Hepatotoxicity Studies of Nimesulide in Litters of Rat

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Abstract : Present study was carried out to evaluate hepatotoxicity of nimesulide by single dose and seven days administration in sub therapeutic, therapeutic and supra therapeutic doses in litters of rat. Single dose and seven days administration hepatotoxicity studies of nimesulide were carried out in litters of rat of either sex. They were further subdivided into sub therapeutic (20 mg/kg), therapeutic (30 mg/kg) and supra therapeutic (100 mg/kg) groups. Effect of nimesulide on liver functions were analysed by serum glutamate oxaloacetate transaminase (SGOT), serum glutamate pyruvate transaminase (SGPT), alkaline phosphatase (ALP) and histopathological examination of liver through scoring system. Histopathological changes in liver were observed in therapeutic and supra therapeutic doses in single dose groups and sub therapeutic, therapeutic and supra therapeutic doses in seven days groups. In single dose of nimesulide in litters, there were significant increases in biochemical parameters ($p< 0.05$) in supratherapeutic doses. However, in seven days studies of nimesulide in litters, there were significant increases in biochemical parameters ($p< 0.05$) in therapeutic and supratherapeutic doses. The present study indicates that nimesulide causes significant hepatotoxicity in litters of rat.

Key words: Words: Nimesulide, Hepatotoxicity, Histopathology of liver, Serum glutamate oxaloacetate transaminase, Serum glutamate pyruvate transaminase, Alkaline phosphatase.

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INTRODUCTION: Nimesulide is a 4-nitro-2-phenoxy-methanesulphonamide, non-steroidal anti-inflammatory Drug (NSAID). It is a preferential COX 2 inhibitor, which acts by inhibiting leukocyte function, PAF synthesis, TNFα release, metalloproteinase activity in cartilage and has strong free radical scavenging effect[1, 2]. It has potent analgesic, anti pyretic and anti-inflammatory activity on oral and rectal administration[3]. It was introduced in Indian market in 1990 as an analgesic and antipyretic agent, for painful musculoskeletal disorders (osteoarthritis), primary dysmenorrhoea, and also for paediatric patient[3]. Adverse effects of nimesulide commonly involve the hepato-biliary, cutaneous and gastrointestinal system. Acute hepatitis, fulminant hepatic failure, cholestatic liver injury, multiple enterocolic perforations and end stage renal failure with nimesulide intake have been reported[4, 5]. After its launch, various case reports of hepatotoxicity[6-10] and even fatal hepatic failure[11, 12-16] leading to the withdrawal of the drug in various countries[11, 16, 17].

Paediatric uses of nimesulide includes mainly as an antipyretics in case of upper respiratory tract infection (URTI), lower respiratory tract infection (LRTI), malaria, enteric fever and urinary tract infection on as and when required basis. It has shown superior efficacy than naproxen, mefenamic acid and paracetamol as an antipyretic and an anti-inflammatory in paediatric patients[18-20]. Systemic reviews done from the randomized trials on the paediatric patients comparing nimesulide with placebo or other NSAIDS fails to conclude whether nimesulide is safe or unsafe as other analgesics-

antipyretics for short-term use in children. Gupta P and Sachdev H P suggested that the drug should be best avoided in known or suspected liver disease and caution is warranted while prescribing nimesulide concomitantly with other hepatotoxic drugs[21]. So, controversy continues about its use in countries where it is available in market. The present study was carried out to evaluate the hepatotoxic effect of nimesulide on single dose and seven days administration in sub therapeutic, therapeutic and supra therapeutic dose in litters of rat.

MATERIAL AND METHODS: The study was started after obtaining clearance from Institutional Animal Ethics Committee.

Experimental animal : Litters of *wistar* albino rats weighing 25-35 g (age 25-30 days) of either sex procured from central animal house of Govt. Medical College, Bhavnagar were used in experiments. They were housed in a temperature-controlled room (24±2 C; relative humidity 60-70%) with 12 h light-dark cycles, housed in clean polypropylene cages under standard condition and were given standard pellet diet and water *ad libitum*. Food was withdrawn 12 h before the experiments.

Chemicals : Nimesulide (Alpha Health Care, Ahmedabad, India) and SGOT, SGPT, ALP (Transgenic, Ahmedabad, India) measurement kits were used for the study.

Study design : Single dose and seven days nimesulide administration studies were carried out on litters. They were divided into seven groups with 6 animals in each group. The dose of nimesulide in rats was extrapolated by referring the table given in Ghosh MN[22] and it is equivalent to the human beings (5 mg/kg).

Groups for Study of nimesulide hepatotoxicity in litter rats:

Group 1: Vehicle control was given distilled water

Single dose groups:

Group 2:
Received sub therapeutic dose of 20 mg/kg p o

Group 3:
Received a therapeutic dose of 30 mg/kg p o

Group 4:
Received a supra therapeutic dose of 100 mg/kg p o

Seven days groups:

Group 5:
Received a sub therapeutic dose of 20 mg/kg p o

Group 6:
Received a therapeutic dose of 30 mg/kg p o

Group 7:
Received a supra therapeutic dose of 100 mg/kg p o

Biochemical Parameters: Blood samples were collected from the intra orbital plexus under pentobarbitone sodium (30 mg/kg ip) anaesthesia at 24 h after the last dose in each group. Serum was separated for estimation of biochemical parameters: serum glutamate oxaloacetate transaminase – SGOT, serum glutamate pyruvate transaminase – SGPT and alkaline phosphatise – ALP by UV kinetic test-optimised International Federation of Clinical Chemistry (IFCC) method in fully automated analyser[23-24].

