

ORIGINAL RESEARCH ARTICLE

Biological activities of *Lycium shawii* Leaves Extract

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ABSTRACT

Medicinal plant extracts used in traditional folk medicine provide a largely unexplored source for identification and development of biologically active compounds. In this report, we have analyzed the biological effects of extracts from *Lycium shawii* leaves, a native plant of the Arabian Gulf region.

Lycium shawii leaves were extracted with methanol and the total extract fractionated by HPLC. Proliferative and cytotoxic activities of the fractionated extract were determined using the cancer cell line HEK293, whereas the anti-inflammatory assays were carried out by using an NF- κ B-luciferase reporter assay and stimulation with TNF α . Our data indicates that selective fractions of *Lycium shawii* leaves extract contain proliferative, cytotoxic and anti-inflammatory activities. These results indicated the possible application of *Lycium shawii* leaves as a source of bioactive compounds, potent as proliferative, anti inflammatory and cytotoxic agents.

Key words: Medicinal plant, HPLC, NF- κ B-luciferase, TNF α .

INTRODUCTION

Medicinal plant extracts used in traditional folk medicine provide an interesting and still largely unexplored source for the identification and development of biologically active compounds. For this, in recent years research on medicinal plants has drawn enormous global attention for their use as a natural source of medicinal agents [1]. Plants are rich in a wide variety of secondary metabolites such as tannins, terpenoids, alkaloids, flavonoids, etc., and their antioxidant, anti-inflammatory, antimicrobial or cytotoxic properties are used to developed drugs, dietary supplements and cosmaceuticals [2-4]. Therefore, it is of great interest to carry out a biochemical screening of these plants in order to validate their use in folk medicine and to reveal their biologically active principles by isolation and characterization of their constituents.

In the Saudi Arabia region, the plant *Lycium shawii* (Solonaceae), locally called 'Gul Gaider', is used in the form of dry powder by traditional healers as anti-diabetic and hypotensive agent [5, 6].

In fact, recent experiments have indeed demonstrated that *Lycium shawii* extract possesses hypoglycemic activity in vivo [7]. *Lycium shawii* extract has also been reported to possess antiplasmodial and antitrypanosomal activity [8]. However, it is important to note that several herbs and plant extracts used in traditional medicine were later shown to contain cytotoxic activities, or other unwanted biological activities.

In the present manuscript, we present data on the biological characterization of extracts from *Lycium shawii* leaves in order to verify their safety, and thus legitimizing its use either in popular medicine either in other areas, such as nutraceuticals and/or cosmaceuticals.

METHODS

Plant material and preparation:

Leaves of *Lycium shawii* were collected from the wild surrounding Qatar University Campus. The leaves were authenticated at the Department of Biology, Qatar University. Plant material was

washed several times with distilled water and oven-dried overnight at 60°C. The leaves were ground and 5 grams of ground material was extracted with methanol at 1:5 plants to solvent ratio through overnight rotation. The suspension was centrifuged at 4000 rpm for 10 minutes at 25°C and the resulting supernatant filtered, concentrated using a rotavapor and stored at -20°C.

HPLC fractionation:

Fractionation by HPLC was carried out using a Waters Xbridge C18 column (19x150 mm; 5 µm particle). Compounds were eluted with a gradient of water and acetonitrile at 1 ml/min. For each fractionation run, 350µl of the solubilized residue in methanol/DMSO was injected.

Cytotoxic Activity:

HEK-293 (Human embryonic kidney), MCF7 (Human breast adenocarcinoma cell line) and HeLa (Human cervical cancer cell line) obtained from the American Type Culture Collection (ATCC) were used in this study.

