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RESEARCH ARTICLE

A STUDY ON TOXIC EFFECTS OF NIMESULIDE IN PREGNANT *SPRAGUE DAWLEY* RATS

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ABSTRACT

The present study was aimed to evaluate the organ toxicity and teratogenic effects, if any,respectively in the dams treated with nimesulide in gestation period at different dose levels and in progeny. Thirty six female albino rats of *Sprague dawley* strain were equally divided into 3 groups and treated as follows. Group 1 served as control, group 2 received nimesulide @ 20 mg/kg body weight and group 3 received nimesulide @ 60 mg/kg body weight via intramuscular route from 7th to 17th day of gestation. In each group, pregnant rats were subjected to caessarian section on 19th day of gestationfor uterine weights with progeny, resorption sites, inborn progeny body weight, litter size, live and dead numbers, male: female progeny numbers, skeletal staining of progeny with Alizarin-Red S, Alcian blue-Alizarin Red S stains and soft tissue developmental anomalies.Thiobarbituric acid reacting substances (TBARS) andreduced glutathione (GSH) were estimated in kidney and liver on 19th day of gestation. Hepatic and renal biomarkers were estimated on 19th day of gestation.There was a significant difference in sero-biochemical profiles of dams and was more evident with nimesulide treated @ 60 mg/kg body weight. Treatment with nimesulide at higher dose induced oxidative stress and tissue damage of liver and kidney as evident from altered biochemistry and histology. It is concluded that nimesulide at 60 mg/kg body weight in pregnant dams showed more significant damage to liver and kidney as compared to the dose of 20 mg/kg body weight and control, while no teratogenicity was reported in any of the tested doses.

INTRODUCTION

The Non Steroidal Anti-Inflammatory Drugs (NSAIDs) are the most commonly prescribed drugs world-wide in the treatment of acute and chronic painful inflammatory musculoskeletal conditions and also in the management of pain and fever (Suleyman *et al*., 2007). The number of pregnant women and women of rheumatic diseases in child bearing age are receiving drugs during pregnancy to control maternal disease activity and to ensure a successful pregnancy outcome (Ostensen *et al*., 2006). Cyclooxygenase (COX) inhibitors are one of the most ingested drugs during pregnancy (Burdan, 2005). Treatment with NSAIDs is possible at some stages of pregnancy as well as during lactation (Ostensen, 2006). Sometimes such drugs cross the placental barrier, enter the fetal circulation and potentially alter fetal development, particularly the development of the kidneys. Increased incidences of maternal toxicity, intrauterine growth retardation and adverse renal effects have been reported. The fetus and the newborn infant may thus experience renal failure, varying from transient oligohydramnios to severe neonatal renal insufficiency leading to death. Such adverse effects may particularly occur when fetuses are exposed to NSAIDs (Balasubramaniam, 2000; Farid *et al*., 2006).

Although NSAIDs are effective, they are associated with adverse effects that predominantly affect the gastrointestinal tract (GIT), acute hepatitis and fulminant hepatic failure (Tan *et al*., 2007). In addition, they may decrease renal and platelet function (Saillant, 2009).

Nimesulide is a preferential COX-2 inhibitor with anti-inflammatory, analgesic and antipyretic effects (Thawani *et al*., 2003). Similar to other NSAIDs, prolonged use and more than therapeutic doses of nimesulide causes adverse effects (Goyal *et al*., 1998). Its adverse effects commonly involve the hepato-biliary, renal, cutaneous and gastrointestinal systems (Sbeit *et al*., 2001; Burke *et al*., 2005).

MATERIALS AND METHODS

Female albino rats of *Sprague dawley* strain weighing about 200-250 g were procured from NCLAS, National Institute of Nutrition, Hyderabad. The animals were housed in solid bottom polypropylene cages in Laboratory Animal House of Department of Pharmacology & Toxicology, College of Veterinary Science, Rajendranagar, Hyderabad, with 12 h –12 h dark and light cycle and temperature of 22-24°C. Animals were placed on commercial standard mash feed for rat and provided water *ad libitum.* Before conducting the experiment, rats were acclimatized for 1 wk. Experiment was conducted according to the guidelines of Institutional Animal Ethics Committee with a single batch of 36 female rats. These rats

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were mated and after confirming pregnancy, 36female rats were divided into 3 groups consisting of 12 in each group. Group 1 was control maintained on basal diet, groups 2 and 3 were given nimesulide @ 20 and 60 mg/kg body weight, respectively via IM route daily from 7^{th} to 17^{th} day of gestation. Pregnant rats were subjected to caessarian section on 19^{th} day of gestation. Litter was screened for soft tissue and skeletal developmental anomalies.

