SUB-ACUTE TOXICITY STUDIES OF PARACETAMOL INFUSION IN MUS MUSCULUS MICE

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ABSTRACT

The objective of the present study was to evaluate the sub-acute toxicity of paracetamol infusion in Mus musculus mice (male and female) at different dose levels, ranging from 16 to 66 mg/kg body weight. There were no major changes in physiological, hematological and biochemical parameters at any dose level used in this study. No mortality was observed in any of the treatment groups during the course of whole study. It has been concluded from the present study that paracetamol infusion is safe even at higher dose. Overall safety and tolerability profile of paracetamol infusion has been proved good and does not appear to carry risk of serious adverse effects in Mus musculus mice.

Introduction

Acetaminophen (paracetamol) was approved by the U.S. Food and Drug Administration (FDA) in 1955. Acetaminophen is being a promoted as the preferred analgesic antipyretic particularly for pediatric use with suggestion that it is safer and perhaps more efficacious than aspirin [1]. The acetaminophen is less toxic than aspirin is based on a lower incidence of side effects and toxic manifestations observed with acetaminophen than with aspirin. It is commonly used for the relief of fever, headaches, and other minor aches and pains, and is a major ingredient in numerous cold and flu remedies [2]. Acetaminophen and aspirin given in equal miligram doses are equally effective in relieving pain and fever [3,4]. In combination with non-steroidal anti-inflammatory drugs (NSAIDs) and opioid analgesics, paracetamol is used also in the management of more severe pain (such as postoperative pain) [5]. The analgesic effect of paracetamol is probably dependent on the rate and amount of active drug reaching the CNS, where its analgesic effect takes place [6]. Paracetamol is available as oral, rectal and injectable formulation [7].

It has been established that acetaminophen, taken in therapeutic doses, is metabolized by the liver through two pathways. Most of the drug (80–90%) is conjugated with either glucuronic acid or sulphates, yielding the nontoxic conjugates that are excreted by the kidney. A small proportion (5%) is metabolized to a reactive electrophilic intermediate by the cytochrome P-450 system. This metabolite is rendered nontoxic by conjugation with glutathione to form mercapturic acid and related conjugates that
are also excreted in the urine. If the drug is taken in excessive doses, an augmented amount is converted by cytochrome P-450 to the highly reactive, toxic intermediate metabolite [8,9]. It then may reach a level that overwhelms the protective mechanism of glutathione conjugation and ultimately, through covalent binding to hepatocyte proteins, leads to hepatocellular necrosis [10].

Acetaminophen has long been recognized as potentially lethal because of dose-related hepatic, and often renal, injury [11,12]. The acute liver failure (ALF) study group has compiled data on more than 500 acetaminophen-related ALF cases showing that the number of cases has increased considerably since 1998 [13]. The overall incidence of acute renal failure in patients with paracetamol poisoning is less than 2% [14] and acute renal failure occurs in 10 to 40 % of patients with severe hepatic necrosis [15]. The mechanism of acetaminophen toxicity remains somewhat a mystery with recent evidence suggesting that multiple cytotoxic pathways are involved [16].

Toxicity studies on other formulation of paracetamol are available. However, there are scanty reports on the toxicity of paracetamol infusion therefore the present study was designed to evaluate the sub-acute toxicity of Paracetamol infusion in Swiss Mus musculus mice.

Materials and methods

Study Conduct

The study was conducted in Venus Medicine Research Centre, Baddi, HP,India.

Chemicals

All the chemicals used in the present study were procured from Sigma, St. Louis, MO, USA. Other chemicals purchased locally were of analytical grade. Paracetamol infusion was obtained from Venus Remedies Ltd., India.

Animals

Total Forty eight healthy Swiss Mus musculus mice (24 male and 24 female mice, weight 20-25 gms) were selected for the present study. All the animals were acclimatized to laboratory conditions for a week before commencement of experiment. The animals were grouped and housed in polycarbonate cages (6 in each cage) at controlled room temperature of 27-29 °C and a relative humidity between 30 to 70%, and a constant light-dark schedule (12 hours light and 12 hours dark cycle). Animals were fed with Nutrilab brand extruded pelleted mouse feed supplied by (Tetragon Chemie, Pvt. Ltd, Bangalore, India) and fresh water ad libitum. All procedures were reviewed and approved by the Venus Remedies Research Center Animals Ethical committee.

