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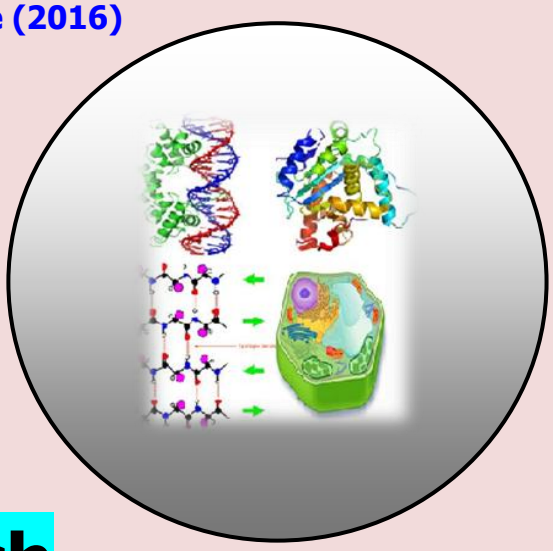

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RESEARCH PAPER

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Anti-Inflammatory Activity of the *Abelmoschus moschatus, Tephrosia purpurea, Caesalpinia pulchaerrima* and *Gloriosa superba* Flowers Extract

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ABSTRACT

Anti-inflammatory activity of Abelmoschus moschatus, Tephrosia purpurea, Caesalpinia pulchaerrima and Gloriosa superba flowers extract evaluated using protein denaturation method. 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 the Abelmoschus moschatus, Tephrosia purpurea, Caesalpinia pulchaerrima and Gloriosa superba flowers extract. The effect of Abelmoschus moschatus, Tephrosia purpurea, Caesalpinia pulchaerrima and Gloriosa superba flowers extract (500µg/ml) was found to be close to diclofenac sodium.
Keywords: Anti-inflammatory activity, Abelmoschus moschatus, Tephrosia purpurea, Caesalpinia pulchaerrima and Gloriosa superba flowers extract.

INTRODUCTION

Inflammation is caused by a variety of stimuli including physical damage, ultra-irradiation, microbial invasion, and immune reactions (Zhang and Kaufman, 2008). The classical key features of inflammation are redness, warmth, swelling, and pain. Inflammation cascades can lead to the development of diseases such as chronic asthma, rheumatoid arthritis, multiple sclerosis, inflammatory bowel disease, and psoriasis (Zhang *et al*., 2011). Many of these diseases are debilitating and are becoming increasingly common in our aging society. Rheumatoid arthritis and osteoarthritis are the major inflammatory diseases affecting people worldwide (Bulua *et al*., 2011).

Medicinal plants are a major source of biodynamic compounds of the therapeutic values (Havsteen, 2002). Use of plants for treating various elements of both man and animal is as old practice as man himself. India is richly endowed with a wide variety of plants having medicinal value. These plants are widely used by all sections of the society as pharmaceutical preparation of modern medicine (Bhagawati Uniyal, 2003). A number of natural products are used in the traditional medical systems in many countries. Alternative medicine for treatment of various diseases is getting more popular. Making medicinal plants provide relief of symptoms comparable to that obtained from allopathic medicines. The majority of clinically important medicines belong to steroidal or

non-steroidal anti-inflammatory chemical therapeutic for treatment of various inflammatory diseases. Though these drugs have potent activity, they have various and severe adverse effects. Therefore, agents of natural origin with very little side effects are required as substitute of chemical therapeutics. In order to investigate the anti-inflammatory activity of *Abelmoschus moschatus, Tephrosia purpurea, Caesalpinia pulchaerrima* and *Gloriosa superba* flowers extract.

MATERIALS AND METHODS

Plant materials

The fully mature flowers of *Abelmoschus moschatus, Tephrosia purpurea, Caesalpinia pulchaerrima* and *Gloriosa superba* were collected in January 2015 from Thanjavur, Tamil Nadu, Kerala and Karnadaka states, India from a single herb. The flowers were identified and authenticated by Dr. S. John Britto, The Director, the Rapiant Herbarium and centre for molecular systematics, St. Joseph's college Trichy-Tamilnadu. India. A Voucher specimen has been deposited at the Rabinat Herbarium, St. Josephs College, Thiruchirappalli, Tamil nadu, India.

