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Sub-Acute Toxicity Profile of Fixed Dose Combination of Pirotum (Cefpirome-Sulbactam) in Swiss Albino Mice and Wistar Rat

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Abstract: The objective of current study was to evaluate deleterious effects of a potential combination of cefpirome, a member of the latest class of broad-spectrum cephalosporins, in combination of β-lactamase inhibitor, sulbactum. To assess the toxicity profile of fixed dose regimen Pirotum (Cefpirome+Sulbactum in 2:1 ratio), a repeated dose subacute toxicity study was conducted on Swiss albino mice and Wistar rat (male and female). Three different dose levels (30, 60 and 120 mg kg^{-1}) of combination were administered for twenty eight days. Physical parameters, hematological parameters and biochemical parameters related to liver toxicity and nephrotoxicity were evaluated as end point parameters. Findings of present study were also supported by hematological as well as histopathology parameters. Data of current study indicated that Pirotum exerted no deleterious effect on blood, liver and kidney function as no alteration was observed in biochemical parameters at any dose level.

Key words: Sub-acute toxicity, pirotum, cefpirome-sulbactam, beta-lactamase

INTRODUCTION

Cephalosporins have been classified into different generations depending upon their activity against Gram-positive and Gram-negative organisms. Cefpirome, a member of the latest class of broad-spectrum cephalosporins, is characterized by a high degree of stability against hydrolytic bacterial enzymes (Hancock and Bellido, 1992). It has been classified as a fourth generation cephalosporin and is considered to be highly active against most of the Gram-negative organisms including staphylococci and Gram-positive bacteria (Hancock, 1992). This antibiotic does not harm anaerobic bacteria, hence spares the intestinal flora, unlike other antibiotics (Lipman *et al*., 2003; Wiseman and Lamb, 1997). Evaluation of its antibacterial activity, pharmacokinetic properties and clinical efficacy in the treatment of severe nosocomial infections and febrile neutropenia is well reported by Wiseman and Lamb (1997), Sauermann *et al*. (2005) and Muller *et al*. (2004). Due to all these favorable properties, cefpirome is frequently used for empirical therapy in severely ill patients in intensive care, oncology and transplantation units (Wiseman and Lamb, 1997; Lewis *et al*., 1999) but emergence of resistance in range of bacteria limits future therapeutic choices and is associated with increased rates of mortality and morbidity and higher costs. Many microorganisms initially susceptible to cefpirome have become resistant due to the production of β-lactamases (Lipman *et al*., 2001). It was concluded from available studies of emerging resistance that a mechanistic combination of at least two antibiotics may provide better results in order to reduce resistance.

To address this rapidly emerging challenge, a potential combination of Cefpirome and Sulbactam was introduced as Pirotum. Sulbactam acts primarily by irreversible inactivation of β-lactamases produced by many bacteria (Muller *et al*., 2004) thus reducing β-lactamases mediated drug destruction and development of bacterial resistance. Since, Sulbactam is poorly absorbed after oral administration (Korvick *et al*., 1992) it is combined with cefpirome for intravenous treatment of various infections. Though reports are available on toxicity of Cefpirome alone (Deki *et al*., 1990; Donaubauer *et al*., 1990) and there is well established studies to prove the efficacy of Pirotum, there is a gap about the safety profile of drug.

Against this background a study was conducted to evaluate toxicity and safety profile of Pirotum (fixed dose combination of cefpirome and sulbactam) in rat and mice.

MATERIALS AND METHODS

Healthy Swiss albino mice (male and female mice, 20-25 g weight) and Wistar rats weighing between 150-160 g were divided into four groups (three treatment

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groups and one control group). Each group is further sub divided into two groups depending on sex of mice having 6 animals of each sex. Mice and rats were housed in polypropylene cages with stainless steel grill tops, under hygienic conditions and acclimatized to the laboratory conditions for a period of seven days prior to initiation of dosing. Animals were kept at temperature between 20-25°C having 12 h light and dark cycle. Animals were given Nutrilab brand extruded pelleted mouse feed (Tetragon Chemie Pvt. Ltd., Bangalore, India) and portable water *ad libitum*. Animals were given freshly prepared intramuscular injection of cefpirome-Sulbactam combination for 28 days. The study was carried out from 10 May 2007 to 31 July 2007 at Biochemical Laboratory, Venus Medicine Research Centre, Baddi, India.

