaglandins by inhibiting COX enzyme [27]. The topical injury occurs because NSAIDs are weak acids, so they are non-ionized in the acidic environment of the stomach allowing them to diffuse freely across cell membranes and accumulate in the neutral environment of the mucosal cells [27].

Non-oral routes of administration of NSAIDs do not avoid the gastric ulceration risk completely but only it decreases the part of risk that is due to topical injury of the gastric mucosa [13,27]. In addition, generation of reactive oxygen species [ROS] is also considered as a major factor contributing to ulcer pathogenesis. The pathogenesis of experimental mucosal damage in rat stomach includes the generation of ROS that seem to play an important role, namely due to generation of lipid peroxides, accompanied by impairment of antioxidative enzyme activity of cells [14].

Aspirin, being the prototype of NSAIDs, was found to significantly lower the gastric juice prostaglandins [PGs]. It caused gastric ulceration in healthy subjects even after oral administration of 30mg/ day of aspirin for one week [27]. In addition, it was found that as the dose of aspirin increases, the risk of gastric ulcers also increases in parallel manner [27]. For these reasons, aspirin is widely used in clinical researches or acute induction of gastric ulcers [16, 27].

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Usually mechanisms of treatment of gastric ulcers are confined in neutralizing the gastric acids by using antacids, decreasing gastric acids secretion by using H_2-receptor blockers, eradicating *Helicobacter pylori* bacteria by forming a protective barrier from gastric acids by using sucralfate. Also patient has to stop using anti-inflammatory drugs, alcohol ingestion, tobacco smoking and learn to control emotional stress [12].

Artemisia herba alba [Asso.] is widely distributed in west southern France, Spain, Morocco, Algeria, Tunisia, Libya, Egypt, Syria and Iran. The use of its leaves and flowers in folk medicine was reported [7, 9]. Many phytochemical studies were carried out on the *Artemisia herba alba* [*AH*], and about four essential oils were isolated from its leaves such as armoise, artemisine, togone and the well characterized santonin [7,8]. Two polyphenols were early recognized, they are beta sterols [7]. Recently, essential oil compositions as cineole, thujones, chrysanthenone, camphor, borneol, chrysanthenyl acetate, sabinyl acetate, davana ethers and davanone were identified [29,17,21]. The major components are monoterpenes and sesquiterpenes [17]. A recent study suggests that flavonoids extracted from *Artemisia herba alba* could have potential immuno-modulatory effects [14]. The aqueous and alcoholic extracts of *AH* were found to have gastroprotective effects on ethanol induced gastric ulcer [10]. However, the mechanism underlying the protective effects of *AH* against gastric damage is unclear. Here in, we investigated the gastroprotective effects of *AH* aqueous leaves extract on aspirin-induced gastric ulcer model of rats.

2. Materials and Methods

Plant materials: *Artemisia herba alba* [Asso] belongs to family *Asteraceae*. It is an aromatic, low growing, up to 30 cm high, much branched, shrubby perennial plant [6]**.** Fresh leaves of *AH* were collected during 2015 from North West Libya.

2.1 Preparation of Artemisia herba alba aqueous extract

The powdered dry leaves of *AH* [3g] were boiled in 100 mL distilled water for 10 min. The leaves were then filtered off and the filtrate was collected and diluted three times with distilled water. The collected yellowish brown aqueous leaves extract was then freeze-stored and kept until used.

2.2 Preparation of aspirin solutions

Acidified aspirin solution was prepared by dissolving 1 g of aspirin [Sigma Chemical Co.] in 100 ml of 0.2 M HCl to give [1%] solution of acidified aspirin.

Animals: 30 male Wistar rats [bred at the Animal Care Unit, Department of Pharmacology and Clinical Pharmacy, University of Tripoli] at about six weeks of age weighing 185-215 g were used in this study. The rats were fasted for 48 h before the experiments while water was allowed *ad libitum*. During the fasting period, the animals were kept in metabolic cages with wide mesh wire bottoms to prevent coprophagy.. The study was approved by the faculty and the experiments were done according to the ethics guidelines of University of Tripoli.

