Analysis of Photochemical and Anti-inflammatory activity of selected medicinal plants in rats

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Abstract
To identify the effectiveness of Adhatoda vasica, Garcinia mangostana and Sesbania sesban on anti-inflammatory activity by following histamine-induced paw edema, carrageen an induced paw edema in mice, yeast induced pyrexia, inhibition of albumin denaturation and finally toxicity analysis to know their safety with the objective to use these natural agents with no side effects for anti-inflammatory activity instead of chemicals which has got more side effects and costlier. All the results have clearly indicated that the selected three plants have got a good anti-inflammatory activity in a dose dependent manner and as there is no toxicity the extracts could effectively be used as alternative medicines in the place of general allopathic anti-inflammatory drugs.

Keywords: Adhatoda vasica, Garcinia mangostana, Sesbania sesban, Pyrexia

INTRODUCTION
Many botanical extracts have been found to contain phytochemical such as alkaloids, tannins, saponins, flavonoids, diterpenes, glucosinolates, acetylenes and thienyls [1-2]. Medicinal plants play an important role in the development of potent therapeutic agents. There are over 1.5 million practitioners of traditional medicinal system using medicinal plants in preventive, promotional and curative applications [3]. The medicinal values of the plants lie in these phytochemicals, which produce definite physiological actions on the human body. The most important of these phytochemicals are alkaloids, tannins, flavonoids and phenolic compounds. The leaves also serve as the source of many bioactive components which are used as anti-inflammatory agents [4].

Antioxidants protect cells against damage caused by molecules known as free radicals. The antioxidant effects in plants are mainly due to the presence of phenolic compounds such as flavonoids, phenolic acids, tannins and phenolic diterpenes [5]. In the recent years, there is a tremendous research interest in the possible role of phytochemical in the prevention and treatment of many diseases. In this context, antioxidants especially those that are derived from natural sources such as Indian medicinal plants and herbal drugs derived from plants require special attention.

The use of herbal medicines is becoming popular due to toxicity and side-effects of allopathic medicines. Medicinal plants play an important role in the development of potent therapeutic agents. There are over 1.5 million practitioners of traditional medicinal system using medicinal plants in preventive, promotional and curative applications. Phytochemicals are naturally occurring in the medicinal plants, leaves, vegetables and roots that have defense mechanism and protect from various diseases. Phytochemicals are primary and secondary compounds. Chlorophyll, proteins and common sugars are included in primary constituents and secondary compounds have terpenoid, alkaloids and phenolic compounds [6].

MATERIALS AND METHODS
All chemicals, reagents and solvents used in this study were of analytical grade (Sigma-Aldrich, Mumbai). Double distilled, deionized water was used for all the experiments.

Following are the indigenous plants subjected in the following investigation:
1. Adhatoda vasica
2. Garcinia mangostana
3. Sesbania sesban

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PHYTOCHEMICAL SCREENING
Phytochemical examinations were carried out for all the extracts, as per the standard [7].

IN VIVO ANTI-INFLAMMATORY ACTIVITY
Animals
Animals are divided into seven groups, each group containing 6 animals. Adult female albino rat of Wistar strain weighing around 250 to 300 gms was procured from Tamil Nadu Veterinary and Animal Sciences University, Chennai. The animals were kept in polypropylene cages (four in each cage) at an ambient temperature of 25±2°C and 55-65% relative humidity. A 12±1hr light and dark schedule were maintained in the animal house till the animals were acclimatized to the laboratory conditions, and were fed with commercially available rat chow (Hindustan Lever Ltd., Bangalore, India) [8] and had free access to water. The experiments were designed and conducted in accordance with the institutional animal ethics committee.

Sample Collection and Preparation
The rats were sacrificed at the end of the experimental period and the venous blood was collected in clean sample bottles. This was allowed to clot and then centrifuged at 3000 rpm for 5 minutes, after which the serum was separated and stored frozen until needed for analysis.