Pirotum (Cefpirome: Sulbactam; 2:1 ratio) was injected at following dose levels i.e., 30 mg kg^{-1} (Cefpirome 20 mg: Sulbactam 10 mg), 60 mg kg^{-1} (Cefpirome 40 mg: Sulbactam 20 mg), 120 mg kg^{-1} (Cefpirome 80 mg: Sulbactam 40 mg) throughout the study period (28 days) on once daily basis. Control group was injected 0.9% NaCl only. All the animals have been observed for physical, biochemical and hematological alterations. Overnight fasted animals were sacrificed on 29th day, blood and tissues samples were collected.

The Institutional Animal Ethics Committee of Institute for Toxicological Studies, Pune, India had approved the study protocol.

Reagents: All chemicals were purchased from Sigma, St. Louis, MO, USA. Pirotum (Cefpirome Sulbactam combination in 2:1 ratio) was procured from Venus Remedies Limited, Baddi (India).

Hematological and biochemical parameters: Hemogram was performed on ACT diff-2 Hematology Analyzer (Beckman Coulter India Ltd., Mumbai, India).

Biochemical parameters: Serum Gluatmic Oxaloacetic Transaminase (GOT), Gluatmic Pyruvic Transaminase (GPT) activities, serum alkaline phosphatase, blood urea nitrogen and plasma sugar levels were estimated using standard diagnostic kits (Transasia Biomedicals Ltd., Mumbai India) on Erba Chem-5 plus (Transasia, India) semi-autoanalyzer.

Histological examination: Liver, kidney, stomach, lungs and gonads were removed from the sacrificed animals and were preserved in 10% buffered formalin for histological examination (Tikoo *et al*., 2008).

Statistical analysis: Results are shown as Mean±SD. Significance of difference between groups was evaluated by using ANOVA. If ANOVA shows significant differences, post hoc analysis was performed with Dunnet test using Sigma stat 7.0 version. $p<0.05$ was considered as statistically significant.

RESULTS

Physical parameters: There were no physical changes observed throughout the dosing period. No significant change group mean body weight was observed in all the groups as compared to control group on 29th day. No signs of local damage at injection site was observed.

Hemogram: In male and female mice groups, no significant change was observed in hemoglobin (Hb), Red Blood Cell (RBC) counts, white blood cell counts and platelet counts in all the treated groups as compared to control group. Hematocrit including MCH, MCV was found comparable to control (Table 1, 2).

In pirotum treated rat (male as well as female groups) also no significant change in different hematological parameters were observed as compared to respective control group at any dose level (Table 3, 4).

Biochemical parameters: There were no clinically significant changes were observed in case of male and female mice treated with pirotum as compared to untreated control. Alkaline phosphatase level has been changed which is statistically insignificant except at highest dose (Table 5, 6). Other parameters i.e., SGOT, SGPT, sugar and protein levels in pirotum treated groups of all three doses were observed at par to untreated control. Kidney function unaltered as BUN level in treated groups was similar to control animals.

Table 1: Effect of sub acute dose of cefpirome-sulbactam FDC on hemogram in male mice

