Effect of *Melia Azedarach* Fruits on Gipsing-Restraint Stress-Induced Ulcers in Rats

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Abstract—A study was undertaken on the antiulcer effect of some active ingredients present in the lipid part of the fruits of *M. azedarach* administered p.o. to male rats. Acute gastric ulcers were induced by gipsing the rats for 22 hr preceded by 24 hr starvation to obtain the maximum stress. The free HCl, total HCl and total acidity were also measured. The total lipid (TLP), 1.0, 2.5 and 5.0 g/kg, reduced the ulcer index by 25–41.8% and 50–58% when given daily for 5 and 10 days, respectively. The saponifiable fraction (SP), 0.85, 2.0 and 4.0 g/kg, given for 10 days reduced the ulcer index by 41.8–50%, while the nonsaponifiable (NSP), 0.075, 0.150 and 0.50 g/kg, for 10 days reduced it by 50–83.5%. The 70% ethanol extract of the defatted residue showed no antiulcer effect. Analysis of the gastric juice showed a significant decrease in free HCl (P<0.001) induced by TLP; the total HCl and total acidity were reduced only at 5 g/kg. The results revealed the antiulcer effect of the lipid components of *M. azedarach* fruits which is mainly due to the phytosterol fraction.

*Melia azedarach* (Fam. Meliaceae) is widely distributed all over the world, and some parts of the plant, including the fruits, have been used in the traditional medicine of many countries as an antiulcer agent, resolvent and antiseptic (1), vermifuge and antiseptic (2), insecticide (3, 4), and fungicide (5).

Several factors were used to induce gastric ulcers experimentally such as restraint, starvation, environmental changes and drugs (6). Among these, stress was the simplest one to be induced by various methods: by tying fore- and hind-limbs together or by wrapping the rat with a towel for 24–48 hr (7). The factors involved in stress were suggested to be age, presence of adrenal and hypophysis and starvation (7, 8).

The present study is part of a series of studies to obtain new natural sources of drugs from medicinal plants. *M. azedarach* was selected to study its antiulcer effect because of its wide distribution and popularity in Iraq. Moreover, the validity of a scoring system to study the effect on gastric ulcer induction of some of its active ingredients, i.e., the alcoholic extract and lipid part of the fruit, was evaluated.

Materials and Methods

Animals: Male albino rats 150–200 g were used. Animals were housed in a temperature controlled room at 22±3°C and 50±5% humidity with a 12:12 light-dark cycle (lights on 07:00–19:00). Rats were kept in groups of 5 per cage (35×30×30) with food and water ad lib. till starvation.

Procedure for extraction of samples: The lipid fractions were extracted by defatting samples of 100 g dried powdered ripe fruits with petroleum ether (300 ml) (b.p. 60–80°C) in a Soxhlet for 8 hr at 40°C. The solvent system was evaporated under reduced pressure: the extract was concentrated and
amassed to 4.8% of the total dry weight. Samples of 1 g each of the lipid residue were subjected to saponification (9). The non-saponifiable fraction (NSP), containing phytosterols, and the saponifiable fraction (SP), containing fatty acids, were obtained and amounted to 15% and 85% of the total lipid, respectively. They were flushed with nitrogen and stored in screw cap vials at -20°C. The alcoholic extract was obtained by extracting the defatted residue with 300 ml of 70% ethanol in a Soxhlet for 24 hr at 60°C and then evaporated under reduced pressure. It amounted to 15% of the total defatted fruit weight.

Experimental induction of gastric ulcers:
Stress procedure was performed according to the method of Shumpelich and Paschen (10) with some modifications. Briefly, it was performed by gipsing the extracts-pretreated rats for 22 hr preceded by 24 hr food deprivation to induce acute ulcers. For recording the gastric ulceration, an ulcer index was applied which was taken from two scoring systems.

Score I: This score depends on the macroscopic appearance of the ulcer which was seen by lens and the numericals were given according to the size of the ulcer as: score 1: small; 2: medium; 3: large; 4: very large or stretch. Then multiplying the number of each observed ulcer with score numericals and adding the products, score 1 was obtained.

Score II: It depends on the sum of the values of score I (Table 1).

The effects of the tested ingredients were studied by orally administering the TLP (1.0, 2.5 and 5.0 g/kg; 5 and 10 days), the NSP (0.075, 0.150 and 0.500 g/kg), SP (0.85, 2.00 and 4.00 g/kg) and the alcoholic extract (0.10, 0.25 and 0.500 g/kg) daily for 10 days followed by application of stress.

Histological study of the stomach: The specimens of stomach tissue, after scoring, were preserved in 10% formalin, and 6 μm thick sections were prepared and stained with hematoxyline-eosine.

Statistical analysis: Student's t-test was used to analyze the data.

