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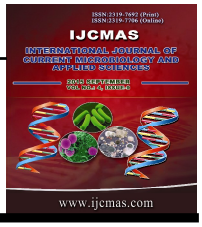

Original Research Article

Evaluation of *invitro* Anti-Arthritic Activity of *Vitex negundo* against the Denaturation of Protein

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A B S T R A C T

Vitex negundo was traditionally used as antiarthritic, antileprotic, anti-inflammatory, anti-spasmodic, anti-asthmatic, astringent and anthelmintic agents. So our aim was to investigate in vitro antiarthrtic activity of aqueous extract of *Vitex negundo* by denaturation of protein methods. Aqueous extract of *Vitex negundo* was screened for antiarthritic activity by denaturation of egg albumin and bovine albumin proteins. The extract at different concentrations was incubated with egg albumin and bovine albumin in controlled experimental conditions and subjected to determination of absorbance to assess the anti-inflammatory property. Diclofenac sodium was used as the reference drug. The present findings exhibited a concentration dependent inhibition of protein (albumin) denaturation by aqueous extract of *Vitex negundo.* The effect of diclofenac sodium was found to be less when compared with the *Vitex negundo* (1000 µg/ml). From the present study it can be concluded that *Vitex negundo* showed marked *in vitro* antiarthritic effect against the denaturation of protein.

Keywords

Vitex negundo, Antiarthritic activity, Denaturation of protein

Introduction

Rheumatoid arthritis is a chronic inflammatory disease of joints that results in joint pain, swelling and destruction. It affects an estimated 1% of the adult population throughout the world. Progression of the disease results in joint destruction deformity and significant disability (Page *et al.*, 2002).

Vitex negundo belongs to the family *Verbenaceae* is a large aromatic shrub or a small tree of about 3 meters in height and it is claimed to possess anti-arthritic, antileprotic, anti-inflammatory, anti-spasmodic, anti-asthmatic, laxative, astringent, anthelmintic and promotes the growth of hair and useful in eye diseases (Kirtikar and Basu, 2008; Nadkarni, 2002; Meena *et al.*, 2011; Sharma *et al.*, 2005; Tandon and Gupta, 2006).

The present study is aimed to investigate antiarthritic activity of *Vitex negundo* by protein denaturation method.

Materials and Methods

Plant material and extraction

Dried leaf of *Vitex negundo* was reduced to coarse size and subjected to extraction by using water. The aqueous extract of *Trichosanthes dioica* was screened for the presence of various phytoconstituents like flavonoids, saponins, glycosides, terpenoids aminoacids, alkaloids, carbohydrates, phenolic compounds and proteins as described by Kokate (1986).

Evaluation of *in vitro* anti-arthritic activity

Protein denaturation by using egg albumin (Sangita Chandra et al., 2012)

The reaction mixture (5 ml) consisted of 0.2 ml of egg albumin (from fresh hen's egg), 2.8 ml of phosphate buffered saline (PBS, pH 6.4) and 2 ml of varying concentrations of aqueous extract of *Vitex negundo* so that final concentrations become 100,200,400,800 and 1000 µg/ml. Similar volume of double-distilled water served as control. Then the mixtures were incubated at 37±2ºC in a BOD incubator for 15 min and then heated at 70ºC for 5 min. After cooling, their absorbance was measured at 660 nm. Diclofenac sodium was used as reference drug. The percentage inhibition of protein denaturation was calculated by using the following formula:

Percentage inhibition = (Abs control – Abs sample) X 100/ Abs control

Protein denaturation by using Bovine albumin (Mizushima and Kobayashi, 1968)

The reaction mixture was consisting of aqueous extract of *Vitex negundo* at different concentrations and 1 % of aqueous solution of bovine albumin. The samples were incubated at 37ºC for 20 min and then heated at 57ºC for 20 min. after cooling the samples. Absorbance of turbidity was measured at 660 nm. Percentage of inhibition of protein denaturation was calculated as follows:

Percentage inhibition = (Abs control – Abs sample) X 100/ Abs control

Results and Discussion

The effect of aqueous extract of *Vitex negundo* was evaluated against denaturation of egg albumin and bovine albumin. The results are summarized in table 1 and 2. The presenting findings exhibited a concentration dependent inhibition of protein denaturation by *Vitex negundo* throughout the concentration range of 100 to 1000 µg/ml. diclofenac sodium at concentration 100 µg/ml showed less effect compared to *Vitex negundo* at concentration (1000 µg/ml).

In the present study the protein denaturation bioassay was selected for *in vitro* assessment of antiarthritic activity of aqueous extract of *Vitex negundo.* Denaturation of tissue proteins is one of the well-documented causes of inflammatory and arthritic diseases. Production of auto antigens in certain arthritic diseases may be due to denaturation of proteins in vivo (Umapathy *et al.,* 2010). Agents that can prevent protein denaturation therefore, would be worthwhile for antiarthritic drug development. The increments in absorbances of test samples with respect to control indicated stabilization of protein i.e. inhibition of heat-induced protein (albumin) denaturation by *Vitex negundo* and reference drug diclofenac sodium (Jagtap *et al.,* 2011). *Vitex negundo* contains alkaloids, flavonoids, tannins and a phenolic acid are known to promote antiarthritic activity (Khanna *et al.,* 2007).

Table.1 Effect of *Vitex negundo* on protein denaturation (Fresh egg albumin)

Treatment	Concentration (µg/ml)	Percentage of inhibition (%)
Aqueous extract of *Vitex negundo*	100	36.11
	200	41.66
	400	57.40
	800	79.62
	1000	88.40
Diclofenac sodium	100	80.24

Table.2 Effect of *Vitex negundo* on protein denaturation (Bovine serum albumin)

Treatment	Concentration (µg/ml)	Percentage of inhibition (%)
Aqueous extract of *Vitex negundo*	100	20.22
	200	44.4
	400	58.6
	800	66.25
	1000	70.88
Diclofenac sodium	100	72.04

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