Experimental treatment

Animals were divided into four groups of 6 animals each as given below:

Group I = treated with vehicle/sterile water (Control).
Groups II = treated with 16.6 mg/kg body weight of paracetamol (low dose)
Group III = treated with 33.6 mg/kg body weight of paracetamol (intermediate dose)
Group IV = treated with 66.6 mg/kg body weight of paracetamol (high dose)

The treatments were given for 28 days according to the body weight of each group of mice. Sterile water was injected intravenously to the animals of control group of mice (0.5 ml sterile distilled water/animal) as sham treatment. Treatment was done once daily for continuous 28 days. At the end of treatment, overnight fasted animals were sacrificed and 1 ml blood and tissues samples were collected on 29th day. Hematological, biochemical and physical parameters were measured in all treated groups as well as in control group in mice. The organs were
quickly blotted, weighed on digital balance and processed for histological studies.

**Physical parameters**

Physical parameters (body weight, food and water intake) and local injury were studied during treatment of animals (mice). Mortality was also recorded during treatment of all groups. Autopsy was done if mice died during the course of treatment.

**Hematological and Biochemical Parameters**

Blood was collected by cardiac puncture. Blood samples were analyzed for routine hematological parameters. Blood cell count was done with blood smears. Hemogram was performed on ACT diff-2 Hematology Analyzer (Beckman Coulter India, Ltd., Mumbai, India).

**Biochemical Parameters**

Biochemical parameters were analyzed in serum sample. Serum glutamic oxaloacetic transaminase (SGOT), serum glutamic pyruvic transaminase activities (SGPT), alkaline phosphatase (ALP), Total serum protein, blood urea nitrogen (BUN) Creatinine and Bilirubin and blood sugar levels were estimated. All parameters were studied by Merck semi auto analyzer by using Merck analytical kits.

**Histological Examinations**

Liver, kidney, Stomach, Heart and Lungs were removed from the sacrificed animals and preserved in 10% buffered formalin for histological examination.

**Statistical Analysis**

Resulting data were represented as mean ± SD. Statistical data were analyzed by Dennett’s test, between control vs all treated groups. p>0.05 was considered as non significant.

**Results**

**Physical Parameters**

No significant changes were observed in Physical parameters (behavioral, food and water intake) in any groups of mice throughout the dosing period. No mortality was observed in all groups of mice throughout the dosing period. There was no significant change in the mean body weight of all the groups as compared with control group on 29th day.

**Hematology**

In male and female mice groups, no significant changes were observed in hemoglobin (Hb), red blood cell (RBC), white blood cell (WBC), platelet counts in all the treated groups as compared to respective control groups (Fig 1 and Fig 2).

![Comparative hematological parameters in male mice in all groups](image-url)
Biochemical Parameters

In male and female mice groups, no significant changes were seen in total serum protein, BUN, SGPT, SGOT, ALP activities, glucose, creatinine and bilirubin in all the groups as compared to respective control group (Tab. 1 and Tab. 2). However, changes were statistically insignificant.

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Group I</th>
<th>Group II</th>
<th>Group III</th>
<th>Group IV</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total serum Protein (g%)</td>
<td>6.70±0.29</td>
<td>6.59±0.19</td>
<td>6.61±0.23</td>
<td>6.68±0.16</td>
</tr>
<tr>
<td>BUN (mg%)</td>
<td>31.25±0.65</td>
<td>30.16±0.72</td>
<td>31.65±1.04</td>
<td>30.90±1.68</td>
</tr>
<tr>
<td>SGPT (IU/L)</td>
<td>57.71±3.13</td>
<td>59.64±2.97</td>
<td>63.51±5.43</td>
<td>62.35±5.89</td>
</tr>
<tr>
<td>SGOT (IU/L)</td>
<td>56.88±1.43</td>
<td>57.87±1.43</td>
<td>57.53±1.45</td>
<td>57.46±1.94</td>
</tr>
<tr>
<td>ALP (IU/L)</td>
<td>366.23±35.61</td>
<td>379.46±13.39</td>
<td>385.93±26.65</td>
<td>393.28±20.51</td>
</tr>
<tr>
<td>Glucose (mg%)</td>
<td>101.10±1.62</td>
<td>102.99±2.02</td>
<td>99.85±2.60</td>
<td>101.57±1.14</td>
</tr>
<tr>
<td>Creatinine (mg/dL)</td>
<td>0.71±0.10</td>
<td>0.74±0.11</td>
<td>0.75±0.12</td>
<td>0.74±0.12</td>
</tr>
<tr>
<td>Bilirubin (mg/dL)</td>
<td>0.77±0.12</td>
<td>0.76±0.14</td>
<td>0.76±0.14</td>
<td>0.72±0.17</td>
</tr>
</tbody>
</table>
Table: 2 Comparative biochemical parameters in female mice in all groups

