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Gastroprotective effect of *Lippia nodiflora* L. extracts in ethanol-induced gastric lesions

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ABSTRACT

Lippia nodiflora L., is a herbaceous, perennial and prostrate plant; containing a group of flavonoids. Accumulating evidence indicates potential therapeutic benefits of flavonoids as anti-oxidative in pathological conditions associated with oxidative stress. Here, we examined the gastroprotective effect of different fractions (n-Hexane, acetylacetate, aqueous and total extract) of *Lippia nodiflora* in ethyl alcohol-induced ulcer in rats.

Our results showed that all these fractions significantly reduced gastric contents of Thiobarbituric acid reactive substances (TBARS), which represent a biochemical marker of oxidative stress associated with lipid peroxidation. This was mirrored by a marked decrease in ulcer index observed for different fractions. These data suggest a potential anti-ulcerative activity for *Lippia nodiflora* extracts and this might be due to, at least partly, their antioxidant property.

KEYWORDS: Antioxidant, gastroprotective, lipidperoxidation, *Lippia nodiflora*.

INTRODUCTION

Lippia nodiflora L., family *Verbenaceae* is known as cape weed, daisy lawn, frog fruit, fog fruit, turkey tangle or mat-grass. It has several synonyms; *Phyla nodiflora, Verbena nodiflora, Zapania nodiflora, Phyla canescens* and *Lippia repens*. Greek *Phyla* is probably referring to the flowers clustered in a tight head or the spreading mat-like growth and ground cover [1].

It is a herbaceous, perennial and prostrate plant; native to India and scattered in Mediterranean region, tropical and south Africa, Madagascar, Ceylon and the warmer region of Asia and America (2, 3, 4). It is cultivated in Egypt in the gardens of Faculty of Agriculture Cairo University and was successfully cultivated in the experimental station of Faculty of Pharmacy, El-Minia University.

The plant is used as aphrodisiac, astringent to the bowels, stomachic, vulnerary, anthelmintic and useful in fevers, colds, urinary concretions and the diseases of the heart, the blood and the eye. The plant is also good for ulcers, wounds, burning sensation, asthma, bronchitis, thirst and loss of consciousness (5).

The major phytochemicals detected in *Lippia nodiflora* L. are flavonoids that are reputed to have analgesic, anti-inflammatory, diuretic, demulcent and antipyretic (6, 7).

Based on several reports describing the antioxidant activity of flavonoids and the effectiveness of antioxidants as gastroprotectives, we have hypothesized that the plant extracts may possess an antioxidant activity and a gastroprotective effect against ethanol-induced ulcers in rats.

MATERIALS AND METHODS

Plant extraction

The air-dried powdered aerial parts (2 kg) of *Lippia nodiflora* L. was extracted with 70% methanol till exhaustion then concentrated under reduced pressure to a syrupy consistency.

The concentrated methanolic extract (200 g) was digested in a least amount of distilled water, transferred to a separating funnel and partitioned with successive portions of n-hexane, chloroform and ethyl acetate. The n-hexane, chloroform and ethyl acetate extracts were concentrated under reduced pressure to give 20, 20 and 50 g, respectively. The steps of the fractionation of the methanol extract are summarized in Figure 1.

Thin layer chromatography (TLC) examination of the aforementioned extracts and fractions revealed that the n-hexane, ethyl acetate and the aqueous extracts contain the major constituents of the plant.

Animals

Rats were fed a standard diet of commercial rat chow and tap water and left to acclimatize to the environment for at least one week prior to inclusion in the experiments. Rats were fasted for 24 hours prior to the experiment in mesh-bottomed cages. All experiments were performed during the same time of the day to avoid variations due to diurnal rhythms of putative regulators of gastric functions (8). Experiments were conducted in accordance with the guidelines for animal care of the United States Naval Medical Research Centre, Unit No. 3, Abbaseya, Cairo, Egypt, accredited by the Association for Assessment and Accreditation of Laboratory Animal Care international (AAALAC international).

Induction of experimental ulcers using 70% ethanol (9)

Rats were randomly divided into the following groups (each group consisted of at least 6 rats each):

