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Sub-Acute Toxicity Studies of Paracetamol Infusion in Albino Wistar Rats

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ABSTRACT
The objective of the present study was to evaluate the sub-acute toxicity of paracetamol infusion in albino wistar rats (male and female) at different dose levels, ranging from 16 to 66 mg/kg body weight. No mortality was seen in any of the treatment groups during the course of study. Various physiological, hematological as well as biochemical parameters were studied and found not to be changed significantly, indicating that paracetamol infusion is non toxic even at higher dose level in wistar rats. Overall safety and tolerability profile of paracetamol infusion is proved good and does not appear to carry risk of serious adverse effects.

Keywords: Paracetamol, toxicity, rat, biochemical parameters.

INTRODUCTION

Paracetamol (N-acetyl-para-aminophenol) is discovered in 1889 and is an active metabolite of phenacetin. [1] It is widely used analgesics (pain reliever) and antipyretic (fever reducer), however, it has minimal anti- inflammatory activity compared with aspirin. [2]

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. [3] It is believed that selective inhibition of the enzyme COX-3 in the brain and spinal cord explains the effectiveness of paracetamol in relieving pain and reducing fever without having unwanted gastrointestinal side effects. [4] The fever reducing action of paracetamol was due to activity in the brain while its lack of any clinically useful anti-inflammatory action was consistent with a lack of prostaglandin inhibition peripherally in the body. [5] However, its mechanism of action is not fully understood, but it is generally accepted that paracetamol is centrally acting drug. [6] Paracetamol is available as oral, rectal and injectable formulation. [7]

Toxicity from paracetamol is not from the drug itself but from one of its metabolites, N-acetyl-p-benzoquinoneimine (NAPQ1). Paracetamol biotransformation involves conjugation with glucoronide and sulphate. A small amount of paracetamol is metabolised by mixed function oxidase enzymes to form highly reactive compound NAPQ1, which is immediately conjugated with glutathione and subsequently excreted as cysteine and mercapturic conjugates. In overdoses, large amounts of paracetamol are metabolised by oxidation because of saturation of the sulphate conjugation pathway [8-9], but once the protective intracellular glutathione stores are depleted hepatic and renal damage may ensue.

Hepatotoxicity is the most remarkable feature of paracetamol overdose. [10] Acute overdoses of paracetamol can cause potentially fatal liver damage and, in rare individuals, a normal dose can do the same; the risk is heightened by alcohol consumption. Paracetamol toxicity is the foremost cause of acute liver failure. [11] Renal effects of paracetamol overdose are less commonly seen than hepatic effects. However, renal impairment may be more common than previously recognised. The overall incidence of acute renal failure in patients with paracetamol poisoning is less than 2 % [12], and acute renal failure occurs in 10 to 40 % of patients with severe hepatic necrosis. [13]

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 albino wistar rats.

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MATERIALS AND METHODS

Study Conduct

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

Animals

Total forty eight healthy wistar rats (24 male and 24 female rats, weight 100-125 g) were selected for the present study. All the animals were acclimatized to laboratory condition 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. Group I treated with vehicle (sterile water) was kept as control. Groups II, III and IV treated with 16.6, 33.3 and 66.6 mg/kg body weight corresponding to low, intermediate and high dose, respectively for 28 days according to body weight of each group rats. Sterile water was injected intravenously to the animals of control group of mice (1.0 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 blood and tissues samples were collected on 29th day. Hematological, biochemical and histological parameters were measured in all treated groups as well as in control group. 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. Mortality was also recorded during treatment of all groups. Autopsy was done if animals died during the course of treatment.

Hematological

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

Serum glutamic oxaloacetic transaminase (SGOT), serum glutamic pyruvic transaminase activities (SGPT), alkaline phosphatase (ALP), Total protein, blood urea nitrogen (BUN) and blood sugar levels were estimated in plasma sample. 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 was analysed by Dennett's test, between control vs all treated groups. P>0.05 was considered statistically significant.

RESULTS

Physical parameters

There was no significant changes were observed in physical parameters in all groups of both mice and rats throughout the dosing period. No mortality was observed in all treatment groups 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 (data not shown).

Hematology

In male and female rats, no significant changes were observed in hemoglobin (Hb), red blood cell (RBC), white blood cell (WBC), platelet counts and erythrocyte sedimentation rate (ESR) in all the treated groups as compared to respective control group (Table 1 and 2).

Biochemical parameters

In male and female rat 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 (Table 3 and 4). However, changes were statistically insignificant.

Histological examination

There were no significant treatment related histopathological changes observed in organs of all the treated groups of male and female rats as compared to control group.