Procedure for gastric analysis: A modification of the method of McColl et al. (11) and Shay et al. (12) was performed. The total lipid residue was given daily per os in doses of 1.0, 2.5 and 5.0 g/kg for 10 days. The treated rats were kept off food for 48 hr before the ligature of the pylorus. Water was given ad lib. Under ether anaesthesia, the pylorus of each animal was ligated, the animals were kept under normal conditions without food and water for 5 hr, then the stomach was removed, and the gastric juice was collected, its volume was measured, and it was centrifugated for 10 min at 3000 r.p.m. The supernatant was discarded, and the juice was analyzed quantitatively (13) to determine the free and bound HCl in addition to the total acidity in mmol/l.

Results
Induction of gastric mucosal lesions by gipsing: The stress obtained by gipsing was found to be enough to induce various mucosal lesions that were observed macroscopically and verified histologically (Fig. 1A and C).

Effect of M. azedarach components on the gastric lesions: The results shown in Fig. 2 revealed that the incidence of gastric ulcers was reduced in the rats treated with the extracts in a dose- and time-dependent manner. As depicted in Table 2, by applying our scoring system, it was possible to

| Table 1. Values of score II determined according to the sum of points of score I range |
|--------------------------------------|-------------------------------|
| score II value | score I range |
|----------------|----------------|
| 0 | No lesions |
| 1 | Medium lesions |
| 2 | Severe lesions |
| 3 | Very severe lesions |
| 4 | Affecting the whole gastric mucosa |

The ulcer index is the mean±S.E. of score II.
### Table 2. Effect of different components of the fruits of *M. azedarach* on gepsing-restraint induced gastric ulcers in rats

| Treatment          | Doses (g/kg, p.o.) | No. of ulcers according to size | Ulcer points |
|--------------------|--------------------|---------------------------------|--------------|
|                    |                    | Small | Medium | Large | Very large | Score I | Score II |
| Control            |                    |       |        |       |            |         |          |
| Total lipid (TLP)  | 1                  | 21.6±1.8** | 2.0±0.3** | 3.0±0.6 | 1.2±0.2 | 39.6±1.3** | 3.0±0.0** |
| (5 days)           | 2.5                | 6.4±3.6*** | 3.0±0.9 | 2.2±1.1 | 1.6±0.2 | 25.4±6.4** | 2.6±0.25** |
|                    | 5.0                | 4.2±1.1*** | 0.0±0.0*** | 0.0±0.0*** | 0.8±0.4 | 7.4±0.75*** | 1.8±0.2*** |
| Control            |                    |       |        |       |            |         |          |
| Total lipid (10 days) | 1                  | 9.2±0.3*** | 0.4±0.2* | 0.4±0.2* | 0.6±0.2* | 13.6±4.5*** | 1.8±0.5*** |
|                    | 2.5                | 3.0±1.0*** | 1.6±0.7* | 0.6±0.2* | 0.0±0.0** | 8.0±1.4*** | 2.0±0.0** |
|                    | 5.0                | 5.0±1.9*** | 0.6±0.4* | 0.4±0.2* | 0.8±0.3* | 10.6±2.8*** | 1.8±0.2*** |
| Saponifiable fraction (SP) | Control | 40.4±5.1 | 6.2±1.1 | 4.2±1.3 | 2.6±1.3 | 75.8±11.0 | 3.8±0.2 |
| (10 days)          | 0.85               | 17.0±3.0** | 0.6±0.2** | 0.4±0.2* | 0.0±0.0* | 19.2±3.6*** | 2.4±0.25** |
|                    | 2.0                | 3.6±1.1*** | 0.0±0.0*** | 1.0±0.5 | 3.4±1.5 | 20.8±5.6** | 2.6±0.25** |
|                    | 4.0                | 8.4±2.7*** | 0.0±0.0*** | 0.0±0.0* | 0.6±0.2 | 10.4±2.3*** | 1.8±0.2*** |
| Nonsaponifiable fraction (NSP) | Control | 38.0±0.4 | 5.0±0.3 | 2.2±0.2 | 2.0±0.5 | 63.0±1.7 | 4.0±0.0 |
| (10 days)          | 0.075              | 16.0±0.8 | 5.6±0.5 | 0.2±0.1*** | 0.0±0.0** | 27.8±1.1*** | 3.0±0.0* |
|                    | 0.15               | 1.6±0.7*** | 0.0±0.0*** | 0.0±0.0** | 0.0±0.0* | 1.6±0.7*** | 0.8±0.2*** |
|                    | 0.50               | 2.6±0.7*** | 0.0±0.0*** | 0.0±0.0** | 0.0±0.0* | 2.6±0.75*** | 0.8±0.2*** |

