Haematinic Activity of Poovarasam pattai kudineer chooranam in Phenylhydrazine Induced Anaemic Rats for Treatment of Vitiligo

K. Kanakavalli¹, P. Parthiban², V. Anbu³ and G. Kala⁴

¹,²Government Siddha Medical College, Chennai, Tamil Nadu, India.
³Department of Pharmacology, Vel’s University, Chennai, Tamil Nadu, India.
⁴P G Scholar, Illyr Pothu Maruthuvam, GSMC, Tamil Nadu, India.

ABSTRACT

Backgrounds objective: Considerable efforts are being made to develop an effective drug for vitiligo as there exist no definite cure. The present study was carried out to examine the effect of Poovarasampattai kudineer chooranam (A Siddha drug) in treating vitiligo and as one of the objective this drug’s haematinic activity is observed.

Methods: Six male albino rats were kept as normal control group (Group 1), while 24 rats were made anaemic by oral intubations of phenylhydrazine (100mg/kg body weight) daily for 8 days. Rats that developed anaemia with haemoglobin concentration <14g / dl were recruited for the study.

Result: The administration of the Poovarasam pattai Kudineer Chooranam produced a significant (p<0.05) increase in the haematological parameters. The phenylhydrazine induced anaemia was significantly (p<0.05) reversed after 14 days treatment with the Poovarasam pattai Kudineer Chooranam at the dose level of 400mg/kg towards almost normal.

Keywords: Vitiligo – Puvarasampattai – Haematinic effect – ‘Pitta’.

INTRODUCTION

Vitiligo is an acquired idiopathic disorder showing white non-sclary macules. An Auto immune aetiology and associated with pernicious anaemia, thyroid disease and Addison’s diseases above 30% of patients have a family history of the disorder. All races are affected, but lesions are most prevalent among dark pigmented individuals¹. It affects 1% of the population worldwide².

In India the prevalence of vitiligo is 0.25 to 2.5%³. The prevalence of vitiligo was 0.38% in 47,033 people in a world. Both sexes were equally affected. Age specific prevalence increased from 0.09% under the age Group of 60 to 69 years. After the age of 70 the prevalence is declined. The common onset being most often between the 4th to 6th decade.

Review of Literature recommended herbs having bitter taste and Haematinic activity are used to combat vitiligo.

List of herbals tested for Haematinic activity and Bitter taste used in Vitiligo are listed below,

1. Brahmi⁵ (Bacopa monnieri)
2. Cherangkottai (Semecarpus anacardium)
3. Karisalankanni (Eclipta prostrata)
4. Pungu (Pongamia pinnata)
5. Puvarasu (Thespesia populnea. Linn.)

Among the above said herbs, trial drug used in treating Vitiligo is Puvarasam pattai (Thespesia populnea Linn. belongs to Malvaceae family. It is a fairly large, quick growing evergreen tree distributed mainly along the coastal Regions throughout India. Bark of the plant is used externally in Scabies, Psoriasis and other Skin diseases. The plant is astringent, acrid, depurative, haemostatic, antidiarrheal and antibacterial. Gossypol was reported to be present in the bark of the plant and it was found to be optically active⁶.

Traditional herbal practitioners have made several claims on numerous herbal preparations with specific claim on the efficacy of parts of Thespesia populnea in the treatment of several disease conditions. According to the literature, the improvement in the haemoglobin level in these diseased conditions will enhance the beneficial treatment outcome. Hence in the present investigations, the traditionally used Siddha medicament poovarasam pattai kudineer chooranam was evaluated for its antianemic property in terms of haematinic action in...
Scientific manner using phenylhydrazine induced anemic animal model. SOP of the trial drug which is used in the treatment of vitiligo is as follows: 210gms of Puvarasam bark (Thespesia populnea Linn.) is boiled in 1400ml of water. This is boiled up to a concentration of one third of the original decoction. Dosage: 60ml Bd

Swetha Kuttam is analogous to Vitiligo. In Yugi Munni Vaithya Chinthamani – 800, Swetha kuttam is one among the 18 types of kuttam. Traditional knowledge makes the diagnosis, with the help of 8 basic tools. Among them Niram (Colour) is one of the diagnostic tools. Red colour represents Pitham and white colouration represents kapham. Any decreased colour of the skin, mucous membrane, and sclera represents kapham. A drug which is bitter in taste and having a hot potency in general increases all divisions of the pitham. The hypothesis is, if Ranjaga pittam is increased prasaga pittam will increase. With the above said hypothesis, haematinic activity of the drug supports the increase of Prasaga pitham which may help in treating vitiligo is assessed.