Yeast-induced pyrexia in rats
Rats were injected subcutaneously with 10 ml/kg of 20% aqueous suspension of baker’s yeast, and the rectal temperatures were recorded initially and at 18 h. Extracts (50 mg/kg) and indomethacin (10mg/kg) were administered orally after the 18 h reading, when the temperature increase was at its peak. The temperature was measured at hourly intervals up to 5 h after administration of drugs [9].

Carrageenan-induced paw edema in mice
Mice were given a subcutaneous injection of 20 μL of 1% carrageenan in sterile saline into the right paw. Extracts and the positive drug, aspirin (250 mg/kg) were administered orally 1 h before carrageenan injection. The paw volume was measured using a plethysmometer, before and at 1, 2, 3, 4 and 5 h after injection. The edema rate percentage = (B/A–1) ×100%, where A and B represent the volume before and after injection respectively.

Histamine-induced paw edema in mice
The tested samples including three extracts and the positive drug chlorophenamine maleate (1 mg/kg) were given orally to the mice. One hour later, mice were given 20 μL of 0.2% histamine solution by intra-plantar injection. Paw volume was measured using a plethysmometer, before and at 1, 2 h after histamine injection. The edema rate percentage and inhibition were calculated as above.

IN VITRO ANTI-INFLAMMATORY ACTIVITY
Inhibition of albumin denaturation
The anti-inflammatory activity of the compound was studied by using inhibition of albumin denaturation technique which was studied according to Mizushima et al (1968) and Sakat et al (2010) [10] followed with minor modifications. The reaction mixture was consists of test extracts and 1% aqueous solution of bovine albumin fraction, pH of the reaction mixture was adjusted using small amount of 1N HCl. The sample extracts were incubated at 37 °C for 20 min and then heated to 51 °C for 20 min, after cooling the samples the turbidity was measured at 660nm(UV Visible Spectrophotometer Model 371, Elico India Ltd) The experiment was performed in triplicate. The Percentage inhibition of protein denaturation was calculated as follows: Percentage inhibition = (Abs Control –Abs Sample) x 100/ Abs control.

TOXICITY ANALYSIS
Analysis of toxicity of extract
In order to identify the harmful systemic effects, the rats were treated with the test Siddha preparations. Male rats (n = 6/group) were treated with 5 g/kg/day of Siddha preparations for 21 consecutive days. After the treatment, these rats were continuously observed for 1 hour for overt clinical signs of acute toxicity or stress. They were daily observed for overt signs of toxicity or stress during the period of treatment. The serum activities of aspartate and alanine transaminases, serum concentrations of glucose, urea and creatinine were determined using respective assay kits.[11] Then, the rats were killed; stomachs were taken out, opened and examined for macroscopic haemorrhagic gastric lesions.

Estimation of urea in serum (DAM Method)
Procedure
All the reagents were brought to the room temperature before using the test. The undiluted
serum sample was used in this method. 3 sets of test tubes were taken and marked as the Blank, Standard and Test. 0.01 mL serum sample was taken in the test tube, and then 0.01 mL of glucose standard reagent was added in standard test tube and 0.01 mL of distilled water in blank. 1.0 mL of urea reagent, acid reagent and DAM reagent was added to all the test tubes. These solutions were mixed well and kept in boiling water bath (100ºC) for 10 minutes and cooled in running tap water. The absorbance was read at 520 nm against a reagent blank.

Normal Reference Value Serum Urea: 15-40 mg/dL

Estimation of creatinine in serum (Alkaline picrate method)
Procedure
Deproteinization of sample
2.0 mL of picric acid reagent was added in 0.2 mL of the serum sample. It was mixed well and centrifuged at 2500 – 3000 rpm for 10 minutes to obtain a clear supernatant. The supernatant obtained was used for the estimation.
All the reagents were brought to the room temperature before the test. The supernatant was used in this method. Three set of test tubes and marked as the Blank, Standard and Test. 1.1 mL of supernatant was taken in the test tube, then 0.1mL of creatinine standard was added in a standard test tube. Finally 0.1 mL of distilled water was added to the Blank test tube. 1.0 mL of picric acid reagent was added in the blank and standard test tubes alone. 0.1 mL of buffer reagent was added to all the test tubes. These solutions were mixed well and kept at room temperature for 20 minutes. The absorbance was read at 340nm. The absorbance reading was repeated after every 1, 2, & 3 minutes. The mean absorbance was calculated change per minute (Δ A / min) [13].
Normal Reference Value: Serum SGOT: 39 - 92 Units/L

