ACUTE AND SUB-ACUTE TOXICITY STUDY OF POLYHERBAL FORMULATION IN WISTAR RATS

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ABSTRACT
The renowned eight herbs considered in the use of traditional system of medicine for the treatment of piles are the main constituents of the PILE RELIEF formulation (PRF). Every single herb is proven as a safest drug but the effects are yet not identified when they are used in combination with each other. Hence, the present investigation is carried out to elucidate toxicological profile of PRF in rats in terms of acute and sub-acute oral toxicity. The OECD guidelines 420 and 407 were used to perform acute and sub-acute oral toxicity study respectively. In case of acute toxicity study, single 2000 mg/kg oral dose of PRF was administered in female albino Wistar rats, while for sub acute oral toxicity study various dose like 50, 250 and 500 mg/kg daily for 28 days were administered in both male and female albino Wistar rats. The effect of PRF on body weight, relative organ weights (liver, kidney, lung, heart, brain), biochemical (cholesterol, triglyceride, urea, AST, ALT, bilirubin, protein, uric acid, RBS), haematological (RBC, WBC, HB, PCV, MCV, MCH, MCHC, PLT, Monocytes, lymphocytes, eosinophils) and histopathological parameters were characterized. No mortality or behavioural changes in rats treated with single dose of 2000mg/kg was observed so PRF is considered to be safe at this dose. No significant changes in body weight, relative organ weights, biochemical, haematological and histopathological parameters with three different dose levels were observed in sub-acute toxicity study for 28 days. The result clearly demonstrated the absence of acute and sub-acute oral toxicity of PRF in order to prove safety evidence for its use in animals. However, further animal and clinical experiments are needed for safety and efficacy of the PILE RELIEF.

KEYWORDS: PILE RELIEF formulation, acute and sub-acute toxicity, biochemical, haematological and histopathological, OECD.

INTRODUCTION
Herbal formulations have attained wide recognition and gained greater importance than ever before, mainly due to their efficacy and easy availability as well as less side effects as compared to the synthetic drugs in the modern era. A World Health Organization survey indicated that 70 to 80% of the global population depends on alternative medicine, predominantly herbal in nature, in their primary health care. The uses of medicinal plants as a source of drugs in primary health care have become popular universally, particularly in developing countries as a safe because of natural source. [1-6]
Suran, Rasanjan, Yashtimadhu, Neem, Sonamukhi, Trifla, Fennel and Methi are renowned herbs available throughout India and widely used in the traditional system of medicine for the treatment of pile and various ailments. The pile relief polyherbal formulation (PRF) is prepared by using above mentioned eight herbs.\[6\]

The safeties of these individual herbs are well known, but the combined effects of these herbs are unclear. Thus, it becomes essential to evaluate the safety and toxicity of the combination of herbs (PRF), before their use in human. Preclinical toxicity studies are necessary for determining a safe dose for human trials. Consequently, the main purpose of the study was to measure the safety of PRF by acute oral toxicity (single dose, 14 days) and sub-acute oral toxicity (repeated doses for 28 days).\[6,7\]

In albino Wistar rats according to the Organization for Economic Co-operation and Development (OECD) guidelines 420 and 407 respectively. \[4,5\]

**MATERIALS AND METHODS**

**Materials**

**Drugs and chemicals**

Name: POLYHERBAL FORMULATION- PILERELIEF

Storage condition: Room Temperature

Route: Oral

**Experimental animals**

Albino Wistar rats of male and female weighing 180-250g±20 were maintained under standard laboratory conditions of temperature (22±2 °C) and humidity 50±15% with 12 h day: 12 h night cycle. Rats had free access to water and rodent pellet diet. Animals were acclimatized to laboratory conditions one week prior to initiation of experiments. The experimental protocol has been approved by the institutional animal ethics committee of BKMGPC, Rajkot and all the animal experiments were carried out according to CPCSEA guidelines.

**Preparation of dose of PILERELIEF**

1% CMC solution was added as a suspending agent to powder of PILERELIEF Capsule with continuous trituration till uniform suspension was formed.

**Toxicity studies**

Organization for Economic Co-operation and Development (OECD) guideline 420 and 407 were used to conduct acute and sub-acute oral toxicity study respectively. \[4,5\]

**Acute oral toxicity**
Groups of animals of a single sex are dosed in a stepwise procedure using the fixed doses of 5, 50, 300 and 2000 mg/kg. The initial dose level is selected on the basis of a sighting study as the dose expected to produce some signs of toxicity without causing severe toxic effects or mortality. Clinical signs and conditions associated with pain, suffering, and impending death, are described in detail in a separate OECD Guidance Document. Further groups of animals may be dosed at higher or lower fixed doses, depending on the presence or absence of signs of toxicity or mortality.