MATERIALS AND METHODS
Drug material
Poovarasam Pattai Kudineer Chooranam was collected from Siddha commercial lab and 2gms of this chooranam was suspended in 10ml of 2% CMC solution to achieve 200mg/ml stock solution and used in this study. The resulting suspension was then filtered. The filtrate was stored in a refrigerator until use.

Animals
Male albino rats (150-180g) and Mice of either sex weighing 25-30g were used for the study. The rats were housed in wire-mesh cages with a 12 h light/dark cycle. They had continuous access to food and water during the entire period of experimentation.

Acute toxicity study
Single dose acute oral toxicity test for the Poovarasam Pattai Kudineer Chooranam was carried out as per OECD Guidelines 425. Animals were observed individually after dosing at least once during the first 30 minutes, periodically during the first 24 hours, with special attention given during the first 4 hours, and daily thereafter, for a total of 14 days, except where they need to be removed from the study and humanely killed for animal welfare reasons or are found dead. All observations are systematically recorded and Observations include changes in skin and fur, eyes and mucous membranes, and also respiratory, circulatory, autonomic and central nervous systems, and somatomotor activity and behaviour pattern. Attention was directed to observations of tremors, convulsions, salivation, diarrhoea, lethargy, sleep and coma. The principles and criteria summarised in the Humane Endpoints Guidance Document taken into consideration. Animals found in a moribund condition and animals showing severe pain or enduring signs of severe distress was humanely killed.

Evaluation of Haematinic Activity
Six rats were kept as normal control group (Group 1), while 24 rats were made anaemic by oral intubations of phenylhydrazine (10 mg/kg body weight) daily for 8 days. Rats that developed anaemia with haemoglobin concentration <14 g/dl were recruited for the study. Anaemic rats were randomly divided into 5 groups (2 to 6) and treated as follows: Group 1: received distilled water (1 ml) daily (normal control), Group 2: received 2% CMC (1 ml) daily (anaemic control), Group 3: received oral single dose of the Poovarasam Pattai Kudineer Chooranam 100mg/kg body weight/day Group 4: received oral single dose of the Poovarasam Pattai Kudineer Chooranam 200mg/kg, Group 5: received oral single dose of the Poovarasam Pattai Kudineer Chooranam 400mg/kg Group 6: received oral single dose of the haematinic syrup 2ml/kg body weight/day. The treatment was continued for 2 weeks.

Haematological investigation
Blood was collected from the retro orbital vein of experimental animals after an overnight fast (T=0) and after 1 and 2 weeks of treatment with Poovarasam Pattai Kudineer Chooranam, was used for the determination of red blood cell count (RBC), haemoglobin (Hb) concentration and packed cell volume (PCV). The mean cell volume (MCV), mean cell haemoglobin (MCH) and the mean cell haemoglobin concentration (MCHC) were calculated.

Statistical analysis
Experimental data was analysed using analysis of variance (ANOVA) and Dunnet’s ‘t’ test to determine significant differences between means. The statistical analysis system (INSTAT-V3) package was used for this analysis.
RESULTS AND DISCUSSION

The phytochemical screening of Poovarasam Pattai Kudineer Chooranam revealed presence of alkaloids, flavonoids, saponins and terpenoids. The acute toxicity testing revealed no significant toxic signs or death up to doses of 2000 mg/kg. In the untreated control rats phenylhydrazine induced significant (p<0.05) decrease in Hb concentration, RBC, WBC level, indicating anaemia. The administration of the Poovarasam Pattai Kudineer Chooranam produced a significant (p<0.05) increase in the haematological parameters. The phenylhydrazine induced anaemia was significantly (p<0.05) reversed after 14 days treatment with the Poovarasam Pattai Kudineer Chooranam at the dose level of 400mg/kg towards almost normal. In the anemic control, the Hb was 12.21±1.34g/dl at day seven and this was improved to 13.00±0.42, 15.36±1.15 and 15.87±1.26g/dl at the dose levels 100, 200 and 400mg/kg respectively. Similarly, After 14 days of treatment with Poovarasam Pattai Kudineer Chooranam 400mg/kg, the Hb level was increased from 10.12 ± 1.00g/dl to 13.11 ± 1.05, 14.52 ± 1.92 and 16.33±1.53g/dl at the doses described earlier. Same kind of beneficial and significant (p<0.05) changes were recorded in the other haematological parameters and at the higher dose of the Poovarasam Pattai Kudineer Chooranam. The effect of commercially availed haematinic syrup was comparable to those of the test drug. There was no change in other blood parameters like MCV, MCHC and MCH. On other hand, red blood cell count (p<0.01) in animals treated with 400mg/kg of Poovarasam Pattai Kudineer Chooranam exhibit a statistically significant elevation when compared with control group. The main importance of this study is to correlate the positive or beneficial effects along with the cofactors like flavonoids and alkaloids in the treatment of various traditional claims of this drug. Factors like alkaloids, flavonoids, saponins, tannins, calcium, zinc, vitamins C and K are involving in the utilization of iron content by our body tissues. Especially vitamin C contributes to the bioavailability of iron in the body. Since, anemia causes important physiologic effects on the cardiovascular system, hormonal and metabolic effects can result in direct myocardial toxicity, myocardial hypertrophy, and salt and water retention, which could be harmful in patients with heart failure.

