Sub-acute Toxicity Studies of Acetaminophen in Sprague Dawley Rats

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The aim of the present study was to evaluate the sub-acute oral toxicity of acetaminophen in Sprague Dawley (SD) rats at 250 to 1000 mg/kg body weight (b.wt.). The following observations were noticed during the study. No mortality in male and female rats, at and up to the dose of 1000 mg/kg b.wt. There were abnormal clinical signs observed on female animals at 1000 mg/kg b.wt. dose level. There were no difference in body weight gain and no effect on the daily feed consumption. No toxicologically significant effect on the haematological parameters but liver and kidney related biochemical parameter showed significant difference at 1000 mg/kg b.wt. in females. No toxicologically significant effect on the urinalysis parameters, absolute and relative organ weights and gross pathological alterations; whereas histopathological alterations were observed in female liver at dose level of 1000 mg/kg b.wt. were observed. Based on the findings of this study, the No Observed Adverse Effect Level (NOAEL) of acetaminophen in SD rats, following oral administration at the doses of 250, 500 and 1000 mg/kg on daily basis was found to be 500 mg/kg b.wt.

Key words acetaminophen; rat; hematology; biochemistry

Acetaminophen (paracetamol) is a widely consumed antipyretic over-the-counter (OTC) drug in India in spite of it was banned in most of the developed countries. Acetaminophen was first evaluated for pharmacologic activity in 1893 by Von Mehring, who discovered its analgesic and antipyretic properties; however, it was not until the work of Brodie in the 1940’s that serious consideration was given to its use in humans.1) Acetaminophen was first introduced as a prescription drug in the United States in 1955 and was approved by the Food and Drug Administration for sale as a non prescription drug in 1960.2) Toxicity from acetaminophen is not from the drug itself but from one of its metabolites, N-acetyl-p-benzoquinoneimine (NAPQI). Acetaminophen biotransformation involves conjugation with glucuronide and sulphate. A small amount of acetaminophen is metabolised by mixed function oxidase enzymes to form highly reactive compound NAPQI, which is immediately conjugated with glutathione and subsequently excreted as cysteine and mercapturic conjugates. In overdoses, large amounts of acetaminophen are metabolised by oxidation because of saturation of the sulphate conjugation pathway.3,4) but once the protective intracellular glutathione stores are depleted hepatic and renal damage may ensue. Hepatotoxicity is the most remarkable feature of acetaminophen overdose.5) Acute overdoses of acetaminophen can cause potentially fatal liver damage and, in rare individuals, a normal dose can do the same; the risk is heightened by alcohol consumption. Acetaminophen toxicity is the foremost cause of acute liver failure. Renal effects of acetaminophen overdose are less commonly seen than hepatic effects. However, renal impairment may be more common than previously recognised. There are extensive toxicity studies on acetaminophen are available. However, there are few reports on the toxicity of acetaminophen in India is available.6) Therefore the present study was designed to evaluate the sub-acute toxicity of acetaminophen in order to find out no observed effect level, target organ toxicity and reversibility of signs of toxicity after the recovery period in Sprague Dawley (SD) rats.

MATERIALS AND METHODS

The methods and test procedure were followed as per the OECD test guideline No. 407.7)

Test System Total seventy two healthy SD rats (36 male and 36 female rats) of age 6–8 weeks were selected for the present study. All the animals were acclimatized to laboratory condition for a week before commencement of experiment. Prior to final assignment to the study, it was ensured that the selected rats were in a good state of health, females were nulliparous and nonpregnant. Rats were housed in sterilized suspended polycarbonate cages, with stainless steel top grills having facilities for holding pellet feed and drinking water in bottle with stainless steel sipper tube. All rats were freely accessible to reverse osmosis (RO) generated potable water and standard pellet laboratory animal diet ad libitum (Tetragon Vetcare, Bangalore). Autoclaved corn cob was used as bedding material. Throughout the acclimatization and treatment period, animal room temperature and relative humidity were maintained at 19 to 25°C and 30 to 70%, respectively. Illumination was controlled to give 12 h light and 12 h dark cycle during the 24-h period. The study protocol was approved by Institutional animal ethics committee.