Histopathological Analysis: The animals were sacrificed, and abdomen was cut open to remove liver, kept in 10% formalin for 24 h. Then 5 mm thick pieces of the liver were embedded in paraffin and cut into 5 µm thick sections and stained using haematoxylin-eosin dye[25]. Each histopathology slide was coded and observer masked evaluation performed for histopathological changes in liver architecture. Ballooning degeneration, inflammation, apoptotic cells and fibrosis were analysed according to scoring criteria as described in Table-1 and their photomicrographs were taken.

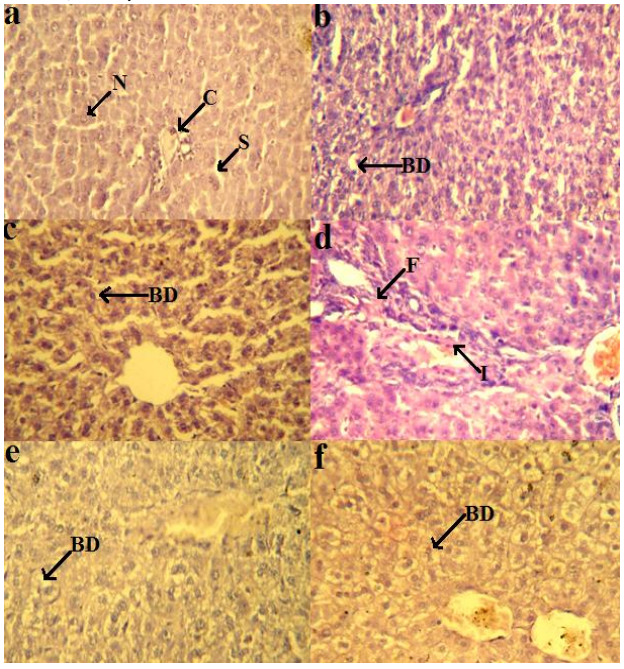
Data analysis: The results analysed according to scoring criteria were expressed as median (Interquartertile Range). The results obtained for biochemical parameters were expressed as mean ± SEM. One way ANOVA and Dunnett's Multiple Comparison Test was performed using GraphPad prism 5 demo version software. P value <0.05 was considered significant.

Table-1 Scoring criteria for the evaluation of histopathological changes in liver architecture

Ballooning degeneration	
Score 0	No ballooning degeneration
Score 1+	Minimal enlargement in few hepatocytes
Score 2+	Mild enlargement in many hepatocytes
Score 3+	Moderate enlargement in most hepatocytes
Score 4+	Severe enlargement in most hepatocytes
Inflammation	
Score 0	No inflammatory foci
Score 1+	1 inflammatory foci per 200 hpf
Score 2+	2-4 inflammatory foci per 200 hpf
Score 3+	>4 inflammatory foci per 200 hpf
Apoptotic cells	
Score 0	No apoptotic cells
Score 1+	Few apoptotic cells
Fibrosis	
Score 0	No fibrosis
Score 1 +	Portal / sinusoidal minimal fibrosis
Score 2+	Portal / sinusoidal mild fibrosis
Score 3+	Bridging fibrosis
Score 4+	Cirrhosis

hpf: high power field.

Figure-1 Photomicrographs of liver (Haematoxylin & Eosin, 40X)



RESULTS: In control group, histology of liver in litters showed normal hepatic cells with well preserved cytoplasm, nucleus, nucleolus and central vein (Figure-1a). The histopathological scoring systems were grouped into four categories: ballooning degeneration, inflammation, fibrosis and apoptotic cells (Table-1). In single dose, there were a few apoptotic cells and minimal enlargement in few hepatocytes (ballooning degeneration) in therapeutic (Figure-1b) group and mild enlargement in many hepatocytes in supra therapeutic (Figure-1c) group. In seven days study, there were a few apoptotic cells, one inflammatory foci per 200 high power field and minimal fibrosis in sub therapeutic (Figure-1d) group, while it caused minimal fibrosis and moderate enlargement in most hepatocytes in therapeutic (Figure-1e) group and severe enlargement in most hepatocytes in supra therapeutic (Figure-1f) group (Table-2).

In single dose, elevations in biochemical parameters were significant for SGOT and ALP in supra therapeutic group. Other groups were showing non significant elevation in biochemical parameters for single dose (Table-3). In seven days group, nimesulide caused significant elevation in SGOT, SGPT in therapeutic group and SGOT, SGPT, ALP in supra therapeutic group. Elevations in biochemical parameters were non significant for sub therapeutic group (Table-3).