Scientific studies failed to give any valid evidence of an association between circulating hemoglobin level and the severity of symptoms. Number of other conditions, such as malaria and haemoglobinopathies are also responsible because the RBC plays a primary role of transporting substances including nutrients, respiratory gases and other waste materials throughout the body. The WBC defends the body against pathogens and other foreign bodies. The platelets play the role of preventing blood loss. Therefore, severe alteration in the concentration of any of these haemopoietic components may be detrimental.

CONCLUSION

From the toxicity study, no treatment related toxic signs and symptoms was observed and the Poovarasam Pattai Kudineer Chooranam, at a dose of 400 mg/kg (p.o.), significantly increased the haemoglobin, and RBC count in anaemic rats indicating the haematinic effect. The presence of alkaloids, saponins, tannins was confirmed by the preliminary phytochemical study. These phytochemicals could be responsible for their anti-anaemic effects.

| Table 1: Dose finding experiment and its behavioral Signs of Toxicity |
|----------------|----------------|----------------|----------------|----------------|----------------|----------------|----------------|----------------|----------------|----------------|----------------|----------------|----------------|----------------|----------------|----------------|----------------|----------------|
| No | Dose mg/kg | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 | 13 | 14 | 15 | 16 | 17 | 18 | 19 | 20 |
| 1   | 1000       | + | - | - | + | + | + | - | - | - | - | - | - | - | - | - | - | - | - | - |
| 2   | 2000       | + | - | - | + | + | + | - | - | - | - | - | - | - | - | - | - | - | - | - |
| 3   | 5000       | + | - | - | + | + | + | - | - | - | - | - | - | - | - | - | - | - | - | - |

Table 2: Effect of Phenylhydrazine (10mg/kg, p.o. daily for 7 days) alone on Hematological parameters

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Group 1 (Control)</th>
<th>Group 2 (anemic)</th>
<th>Group 3 (anemic)</th>
<th>Group 4 (anemic)</th>
<th>Group 5 (anemic)</th>
<th>Group 6 (anemic)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hb (g/dl)</td>
<td>18.00 ± 0.48</td>
<td>13.21±0.32**</td>
<td>13.56±0.24**</td>
<td>13.41±0.36**</td>
<td>14.12±0.31**</td>
<td>13.55 ± 0.29**</td>
</tr>
<tr>
<td>PCV (%)</td>
<td>56.42±1.66</td>
<td>41.92 ± 2.74**</td>
<td>40.28 ± 2.26**</td>
<td>41.22 ± 2.44**</td>
<td>40.51 ± 2.15**</td>
<td>40.34 ± 2.48**</td>
</tr>
<tr>
<td>RBC (x10^6/ml)</td>
<td>6.30 ± 0.21</td>
<td>4.28 ± 0.24**</td>
<td>4.28 ± 0.21**</td>
<td>4.54 ± 0.25**</td>
<td>4.47 ± 0.20**</td>
<td>4.20 ± 0.32**</td>
</tr>
<tr>
<td>MCV (fl)</td>
<td>74.11 ± 2.72</td>
<td>82.45±4.02</td>
<td>86.18±5.35</td>
<td>85.55±4.24</td>
<td>86.11±5.65</td>
<td>81.99±4.26</td>
</tr>
<tr>
<td>MCH (pg)</td>
<td>22.65 ± 1.56</td>
<td>31.45±5.22*</td>
<td>30.15±2.28</td>
<td>30.12±1.88</td>
<td>30.00 ± 2.23</td>
<td></td>
</tr>
<tr>
<td>MCHC (g/dl)</td>
<td>35.10 ± 0.52</td>
<td>33.270.80</td>
<td>34.11±0.98</td>
<td>34.15±1.06</td>
<td>30.75 ± 1.00*</td>
<td></td>
</tr>
</tbody>
</table>

Values are mean of 6 animals ± S.E.M. (Dunnet’s test). *P<0.05; **P<0.01 Vs Control; *P<0.05; **P<0.01 Normal Vs Control.

