Analysis on creatinine reducing herbals renal as protective agents in rat

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Abstract

*Tribulus terrestris* (Nerunji mullu), *Butea monosperma* (Parasa pattai) and *Chicorium intybus* (Kasini keerai) are the traditional herbal medicines used in Siddha and Ayurvedic system. They are analyzed for their creatinine reducing activity along with other related parameters. All the three plants showed high potent phytochemicals such as Alkaloids, Phenolics, and Flavonoids, the chemicals known for antimicrobial, antioxidant, immune modulatory activity, and anticancer activities. Metabolism correction is the common features of chemicals having antioxidant activity. Most of them including others responsible for nephron protective activity by controlling creatinine level from deleterious level to non-dangerous level. To identify the effectiveness of *Tribulus terrestris*, *Butea monosperma* and *Chicorium intybus* on Creatinine reducing activity in KMnO₄ induced rats where Simvastatin used as a positive standard by following hematological, biochemical and histopathological analysis with the objective to use these natural agents with no side effects for Creatinine reducing activity instead of chemicals which has got more side effects and costlier.

Keywords: *Tribulus terrestris*, *Butea monosperma*, *Chicorium intybus*, Alkaloids

INTRODUCTION

*Tribulus terrestris* is an annual plant in the caltrop family (Zygophyllaceae) widely distributed around the world, that is adapted to grow in dry climate locations in which few other plants can survive. Its extracts are widely used by body builders.

*Butea* is a genus of flowering plants belonging to the pea family, Fabaceae. It is sometimes considered to have only two species, *B. monosperma* and *B. superba*, or is expanded to include four or five species. *Butea monosperma* is used for timber, resin, fodder, medicine, and dye. *Butea* is also a host to the Lac insect, which produces naturallacquer.

South Asian cuisine encompasses a delectable variety of sub-cuisines and cooking styles that vary very widely, reflecting the diversity of the Indian subcontinent, even though there is a certain centrality to the general ingredients used. Terms used the recipes of varied Indian and other South Asian sub-cuisines sometimes tend to be multi-lingual and region-specific, mostly based on the author's specific sub-ethnicity, the popularity of a given vegetable/spice in a given sub-cuisine within South Asia, etc [1].

Indian cuisine is overwhelmingly vegetarian friendly and employs a variety of different fruits, vegetables, grains, and spices which vary in name from region to region within the country. Most Indian restaurants serve predominantly Punjabi/North Indian cuisine, while a limited few serve a very limited choice of some South Indian dishes like Dosa. But for the connoisseurs, India offers a complex and eclectic array of sub-cuisines to explore, which are equally vegetarian friendly and a delight to the taste buds.

MATERIALS AND METHODS

Phytochemical Analysis

Phytochemical examinations were carried out for all the extracts, as per the standard methods [2].

Detection of Alkaloids

Extracts were dissolved individually in dilute Hydrochloric acid and filtered.

Detection of carbohydrates

Extracts were dissolved individually in 5 ml distilled water and filtered. The filtrates were used to test for
the presence of carbohydrates.

Detection of glycosides
Extracts were hydrolysed with dil. HCl, and then subjected to test for glycosides.

Detection of saponins
a) Froth Test: Extracts were diluted with distilled water to 20 ml and this was shaken in a graduated cylinder for 15 minutes. A formation of 1 cm layer of foam indicates the presence of saponins.

b) Foam Test: 0.5 gm of the extract was shaken with 2 ml of water. If foam produced persists for ten minutes it indicates the presence of saponins.

Detection of phytosterols
a) Salkowski’s Test: Extracts were treated with chloroform and filtered. The filtrates were treated with a few drops of Conc. Sulphuric acid, shaken and allowed to stand. Appearance of golden yellow colour indicates the presence of triterpenes.

b) Libermann Burchard’s test: Extracts were treated with chloroform and filtered. The filtrates were treated with a few drops of acetic anhydride, boiled and cooled. Conc. Sulphuric acid was added. The formation of brown ring at the junction indicates the presence of phytosterols [3].

Detection of phenols
Ferric Chloride Test
Extracts were treated with 3-4 drops of ferric chloride solution. Formation of bluish black colour indicates the presence of phenols.

Detection of tannins
Gelatin Test
To the extract, 1% gelatin solution containing sodium chloride was added. Formation of white precipitate indicates the presence of tannins.