Estimation of SGPT (ALAT) in serum (Modified IFCC Method)
Procedure
All the reagents were brought to the room temperature before the test. The undiluted serum sample was used in this method. 0.8 mL of enzyme reagent and, 0.1 mL of sample was added in a test tube marked as T. The tube was incubated at 37º C for 1 minute and then 0.2 mL of starter reagent was added. These solutions were mixed well and the absorbance was read at 520 nm against a reagent blank.

Normal Reference Value: Serum SGPT: 17-50 Units/L

RESULTS AND DISCUSSION
Adhatoda vasica, Garcinia mangostana and Sesbania sesban were the three plants chosen for the anti-inflammatory activity study. Alkaloids, Phenol, Flavonoids, Tannins and Saponins were identified in photochemical analysis in the extracts of those 3 plants and they are the chemicals commonly involved in anti-inflammatory activity, anticancer activity, antiviral activity, Antioxidant activity, and immune modulatory activity (Table 1). Effect of test extracts on histamine-induced paw edema in mice and Effect of test extracts on carrageenan induced paw edema in mice were analyzed and all the extracts were found to be better anti-inflammatory activity comparable to control. Subcutaneous injections of yeast suspension markedly increased the rectal temperature 18 h after its administration. The temperature was raised to 40°C. Treatment with extracts after 18 h showed a marked decrease in temperature. Administration of Adhatoda vasica showed 1ºC, Garcinia mangostana has reduced 1°C and Sesbania sesban has reduced 2ºC. Administration of indomethacin decreased the temperature to 37°C after 5 h.

TOXICITY STUDIES
The results of Inhibition of albumin denaturation by the extracts Adhatoda vasica showed 37%, Garcinia mangostana 47% and Sesbania sesban has shown 57%. Compared to control it is lesser but
encouraging. In order to identify whether any harmful systemic effects caused by the extracts tested a toxicological study has been undertaken in rats. No overt signs or symptoms of stress were observed for any of the test extracts. The serum sample has been analyzed for liver function tests such as SGOT, and SGPT and, for kidney function tests such as glucose, creatinine and urea have been analyzed. All the extracts were found to be non-toxic, which was evident in the biochemical analysis of serum that they have shown no marginal changes compared to the control. In order to identify the haemorrhage in the intestine, the animals were sacrificed and opened [15]. The results are presented in Table and in Plate. No haemorrhage has been observed for any of the test extracts.