Table 3: Hematological parameter of rats after Seven days treatment with Poovarasam Pattai Kudineer

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Group 1 (control)</th>
<th>Group 2 (anemic control)</th>
<th>Group 3 (5ml/kg)</th>
<th>Group 4 (10ml/kg)</th>
<th>Group 5 (20ml/kg)</th>
<th>Group 6 (Heamatinic syrup)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hb (g/dl)</td>
<td>18.56 ±1.33</td>
<td>12.21±1.34**</td>
<td>13.00±0.42*</td>
<td>15.36±1.15</td>
<td>15.87 ± 1.26</td>
<td>20.52 ±1.88</td>
</tr>
<tr>
<td>PCV (%)</td>
<td>52.18±1.22</td>
<td>43.85±2.26</td>
<td>46.74±2.41</td>
<td>47.00±2.48</td>
<td>49.39±2.98</td>
<td>54.65 ±2.61</td>
</tr>
<tr>
<td>RBC (x10^6/ml)</td>
<td>7.62±0.20</td>
<td>5.55±0.20**</td>
<td>6.29±0.14**</td>
<td>6.54±0.17**</td>
<td>7.26±0.15</td>
<td>8.55±0.19**</td>
</tr>
<tr>
<td>MCV (fl)</td>
<td>77.24±2.22</td>
<td>78.10±2.47</td>
<td>80.32±2.83</td>
<td>80.12±2.14</td>
<td>80.00±3.00</td>
<td>76.52±2.68</td>
</tr>
<tr>
<td>MCH (pg)</td>
<td>25.88±1.74</td>
<td>28.1±1.52</td>
<td>23.20±1.41</td>
<td>24.16±1.56</td>
<td>24.22±1.19</td>
<td>26.24±1.50</td>
</tr>
<tr>
<td>MCHC (g/dl)</td>
<td>34.26±1.33</td>
<td>32.05±1.24</td>
<td>27.64±1.65*</td>
<td>26.00±1.21*</td>
<td>29.54±1.75</td>
<td>30.42±2.00</td>
</tr>
</tbody>
</table>

Values are mean of 6 animals ± S.E.M. (Dunnet’s test). *P<0.05; **P<0.01 Vs Control; *P<0.05; **P<0.01 Normal Vs Control.

Table 5: Hematological parameters of rats after 14 days treatment with Poovarasam Pattai Kudineer

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Group 1 (control)</th>
<th>Group 2 (anemic control)</th>
<th>Group 3 (5ml/kg)</th>
<th>Group 4 (10ml/kg)</th>
<th>Group 5 (20ml/kg)</th>
<th>Group 6 (Heamatinic syrup)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hb (g/dl)</td>
<td>18.48±1.85</td>
<td>10.12±1.00**</td>
<td>13.11±1.05</td>
<td>14.52±1.92</td>
<td>16.33±1.53</td>
<td>22.71±2.04</td>
</tr>
<tr>
<td>PCV (%)</td>
<td>46.24±1.27</td>
<td>40.32±1.28</td>
<td>42.35±1.62</td>
<td>41.22±1.38</td>
<td>42.38±1.12</td>
<td>55.22±1.32**</td>
</tr>
<tr>
<td>RBC (x10^6/ml)</td>
<td>4.88±0.26</td>
<td>3.52±0.30*</td>
<td>3.40±0.22**</td>
<td>3.86±0.31</td>
<td>4.10±0.36</td>
<td>5.63±0.38</td>
</tr>
<tr>
<td>MCV (fl)</td>
<td>73.43±2.52</td>
<td>90.02±2.45**</td>
<td>84.22±2.12**</td>
<td>81.35±1.62</td>
<td>78.56±2.19</td>
<td>74.05±2.37</td>
</tr>
<tr>
<td>MCH (pg)</td>
<td>27.54±1.61</td>
<td>34.12±1.69</td>
<td>30.33±1.56</td>
<td>30.78±2.04</td>
<td>30.00±2.11</td>
<td>29.13±2.82</td>
</tr>
<tr>
<td>MCHC (g/dl)</td>
<td>31.29±2.45</td>
<td>35.05±1.32</td>
<td>33.52±1.08</td>
<td>32.16±1.12</td>
<td>32.24±2.00</td>
<td>31.98±2.28</td>
</tr>
</tbody>
</table>

Values are mean of 6 animals ± S.E.M. (Dunnet’s test). *P<0.05; **P<0.01 Vs Control; *P<0.05; **P<0.01 Normal Vs Control.

Effect of Phenylhydrazine alone on Hematological parameters
HISTOPATHOLOGY SLIDES

BONE

BRAIN

HEART

INTESTINE

KIDNEY

LIVER
REFERENCES
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