Study Design The rats were distributed into 6 groups each consisting of 12 rats/group (6 male+6 female/group). Groups I, II, III and IV rats were received doses of 0, 250, 500 and 1000 mg/kg body weight (b.wt.) of acetaminophen respectively. Groups V and VI were administered doses of 0 and 1000 mg/kg b.wt. of control and acetaminophen respectively as a recovery group. The rats were examined twice daily for signs of toxicity, moribund status and mortality. Further they were also subjected to detailed clinical examination before initiation of the study and daily thereafter during the exposure period. Body weights and food consumption were recorded daily. Laboratory investigations were performed on day 29 for main and on day 43 for recovery groups. All rats in main and
recovery groups were sacrificed at termination on days 29 and 43, respectively and subjected to a detailed gross pathology and organ weights were recorded.

**Physical Parameters** General clinical observations, moribund condition and mortality were observed daily for all the groups until 42nd day. Feed intake and body weight were measured on days 8, 15, 22 and 28 during treatment and recovery period (days 35, 42).

**Laboratory Investigations** The following haematological parameters were determined; red blood cell count, white blood cell differential count, hemoglobin, platelets count, packed cell volume (PCV/HCT), and Red blood cell distribution width (RDW). The following biochemical parameters were analyzed with the help of automatic biochemical analyzer, using standard reagent kits (Siemens) and standard laboratory methodology; alanine aminotransferase (ALT), albumin (Alb), creatinine (Creat), aspartate aminotransferase (AST), blood urea nitrogen (BUN), glucose (Glu), total plasma protein (T.Pro), alkaline phosphatase (ALP), gamma glutamate transferase (GGT), triglycerides (TG), cholesterol, bilirubin, globulin (Glob), albumin/globulin ratio (A/G ratio). The serum electrolytes viz., sodium, potassium, calcium, phosphorus were analyzed with the help of automatic biochemical analyzer, using standard reagent kits.

Urine samples were collected from animals for main and control groups at the end of the treatment (day 29) and for recovery groups after recovery period (day 43) by using metabolic cages. Urine samples were analyzed for approximate color, appearance, specific gravity, pH, albumin, glucose, ketone bodies, urobilinogen, bilirubin and erythrocytes using uriscan pro+, Merck India, diagnostic strips as qualitative indicators of analyte concentration.

**Pathological Examination** The animals were subjected to detailed gross pathological observation during necropsy (adrenal glands, aorta, brain, epididymides, esophagus, eyes (with optic nerve), heart, kidneys, large intestine (cecum, colon, rectum), liver, lung, mammary gland, ovaries, skin, small intestine (duodenum, jejunum, ileum), spleen, stomach (cardia, fundus, pylorus), testis, thymus, thyroid gland, urinary bladder, uterus, thymus, skeletal muscle, mesentric lymph nodes). The following organs (adrenals, brain, heart, lung, kidneys, liver, spleen, testes/uterus) from main, control and recovery group rats were sacrificed as scheduled, dissected free of fat and weighed wet as soon as possible to avoid drying. The absolute organ weights were used to estimate the organ-body weight ratio (relative) by using the terminal body weights.

**Statistical Analysis** Group mean and standard deviation of mean were calculated for all generated data using GraphPad Prism 6 for Windows Version 6.03 software. One way ANOVA and Dunnett’s multiply comparison test was employed to confirm significance difference between control and treated groups. All statistical analysis and comparisons will be determined at \( p<0.05 \) level.

**RESULTS**

**Physiological Parameters** There was no incidence of mortality among the male and female rats exposed to acetaminophen at 250 mg/kg, 500 mg/kg and 1000 mg/kg b.wt. did not induce any abnormal clinical signs in both male and female rats during the study and reversal period. In female at group 4 (1000 mg/kg b.wt.) is significant in day 15 and day 28 when compare to control group; whereas in male at and up to 1000 mg/kg b.wt. dosage did not result in any significant change in the group mean body weight and mean cumulative net body weight gain when compared to control and treatment groups (Figs. 1, 2). The food consumption in both male and female rats exposed to acetaminophen at and up to the dose of 1000 mg/kg b.wt. was found to be comparable to the control groups (Figs. 3, 4).

**Laboratory Investigations** Rats treated with acetaminophen up to 1000 mg/kg b.wt. did not exhibit any significant treatment related effects, on haematology parameters and the results of the treatment groups were found to be comparable to the control groups (Tables 1, 2). In biochemical parameters male rat showed significant in total cholesterol and creatinine at group 3 and other parameters were not significant and comparable with control group (Table 3). In female group 2 showed significant level in BUN; group 3 showed significant levels in glucose, BUN and ALT; group 4 showed significant

![Fig. 1. Mean Body Weight in Male](image)

Mean values \( (n=6) \) were presented over the bar and line depicts standard deviation.
Fig. 2. Mean Body Weight in Female
Mean values ($n=6$) were presented over the bar and line depicts standard deviation.