(a) Control group: showing normal architecture, central vein (C), nucleus (N) and sinusoids (S). (b) Therapeutic group (single dose): showing minimal enlargement in few hepatocytes [1+ ballooning degeneration (BD)]. (c) Supra therapeutic group (single dose): mild enlargement in many hepatocytes (2+ BD). (d) Sub therapeutic group (seven days): 1 inflammatory foci per 200 hpf (I), minimal fibrosis (F). (e) Therapeutic group (seven days): minimal fibrosis (F), moderate enlargement in most hepatocytes (3+ BD). (f) Supra therapeutic group (seven days): severe enlargement in most hepatocytes (4+ BD).

Table-2 Effect of single dose and seven days administration of nimesulide in histopathological scoring of liver in litters of rat. (n=6, in each group)

Groups	Scoring of liver lesions			
	Ballooning degeneration	Inflammation	Fibrosis	Apoptotic cells
Control	0 (0,0)	0 (0,0)	0 (0,0)	0 (0,0)
Single dose treatment group				
Sub therapeutic (20mg/kg)	0 (0,0)	0 (0,0)	0 (0,0)	0 (0,0)
Therapeutic (30mg/kg)	1.5 (0,4)	0 (0,0)	0 (0,0)	0 (0,1)
Supra therapeutic (100mg/kg)	2 (2,4)	0 (0,0)	0 (0,0)	0 (0,0)
Seven days treatment group				
Sub therapeutic (20mg/kg)	0 (0,0)	1 (1,1)	1 (0,1)	1 (0,1)
Therapeutic (30mg/kg)	2 (1,4)	0 (0,1)	1 (0,1)	0 (0,0)
Supra therapeutic (100mg/kg)	4 (4,4)	0 (0,0)	0 (0,0)	0 (0,0)

Data are presented as Median (Interquartertile Range)

Table-3 Effect of single dose and seven days administration of nimesulide in sub therapeutic, therapeutic and supra therapeutic groups on SGOT, SGPT and ALP in litters of rat. (n= 6, in each group)

Groups			
	SGOT (U/L)	SGPT (U/L)	ALP (U/L)
Group 1 (control)	148.3±2.9	45.5±3.6	387.4±205.7
Single dose treatment group:			
Group 2 (sub therapeutic dose - 20mg/kg)	154.2±13.0	57.5±5.4	453.0±51.9
Group 3 (therapeutic dose - 30mg/kg)	156.2± 9.3	65.0±5.8	725.2±40.5
Group 4 (supra therapeutic dose - 100mg/kg)	237.5±40.3*	76.3±17.9	853.2±108.4*
Seven days treatment group:			
Group 5 (sub therapeutic dose - 20mg/kg)	176.5±9.1	60.5±5.8	715.7±91.3
Group 6 (therapeutic dose - 30mg/kg)	202.7±19.9#	70.7±8.72#	777.3±106.4
Group 7(supra therapeutic dose - 100mg/kg)	201.1±3.1#	72.3±8.1#	903.7±40.2#

Data are presented as Mean ± SEM.

*P < 0.05 compared to their corresponding control value, # P < 0.05 compared to their corresponding control value (One way ANOVA and Dunnett's multiple comparisons test).

DISCUSSION: Present study in litters of rat suggest that single dose administration of nimesulide in supra therapeutic dose of 100 mg/kg (equivalent human dose 16 mg/kg) caused mild hepatotoxicity both by histopathological scoring and biochemical studies (increases in SGOT and ALP-P<0.05). However, in therapeutic dose of 30 mg/kg (equivalent human dose 5 mg/kg), there was minimal hepatotoxicity in rats by histopathological scoring but biochemical parameters were not statistically significant. In sub therapeutic dose of 20 mg/kg (equivalent human dose 3.2 mg/kg), both histopathological scoring and biochemical parameters suggest that single dose administration does not caused hepatotoxicity in litters.

Nimesulide was administered for 7 days in litters to simulate the clinical use as an antipyretic for the short duration in paediatric patients. Seven days administration of nimesulide in litters of rat in therapeutic and supra therapeutic doses caused significant hepatotoxicity both by histopathological scoring and biochemical parameters (increase in SGOT, SGPT and ALP- P<0.05). However, in sub

therapeutic dose, there were few apoptotic cells, inflammatory changes and fibrosis in rats by histopathological scoring but increase in biochemical parameters was not statistically significant. This suggests that litters are susceptible to hepatotoxicity of nimesulide on prolonged administration even at sub therapeutic dose.

Nimesulide caused injury to the isolated rat and mouse liver cells by impairment of ATP production by mitochondria due to uncoupling on account of the activity of its nitro group[26-28]. Suggested molecular mechanisms of underlying liver injury are: covalent modification of target protein, oxidoreductive stress, immune-mediated reactions, interference with hepatobiliary export and mitochondrial injury[28]. Bioreductive metabolism of the nitroarene group of nimesulide to reactive intermediates have been implicated in oxidative stress, covalent binding and mitochondrial injury [29].

Nimesulide may cause asymptomatic to symptomatic elevation in liver function parameters in paediatric patients. Liver function monitoring should be done with it. Epidemiological studies should be carried out in countries where it is available for use.

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