Acute toxicity is caused by an agent when it is administered in one or more doses over a period not exceeding 24 hour and involves harmful effects to the organism through a single or short-term exposure. Acute toxicity studies have also been used during the selection of starting doses for phase-I human and animal studies, and provide information relevant to acute overdosing in Humans and animals. [4]

**Sub-acute oral toxicity**

The repeated doses for oral toxicity studies were carried out in rats, according to the OECD test guideline 407. The rats were divided randomly into 4 groups of 10 animals each (5 males and 5 females). Group I served as a vehicle control and received only 1% CMC suspension. Groups II, III and IV received PRF with 1% CMC suspension orally at the doses of 50, 250, 500 mg/kg, respectively, every day for 28 days. The PRF was administered orally by gavage, as a single dose at similar times each day. For all the dose groups, volume (10 ml/kg) was adjusted and rounded up to single decimal point as per the body weight for an individual animal throughout the treatment period. During this period, all the animals were observed daily for signs of toxicity and mortality. The changes in body weight, food and water intake and clinical signs were also observed and recorded. [5]

**Haematological and biochemical examination**

All experimental animals were humanely sacrificed at the end of the experiment by anaesthetic ether in desiccators. Blood samples were collected by retro orbital method for haematological and biochemical analysis. The haematological parameters were analysed such as haemoglobin, PCV, platelet count, RBC, WBC, neutrophils, eosinophils, basophils, lymphocyte and monocyte. [2]

The biochemical parameters were also analysed such as glucose, total bilirubin, cholesterol and triglyceride, markers of renal function (urea and creatinine) and liver (ALT, AST) and protein profile (total protein). [2]
Histopathological examination

All experimental animals were humanely sacrificed at the end of the experiment by anaesthetic. After collecting a blood sample, the vital organs (heart, lung, kidney, liver, and brain) and Organ weight was calculated based on the fasted animal's body weight. Then samples of heart, lung, kidney, liver, and brain were fixed in 10% neutral buffered formalin, embedded in paraffin, sectioned at 5 μm, and stained with haematoxylin and eosin, following the standard laboratory procedures. The stained sections were examined under a microscope for any cellular damage or change in morphology. [2]

Statistical analysis

Results were expressed as mean±standard error of the mean (SEM). Student’s t-test was used for comparison between the two experimental groups (acute toxicity). The differences between groups of sub-acute toxicity were determined by analysis of variance (one-way ANOVA) followed by multiple comparison tests (Graph pad prism-version 7.00). Probability values of 0.05 (p<0.05) or less were considered statistically significant.

RESULTS

Acute oral toxicity

Effect of PRF on body weight and behaviour

As per OECD 420 guideline, the acute toxicity study of PRF was performed in the female rats after administration of single dose of 2000mg/kg. There was no difference seen in the body weight as well as no visible changes in the general behaviour of treated groups when compared to control group. However, minor behavioural changes were observed at the end of 14 days. Moreover, the treated and control groups seemed healthy during the study which found to be safe at the 2000 mg/kg dose level (Table1).

<table>
<thead>
<tr>
<th>Day/s</th>
<th>Groups (n=5)</th>
<th>Body Weight(gm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>Control</td>
<td>190±8.94</td>
</tr>
<tr>
<td></td>
<td>PRF 2000 mg/kg</td>
<td>232±12.4</td>
</tr>
<tr>
<td>7</td>
<td>Control</td>
<td>190±8.94</td>
</tr>
<tr>
<td></td>
<td>PRF 2000 mg/kg</td>
<td>214±8.71</td>
</tr>
<tr>
<td>14</td>
<td>Control</td>
<td>186.8±8.35</td>
</tr>
<tr>
<td></td>
<td>PRF 2000 mg/kg</td>
<td>196±6.78</td>
</tr>
</tbody>
</table>

All values represented by MEAN±SEM
Sub-acute oral toxicity

The daily oral administration of PRF at doses of 50, 250 and 500 mg/kg for 28 consecutive days did not produce any abnormality and sign of toxicity in rats of either sex. The selection of doses was done based on the data of acute toxicity study.

Effects of PRF on clinical signs of toxicity and mortality

The animals were found active and responsive to stimuli during 28 days study. Furthermore, there were no clinical signs of toxicity, mortality and no changes in general behaviour which appears to the survival at three dose level throughout the study.

Effect of PRF on body weight, water, and food intake

The body weight data of wistar rats of either sex clearly showed there was no any significant difference (p<0.05) observed in treated groups with repeated oral doses of PRF (50, 250 and 500 mg/kg), when compared to the control group, however there was no significant changes in water and food consumption in treated groups when compared to the control group of wistar rats (Table 2).