Preparation of alcoholic extract

The collected *Abelmoschus moschatus, Tephrosia purpurea, Caesalpinia pulchaerrima* and *Gloriosa superba* flowers were washed several times with distilled water to remove the traces of impurities from the leaves. The flowers were dried at room temperature and coarsely powdered. The powder was extracted with 70% ethanol for 48 hours. A semi solid extract was obtained after complete elimination of alcohol under reduced pressure. The *Abelmoschus moschatus, Tephrosia purpurea, Caesalpinia pulchaerrima* and *Gloriosa superba* flowers extract was stored in refrigerator until used. Doses such as 20, 40, 60 and 80µg/ml were chosen for *in vitro* anti-inflammatory activity.

Evaluation of *in vitro* anti-inflammatory activity

Anti-inflammatory activity of the *Abelmoschus moschatus, Tephrosia purpurea, Caesalpinia pulchaerrima* and *Gloriosa superba* flowers extract was evaluated by protein denaturation method as described by Padmanabhan and Jangle (2012). Diclofenac sodium, a powerful non-steroidal anti-inflammatory drug was used as a standard drug. The reaction mixture consisting of 2 mL of different concentrations of *Shorea robusta* (100-500 µg/mL) or standard diclofenac sodium (100 µg/mL) and 2.8 mL of phosphate buffered saline (pH 6.4) was mixed with 0.2 mL of egg albumin (from fresh hen's egg) and incubated at (37±1) °C for 15 min. Denaturation was induced by keeping the reaction mixture at 70°C in a water bath for 10 min. After cooling, the absorbance was measured at 660 nm by using double distilled water as blank. Each experiment was done in triplicate and the average was taken

RESULTS

Anti-inflammatory activity of *Abelmoschus moschatus, Tephrosia purpurea, Caesalpinia pulchaerrima* and *Gloriosa superba* flowers extract evaluated using protein denaturation method. 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 the *Abelmoschus moschatus, Tephrosia purpurea, Caesalpinia pulchaerrima* and *Gloriosa superba* flowers extract. The effect of *Abelmoschus moschatus, Tephrosia purpurea, Caesalpinia pulchaerrima* and *Gloriosa superba* flowers extract (500µg/ml) was found to be close to diclofenac sodium. Among the various plants, the anti-inflammatory activityis higner in *Gloriosa superba* followed by *Caesalpinia pulchaerrima, Abelmoschus moschatus* and *Tephrosia purpurea.*

DISCUSSION

There are certain problems in using animals in experimental pharmacological research, such as ethical issues and the lack of rationale for their use when other suitable methods are available or could be investigated. Hence, in the present study the protein denaturation bioassay was selected for *in vitro* assessment of anti-inflammatory property *Abelmoschus moschatus, Tephrosia purpurea, Caesalpinia pulchaerrima* and *Gloriosa superba* flowers extract. Denaturation of tissue proteins is one of the well-documented causes of inflammatory diseases. Production of auto antigens in certain inflammatory diseases may be due to *in vivo* denaturation of proteins.

The mechanism of denaturation probably involves alteration in electrostatic, hydrogen, hydrophobic and disulphide bonding (Grant *et al.,* 1970). Agents that can prevent protein denaturation therefore, would be worthwhile for anti-inflammatory drug development. The increments in absorbance of test samples with respect to control indicated stabilization of protein (Egg & bovine albumin) denaturation by and reference diclofenac sodium. *Abelmoschus moschatus, Tephrosia purpurea, Caesalpinia pulchaerrima* and *Gloriosa superba* exhibited anti-inflammatory activities in dose dependent manner (Table 1 and 2, figure 1and 2).