Fig. 3. Comparative Feed Intake between Groups on Different Days
The mean values ($n=6$) presented above the bar.

Fig. 4. Comparative Feed Intake between Groups on Different Days
The mean values ($n=6$) presented above the bar.
in glucose, total protein, globulin, BUN, creatinine, AST, ALP and total bilirubin (Table 4).

**Pathological Examination** There was no statistically significant change in terminal absolute and relative organ weights in male and female rats treated at and up to the dose of 1000 mg/kg b.w.t. when compared to control groups. Acetaminophen up to the level of 1000 mg/kg b.w.t. did not induce any treatment related gross pathological alterations in any of the organs/tissues. In histopathology, the high dose group of female showed pathological alteration in liver.

**No Observed Adverse Effect Level (NOAEL)** Based on the findings of this study, the NOAEL of acetaminophen in SD rats, following oral administration daily at 500 mg/kg b.w.t. of male and female rat.
Acetaminophen is a widely used over the counter analgesic and antipyretic drug. Oral administration of acetaminophen has been shown to be at least as effective as intravenous administration of an equivalent dose of acetaminophen, and the target concentration achieved more rapidly and with less variability in plasma concentrations compared with enteral formulations. In the present investigation, there was no signs of behavioural changes were observed during the study period in all the treatment groups. Increase in body weights and growth of treated animals of either sex were of similar pattern as in control groups. Blood was evaluated for hematological toxicity of acetaminophen administration. Hemogram was estimated and results showed no deleterious effect on blood cell count, haemoglobin and other related parameters.

The liver is the vital organ of paramount importance involved in the maintenance of metabolic function and detoxification of drugs. After the intake of toxic dose of acetaminophen causes P450-dependent hepatotoxicity in man and various laboratory animals as observed by the release of serum ALT into the serum. Liver damage is always

**Table 4. Mean Values of Clinical Chemistry Parameters with Standard Deviation (S.D.): Females**

<table>
<thead>
<tr>
<th>Group No.</th>
<th>dose (mg/kg)</th>
<th>Glu (mg/dL)</th>
<th>T.Pro (g/dL)</th>
<th>Alb (g/dL)</th>
<th>Glo (g/dL)</th>
<th>A/G</th>
<th>T.Cho (mg/dL)</th>
<th>Trig (mg/dL)</th>
<th>BUN (mg/dL)</th>
<th>Creat (mg/dL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>G-I, 0</td>
<td>Mean</td>
<td>70.70</td>
<td>6.50</td>
<td>3.50</td>
<td>3.00</td>
<td>0.90</td>
<td>102.50</td>
<td>22.80</td>
<td>18.70</td>
<td>0.40</td>
</tr>
<tr>
<td></td>
<td>S.D.</td>
<td>11.71</td>
<td>0.12</td>
<td>0.14</td>
<td>0.05</td>
<td>0.06</td>
<td>4.51</td>
<td>5.71</td>
<td>1.37</td>
<td>0.08</td>
</tr>
<tr>
<td>G-II, 250</td>
<td>Mean</td>
<td>109.20</td>
<td>6.40</td>
<td>3.50</td>
<td>3.00</td>
<td>1.10</td>
<td>92.20</td>
<td>27.70</td>
<td>13.30</td>
<td>0.40</td>
</tr>
<tr>
<td></td>
<td>S.D.</td>
<td>27.13</td>
<td>0.34</td>
<td>0.35</td>
<td>0.06</td>
<td>0.08</td>
<td>13.98</td>
<td>6.92</td>
<td>2.34</td>
<td>0.00</td>
</tr>
<tr>
<td>G-III, 500</td>
<td>Mean</td>
<td>116.20*</td>
<td>6.20</td>
<td>3.30</td>
<td>2.90</td>
<td>1.10</td>
<td>102.30</td>
<td>44.00</td>
<td>15.50**</td>
<td>0.30</td>
</tr>
<tr>
<td></td>
<td>S.D.</td>
<td>22.13</td>
<td>0.22</td>
<td>0.19</td>
<td>0.09</td>
<td>0.08</td>
<td>7.37</td>
<td>30.84</td>
<td>1.87</td>
<td>0.05</td>
</tr>
<tr>
<td>G-IV, 1000</td>
<td>Mean</td>
<td>181.50**</td>
<td>8.30*</td>
<td>4.60</td>
<td>3.80*</td>
<td>1.00</td>
<td>96.50</td>
<td>31.70</td>
<td>38.20**</td>
<td>1.10*</td>
</tr>
<tr>
<td></td>
<td>S.D.</td>
<td>8.83</td>
<td>0.94</td>
<td>0.92</td>
<td>0.51</td>
<td>0.13</td>
<td>15.11</td>
<td>4.89</td>
<td>8.26</td>
<td>0.36</td>
</tr>
<tr>
<td>G-V, 0</td>
<td>Mean</td>
<td>82.67</td>
<td>6.60</td>
<td>3.60</td>
<td>3.00</td>
<td>0.93</td>
<td>112.17</td>
<td>32.67</td>
<td>16.00</td>
<td>0.33</td>
</tr>
<tr>
<td></td>
<td>S.D.</td>
<td>23.04</td>
<td>0.14</td>
<td>0.14</td>
<td>0.08</td>
<td>0.15</td>
<td>8.59</td>
<td>5.75</td>
<td>2.19</td>
<td>0.05</td>
</tr>
<tr>
<td>G-VI, 1000</td>
<td>Mean</td>
<td>90.83</td>
<td>6.70</td>
<td>3.62</td>
<td>3.08</td>
<td>0.97</td>
<td>109.17</td>
<td>23.83</td>
<td>21.00*</td>
<td>0.33</td>
</tr>
<tr>
<td></td>
<td>S.D.</td>
<td>10.28</td>
<td>0.15</td>
<td>0.09</td>
<td>0.11</td>
<td>0.09</td>
<td>12.09</td>
<td>3.66</td>
<td>1.55</td>
<td>0.05</td>
</tr>
</tbody>
</table>