Parameters	Control	Cefpirome sulbactam 30 mg kg^{-1}	Cefpirome sulbactam 60 mg kg^{-1}	Cefpirome sulbactam 120 mg kg^{-1}
Haemoglobin (g %)	14.43±0.72	14.45±0.66	14.50±0.62	14.47±0.87
Total RBC ($X10^6$/cmm)	6.27±0.60	6.94±0.96	6.99±0.64	7.12±0.40
Rt (%)	1.37±0.26	1.18±0.23	1.33±0.19	1.20±0.14
HCT (%)	42.40±1.07	42.87±0.97	42.47±1.24	42.97±1.22
MCV (μm^3)	53.62±1.16	51.92±2.09	52.42±1.74	52.30±1.29
MCH (pg)	17.68±0.91	17.00±0.82	16.72±0.60	17.37±0.78
MCHC (%)	32.28±1.44	29.05±5.28	32.42±1.33	32.25±1.57
Platelets ($X10^5$/cmm)	3.30±0.24	3.26±0.29	3.37±0.20	3.28±0.31
Total WBC ($X10^3$/cmm)	6.81±0.72	6.97±0.54	6.97±0.26	7.09±0.80
Differential (%)				
N	16.67±4.80	21.67±2.07	19.83±3.13	21.17±3.25
L	79.17±5.49	73.83±0.98	75.17±2.56	75.33±2.58
E	2.33±1.51	2.67±1.03	2.83±0.75	2.17±0.75
M	1.83±1.17	1.83±0.98	2.17±0.98	1.50±0.55

All the values are expressed as Mean±SD (n = 6 in each group). Rt: Reticulocytes, HCT: Hematocrit, MCV: Mean corpuscular volume, MCH: Mean corpuscular hemoglobin, MCHC: Mean corpuscular hemoglobin concentration, WBC: White Blood cells

Table 2: Effect of sub acute dose of cefpirome-sulbactam FDC on hemogram in female mice

Parameters	Control	Cefpirome sulbactam 30 mg kg^{-1}	Cefpirome sulbactam 60 mg kg^{-1}	Cefpirome sulbactam 120 mg kg^{-1}
Haemoglobin (g %)	14.35±0.60	14.18±0.92	14.25±0.74	14.63±0.71
Total RBC (X10^6/cmm)	7.66±0.41	7.53±0.48	7.65±0.43	7.32±0.49
Rt (%)	1.15±0.19	1.18±0.15	1.35±0.26	1.38±0.17
HCT (%)	42.40±0.73	43.12±0.81	42.55±0.87	43.22±0.84
MCV (μm^3)	53.43±1.54	52.53±1.43	53.10±1.02	3.62±1.53
MCH (pg)	17.05±0.43	19.60±0.53	17.32±0.53	17.30±0.64
MCHC (%)	33.48±0.66	33.05±0.69	33.72±0.85	34.35±1.04
Platelets (X10^5/cmm)	3.57±0.30	3.48±0.28	3.54±0.33	3.47±0.19
Total WBC (X10^3/cmm)	7.87±0.32	7.42±0.63	7.64±0.44	6.79±0.59
Differential (%)				
N	21.17±1.17	21.67±1.51	22.33±4.13	19.67±4.68
L	75.33±1.37	74.17±1.17	73.50±4.23	74.83±3.60
E	2.17±0.75	2.83±0.75	2.83±1.17	2.00±0.63
M	1.33±0.82	1.30±0.52	1.34±0.82	1.83±0.41

All the values are expressed as Mean±SD (n = 6 in each group). Rt: Reticulocytes, HCT: Hematocrit, MCV: Mean corpuscular volume, MCH: Mean corpuscular hemoglobin, MCHC: Mean corpuscular hemoglobin concentration, WBC: White Blood cells

Table 3: Effect of sub acute dose of cefpirome-sulbactam FDC on hemogram in male rat

Parameters	Control	Cefpirome sulbactam 30 mg kg^{-1}	Cefpirome sulbactam 60 mg kg^{-1}	Cefpirome sulbactam 120 mg kg^{-1}
Haemoglobin (g %)	14.38±0.86	14.92±0.84	14.45±0.27	14.63±0.77
Total RBC (X10^6/cmm)	7.07±0.81	7.27±0.42	7.65±0.38	7.40±0.83
Rt (%)	1.15±0.19	1.30±0.26	1.43±0.23	1.30±0.24
HCT (%)	42.78±1.01	43.02±1.00	44.35±1.36	42.38±0.98
MCV (μm^3)	51.97±1.59	52.43±1.38	53.77±2.05	52.35±1.34
MCH (pg)	16.92±0.39	17.05±0.21	17.02±0.50	17.55±0.58
MCHC (%)	32.73±0.76	32.72±1.00	32.50±1.09	33.55±0.81
Platelets (X10^5/cmm)	3.17±0.16	3.09±0.57	3.23±0.27	3.37±0.16
Total WBC (X10^3/cmm)	7.38±0.90	7.13±0.87	7.17±1.60	6.91±0.81
Differential (%)				
N	22.33±1.97	21.33±1.67	74.50±0.55	21.50±1.38
L	73.17±1.72	74.50±0.55	74.50±0.55	74.33±1.86
E	2.83±0.98	2.67±0.82	2.67±1.03	2.33±0.52
M	1.67±0.82	1.50±0.84	1.67±1.03	1.67±0.52