3. Experimental Design

3.1 Effects of Artemisia herba alba on aspirin-induced gastric ulcer

The 48 h fasted rats were randomly divided into 6 groups of 6 rats. The normal group [control, G1] received only distilled water [10 ml/kg b.w.] and the negative control group [G2] was given distilled water [10ml/kg b.w.] followed by acidified aspirin solution [10 ml/kg b.w.]. The positive control group [G3] received ranitidine chloridrate [oral solution 15 mg/ml [Ache, trade name Label]. Three treatment groups [G4, G5 and G6] received 2.5, 5.0 and 10 ml/kg b.w. of *Artemisia herba alba* aqueous extract by gastric gavage, respectively. Thirty minutes later, all animals were orally given acidified aspirin to induce gastric ulcer except the control group. Thirty minutes after aspirin administration all animals were killed by a sharp blow on the head. Stomachs of the rats were rapidly removed after clamping both the esophageal and pyloric ends, then stomachs were opened along their greater curvature, its content was drained on a glass funnel placed in a measuring cylinder and mucosal surface was rinsed with 10 ml distilled water to recover the fluid gastric content completely. The fluid content was recorded after subtracting the 10 ml distilled water added previously. Each stomach was photographed after it fixed with 10% formalin. Ulcer size was measured along its greatest length using a mm scale and the mean ulcer index was calculated. The percentage protection was calculated in each group and was analyzed for difference against the control.

3.2 Quantification of the gastric hemorrhagic ulcer

The photographs of the stomach [about 2.5 magnification] were taken for measuring numbers, length and size of ulcer using [mm] scale [Figures were not shown].

3.3 Statistical analysis

The data were expressed as means ± S.E.M and analyzed statistically using one-way analysis of variance [ANOVA] followed by Tukey's test. *P* values less than 0.05 were considered significant.

4. Results

4.1 Aspirin-induced gastric hemorrhagic ulcer formation

The macroscopic findings of the opened stomachs of treated and untreated animals showed that hemorrhagic gastric ulcers covered with coagulated blood were more apparent in the aspirin-administered group [Group 2] than the control [Group 1] and ranitidine [Group3]. The administration of different doses of *AH* prior to aspirin [Group 4, 5 and 6] inhibited aspirin-induced ulcer formation [Table 1]. An analysis of data between the groups indicated a decrease in the number and lenght of gastric lesions compared to the control [Table 1]. Animals treated with water prior to aspirin administration showed consistent and extensive macroscopical damage, which was characterized by presence of elongated haemorrhagic lesions confined in most cases to the glandular region. The gastric protection of *AH* was observed as a decrease in the lesions number and size [Table 2]. The net decrease in the fluid content of the stomach by different doses of *AH* were also showed in Table 2.

5. Discussion

Peptic ulcer disease is a chronic pathology that affects millions of people worldwide. Many populations will develop this condition at some point in their lives [32]. The disease process of peptic ulcers is multifactorial based on etiology and risk factors. Ulcers mainly caused by a disruption in the balance between aggressive factors [pepsin and hydrochloric acid] and mucosal defensive factors, such as blood flow, mucus and bicarbonate secretion. Agents that present gastroprotection against induced gastric lesions act mainly either by stimulation of defense mechanisms [cytoprotective effect] or by the inhibition of aggressive factor production or release [anti-secretory effect].

The gastric ulcer treatment options include antacids, cytoprotective agents; muscarinic antagonists, antimicrobial agents for eradication of *H. pylori*, H_2-receptor antagonists, and proton pump inhibitors that act through severe alteration of pH gradients regulation, including ROS production [14]. Since the effect of proton pump inhibitors was mediated by an early production of ROS, preceded the alkalinization of lysosomal pH, lysosomal membrane permeabilization, and cytosol acidification, suggesting an early destabilization of the acidic vesicular compartment [14].

Table 1. Effect of simultaneous oral administration of different doses of *Artemisia herba alba* extract on aspirin (10ml/Kg) induced gastric damage in rats.

Treatments (n=6)		Number of ulcer spot	Length of ulcer (mm)	Mean of fluid content (ml)
Control	**(water)**	0	0	0.5 ± 0.047
Aspirin	**(10mg/kg)**	5.5 ± 1.02***	83.8 ± 14.71**	0.87 ± 0.095*
Ranitidine	**(50mg/kg)**	2.1 ± 0.4[a]	0	0.3 ± 0.045*[a]
Artemisia	**(2.5 mg/kg)**	4.2 ± 0.54***	69.8 ± 7.19**[a]	0.9 ± 0.132*
extract	**(5 mg/kg)**	3.0 ± 0.63*	36.5 ± 2.07*[a]	0.4 ± 0.047[a]
	(10 mg/kg)	2.2 ± 0.54[a,b]	21.2 ± 4.80*[a,b]	0.4 ± 0.044[a]

One way ANOVA followed by the *Tukeys test* (mean ± S.E.M); ****P<0.0001 **P<0.001 and *P<0.05)* in relation to control (water) group. [a] *P<0.001 in relation to aspirin group,* [b]*P<0.001 in relation to ranitidine group.*

Table 2. Effect of simultaneous oral administration of different doses of *Artemisia herba alba* extract on aspirin (10ml/Kg) induced gastric damage in rats.