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Group I</th>
<th>Group II</th>
<th>Group III</th>
<th>Group IV</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total serum Protein (g%)</td>
<td>6.68±0.27</td>
<td>7.06±0.25</td>
<td>6.48±0.49</td>
<td>6.51±0.37</td>
</tr>
<tr>
<td>BUN (mg%)</td>
<td>30.49±0.62</td>
<td>29.67±1.39</td>
<td>29.7±0.94</td>
<td>29.08±1.18</td>
</tr>
<tr>
<td>SGPT (IU/L)</td>
<td>59.42±2.08</td>
<td>57.06±2.20</td>
<td>59.65±3.23</td>
<td>61.35±2.25</td>
</tr>
<tr>
<td>SGOT (IU/L)</td>
<td>57.79±1.57</td>
<td>56.98±1.62</td>
<td>57.06±1.85</td>
<td>56.1±1.30</td>
</tr>
<tr>
<td>ALP (IU/L)</td>
<td>359.71±23.23</td>
<td>367.79±16.84</td>
<td>351.89±20.90</td>
<td>356.97±19.58</td>
</tr>
<tr>
<td>Glucose (mg%)</td>
<td>101.06±1.63</td>
<td>98.55±2.38</td>
<td>103.23±2.25</td>
<td>99.63±2.58</td>
</tr>
<tr>
<td>Creatinine (mg/dL)</td>
<td>0.77±0.12</td>
<td>0.76±0.14</td>
<td>0.72±0.17</td>
<td>0.76±0.11</td>
</tr>
<tr>
<td>Bilirubin (mg/dL)</td>
<td>0.71±0.1</td>
<td>0.75±0.16</td>
<td>0.74±0.13</td>
<td>0.63±0.14</td>
</tr>
</tbody>
</table>

Histological Examination

There were no significant treatment related histopathological changes observed in organs of all the treated groups of male and female mice as compared to control group and there was no significant variations in the relative organs weight in comparison to respective controls.

Discussion

Acetaminophen is one of the most widely used analgesics and antipyretics worldwide [17]. Although generally considered a safe drug, it continues to be a cause of death either through overdose, idiopathic reaction, or synergism with alcoholic liver disease. Death from acetaminophen overdose is thought to be secondary to liver failure, which is caused by massive hepatic necrosis, the hallmark pathological feature of acetaminophen toxicity. In addition to liver, however, many organ systems may fail under acute overdose such as renal, cardiac, and central nervous systems [18].

There was no signs of local injury and inflammatory response at site of injection in the all treated groups of mice. No behavioral changes were observed during the study period in all the treatment groups. Increase in body weights and growth of treated animals were of similar pattern as in control groups.

Blood was evaluated for hematological toxicity of Paracetamol infusion. Hemogram was estimated and results showed no deleterious effect on blood cell count, haemoglobin and other related parameters. This confirms the safety profile of paracetamol infusion for infusion in blood related aspects.

Liver is a vital organ and is thought that the liver is the target organ for acetaminophen toxicity because this is primarily where the drug is detoxified [8]. Acetaminophen is potentially hepatotoxic resulting in a dose dependent destruction of hepatocytes through its metabolite N-acetyl-P-benzoquinone-imine (NAPQI) [19]. The Liver function indicators such as SGOT, SGPT, ALP are increased in the situation of hepatotoxicity [20]. However, in the present study, there was no significant changes in the hepatic enzymes, SGOT, SGPT and ALP in paracetamol infusion treated groups of both sex as compared to the control group suggesting that paracetamol infusion is not causing hepatotoxicity.

Acetaminophen is the recommended analgesic for subjects with renal dysfunction [21]. Acetaminophen may not have a deleterious effect on the kidney also has no effect on glomerular filtration rate in normal subjects [22]. The development of acetaminophen-induced nephrotoxicity in male Fischer 344 rats of different ages were studied and not found any evidence of renal damage in any age group 6 h after APAP. In the present study, biochemical parameters related to kidney function were evaluated and no significant differences were observed in blood urea nitrogen, creatinine, bilirubin glucose and proteins with...
respect to control, suggesting that paracetamol infusion is not causing nephrotoxicity [23].

There were no signs of toxicity were seen in any of organs in histopathological analysis. Thus histopathological studies also provides supports to the safety data of other physiological, biochemical and hematological parameters of Paracetamol infusion.

In conclusion, our data suggest that paracetamol infusion is safe even at higher dose level than intended to be used for human treatment as there is no clinically relevant alterations in any of the physiological and biochemical parameters.

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References


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