Table 1. Qualitative Photochemical Analysis

<table>
<thead>
<tr>
<th>Phytochemical Analysis</th>
<th>Adhatoda vasica</th>
<th>Garcinia mangostana</th>
<th>Sesbania sesban</th>
</tr>
</thead>
<tbody>
<tr>
<td>Alkaloids</td>
<td>+VE</td>
<td>+VE</td>
<td>+VE</td>
</tr>
<tr>
<td>Carbohydrate</td>
<td>+VE</td>
<td>-VE</td>
<td>-VE</td>
</tr>
<tr>
<td>Glycosides</td>
<td>+VE</td>
<td>-VE</td>
<td>+VE</td>
</tr>
<tr>
<td>Phytosterols</td>
<td>+VE</td>
<td>+VE</td>
<td>-VE</td>
</tr>
<tr>
<td>Phenol</td>
<td>+VE</td>
<td>+VE</td>
<td>+VE</td>
</tr>
<tr>
<td>Flavonoids</td>
<td>+VE</td>
<td>+VE</td>
<td>+VE</td>
</tr>
<tr>
<td>Proteins</td>
<td>+VE</td>
<td>+VE</td>
<td>+VE</td>
</tr>
<tr>
<td>Amino acids</td>
<td>+VE</td>
<td>+VE</td>
<td>-VE</td>
</tr>
<tr>
<td>Tannins</td>
<td>+VE</td>
<td>+VE</td>
<td>+VE</td>
</tr>
<tr>
<td>Steroid</td>
<td>+VE</td>
<td>-VE</td>
<td>+VE</td>
</tr>
<tr>
<td>Saponin</td>
<td>+VE</td>
<td>+VE</td>
<td>-VE</td>
</tr>
<tr>
<td>Catechin</td>
<td>+VE</td>
<td>+VE</td>
<td>-VE</td>
</tr>
<tr>
<td>Alkaloids</td>
<td>+VE</td>
<td>+VE</td>
<td>+VE</td>
</tr>
</tbody>
</table>

Table 2. Toxicity effect of selected solvent extracts of Adhatoda vasica in rat based on biochemical markers

<table>
<thead>
<tr>
<th>Concentration of extract</th>
<th>Glucose (mg/dL)</th>
<th>Urea (mg/dL)</th>
<th>Creatinine (mg/dL)</th>
<th>SGOT (Units/L)</th>
<th>SGPT (Units/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hexane</td>
<td>114.6±4.45a</td>
<td>32.7±2.88a</td>
<td>1.10±0.00a</td>
<td>69.5±1.95a</td>
<td>49.4±0.26a</td>
</tr>
<tr>
<td>Chloroform</td>
<td>106.3±3.80a</td>
<td>37.3±1.76b</td>
<td>1.40±0.10b</td>
<td>54.1±1.50b</td>
<td>56.0±2.00b</td>
</tr>
<tr>
<td>Ethyl acetate</td>
<td>88.8±2.60b</td>
<td>30.8±1.52a</td>
<td>1.03±0.05c</td>
<td>65.7±1.05c</td>
<td>59.7±2.01c</td>
</tr>
<tr>
<td>Methanol</td>
<td>117.7±2.35c</td>
<td>35.0±0.79ab</td>
<td>0.90±0.00d</td>
<td>70.5±1.95db</td>
<td>49.4±2.00db</td>
</tr>
<tr>
<td>Control</td>
<td>107.3±2.66d</td>
<td>35.30±0.05a</td>
<td>1.30±0.05db</td>
<td>54.7±2.05c</td>
<td>34.40±0.10a</td>
</tr>
</tbody>
</table>
CONCLUSION

Adhatoda vasica, Garcinia mangostana and Sesbania sesban were the three plants chosen for the anti-inflammation study. Alkaloids, Phenol, Flavonoids, Tannins and Saponins were identified in photochemical analysis in the extracts of those 3 plants and they are the chemicals commonly involved in anti-inflammatory activity etc. Effect of test extracts on histamine-induced paw edema in mice and Effect of test extracts on carrageenan induced paw edema in mice were analyzed and the all the extracts were found to be better anti-inflammatory activity comparable to control. Treatment with extracts after 18 h showed a marked decrease in temperature in yeast induced pyrexia. The results of Inhibition of albumin denaturation by the extracts was lesser compared to control but encouraging. In order to identify whether any harmful systemic effects caused by the extracts tested, a toxicological study has been undertaken in rats. No overt signs or symptoms of stress were observed for any of the test extracts. All the extracts were found to be non-toxic. All the results have clearly indicated that the selected three plants have got a good anti-inflammatory activity in a dose dependent manner and as there is no toxicity the extracts could effectively be used as alternative medicines in the place of general allopathic anti-inflammatory drugs. Anyway further detailed analysis in other aspects are needed for promoting this as a therapeutic agent.

AKNOWLEDGEMENT

None

CONFLICT OF INTEREST

No conflict of interest.

REFERENCES