Table 1: Hematological parameters in male rats

Parameters	Group I	Group II	Group III	Group IV
Hb (g %)	14.05±0.73	13.82±0.88	12.88±0.46	14.28±0.74
Total RBC (x 10^6/cmm)	6.90±0.49	7.02±0.33	6.51±1.28	7.05±0.34
Platelets (x 10^5cmm)	2.90±0.37	3.20±0.25	3.16±0.08	3.60±0.36
Total WBC (x 10^3cmm)	6.79±0.51	6.91±0.45	6.72±0.53	7.51±0.99
ESR (mm/h)	6.50±0.84	6.67±0.82	7.00±0.89	6.67±0.82

Table 2: Hematological parameters in female rats

Parameters	Group I	Group II	Group III	Group IV
Hb (g %)	14.2±1.29	13.22±0.56	13.13±1.09	13.65±0.49
Total RBC (x 10^6/cmm)	7.29±0.22	6.49±1.67	6.95±0.62	7.59±0.40
Platelets (x 10^5cmm)	3.06±0.20	3.09±0.36	3.17±0.19	3.32±0.23
Total WBC (x 10^3cmm)	7.15±0.49	6.89±0.3	6.88±0.80	7.51±0.99
ESR (mm/h)	6.50±1.05	6.83±0.75	7.00±0.89	7.00±0.89

Table 3: Biochemical parameters male rats

Parameters	Group I	Group II	Group III	Group IV
Total serum Protein (g %)	6.97±0.31	6.98±0.51	6.43±1.5	7.15±0.29
BUN (mg %)	41.67±2.07	41.00±1.26	40.00±1.67	41.67±1.75
SGPT (IU/L)	63.26±3.85	63.43±1.21	62.93±2.85	63.12±2.41
SGOT (IU/L)	57.33±1.51	57.1±1.71	57.47±1.52	57.57±1.9
ALP (IU/L)	345.17±15.37	346±11.19	345.17±9.85	345.5±10.45
Glucose (mg %)	97.33±8.96	92.83±6.01	92.17±3.49	91.83±7.96
Creatinine (mg/dL)	0.76±0.11	0.72±0.17	0.76±0.14	0.77±0.12
Bilirubin (mg/dL)	0.73±0.18	0.74±0.14	0.75±0.13	0.71±0.11

DISCUSSION

Paracetamol is an effective simple analgesic and antipyretic drug. Intravenous administration of propacetamol has been shown to be at least as effective as oral administration of an

equivalent dose of paracetamol, and the target concentration achieved more rapidly and with less variability in plasma concentrations compared with enteral formulations. [14-15] In the present investigation, there was no signs of local injury and inflammatory response at site of injection in the treated groups of rat. No behavioral 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 Paracetamol infusion. 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. Liver damage is always associated with cellular necrosis, increase in tissue liquid peroxidation and depletion in the tissue GSH levels. In addition, serum levels of many biochemical markers like SGOT, SGPT, ALP and billirubin are elevated. [16-17] The laboratory features of hepato-toxicity induced by paracetamol resemble other kinds of acute inflammatory liver disease with prominent increase in levels of SGOT, SGPT, and ALP. Hepato-toxicity is the most remarkable feature of paracetamol overdose. [18] However, in the present study, there was no significant change in the levels of hepatic enzymes SGOT, AGPT and ALP in paracetamol infusion treated groups of either sex as compared to the respective control group suggesting that towards the safety profile of paracetamol infusion for injection in hepatic related aspects.

Paracetamol infusion eliminated through renal excretion, thus it is mandatory to estimate effects of paracetamol on kidney functions. Studies in the CD-1 mouse suggest that reactive electrophile contributes to covalent binding and subsequent nephrotoxicity. [19] The overall incidence of acute renal failure in patients with paracetamol poisoning is less than 2 %, and acute renal failure occurs in 10 to 40 % of patients with severe hepatic necrosis. [13, 20] In the present study, biochemical parameters related to kidney function were evaluated and no 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 paracetamol. [21] Paracetamol given in increasing doses to male fischer rats depleted glutathione stores in the liver and kidneys; large amounts of oxidative radio-labelled metabolite bound to hepatic and kidney protein then led to a dose dependent acute hepatic and renal necrosis. [22]

There were no signs of toxicity were seen in any of organ in histopathological analysis. Thus histopathological studies provide supports to the safety data of other physiological, biochemical and heamatological parameters of paracetamol infusion.

In summary, our data suggest that paracetamol infusion is safe at high dose than intended to be use for human treatment as it indicates no clinically relevant alterations of any of physiological and biochemical parameters. In conclusion, our results provide support for safety profile of this potential drug. The data suggest that paracetamol is safe even at maximum dose level and no significant effect was observed on any of physiological and biochemical parameters. Thus, paracetamol infusion is an effective safe NSAID and possessing widely clinical application and worth for wide use.

Table 4: Biochemical parameters female rats

Parameters	Group I	Group II	Group III	Group IV
Total serum Protein (g %)	7.05±0.49	6.98±0.29	7±0.41	7.15±0.23
BUN (mg %)	41.17±1.33	40.17±2.04	42.17±2.14	41.5±1.38
SGPT (IU/L)	63.33±2.70	61.07±4.35	62.62±2.82	62.39±3.70
SGOT (IU/L)	57.68±1.69	57.5±1.89	56.68±1.02	57.45±1.89
ALP (IU/L)	351.17±8.35	338.5±15.93	343±6.78	340.83±12.45
Glucose (mg %)	100.00±9.42	103.50±7.01	91.67±2.8	93.17±10.6
Creatinine (mg/dL)	0.74±0.12	0.75±0.12	0.74±0.11	0.71±0.10
Bilirubin (mg/dL)	0.72±0.17	0.76±0.14	0.76±0.14	0.77±0.12

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