1. **Vehicle-pretreated ethanol (70%) group:** where rats received 0.5% carboxymethylcellulose solution (vehicle for the plant extracts, 5 ml/kg, p.o.) 1 hour prior to administration of 70% ethanol (10 ml/kg, p.o.).
2. **Total extract-pretreated ethanol (70%) group:** where rats received the plant total extract (1.5 gm/kg, p.o.) 1 hour prior to administration of 70% ethanol (10 ml/kg, p.o.).
3. **n-Hexane fraction-pretreated ethanol (70%) group:** where rats received the plant petroleum ether extract (1 gm/kg, p.o.) 1 hour prior to administration of 70% ethanol (10 ml/kg, p.o.).
4. **Ethylacetate fraction-pretreated ethanol (70%) group:** where rats received the plant ethylacetate extract (1 gm/kg, p.o.) 1 hour prior to administration of 70% ethanol (10 ml/kg, p.o.).
5. **Aqueous fraction-pretreated ethanol (70%) group:** where rats received the plant aqueous extract (1 gm/kg, p.o.) 1 hour prior to administration of 70% ethanol (10 ml/kg, p.o.).
6. **Compound 2-pretreated ethanol (70%) group:** where rats received compound 2 (75 mg/kg, p.o.) 1 hour prior to administration of 70% ethanol (10 ml/kg, p.o.).

One hour after alcohol administration rats were killed by overdose of ether. Their stomachs were removed and opened along the greater curvature. Then, they were washed with ice-cold phosphate-buffered saline (PBS) and scored for macroscopic gross mucosal lesions. After that, the stomachs were stored at -80^{o}C until assessment of gastric mucosal levels of thiobarbituric acid reactive substances (TBARS), which are the breakdown products of lipid peroxides.

Assessment of Gastric Mucosal Lesions:

Gastric mucosal lesions in each stomach were measured and the ulcer score for each stomach was expressed as the total length of gastric lesions in that stomach. Afterwards, the mean ulcer score for each group was calculated. The preventive index (P.I.) of a given drug was calculated from the following equation (10):

$$P.I. = \frac{[U.I.\ of\ ulcered\ group] - [U.I.\ of\ treated\ group]}{[U.I.\ of\ ulcered\ group]} \times 100$$

Where P.I. is the preventive index and U.I. is the ulcer index

Determination of lipid peroxides in gastric mucosa:

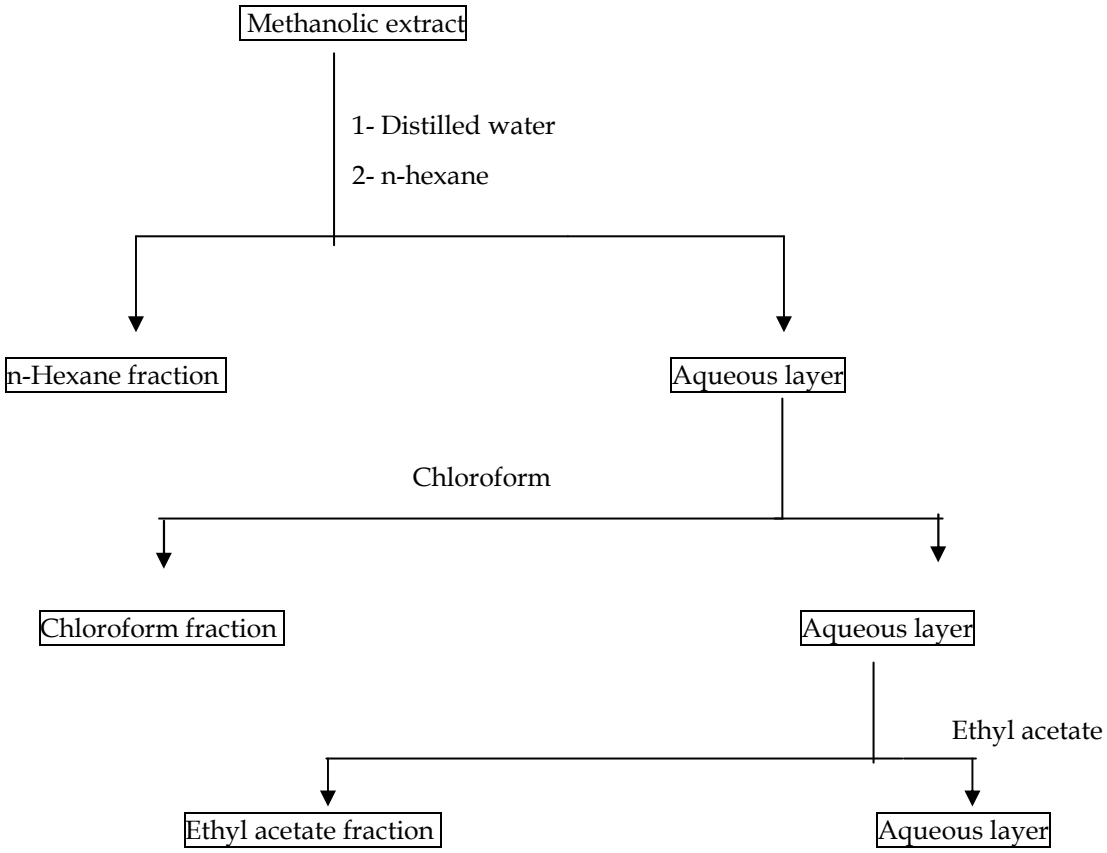
The stomachs isolated from the rats of different groups and stored in temperature of -80^{o}C were used. The mucosa of each stomach was scraped, weighted and homogenized in phosphate buffer saline (PBS) to achieve a concentration of 10% w/v.