Treatments (n=6)		mean ulcer index (mm)	% Protection	% Decrease in gastric content
Control		0	0	-
Aspirin	**(10mg/kg)**	83.8	0	0
Ranitidine	(50mg/kg)	0	91.1	0
Artemisia extract	(2.5 mg/kg)	69.8	16.7	26.8
	(5 mg/kg)	36.5	56.4	59.2
	(10 mg/kg)	21.2	74.8	78.9

Parameters: Mean ulcer no.= mean ± S.E.M; Artemisia extract; Mean ulcer index (MUI) = total ulcer length/no. of rats in each group; Ulcer index represents the mean ± SEM, *%Protection = total ulcer length of treated group/ total ulcer length of control group x 100.*

Some of these treatments have side effects such as increase susceptibility to fractures, pneumonia, and gastric cancer [25]. Thus, research to develop new therapeutic agents for gastric ulcer treatment is necessary. In this context, medicinal plants are known to be important resources of bioactive molecules with anti-ulcerogenic properties potential [33,22]. Among medicinal plants, many species have been used since antiquity because of therapeutic properties that are traditionally attributed to the presence of phytochemical constitutes [5,4].

The main findings in the present study indicate that after oral administration of aqueous leaf extract of *Artemisia herba alba* at a dose of 2.5, 5, 10 ml/kg, a gastroprotective effect was produced. This effect was demonstrated by the decrease in the amount of fluid content, size and number of lesions and an increase in the percentage protection. The protective effect of Artemisia extract was dose dependent as shown in figure 2 and 3. The anti-ulcerative effects of Artemisia extract have previously been investigated in experimental gastric ulcer models [10].

It is known that damage to gastric cells in acute and chronic inflammation is due to the toxicity of reactive oxygen species [ROS] generated in the stomach, so ROS play an important role in the progression of gastric ulcers. The ethanol induction model of gastric lesions is widely used in the investigation of anti-ulcer drugs. Lesions in this model are associated with increased production of ROS, with consequent increased lipid peroxidation, decreased mucus production, and bicarbonate secretion [18].

Previous studies have shown that *AH* is rich in flavonoids. [28]. Flavonoids markedly inhibit the lipopolysaccharide [LPS] induced expression of inducible nitric oxide synthase [iNOS] and cyclooxygenase-2 [COX-2] [11], by acting on cytokines and nitric oxide production resulting in potential immuno-modulatory effects [14]. As several reports showed polyphenols, especially flavonoids exert gastroprotective effects against experimentally induced gastric lesions. The mechanism involved in these gastroprotective effects of polyphenols are likely to act by enhancement of the gastric mucosal barrier by inhibition of the proton pump; also, it works as free radicals scavengers [23]. On the other hand, studies on herbal extract showed that it has a stimulation effect on PGs and mucus secretion [18,3].

Aspirin has also been reported to reduce the gastric juice pH and increase the volume of gastric juice [31]. In the present study, the volume of gastric content reduced by aspirin and recovered by the administration of *Artemisia* extract with aspirin. Taken together, these data suggests that polyphenols present in this extract may participate in enhancing the mucosal barrier and the gastroprotective effect of this substance is related to antisecretory effects. These findings indicate a strong participation of endogenous prostaglandins compounds in the gastroprotective effect of *Artemisia herba alba*. These results may also suggest that *AH* has gastroprotective activity, neutralizes acid secretion, partially inhibits *H. pylori*, and is therefore a potential candidate for treatment of gastritis.

In conclusion, the aqueous leaves extract of Artemisia *herba alba* exerts a reliable protection effect against stomach injury induced by aspirin as based on experimental results. Nevertheless, further investigations are needed to determine any of the active ingredients in the extract and to elucidate, if possible, the mechanisms involved in gastroprotection. Moreover, to clarify the gastroprotective properties of Artemisia *herba alba* further investigations with some selective COX inhibitors, will allow a better understanding of its effects.

We suggest that *AH* extract prevents the aspirin induced gastric ulcer formation most probably by reducing mucosal iNOS activity and the inflammatory cytokines, but does not affect gastric juice or acid production or mucosal PGE_2 content. Thus, the protective effect of *Artemisia herba alba* aqueous extract against gastric ulcers may be attributable to its polyphenols and flavonoids content.

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