All the values are expressed as Mean±SD (n = 6 in each group). Rt: Reticulocytes, HCT: Hematocrit, MCV: Mean corpuscular volume, MCH: Mean corpuscular hemoglobin, MCHC: Mean corpuscular hemoglobin concentration, WBC: White Blood cells

In male and female rat groups, no significant change in serum SGPT, SGOT activities and BUN, were observed in all the treated groups as compared to respective control group. No significant change in plasma sugar levels and proteins were observed in both the groups (Table 7, 8). The Alkaline phosphatase has been altered at 120 mg kg^{-1} dose.

Histological examination: There were no significant and treatment related gross and histopathological changes at and upto the dose of 120 mg kg^{-1} were observed in both animal model. No inflammatory cell infiltration, single cell and piecemeal necrosis, fibrosis, enlarged, swollen hepatocytes with granular cytoplasmic characteristics and

Table 4: Effect of sub acute dose of cefpirome-sulbactam FDC on hemogram in female rat

Parameters	Control	Cefpirome sulbactam 30 mg kg^{-1}	Cefpirome sulbactam 60 mg kg^{-1}	Cefpirome sulbactam 120 mg kg^{-1}
Haemoglobin (g %)	14.35±0.60	14.18±0.92	14.25±0.74	14.63±0.71
Total RBC (X10^6/cmm)	7.66±0.41	7.53±0.48	7.65±0.43	7.32±0.49
Rt (%)	1.15±0.19	1.18±0.15	1.35±0.26	1.38±0.17
HCT (%)	42.40±0.73	43.12±0.81	42.55±0.87	43.22±0.84
MCV (μm^3)	53.43±1.54	52.53±1.43	53.10±1.02	3.62±1.53
MCH (pg)	17.05±0.43	19.60±0.53	17.32±0.53	17.30±0.64
MCHC (%)	33.48±0.66	33.05±0.69	33.72±0.85	34.35±1.04
Platelets (X10^5/cmm)	3.57±0.30	3.48±0.28	3.54±0.33	3.47±0.19
Total WBC (X10^3/cmm)	7.87±0.32	7.42±0.63	7.64±0.44	6.79±0.59
Differential (%)				
N	21.17±1.17	21.67±1.51	22.33±4.13	19.67±4.68
L	75.33±1.37	74.17±1.17	73.50±4.23	74.83±3.60
E	2.17±0.75	2.83±0.75	2.83±1.17	2.00±0.63
M	1.33±0.82	1.33±0.52	1.33±0.82	1.83±0.41

All the values are expressed as Mean±SD (n = 6 in each group). Rt: Reticulocytes, HCT: Hematocrit, MCV: Mean corpuscular volume, MCH: Mean corpuscular hemoglobin, MCHC: Mean corpuscular hemoglobin concentration,WBC: White Blood cells

Table 5: Effect of sub acute dose of cefpirome-sulbactam FDC on biochemical parameters in male mice

Parameters	Control	Cefpirome sulbactam 30 mg kg^{-1}	Cefpirome sulbactam 60 mg kg^{-1}	Cefpirome sulbactam 120 mg kg^{-1}
Total protein (g %)	6.90±0.42	7.00±0.43	6.88±0.45	6.87±0.42
BUN (mg %)	42.70±0.58	42.00±2.19	42.33±2.16	42.83±3.37
SGPT (IU L^{-1})	64.17±1.47	67.33±5.01	62.00±2.37	64.67±4.41
SGOT (IU L^{-1})	107.50±6.75	110.33±5.96	102.67±9.58	107.00±5.10
SAP (IU L^{-1})	313.67±18.24	350.33±30.36	400.00±93.19	583.33±96.36*
Blood sugar (mg %)	104.33±13.57	97.00±10.47	104.50±11.57	106.50±7.23