Gastric mucosal content of lipid peroxides was determined using the thiobarbituric acid method described by Uchiyama and Mihara (11), which measures the thiobarbituric acid reactive substances (TBARS) that are the breakdown products of lipid peroxides.

The procedure was as follows:

To a 0.5 ml of 10% homogenate of the tissue sample, 3 ml of 1% phosphoric acid and 1 ml of 0.6% thiobarbituric acid aqueous solution were added. The mixture was stirred and heated on a boiling water bath for 45 minutes. After cooling, 4 ml of *n*-butanol were added, the mixture was shaken vigorously for 5 minutes, and the butanol layer was separated by centrifugation.

The optical density of the butanol layer was determined at 535 and 520 *n*m (this double wavelength measurement is necessary to avoid interference). The difference in optical density between the two wavelengths was calculated and taken as the thiobarbituric acid value. 1,1,3,3-Tetra-methoxypropane (TMP) was used as an external standard to prepare standard concentrations of 2, 4, 6 and 8 *n*mol/ml and the procedure was repeated to prepare a standard curve using TMP instead of gastric mucosa. From this curve, the peroxide concentration in the unknown sample was deduced from the corresponding absorbance using the regression line from the standard.



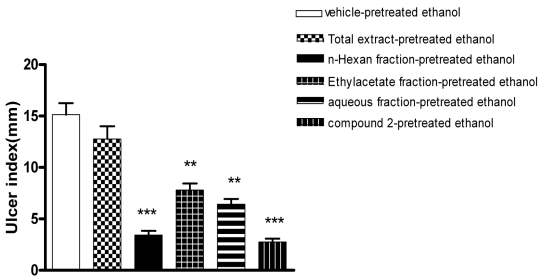


Figure 2

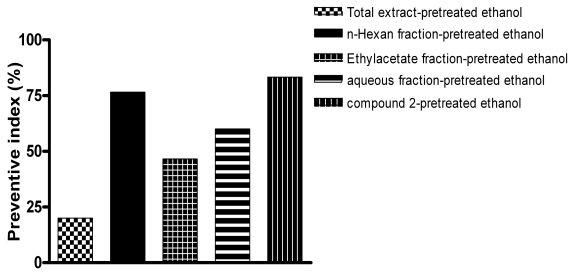


Figure 3

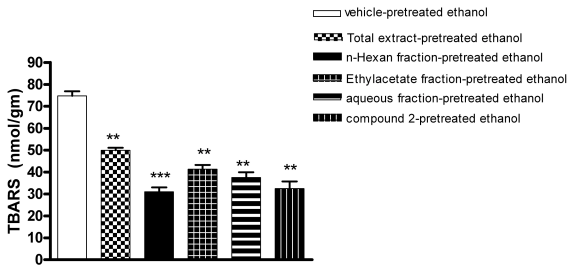


Figure 4

Statistical analysis

Results were expressed as mean ± SEM. and were analysed for statistically significant difference using one-way ANOVA, followed by the Banferroni post-hoc test. P values < 0.05 were considered significant. GraphPad Prism was used for statistical calculations (Version 4 for Windows, GraphPad Software, San Diego, Calif., USA, www.graphpad.com).

RESULTS AND DISCUSSION

As illustrated by figures 2 and 3, all of the extracts except for the total extract significantly mitigated gastric lesions formation (as measured by the ulcer index) demonstrating a prominent gastrprotective effect against gastric lesions induced by 70% ethanol in rats. Compound 2 and n-hexane extract demonstrated the greatest potency, while the aqueous and ethyl acetate extracts showed lower potencies in protection against gastric damage.

As illustrated in figure 4, all of the extracts were observed to significantly reduce gastric mucosal TBARS (as a measure of lipid peroxides) as compared to the vehicle-pretreated group. The n-hexane extract caused the most potent decrease in lipid peroxides, followed by the total, ethyl acetate, and aqueous extracts and compound 2.

An increasing number of evidence indicates a gastroprotective effect for antioxidants, including flavonoids, against experimentally-induced ulcers, of which is ethanol-induced gastric ulceration (12-15).

These results show good correlation with the reduction in ulcer index observed for the different fractions raising the assumption that the extracts may possess anti-ulcer activity due to, partly or totally, their antioxidant activity, but further investigations are needed to elucidate the exact mechanism for gastroprotection.

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