Anti-Inflammatory Activity of Pomegranate Peel Extract

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Abstract—The possible anti-inflammatory activity of Punica granatum peel extract was in vitro evaluated by measuring Nitric Oxide (NO) production by the inflammagen lipopolysaccharide. The extract showed moderate anti-inflammatory activity as shown in the amount of (NO) produced with a level of 4.4 μ mole/ml (67 % inhibition), in comparison to the potent anti-inflammatory drug Dexamethasone (95 % inhibition).

Keywords—Anti-inflammatory, lipopolysaccharide, Punica granatum, peels.

I. INTRODUCTION

The field of natural product biology, ethnopharmacology, as well as bio-prospecting approaches, have received renewed attention in recent years [1, 2]. Natural products and herbal remedies used in traditional folk medicine have been the source of many medically beneficial drugs.

The pomegranate, botanical name Punica granatum is a fruit-bearing small shrub or tree growing shrub or tree growing between 5–8 meters (16–26 ft) tall. The pomegranate is widely considered to have originated in the vicinity of Iran and has been cultivated since ancient times. Today, it is widely cultivated throughout the Mediterranean region of southern Europe, the Middle East and Caucasus region, northern Africa and tropical Africa, the Indian subcontinent, Central Asia and the drier parts of southeast Asia. Pomegranates are used in cooking, baking, juices, smoothies and alcoholic beverages, such as martinis and wine. The Punica granatum leaves are opposite or sub-opposite, glossy, narrow oblong, entire, 3–7 cm long and 2 cm broad. The flowers are bright red, 3 cm in diameter, with four to five petals (often more on cultivated plants). Some fruitless flowers are bright red, 3 cm in diameter, with four to five narrow oblong, entire, 3 cm petals (often more on cultivated plants).

II. MATERIALS AND METHODS

A. Plant Material

Fresh fruits of Punica granatum (Punicacea) were purchased from the local market in Cairo, Egypt.

B. Extraction

Fruit peels (1 kg) of P. granatum were dried in the shadow in air draft and comminuted to powder and exhaustively extracted under reflux over a boiling water bath by 2 liters of an ethanol / bidistilled water (3: 1) mixture for 8 hours. The extract was filtered and the process was repeated 3 times. The solvent was removed under reduced pressure at 45°C. The process yielded finally 100 g of a sticky dark brown material.

C. In vitro Anti-inflammatory assay by Estimation of Nitric Oxide (NO) production

Nitrite accumulation was used as an indicator of Nitric Oxide (NO) production using a microplate assay based on the Griess reaction. The Griess reaction is based on a two-step diazotization reaction in which acidified nitrates generate a nitrosating agent that reacts, with sulfanilic acid to form diazonium ion.

This ion is then coupled to N-(1-naphthyl) ethylene-diamine to produce the chromophoric pink azo-derivative that can be determined spectrophotometrically at λ540 nm [3].

Reagents preparation: Griess reagent 40 mg Griess powder was dissolved in 1 ml deionized water.

In each well of a flat bottom 96 well- microplate, 40 μl freshly prepared Griess reagent was mixed with 40 μl cell supernatant or different concentrations of sodium nitrite ranging from 0-50 μ mole/ml. The plate was incubated for 10 min in the dark and the absorbance of the mixture at 540 nm was determined using the microplate ELISA reader. A standard curve relating (NO) in μmole/ml to the absorbance is constructed, from which the (NO) level in the cell supernatant is computed by interpolation. The (NO) level of each of the tested cell supernatant was expressed as:

NO level of the tested cell supernatant x100/ NO level of the control.

III. RESULTS AND DISCUSSION

Anti-inflammatory Assay

Results of nitric oxide index:
A standard curve, relating NO in μmole/ml of sodium nitrite to the absorbance, was constructed, (Figure 1), from

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which the NO level in the cell supernatant is computed by interpolation (Figure 2).

Fig. 1 A standard curve of sodium nitrite μmole/ml

The results indicated that the inflammmagen lipopolysaccharide (LPS 100 μg/ml) induced nitric oxide production up to 2 folds of the control, while that the potent anti-inflammatory Dexamethasone (50 ng/ml) inhibited nitric oxide production to 3.5 μM/ml compared to 6.5 μM/ml of that of the LPS with level of 95 % inhibition, very close to the control cells with 3.2 μM/ml NO as shown in Figures (2) and (3).

P. granatum peels showed intermediate anti-inflammatory effect as shown in the amount of NO produced with a level of 4.4 μM/ml, leading to 67 % inhibition, in comparison to the potent anti-inflammatory drug Dexamethasone (95 % inhibition).

Fig. 2 The level of Nitric oxide in RAW 264.7 cells supernatant after the treatment with the P. granatum peels extract (25μg/ml) for 24 hours compared with LPS treated cells.

Fig. 3 The percentage of inhibition of Nitric oxide in LPS-stimulated RAW 264.7 cells supernatant after the treatment with the peels extract (25μg/ml) for 24 hours compared LPS treated cells, as measured by Griess assay.

REFERENCES