All the values are expressed as Mean±SD (n = 6 in each group), *p<0.05 versus control. BUN: Blood urea nitrogen, SGPT: Serum glutamic pyruvic transaminase, SGOT: Serum glutamic oxaloacetic transaminase, SAP: Serum alkaline phosphatase

Table 6: Effect of sub acute dose of cefpirome-sulbactam FDC on biochemical parameters in female mice

Parameters	Control	Cefpirome sulbactam 30 mg kg^{-1}	Cefpirome sulbactam 60 mg kg^{-1}	Cefpirome sulbactam 120 mg kg^{-1}
Total protein (g %)	6.93±0.34	7.03±0.38	6.57±0.51	6.58±0.44
BUN (mg %)	42.28±1.46	45.17±3.76	41.67±1.51	41.83±1.72
SGPT (IU L^{-1})	65.83±4.96	67.00±6.10	66.00±4.47	65.00±6.07
SGOT (IU L^{-1})	114.67±9.89	119.67±5.47	109.83±7.78	112.83±3.66
SAP (IU L^{-1})	306.00±10.10	339.67±22.77	369.33±23.46	517.00±11.80*
Blood sugar (mg %)	106.83±5.56	99.50±11.40	108.00±7.85	98.17±9.33

All the values are expressed as Mean±SD (n = 6 in each group), *p<0.05 versus control. BUN: Blood urea nitrogen, SGPT: Serum glutamic pyruvic transaminase, SGOT: Serum glutamic oxaloacetic transaminase, SAP: Serum alkaline phosphatase

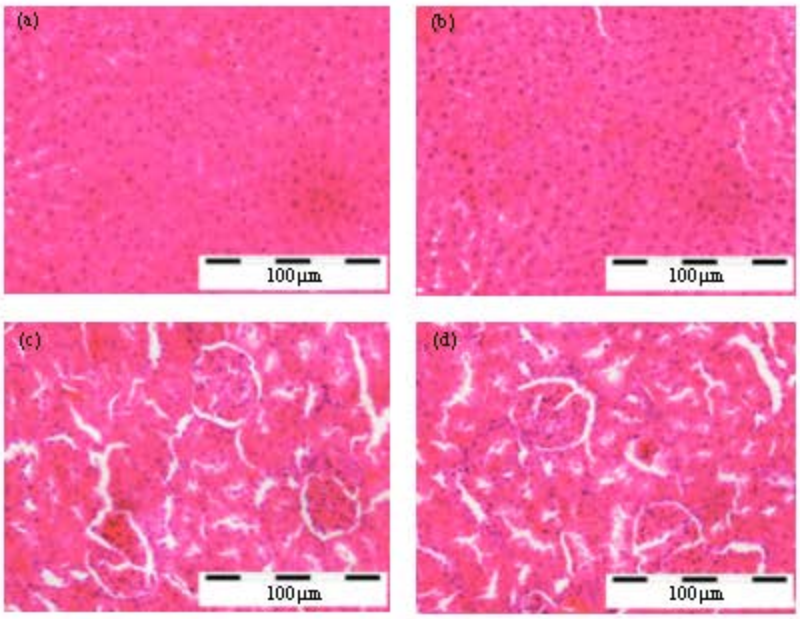

Fig. 1: Representative microphotographs of Liver and kidney sections of control rat and treated with Pirotum (Cefpirome sulbactam) 120 mg kg^{-1}. Magnification is 200 X and micron bar = 100 µm. The sections were stained with Haematoxylin-Eosin staining. (a) liver of control, (b) liver of pirotum 120 mg kg^{-1}, (c) kidney of control and (d) kidney of pirotum 120 mg kg^{-1}