The plant extracts were prepared from the stock solutions by serial dilution in DMEM to give a volume of 100 µl. Cells were plated in 96-well microtiter plates. Each well was filled with 100 µl of cells at 2×10^5 cells/ml. The assay was performed in triplicates and the culture plates were kept at 37°C with 5% (v/v) CO₂. At the end of each testing time, the culture supernatants were removed and a 3-(4, 5-dimethylthiazole-2-yl)-2, 5-diphenyl-tetrazolium bromide (MTT) solution (0.5 mg/mL) was added to each well and the plates were incubated for 1 h at 37 °C. The MTT solution was removed and isopropanol was added to dissolve formazan crystals. The absorbance at 570 nm was read on a microplate spectrophotometer (Applied Biosystem). The percentage of viability was calculated as (AT/AC)x100, where AT and AC are the optical densities of treated and control cells, respectively.

Cell Culture, Plasmids and Antibodies:

Cells were cultured in Dulbecco's modified Eagle's medium supplemented with 10% FCS and transfected by calcium phosphate precipitation. Anti-caspase 3 and anti-caspase 8 antibodies were from Santa Cruz Biotechnology; TNFα was from Sigma. The reporter plasmid pNF-κB -luc was from Clontech.

Immunoblot analysis:

Cell lysates were made in lysis buffer (150 mM NaCl, 20 mM Hepes, pH 7.4, 1% Triton X-100, 10% glycerol and a mixture of protease

inhibitors). Proteins were separated by SDS-PAGE, transferred onto nitrocellulose membrane and incubated with primary antibodies followed by horseradish peroxidase-conjugated secondary antibodies (Amersham Biosciences). Blots were developed using the ECL system (Amersham Biosciences).

RESULTS AND DISCUSSION

In the experiments described below, we have evaluated the cytotoxic and anti-inflammatory activity of methanol extracts of *Lycium shawii* leaves. Because we thought that the whole extract could contain principles with antithetical activities, for instance, both cytotoxic and cytoprotective, pro inflammatory and anti-inflammatory activities, we decided to fractionate the whole lysate by HPLC, and test the biological effects of the purified fractions. Thus, we first evaluated the biological effect of 30 hrs continuous treatment with *Lycium shawii* leaves fractionated extract on proliferation of the human embryonic kidney cancer cell lines HEK-293. (Fig 1) shows that the different fractions of fractionated *Lycium shawii* leaves extracts have a different biological effect on cell proliferation. In fact, while fraction 4 has a significant effect in promoting cellular proliferation ($p < 0.0001$), fraction 10 has a clear cytotoxic effect ($p = 0.005$).

It is known that many cytotoxic substances derived from plants exert their cytotoxic effect through induction of apoptosis^[9, 10], a process of programmed cell death that requires activation of aspartate-specific cysteine proteases also known as caspases^[11, 12]. These proteases are normally present as inactive precursors in the cell and are activated by proteolytic cleavage upon induction of the apoptotic stimulus^[11, 12]. Thus, we monitored the activation state of caspase-3 and caspase-8 following exposure to fractionated *Lycium shawii* extract. As shown in (Fig 1B), fraction 10, which displayed cytotoxic effect when tested on cell cultures, it also induces activation of caspase-3 and caspase-8. Thus, selective components of *Lycium shawii* display cytotoxic activity, which is explicated, most likely, through the induction of the apoptotic cascade.

The transcription factor nuclear factor-kappa B (NF-κB) plays a critical role in immune and inflammatory responses through the regulation of genes encoding pro-inflammatory cytokines, adhesion molecules, chemokines, growth factors and inducible enzymes such as cyclooxygenase-2 (COX-2) and nitric oxide synthase (iNOS)^[13, 14].

In addition, numerous studies have established that active constituents of many medicinal plants exert anti-inflammatory activity by regulating NF- κ B activation [15-17]. Therefore, NF- κ B activation has been considered to be a molecular target for the screening of anti-inflammatory activity of lead compounds. Thus, we monitored whether *Lycium shawii* leaves extract can inhibit NF- κ B activation following cell exposure to the NF- κ B-inducing cytokine TNF- α . For this, HEK293 were transfected with a plasmid encoding for a luciferase reporter gene under the control of an NF- κ B-responsive promoter. 24 hrs after transfection, cells were treated with TNF- α with or without preincubation with fractions of *Lycium shawii* leaves extract. After 1 hr of preincubation cells were stimulated for 5 hrs with TNF- α (10 ng/ml) and total extracts were tested for luciferase activity. As shown in (Fig 2), the TNF-induced NF- κ B activity was completely prevented by preincubation of cells with several fractions of *Lycium shawii* leaves extract.