Table 2: Effect of PRF on body weights, food and water consumption of wistar rats in sub-acute toxicity

<table>
<thead>
<tr>
<th>Groups (n=5)</th>
<th>Body Weight (gm)</th>
<th>Food Intake (gm/day)</th>
<th>Water Intake (ml/day)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0day</td>
<td>28 days</td>
<td></td>
</tr>
<tr>
<td>CONTROL (M)</td>
<td>206±9.20</td>
<td>262±7.69</td>
<td>44.46±1.27</td>
</tr>
<tr>
<td>PRF 50 mg/kg (M)</td>
<td>310±4.00</td>
<td>294±8.29</td>
<td>51.78±2.34</td>
</tr>
<tr>
<td>PRF 250 mg/kg (M)</td>
<td>222±5.21</td>
<td>282±14.53</td>
<td>52.32±1.58</td>
</tr>
<tr>
<td>PRF 500 mg/kg (M)</td>
<td>352±5.93</td>
<td>340±6.32</td>
<td>65.35±2.36</td>
</tr>
<tr>
<td>CONTROL (F)</td>
<td>168±8.67</td>
<td>194±6.69</td>
<td>46.42±1.06</td>
</tr>
<tr>
<td>PRF 50 mg/kg (F)</td>
<td>222±10.35</td>
<td>220±19.59</td>
<td>45.53±1.02</td>
</tr>
<tr>
<td>PRF 250 mg/kg (F)</td>
<td>178±10.35</td>
<td>244±36.11</td>
<td>50.53±1.29</td>
</tr>
<tr>
<td>PRF 500 mg/kg (F)</td>
<td>196±15.38</td>
<td>254±11.52</td>
<td>51.96±2.04</td>
</tr>
</tbody>
</table>

All values represented by MEAN±SEM
Effect PRF on haematological parameters

Figure: 1 Effect of PRF on RBC

Figure: 2 Effect of PRF on WBC

Figure: 3 Effect of PRF on Monocytes

Figure: 4 Effect of PRF on Lymphocytes

Figure: 5 Effect of PRF on Eosinophils

Figure: 6 Effect of PRF on PCV
Figure: 7 Effect of PRF on MCV

Figure: 8 Effect of PRF on MCH

Figure: 9 Effect of PRF on RBC

Figure: 10 Effect of PRF on RBC

Figure: 11 Effect of PRF on HB
Effect PRF on Biochemical parameters

Figure: 12 Effect of PRF on Cholesterol

Figure: 13 Effect of PRF on Triglyceride

Figure: 14 Effect of PRF on Urea

Figure: 15 Effect of PRF on Creatinine

Figure: 16 Effect of PRF on AST

Figure: 17 Effect of PRF on ALT
Figure: 18 Effect of PRF on Bilirubin

Figure: 19 Effect of PRF on Total protein

Figure: 20 Effect of PRF on Uric acid

Figure: 21 Effect of PRF on RBS

Effect PRF on Organ weight

Figure: 22 Effect of PRF on Liver

Figure: 23 Effect of PRF on Kidney
Effect PRF on Histopathology

Figure no: 27 Effect of PRF on LIVER-(A-CONTROL),(B-PR500)
Figure no: 28 Effect of PRF on KIDNEY-(A-CONTROL),(B-PR500)

Figure no: 29 Effect of PRF on LUNG-(A-CONTROL),(B-PR500)

Figure no: 30 Effect of PRF on HEART-(A-CONTROL),(B-PR500)
The data suggest that there was no statistically significant difference in levels of RBCs, WBCs, Monocytes, Lymphocytes, Eosinophils, PCV, Platelets, MCV, MCH, MCHC, and HB levels of PILE RELIEF treated groups and control groups during 28 days. Thus formulation is considered to be safe even at ten times dose the therapeutic dose i.e. 50 mg/kg body weight. Oral administration of the PILE RELIEF formulation for 28 days at a dose of 50, 250 and 500 mg/kg did not cause significant changes in serum biochemical parameters such as RBS, AST, ALT, total bilirubin, total protein, urea, uric acid, creatinine, cholesterol and triglyceride, when compared to control group. However, treatment of PILE RELIEF formulation was shown in no significantly difference in female and male rats, when compared with the respective control group. There were no significant differences in relative organ weight between control rats and PILE RELIEF formulation treated rats at a dose of 50, 250 and 500 mg/kg. The relative organ weight of control rats and PILE RELIEF formulation treated rats statistically insignificant. The results revealed that the vital organs such as liver, kidney, heart, lung, and brain were not adversely affected throughout the treatment by PILE RELIEF formulation. In the histopathological examination no changes in liver, kidney, lung, heart and brain morphology at the dose of PR50mg/kg, PR250mg/kg and PR500mg/kg as compared to CONTROL group. Moreover, histopathological examinations of the internal organs after hematoxylin-eosin staining reveal no pathological abnormalities for rats in any group (Figure no-32 to 36). Based on biochemical and hematological findings as well as findings of the pathological analysis of the major organs, it was concluded that the PILE RELIEF formulation did not show significant toxicity in this experiment.
CONCLUSION

The acute and sub-acute toxicity study of PILE RELIEF formulation revealed no toxicity by oral route over a period of 28 days. So, it can be concluded that the PILE RELIEF formulation prescribed for therapeutic use. PILE RELIEF formulation produced no more difference seen in food and water consumption, body weight, hematological parameters, biochemical parameters, histopathology in period of 28 days. The findings of acute study revealed that this polyherbal formulation is non-toxic with single oral dose of 2000 mg/kg/day. These results exhibit the absence of acute and sub-acute oral toxicity after treatment of PILE RELIEF formulation in rats. However, further studies in animals and in humans are needed in order to have sufficient safety evidence for its use in humans.

ACKNOWLEDGEMENT

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