Table 7: Effect of sub acute dose of cefpirome-sulbactam FDC on biochemical parameters in male rat

Parameters	Control	Cefpirome sulbactam 30 mg kg^{-1}	Cefpirome sulbactam 60 mg kg^{-1}	Cefpirome sulbactam 120 mg kg^{-1}
Total protein (g %)	6.63±0.51	6.87±0.26	6.45±0.48	6.87±0.47
BUN (mg %)	42.50±1.05	41.50±1.64	40.83±1.17	41.50±1.87
SGPT (IU L^{-1})	67.17±3.31	63.00±3.85	64.00±3.58	64.00±4.47
SGOT (IU L^{-1})	111.00±2.19	109.83±5.49	118.33±5.68	111.00±1.90
SAP (IU L^{-1})	345.50±27.73	354.50±5.24	364.00±10.24	588.67±11.86*
Blood sugar (mg %)	100.00±7.80	102.83±8.68	104.17±9.99	99.17±9.56

All the values are expressed as Mean±SD (n = 6 in each group), *p<0.05 versus control. BUN: Blood urea nitrogen, SGPT: Serum glutamic pyruvic transaminase, SGOT: Serum glutamic oxaloacetic transaminase, SAP: Serum alkaline phosphatase

Table 8: Effect of sub acute dose of cefpirome-sulbactam FDC on biochemical parameters in female rat

Parameters	Control	Cefpirome sulbactam 30 mg kg^{-1}	Cefpirome sulbactam 60 mg kg^{-1}	Cefpirome sulbactam 120 mg kg^{-1}
Total protein (g %)	6.67±.62	6.72±.35	6.73±.40	6.90±.28
BUN (mg %)	43.33±1.03	42.50±2.43	42.50±2.43	40.50±1.87
SGPT (IU L^{-1})	63.50±4.64	63.83±3.31	63.00±3.41	66.83±3.06
SGOT (IU L^{-1})	109.83±5.53	110.50±7.09	124.67±7.81	105.00±7.32
SAP (IU L^{-1})	335.17±30.67	334.83±27.56	343.83±29.25	498.17±53.69*
Blood sugar (mg %)	103.17±5.71	97.33±16.23	104.33±8.59	109.33±9.37

All the values are expressed as Mean±SD (n = 6 in each group), *p<0.05 versus control. BUN: Blood urea nitrogen, SGPT: Serum glutamic pyruvic transaminase, SGOT: Serum glutamic oxaloacetic transaminase, SAP: Serum alkaline phosphatase

vascular abnormalities resembling veno-occlusive changes were found in liver of treated rat and mice as compared to control (Fig. 1a, b). Similarly, there were no occurrence of glomerular, tubular and interstitial lesions in the kidney of Pirotum exposed group (Fig. 1c, d). Other organs i.e., stomach, lungs and gonads have also not shown any pathological signs in treatment groups as compared to control.

DISCUSSION

The β-lactams are a family of antimicrobial agents consisting of four major groups: penicillins, cephalosporins, monobactams and carbapenems. These all have a β-lactam ring, which can be hydrolysed by β-lactamases (Horstmann and Engelbart, 1990; Rice *et al*., 1991). To bypass the antimicrobial action, bacteria resist by producing β-lactam inactivating enzymes (β-lactamases) or mutated types of penicillin binding proteins (Hancock, 1992; Kinzig *et al*., 1992). Sulbactam like other β-lactamase inhibitors can be combined with β-lactam antibiotics to prevent their destruction by β-lactamases for the treatment of many bacterial infections (Rice *et al*., 1991). Sulbactam therefore enhances the activity of penicillins and cephalosporins against many resistant strains of bacteria. We here

by analyzed the toxicity profile of this potential fixed dose combination pirotum on sub acute dosing in mice as well as rat.

