Original article

In vitro Anti-Inflammatory Activity of Methanol Extracts of *Balanites aegyptiaca* and *Tamarindus indica*

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Abstract

Balanites aegyptiaca and *Tamarindus indica* are well known and widely used herbs, which possess health promoting properties as well as several other interesting bioactive constituents. The aim of the current research is to evaluate the in vitro anti-inflammatory effects of methanolic extracts of both plants against the denaturation of protein. Different concentrations of the extracts were incubated with egg albumin in controlled experimental conditions and subjected to determination of absorbance and viscosity to assess the anti-inflammatory property. Emifenac, a widely used anti-inflammatory drug was used as a reference. The results showed a concentration-dependent inhibition of protein (albumin) denaturation by *B. aegyptiaca* and *T. indica* extracts. *T. indica* extract expressed a higher activity compared to *B. aegyptiaca*. Hence, both *B. aegyptiaca* and *T. indica* extracts possessed marked anti-inflammatory properties.

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Introduction

Inflammation, usually characterized by redness, swelling, pain and a sensation of heat, is one of the body's self-defense systems. This biological response is a protective mechanism of organisms for defense against noxious physical or chemical stimuli. However, chronic inflammation has been reported to be involved in the development of various diseases such as allergic rhinitis (Weninger *et al.,* 2001), atopic dermatitis (Flavell, 2002), rheumatoid arthritis (Christodoulou et al., 2006, Rajasekaran *et al.,* 2005), cancer (Asbun et al., 2006, Rajput *et al.,* 2010), multiple sclerosis (Poitout *et al.,* 2002, Guzik et al., 2003), inflammatory bowel disease (Nathan, 2009), bronchial asthma (Kamimura *et al.,* 2003) and atherosclerosis (Rankin, 2004, Gazdik *et al.,* 2008) and increase of protein denaturation and membrane alterations (Umapathy *et al.*, 2010), etc. Inflammation can be initiated by complex processes triggered by microbial pathogens or by the release of several soluble mediators of inflammation, reactive oxygen species (ROS), lipid mediators, host proteins such as proteases, and cytokines (Rankin, 2004, Smith *et al.*, 2004, Huerre *et al.*, 1996) or by the release of chemical mediators from injured tissue and migrating cells (Chandra *et al.*, 2012). These inflammatory mediators come from plasma proteins or cells including mast cells, platelets, neutrophils and monocytes /macrophages. The commonly used drugs for management of inflammatory conditions are non-steroidal anti-inflammatory drugs (NSAIDs), which have several adverse effects especially gastric irritation and ulcer. As a result, a search for other alternatives seems necessary and beneficial. For quite some times now, traditional medicine worldwide is being re-evaluated by extensive research on different plant species and their active therapeutic principles (Segismundo *et al.*, 2008, Arivazhagan *et al.*, 2000). The major merits of herbal medicine seem to be their perceived efficacy

and low incidence of serious adverse effects. This explains the reason for which this work was undertaken.

Balanites aegyptiaca Del., also known as 'Desert date', a member of the family *Zygophyllaceae* (Hall *et al.*, 1991), is one of the most common but neglected wild plant species of the dry land areas of Africa and South Asia (Hall *et al*., 1992). This tree is native to much of Africa and parts of the Middle East and the most common trees in Senegal (Ndoye *et al.,* 2004). It can be found in many kinds of habitat, tolerating a wide variety of soil types, from sand to heavy clay, and climatic moisture levels (Pandey, 2005). *Tamarindus indicia* L., (tamarind), is a dicotyledonous plant (Maiti *et al.,* 2004). It belongs to Family: *Caesalpiniaceae*, (Soni *et al.,* 2012). The tree is indigenous to tropical Africa but has become naturalized in North and South America from Florida to Brazil, and is also cultivated in subtropical China, India, Pakistan, Philippines, Java and Spain (Komutarin *et al.,* 2004).

Materials and Methods

Plant Materials

The *Balanites aegyptiaca* and *Tamarindus indica* plants were both identified by the herbarium and then dried, grind mechanically by mortar and pestle, which then kept into an air-tight container for use in the study.

Chemicals and Drugs

All the chemicals were of analytical grade obtained commercially. Distilled water from all-glass still was used throughout the study. The standard reference drug, Emifenac was obtained commercially.

Preparation of Extracts

The powder plant materials (25 g) were extracted with 250 mL methanol by boiling for 2 hours in the Soxhlet system. The extracts were filtered and evaporated to dryness to yield the dry extracts. The dry extract was kept in a vacuum desiccator until use.

In-vitro Anti-Inflammatory Activity

The screening for anti-inflammatory activity was carried out according to a modification of the *in-vitro* protein denaturation bioassay methods of Jagtap *et al*. (2011) and Shallangwa *et al.* (2013). Separately, 10 mg extracts of *B. aegyptiaca* and *T. indica* were dissolved in minimum quantity of dimethylsulphoxide (DMSO) and diluted with phosphate buffer solution (0.2M, pH 7.4). The final concentration of DMSO in all solution was less than 5%. For drug preparation, one tablet was dissolved in 50 ml distilled water (DW). While 5 ml egg albumin (from fresh hen's egg) were mixed with 70 ml phosphate buffer saline (PBS).