* Significance level at \( p < 0.05 \). ** Significance level at \( p < 0.01 \). \( n = 6 \).

**Table 5. Mean Relative Organ Weight (g) Values with Standard Deviation (S.D.): Males**

<table>
<thead>
<tr>
<th>Group No.</th>
<th>dose (mg/kg)</th>
<th>Liver</th>
<th>Kidneys</th>
<th>Lungs</th>
<th>Heart</th>
<th>Spleen</th>
<th>Brain</th>
<th>Adrenals</th>
<th>Testes</th>
</tr>
</thead>
<tbody>
<tr>
<td>G-I, 0</td>
<td>Mean</td>
<td>3.81</td>
<td>0.89</td>
<td>0.73</td>
<td>0.39</td>
<td>0.22</td>
<td>0.77</td>
<td>0.02</td>
<td>1.17</td>
</tr>
<tr>
<td></td>
<td>S.D.</td>
<td>0.21</td>
<td>0.08</td>
<td>0.23</td>
<td>0.03</td>
<td>0.03</td>
<td>0.05</td>
<td>0.00</td>
<td>0.15</td>
</tr>
<tr>
<td>G-II, 250</td>
<td>Mean</td>
<td>4.31</td>
<td>0.88</td>
<td>0.72</td>
<td>0.37</td>
<td>0.29</td>
<td>0.72</td>
<td>0.02</td>
<td>1.19</td>
</tr>
<tr>
<td></td>
<td>S.D.</td>
<td>0.35</td>
<td>0.06</td>
<td>0.09</td>
<td>0.02</td>
<td>0.19</td>
<td>0.13</td>
<td>0.00</td>
<td>0.19</td>
</tr>
<tr>
<td>G-III, 500</td>
<td>Mean</td>
<td>4.31</td>
<td>0.85</td>
<td>0.68</td>
<td>0.37</td>
<td>0.20</td>
<td>0.74</td>
<td>0.02</td>
<td>1.17</td>
</tr>
<tr>
<td></td>
<td>S.D.</td>
<td>0.24</td>
<td>0.07</td>
<td>0.10</td>
<td>0.01</td>
<td>0.01</td>
<td>0.07</td>
<td>0.00</td>
<td>0.15</td>
</tr>
<tr>
<td>G-IV, 1000</td>
<td>Mean</td>
<td>3.65</td>
<td>0.88</td>
<td>0.77</td>
<td>0.37</td>
<td>0.21</td>
<td>0.75</td>
<td>0.02</td>
<td>1.03</td>
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<tr>
<td></td>
<td>S.D.</td>
<td>0.29</td>
<td>0.04</td>
<td>0.11</td>
<td>0.02</td>
<td>0.02</td>
<td>0.06</td>
<td>0.00</td>
<td>0.37</td>
</tr>
<tr>
<td>G-V, 0</td>
<td>Mean</td>
<td>3.18</td>
<td>0.76</td>
<td>0.66</td>
<td>0.33</td>
<td>0.17</td>
<td>0.67</td>
<td>0.02</td>
<td>1.00</td>
</tr>
<tr>
<td></td>
<td>S.D.</td>
<td>0.31</td>
<td>0.10</td>
<td>0.14</td>
<td>0.05</td>
<td>0.02</td>
<td>0.03</td>
<td>0.00</td>
<td>0.08</td>
</tr>
<tr>
<td>G-VI, 1000</td>
<td>Mean</td>
<td>3.56</td>
<td>0.84</td>
<td>0.64</td>
<td>0.36</td>
<td>0.21</td>
<td>0.68</td>
<td>0.02</td>
<td>1.01</td>
</tr>
<tr>
<td></td>
<td>S.D.</td>
<td>0.12</td>
<td>0.05</td>
<td>0.09</td>
<td>0.02</td>
<td>0.02</td>
<td>0.03</td>
<td>0.00</td>
<td>0.08</td>
</tr>
</tbody>
</table>