Natural products play a dominant role in the discovery of such new nutraceuticals, cosmeceuticals and bioactive compounds in general. In fact, plants constitute a tremendous source for the discovery of new products with medicinal importance in drug development. In addition to drugs, secondary metabolites of plants are also used to develop flavor and fragrances, dye and pigments, pesticides, and food additives [18].

In this report, we have investigated the biological effects of methanolic extracts from leaves of *Lycium shawii*, a native plant of the Arabian Gulf region and used in folk medicine for treating a variety of ailments [4, 7]. *Lycium shawii* extracts were found to contain both a component that stimulate cell proliferation and a component displaying cytotoxic activity. The latter appears to be mediated by apoptotic mechanisms, as suggested by activation of caspases following cell exposure to the extracts. In addition, we also found that *Lycium shawii* extracts possess activities capable of inhibiting activation of NF- κ B transcription factor, one of the most potent mediators of inflammatory processes.

Given all this evidence, it would be useful and important in the future to isolate and identify the metabolites responsible for the multiple biological effects exerted by *Lycium shawii* leaves extracts.

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Fig 1: Cytotoxic effect of fractionated *Lycium shawii* extract

Fig 1 A:

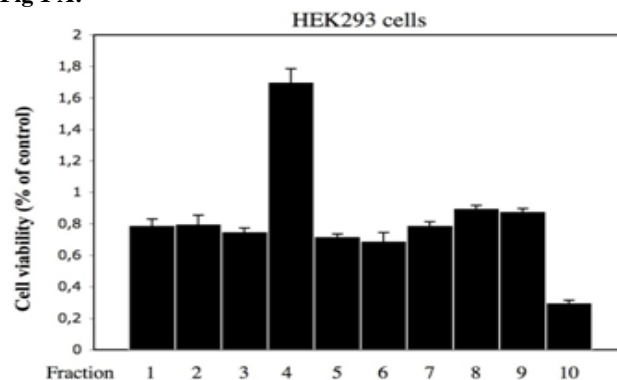


Fig 1B:

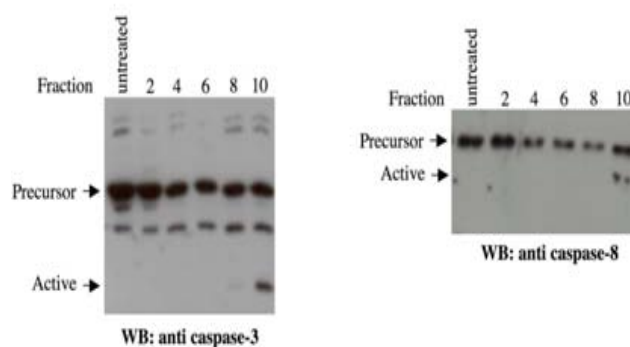
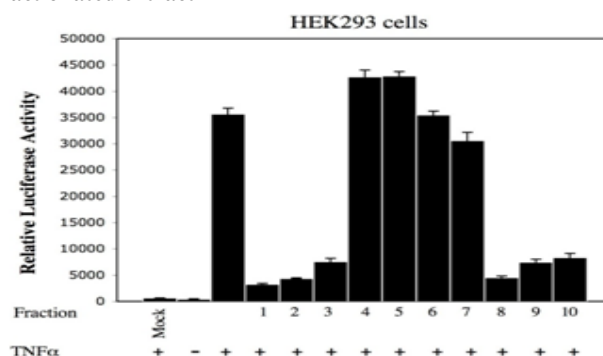


Fig 2: Inhibition of NF- κ B activation by *Lycium shawii* fractionated extract



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