Three different tubes containing 3 mL of egg albumin/PBS mixture were prepared, then 2 mL of plant extracts and 2 ml of drug were added the mixture, separately. Test solutions were incubated at 37° C in Corsair Heating & Catering Limited incubator for 15-20 min. Then albumin denaturation was induced by keeping the reaction mixture at 60°C in water bath for 10-15 min. After cooling, turbidity was measured at 660 nm UV-Visible Spectrophotometer. Percentage of denaturation inhibition was calculated from control where no drug was added and compared to the treated ones. Each experiment was done in triplicate and the average was taken. The percentage inhibition of denaturation was calculated by using the following formula:

$$\% \text{ Inhibition} = 100 \times [Vt / Vc - 1]$$

Where,

Vt = Mean absorbance of test sample, Vc = Mean absorbance of control

Results are expressed as Mean ± SD.

Results and Discussion

In some circumstances, denaturation of tissue proteins occurred (Chandra et al., 2012), which is one of the well-known causes of immunological illnesses, i.e., inflammatory and arthritis. Production of autoantigens in some arthritic diseases may be due to protein denaturation (Opie, 1962 and Umapathy et al., 2010). Therefore, any material that can block or inhibit denaturation of protein, would be useful for the development of anti-inflammatory drugs. In the present study, the evaluation of anti-inflammatory properties was carried out to assess the effect of extracts of *B. aegyptiaca* and *T. indica* on protein denaturation process.

The present results revealed an increase in absorbance of treated samples compared to control (Fig. 1). *B. aegyptiaca* and *T. indica* extracts were more active when compared with Emifenac,

even at low concentrations. According to Jagtap et al., (2011), this indicates the inhibition of heat-induced protein denaturation by plant extracts and reference drug, Emifenac.

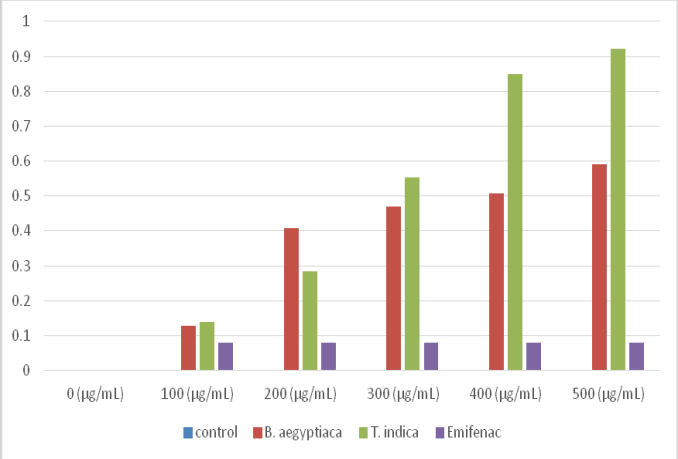

Figure 1: Absorbance of *B. aegyptiaca*, *T. indica* extracts and Emifenac

These anti-denaturation effects were further supported by the changes in viscosities. It's been stated that the denaturation increases viscosities of protein solutions (Anson, 1932). In the current study, the quite high viscosity of control supported this fact. Presence of both plant extracts prevented this, implying inhibition of protein denaturation. Here, the viscosities decreased in comparison to control where no test extract or drug was added. However, the viscosities were found to decrease in relation to decrease in concentration of test-extracts and reference drug as well (Fig. 2).

These results are in agreement with Anupama *et al.,* (2012) who stated that the methanolic extract of *T. indica* seeds exhibited significant analgesic anti-inflammatory activities.

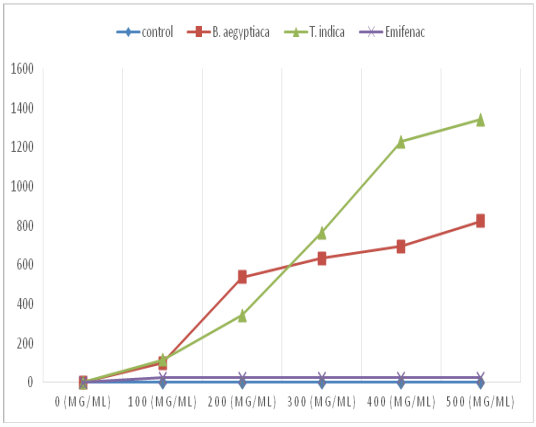

Figure 2: Anti-inflammatory data of *B. aegyptiaca*, *T. indica* extracts and Emifenac

Conclusion

The present study revealed that the methanolic extracts of both *Balanites aegyptiaca* and *Tamarindus indica* were capable of limiting the denaturation of protein process in vitro, hence, *Balanites aegyptiaca* and *Tamarindus indica* extracts possess marked anti-inflammatory properties and can be used for pharmaceutical purposes.

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