Table 1. *In vitro* anti-inflammatory activity of *Abelmoschus moschatus, Tephrosia purpurea, Caesalpinia pulchaerrima* and *Gloriosa superba* flowers extract using Egg albumin.

Concentrations	100 µg/ml	200 µg/ml	300 µg/ml	400 µg/ml	500 µg/ml	IC_{50}
Abelmosechus moschatur	15.79 ± 1.11	26.31 ± 1.84	43.42 ± 3.04	63.15 ± 4.42	84.21 ± 5.89	319.67
Tephrosia purpurea	13.15 ± 0.92	23.68 ± 1.66	40.78 ± 2.85	61.84 ± 4.33	80.26 ± 5.62	335.10
Caesalpinia pulcherrima	17.10 ± 1.20	28.94 ± 2.03	48.68 ± 3.41	69.73 ± 4.88	88.16 ± 6.17	297.16
Gloriosa superba	19.74 ± 1.38	34.21 ± 2.39	52.63 ± 3.68	75.00 ± 5.23	90.79 ± 6.36	275.52
Diclofenac sodium (Std.)	21.37 ± 1.50	36.45 ± 2.55	55.94 ± 3.92	79.45 ± 5.56	93.45 ± 6.54	260.76

Values are expressed as Mean± SD for triplicates

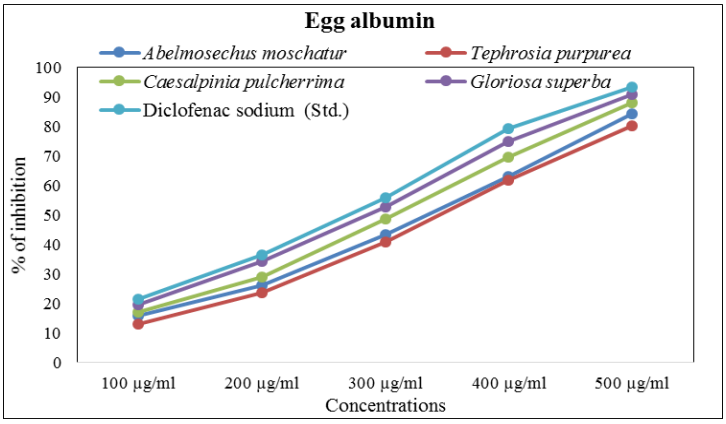

Figure 1. *In vitro* anti-inflammatory activity of *Abelmoschus moschatus, Tephrosia purpurea, Caesalpinia pulchaerrima* and *Gloriosa superba* flowers extract using Egg albumin.

Table 2. *In vitro* anti-inflammatory activity of *Abelmoschus moschatus, Tephrosia purpurea, Caesalpinia pulchaerrima* and *Gloriosa superba* flowers extract using Bovine serum albumin.

Concentrations	100 µg/ml	200 µg/ml	300 µg/ml	400 µg/ml	500 µg/ml	IC_{50}
Abelmosechus moschatur	14.94±1.05	29.58±2.07	48.63±3.40	69.73±6.77	85.37±5.97	301.95
Tephrosia purpurea	13.78±0.96	25.47±1.78	44.74±3.13	65.79±4.61	82.84±5.80	319.55
Caesalpinia pulcherrima	17.05±1.19	31.36±2.20	50.63±3.54	71.05±4.97	87.78±6.14	290.01
Gloriosa superba	20.84±1.46	34.00±2.38	53.26±3.73	75.57±5.29	90.52±6.34	273.30
Diclofenac sodium (Std.)	22.17±1.55	33.65±2.36	58.34±4.08	77.28±5.41	94.67±6.62	261.75