Detection of flavonoids
a) Alkaline Reagent Test: Extracts were treated with a few drops of sodium hydroxide solution. Formation of intense yellow colour, which becomes colourless on the addition of dilute acid, indicates the presence of flavonoids.

b) Lead acetate Test: Extracts were treated with a few drops of lead acetate solution. Formation of a yellow color precipitate indicates the presence of flavonoids.

HISTOPATHOLOGICAL ANALYSIS
Procedure
Fixation
Fixation is a process by which the cells of tissue and its components are fixed in physical and partly in the chemical states. It is the process of killing and hardening the tissue so has to withstand the subsequent processing fluid [4].

It is carried out by immersion of tissue in the fixative (a chemical substance) to prevent autolysis and bacterial attack. The fixative used to be aldehyde fixative:

10% buffered formalin
10 ml commercial formaldehyde, 6.5g di-sodium hydrogen phosphate, 4g dihydrogen phosphate was added and made up to 100 ml with distilled water.

Tissue processing
Tissue when satisfactory fixed, it is to be processed for further, various procedures such as dehydration, clearing, infiltration and embedding [5].

Dehydration
Tissue contain large amounts of water both intracellular and extracellular are removed. So it will be replaced by embedding medium. Dehydration medium used was ethyl alcohol.

Clearing
The essential requirement of a clearing agent (chloroform) is that it should be miscible with both dehydrating agent and filtration medium. Many of these fluids have a similar refractive index of protein. By virtue of high refractive index, clearing is achieved.

Infiltration and embedding
This process involves the impregnation and tissues with a medium that will fill all natural cavities, spaces and the interstices of tissues and that will set into firm consistency to allow cutting of suitable thin sections [5]. Paraffin wax is the most routinely used for embedding and infiltration.

RESULTS AND DISCUSSION
*Tribulus terrestris* (Nerunji mullu), *Butea monosperma* (Parasa pattai) and *Chicorium intybus* (Kasini keerai [4]) are the traditional herbal
medicines used in Siddha and Ayurvedic system. They are analyzed for their creatinine reducing activity along with other related parameters. All the three plants showed high potent phytochemicals such as Alkaloids, Phenolics, and Flavonoids, the chemicals known for antimicrobial, antioxidant, immune modulatory activity, and anticancer activities (Table 1). Metabolism correction is the common features of chemicals having antioxidant activity. Most of them including others responsible for nephron protective activity by controlling creatinine level from deleterious level to non-dangerous level.

The hematological analysis results showed that all the study parameters were increased, except haemoglobin and lymphocyte, following experimental induced with KMnO₄. The haemoglobin and lymphocyte levels have fallen down when compared to control animal. Tribulus terrestritis has not only restored the hemoglobin level but increased the level by 0.9 gms% than the control animals. But Butea monosperma and Chicorium intybus has reduced the haemoglobin count lesser than that caused by KMnO₄. The TRBC counts were very much lower than the control animals and experimentally induced animals. The RBC count was restored on par with control animals by Butea monosperma [6] but Chicorium intybus and Tribulus terrestris have increased the count by 2.4 and 2.8 times than the control level. The TLC levels were reported at very much higher level than the control study and KMnO₄studies. Similar were the results for lymphocyte levels (Table 2).

**Histopathological findings**

Histopathological findings of control animal’s kidney showed no visible damages up to 8 days. The kidney showed normal architecture with vacuolated with normal histological structure. Sections from saline-treated control animals did not have histopathological changes. The Histopathological findings of KMnO₄ induced Kidney shows mild acute congestion with normal splenic architecture on day -7 and thickening wall of eccentric arteriole of white pulp on day-8 [7]. The dilation of trabecular vessels and thickening of eccentric arteriole wall were observed on 8st day whiles cattered hemorrhagic areas in red pulp were seen on 8th day. Histopathological findings of all the three plants and simvastatin were almost similar to normal control which is evident that they are all nephroprotective. The results have clearly indicated that the test plants could effectively be used as an alternative medicine as antidiabetic agent. Anyway the hypoglycemic activity should further be studied along with its side effects in detail (Fig 1).