**DISCUSSION**

Acetaminophen is a widely used over the counter analgesic and antipyretic drug. Oral administration of acetaminophen has been shown to be at least as effective as intravenous administration of an equivalent dose of acetaminophen, and the target concentration achieved more rapidly and with less variability in plasma concentrations compared with enteral formulations. In the present investigation, there was no signs of behavioural changes were observed during the study period in all the treatment groups. Increase in body weights and growth of treated animals of either sex were of similar pattern as in control groups. Blood was evaluated for hematological toxicity of acetaminophen administration. Hemogram was estimated and results showed no deleterious effect on blood cell count, haemoglobin and other related parameters.

The liver is the vital organ of paramount importance involved in the maintenance of metabolic function and detoxification of drugs. After the intake of toxic dose of acetaminophen causes P450-dependent hepatotoxicity in man and various laboratory animals as observed by the release of serum ALT into the serum. Liver damage is always...
associated with cellular necrosis, increase in tissue liquid peroxidation and depletion in the tissue glutathione (GSH) levels. In addition, serum levels of many biochemical markers like serum glutamic oxaloacetic transaminase (SGOT), serum glutamic pyruvic transaminase (SGPT), ALP and bilirubin are elevated.\textsuperscript{14–16} The laboratory features of hepatotoxicity induced by acetaminophen resemble other kinds of acute inflammatory liver disease with prominent increase in levels of SGOT, SGPT, and ALP. Hepatotoxicity is the most remarkable feature of acetaminophen overdose.\textsuperscript{5} However, in the present study, there was significant change in the levels of hepatic enzymes AST, ALT, and ALP in acetaminophen treated groups of either sex at 1000 mg/kg b.wt. group as compared to the respective control group. Studies in the human and animals reports that overall incidence of acute renal failure with acetaminophen toxicity.\textsuperscript{13,17,18} In the present study, biochemical parameters related to kidney function were evaluated and the high dose group showed significant differences were observed in blood urea, creatinine, glucose and proteins with respect to control. However, it has been reported that certain strains of rats that have high concentrations of microsomal cytochrome P450 in their kidneys developed acute tubular necrosis after a single, nonlethal dose of acetaminophen.\textsuperscript{20} It has been observed that conditions that are associated with glutathione depletion or increased activity of P450 microsomal oxidase enzymes enhance acetaminophen toxicity even at the therapeutic dosages. Examples include chronic alcohol use, starvation, fasting or ingestion of drugs that induce these enzymes, such as anticonvulsants. It has been reported that the proximal tubules are the target of APAP toxicity because of their active absorptive and secretory activities.\textsuperscript{20–22} There were no signs of toxicity were seen in any of organ in histopathological analysis.\textsuperscript{23} The rise in liver enzymes and histopathological liver injury were also reported in experiment conducted in India which accords the results of present study.\textsuperscript{24} Thus histopathological studies provide supports to the safety data of other regulatory requirement in countries like India.

### CONCLUSION

Based on the findings of this study, the NOAEL of acetaminophen in SD rats, following oral administration at the doses of 250, 500 and 1000 mg/kg on daily basis for 28d found to be 500 mg/kg b.wt. and there is no reversibility of toxicity observed after 14d recovery period.

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