Values are expressed as Mean± SD for triplicates

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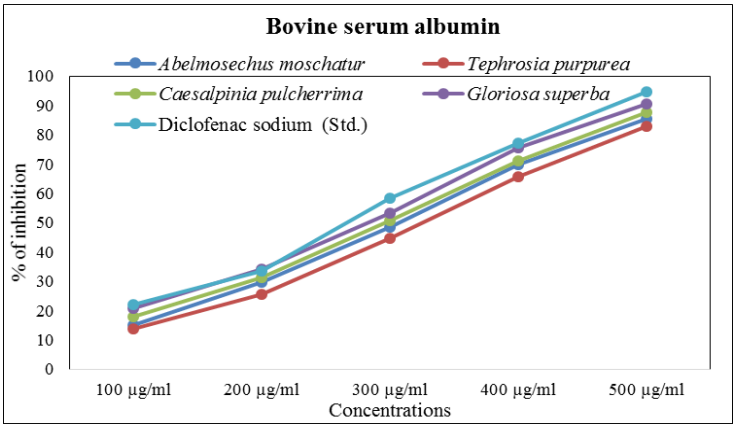

Figure 2. *In vitro* anti-inflammatory activity of *Abelmoschus moschatus, Tephrosia purpurea, Caesalpinia pulchaerrima* and *Gloriosa superba* flowers extract using Bovine serum albumin.

Sakat *et al.* (2010) studied the methanol extract of whole plant of *Oxalis corniculata* Linn (Family: Oxalidaceae) was assessed for its anti-inflammatory activity by in vitro methods. *In vitro* anti-inflammatory activity was evaluated using albumin denaturation assay, membrane stabilization assay and proteinase inhibitory activity at different concentrations. Aspirin was used as a standard drug for the study of anti-inflammatory activity. Results showed that, the extract exhibited significant. Extract also showed *in-vitro* anti-inflammatory activity by inhibiting the heat induced albumin denaturation and Red Blood Cells membrane stabilization with the IC_{50} values of 288.04±2.78 and 467.14±9.56µg/ml respectively. Proteinase activity was also significantly inhibited by the extract (IC50=435.28 ± 5.82µg/ml). From the results, it is concluded that flavonoids and related polyphenols present in the *O. corniculata* extract may be responsible for the activity.

Sangita Chandra *et al.* (2012) evaluated the *in vitro* anti-inflammatory effect of aqueous extract of coffee (*Coffea arabica*) against the denaturation of protein. The extract at different concentrations was incubated with egg albumin in controlled experimental conditions and subjected to determination of absorbance and viscosity 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 the coffee extract. The effect of diclofenac sodium was found to be less when compared with the test extract. He concluded that coffee possessed marked in vitro anti-inflammatory effect against the denaturation of protein.

Sridevi *et al.* (2015) evaluated the anti-inflammatory effect of ethanolic extract of *Pergularia Daemai* (PD) by in vitro method by using membrane stabilization test and protein denaturation test. Membrane stabilization test was done by using human red blood cells (HRBCs). Protein denaturation test was done by using bovine serum albumin (BSA). The results revealed that PD extract was capable of rendering membrane stabilization by inhibiting the hypotonicallyinduced hemolysis of HRBCs in dose-dependent manner (50, 100, 200, 300, 400, 500 and 1000 µg/mL). In lesser concentration (50 µg/mL), the % inhibition of hemolysis was less (26.80%) and in higher concentration (1000 µg/mL), the % inhibition of hemolysis was more (76.30%), which was comparable with that of standard anti-inflammatory drug viz. diclofenac sodium (200 µg/mL – 80.60%). The PD extract was also capable of inhibiting BSA denaturation in dose-dependent manner (50 µg/mL – 20.40%, 1000 µg/mL–83.60%) which was comparable to that of diclofenac sodium (200 µg/mL –86.60%). This finding confirms the potentiality of PD extract as an anti-inflammatory agent and justifies the recommendation of PD extract for the treatment of painful inflammatory conditions.

CONCLUSION

Overall, the *Abelmoschus moschatus, Tephrosia purpurea, Caesalpinia pulchaerrima* and *Gloriosa superba* flowers are a rich source of anti-inflammatory activity that can be important in inflammatory disease prevention including arthritis. In future isolation of lead molecules responsible for the activity will be carried out which may be beneficial for the development of new anti-inflammatory agent.

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