One of the major mechanisms for development of bacterial resistance to β-lactam antibiotics is formation of β-lactamases. Resistance to third generation cephalosporins may also be mediated by mutations in the bacterial gene, resulting in production of more active transcriptional activator or in the structural ampC gene, extending the substrate specificity of the β-lactamase (Meis-Kindblom and Kindblom, 1998; Lewis *et al*., 1999). Cefpirome rapidly penetrate into Gram-negative bacteria, have a high affinity for essential penicillin-binding protein and are stable against attack of chromosome-encoded β-lactamases. Due to this cefpirome shows activity even against derepressed enterobacteria and isolates with mutations. However, resistance to these new cephalosporins has been reported in clinical isolates in which hyperproduction of the β-lactamase was aided with lack of porins in the bacterias (Barnaud *et al*., 2001) and in *in vitro* mutants of Escherichia coli harboring the ampC gene of Enterobacter cloacae (Ishii *et al*., 2008). This was the major reason to incorporate sulbactam along with cefpirone so as to inhibit these β-lactamase, as fixed dose combination.

We evaluated physical, biochemical and histopathological parameters in the control as well as treated groups in rat and mice. In the present study no physical changes were observed during the study period in all the groups. No inflammatory response was observed in the treated groups in either species as compared to respective control groups. Increase in body weights of treated animals of either sex was comparable to control groups.

In a published report cefpirome was intravenously adininistered in dose levels up to 1500 mg/kg/day with good kidney tolerance. Signs of renal functional impairment were observed (800 and 1500 mg kg^{-1}) but histologically no morphological changes could be detected in those groups (Donaubauer *et al*., 1990). The chronic intraperitoneal adininistration (90 day) of cefpirome at dose levels of 400 or 1600 mg/kg/day resulted in some reversible changes in hematology (slight anemia), serum-chemistry parameters (liver), urinalysis (proteinuria) and histopathology (increased numbers and enlargement of lysosomes in proximal tubular epithelia of the kidneys and pigment deposits in follicle epithelia of the thyroids), predominantly in high-dose animals. No effect was observed in 100 mg/kg/day treated group (Donaubauer *et al*., 1990; Horstmann and Engelbart, 1990). However, there were no such changes observed in treatment groups of cefpirome-sulbactum combination.

Cefpirome is excreted principally through kidneys thus it was also important to observe effect on renal system. The biochemical parameters suggested that the fixed dose combination of is not causing any alteration in renal function. The signs of slight, reversible renal impairment including lysosome enlargement and the slight anemia were reported in monkeys on chronic intravenous dosing of cefpirome alone (Horstmann and Engelbart, 1990). While in combination with Sulbactam no such alteration was observed. It has been reported that no significant change in clinical sign were observed in rats on intraperitoneal chronic dosing of cefpirome alone for six months even at a dose level of 120 mg/kg/day (Sirot *et al*., 1987; Deki *et al*., 1990). No significant change in body weight and food intake was observed at all doses. Slight decrease in erythrocyte count, hematocrit and hemoglobin concentration were seen at highest dose level in treated groups which was statistically insignificant (Sauermann *et al*., 2005; Ishii *et al*., 2005, 2008). Similar results have been observed in current study which reflects no additional toxicity with addition of sulbactam in FDC Pirotum.

The biochemical parameters related to liver toxicity were also studied to observe the effect of drug and were found comparable with controls and were within the normal biological and laboratory limits. However there was a change observed in alkaline phosphatase (ALP) level at highest dose level. Many reports suggest increase in ALP level is transient adverse effect of Cefpirome therapy. This may be due to reversible obstruction in hepatocytes (Donaubauer and Mayer, 1992). This may be the possible explanation for the change observed in alkaline phosphate, however no such signs were observed in histopathological findings. Further exploration for the effects on liver may be required at higher doses.

The gross histo-pathological examination of animals revealed no abnormality attributed to the treatment.

It appears to be established that combination regimen Pirotum have not produced any deleterious effects on mice as well as rat at all dose levels used in current study. This study provides clinically relevant data which can be utilized to decide the therapeutic safety of current dosage regimen. It can be concluded that sub acute dosing of the fixed dose combination Pirotum (cefpirome sulbactam for injection) did not cause any haematological, hepato and renal toxicity in rat and mice and may provide a safe alternative for beta lactamase producing resistant bacteria induced infections.

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