Each value is the mean±S.E. Score I and II are calculated from the individual values. Ulcer index is expressed as the mean±S.E. of score II. Five animals were used per group. Statistically significant difference from the control at *P<0.05, **P<0.01, ***P<0.001.
confirm the antiulcer effect and to compare the obtained data quantitatively by statistical analysis. The administration of the TLP for 5 days mainly reduced the small and medium size ulcers, while on prolonging the course of administration to 10 days, the effect was extended to involve the large and stretch ulcers. Moreover, on testing the effect of SP & NSP fractions, it was found that the latter was more potent in exerting the anti-ulcer effect, on the basis of the lower ulcer index, and it completely prevented the
incidence of stretch ulcers in doses of 0.075–5.0 g/kg, and only the small ulcers were observed in doses of 0.15 and 0.5 g/kg. The results were sustained histopathologically (Fig. 1B and D). The alcoholic extract showed no effect on the gastric lesions.

Effect on gastric juice: Table 3 shows that TLP in doses of 1 and 5 g/kg for 10 days increased the volume of the gastric juice along with a significant reduction in total HCl and total acidity only at 5 g/kg. Free HCl was significantly reduced by all doses examined.

Discussion
The prominent finding in the present experiment was that the gipsing-restraint stress-induced ulcer was inhibited, in a dose- and time-dependent manner, by the lipid components of the fruits of *M. azedarach* without causing any observed syndromes during the experiment; and the finds showed the validity of the scoring system used to confirm the antiulcer effect and to compare the potency of the various fractions in this respect.

Gastric acid is regarded as a factor in restraint lesions (14), so the reduction of free and total HCl combined with reduction of total acidity may add to the antiulcer effect of TLP which significantly increased the volume of gastric juice, which was reported to be reduced by stress (15).

The SP contains several fatty acids, myristic, palmitic, stearic, oleic, linoleic and linolenic (16). The fatty acids are well

| Treatment | Dose gm/kg.p.o. | Gastric lesions (% of control) |
|-----------|----------------|--------------------------------|
| control   | 0              | 0                              |
| TLP       | 1.0            | 50                             |
|           | 2.5            | 100                            |
|           | 5.0            |                                |
| control   | 0              | 0                              |
| TLP       | 1.0            |                                |
|           | 2.5            |                                |
|           | 5.0            |                                |
| control   | 0              |                                |
| SP        | 0.85           |                                |
|           | 2.00           |                                |
|           | 4.00           |                                |
| control   | 0              |                                |
| NSP       | 0.075          |                                |
|           | 0.150          |                                |
|           | 0.500          |                                |

Fig. 2. Dose response relationship of various components of the lipid part of *M. azedarach* fruits in preventing gipsing-induced gastric lesions. Each column is the mean±S.E. Five animals were used per group. Abbreviations are explained in the test. Statistically significant difference from the control: *P<0.05, **P<0.01, ***P<0.001.

Table 3. Effect of the total lipid part of the fruits of *M. azedarach* on the gastric juice of rat

| Dose g/kg, p.o. (10 days) | volume (ml) | Free HCl | Bound HCl | Total HCl | Total acidity |
|---------------------------|-------------|----------|-----------|-----------|---------------|
| Control                   | 10±0.4      | 62±7.0   | 80±23.0   | 106±8.0   | 121±9.0       |
| 1.0                       | 11±0.6***   | 11±0.6***| 91±22.0   | 112±12.0  |
| 2.5                       | 10±0.3***   | 19±0.0***| 84±20.0   | 111±26.0  |
| 5.0                       | 19±2.0***   | 14±3.0***| 42±6.0***| 81±11.0***|

Each value is the mean±S.E. Five rats were used per group. * , ** , *** : Significantly different from the control at P<0.05 and P<0.001 levels, respectively.
known as a source of prostaglandins (PGs). A plethora of data shows that PGs have cytoprotective effects (17, 18), and they are reduced in the gastric mucosa of patients with gastric ulcer (19), they also inhibit the pentagastrin-induced acid secretion in dogs (20) and their protective effects are intimately associated with stimulation of gastric chloride transport (21). Whether the SP effect is direct or via conversion to PGs merits verification. The NSP fraction, which contains the phytosterols, stigmasterol, campsterol and β-sitosterol (16), produced the most remarkable reduction of the ulcer index, and the role of phytosterols in this regard needs also to be clarified. No antiulcer effect was exerted by the alcoholic extract of the defatted residue; this proves that the antiulcer effect is confined to the lipid components. Beside the above mentioned components, the fruits also contain various terpenoids (22, 23) which possess antiulcer activity (24, 25) and are involved in synthesis of glyco-proteins which on their own possess cytoprotective activity (26). It is not unusual that by the virtue of the above mentioned components, the examined lipid components induced the antiulcer effect.

This study indicated that the role of sterols, fatty acids and other lipid components in reducing the gastric ulcer should be further clarified and that the medicinal plants are a treasure which must be explored on a scientific base for the constituents of their active ingredients as a resource for medicinal uses.

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