### Table 1. Qualitative analysis of phytoconstituents in different extracts of *Musa paradisiaca*

<table>
<thead>
<tr>
<th>Phytoconstituents</th>
<th>Tribulus terrestris</th>
<th>Butea monosperma</th>
<th>Chicorium intybus</th>
</tr>
</thead>
<tbody>
<tr>
<td>Alkaloids</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Flavonoids</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Polyphenolics</td>
<td>+</td>
<td>+</td>
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</tr>
<tr>
<td>Phytosterol</td>
<td>+</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Saponins</td>
<td>-</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>Fixed oils and fats</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Carbohydrates</td>
<td>-</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>Amino acids and proteins</td>
<td>-</td>
<td>-</td>
<td>+</td>
</tr>
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### Table 2. Hematological Tests Results from rats used in Probiotic study

<table>
<thead>
<tr>
<th>Haematological Tests</th>
<th>Control</th>
<th>KMnO₄ induced</th>
<th>Butea monosperma</th>
<th>Chicorium intybus</th>
<th>Tribulus terrestris</th>
<th>Simvastatin</th>
</tr>
</thead>
<tbody>
<tr>
<td>Haemoglobin gms %</td>
<td>12.0%</td>
<td>11.7%</td>
<td>11.1%</td>
<td>10.3%</td>
<td>12.9%</td>
<td>12.0%</td>
</tr>
<tr>
<td>TRBC millions Cells/cu mm</td>
<td>6.0</td>
<td>6.2</td>
<td>5.0</td>
<td>5.1</td>
<td>6.2</td>
<td>6.0</td>
</tr>
<tr>
<td>RBC distribution width (%(RDW))</td>
<td>12.7</td>
<td>13.5</td>
<td>12.7</td>
<td>14.9</td>
<td>13.9</td>
<td>12.7</td>
</tr>
<tr>
<td>TLC cells/cu mm</td>
<td>7000</td>
<td>13000</td>
<td>14000</td>
<td>11000</td>
<td>12700</td>
<td>9000</td>
</tr>
<tr>
<td>Platelets lakhs cells/ cumm</td>
<td>2.9</td>
<td>5.0</td>
<td>1.8</td>
<td>1.82</td>
<td>1.46</td>
<td>2.6</td>
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CONCLUSION
Cumulative evidence suggests that some herbal medicines, including Tribulus terrestris (Nerunji mullu), Butea monosperma (Parasa pattai) [8] and Chicorium intybus (kasini keeri) have a beneficial role in slowing the progressive renal disease. Their use is often associated with a reduction in creatinine and the amelioration of Acute and Chronic kidney disease. Hematological and Biochemical, and histopathological analysis have clearly revealed their nephron protective activity [9]. The mechanisms are multiple and include anti-inflammation and antioxidant activity of the photochemical present in those plants. Though alternative medicines may be a promising new way to prevent the progression of renal problems, few long-term studies evaluating their therapeutic efficacy or high quality clinical trials have been conducted. Such studies are nevertheless required to confirm the positive effects of these herbs. While not all indigenous herbs are toxic to the kidney, herbal medicines may be hazardous to patients with renal diseases because they may interact with other drugs or contain significant amounts of potassium [10]. The frequency of herb-related nephrotoxicity could be markedly reduced if the herbs are prescribed strictly according to the recommendation of the pharmacopoeia with attention to its origin, dose, way of preparation, and duration of intake. It is obvious that traditional herbal medicines, though natural, are not necessarily safer to use than Western ones [11]. The safety of these herbs needs to be strictly and individually evaluated.

AKNOWLEDGEMENT

CONFLICT OF INTERESET
No conflict of interest.

REFERENCE

<table>
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<tr>
<th>Neutrophil</th>
<th>54%</th>
<th>42%</th>
<th>56%</th>
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<th>51%</th>
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<tbody>
<tr>
<td>Eosinophil</td>
<td>02%</td>
<td>02%</td>
<td>03%</td>
<td>03%</td>
<td>02%</td>
<td>02%</td>
</tr>
<tr>
<td>Basophil</td>
<td>00%</td>
<td>00%</td>
<td>00%</td>
<td>00%</td>
<td>00%</td>
<td>00%</td>
</tr>
<tr>
<td>Lymphocyte</td>
<td>43%</td>
<td>54%</td>
<td>40%</td>
<td>36%</td>
<td>45%</td>
<td>36%</td>
</tr>
<tr>
<td>Monocyte</td>
<td>01%</td>
<td>02%</td>
<td>01%</td>
<td>02%</td>
<td>02%</td>
<td>01%</td>
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<tr>
<td>PCV</td>
<td>33%</td>
<td>31%</td>
<td>28%</td>
<td>16%</td>
<td>09%</td>
<td>33%</td>
</tr>
</tbody>
</table>

Fig 1. AC KID 10x- The glomeruli are normal cellular, irregularly cellular, normocellular the tubules are closely spaced and the interstitium and blood vessels are not prominent
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