Blood samples were collected from the dams on 19^{th} day of gestation in all three groups by retro-orbital puncture and sera samples were separated to estimate sero-biochemical profile (ALP, ALT, AST, BUN, creatinine, GGT and total protein). Dams of all groups at 19^{th} day of gestation were anaesthetized for caessarian section and screened for gravid uterine weights, resorption sites, inborn progeny body weights, litter size, live and dead numbers, male: female sex ratio, skeletal developmental anomalies and soft tissue developmental anomalies. Tissues were collected and stored at -20^0C for further estimation of GSH and TBARS in liver and kidney homogenates. Liver and kidney were immediately excised after sacrifice and rinsed with ice-cold physiological saline for histological studies. The tissues were fixed in 10 % neutral buffered formalin immediately upon removal. They were gradually dehydrated, embedded in paraffin, cut into 5 µm sections, stained with hematoxylin and eosin (H&E) for histological examination according to the standard procedure (Singh and Sulochana, 1997).

Skeletal staining in progeny: Alizarin red-S

All the embryos were eviscerated and cleared by the method of Dawson (1926) and the skeleton stained with alizarin red-S as given below.

1 Embryos were fixed in 95 per cent alcohol for 48-72 h.
2 Embryos were treated in acetone to remove any fat that appears as opaque white mass for 2-4 days.
3 The specimen was returned to 95 % alcohol and kept for 12-24 h.
4 Embryos were placed in 1 per cent KOH until bones were clearly visible through the muscle.
5 Finally they were kept in a solution of 0.1 % alizarin red S in 1 % KOH until bones was stained.
6 When clearing in the initial KOH solution has progressed to the proper stage, the bones took up stain. When the clearing was not complete, the muscle and other tissues took up stain almost as readily as the bone itself. In such cases the specimens were transferred to a fresh KOH solution.
7 Following the staining, embryos were transferred to jars containing
   a) KOH . . 1 g
   b) Distilled water . . 79 ml
   c) Glycerine . . 20 ml
8 When the tissues were properly cleared they were passed through the increasing concentrations of glycerine and finally stored and maintained in pure glycerine.

Alcian Blue – Alizarin Red Skeletal Staining

All the embryos were eviscerated and cleared by the method of Humason (1967) and the skeleton stained with alcian blue-alizarin red-S as given below.

1. Neonatal progeny rats were dissected by removing the skin and organs completely. Fat was removed carefully without damaging the specimen and placed in 15 ml falcon tubes.
2. Specimens were fixed in 95 % ethanol for 12-48 h, slowly rocking at room temperature.
3. Specimens were again placed in 95 % ethanol with Alcian blue staining solution for 1-3 days slowly rocking at room temperature. For vertebral or appendicular skeleton 1-2 days is enough, whereas head staining requires 3 days.
4. Specimens, re-placed in Alcian blue solution with 95 % ethanol for 6 h slowly rocking at room temperature.
5. Specimens re-placed in 95 % ethanol with 2 % KOH solution for 12-24 hours slowly rocking at room temperature. Older animals require more time than younger ones. (Any remaining fat or skin can be removed at this point).
6. Specimen stained in Alizarin Red solution for 12-24 h, slowly rocking at room temperature.
7. Cleared in the solution of 1 % KOH/20% glycerol, until the specimen is completely clear. Do not leave skeleton in the solution for too long because the skeleton still becomes extremely fragile.
8. Replaced clearing solution with 1: 1 glycerol: 95 % ethanol for 1 day.
9. To store skeletons for long periods of time, it was passed through glycerol/ethanol solutions.

Solutions

Alcian Blue (0.03 % Alcian Blue, 80 % ethanoland20 % acetic acid)
Alizarin Red (0.03 % Alizarin Red, 1 % KOH and water)

The data were subjected to statistical analysis by applying one way ANOVA using statistical package for social sciences (SPSS) version 15. Differences between means were tested using Duncan's multiple comparison test and significance was set at $P<0.05$.

RESULTS AND DISCUSSION

Results of sero-biochemistry are presented in Table 1. The activity of serum ALP (IU/L) in group 1 pregnant dams at 19^{th} day was 239.36±10.75, which was significantly ($P < 0.05$) increased in group 2 (382.30±10.10). The group 3 showed a significant ($P < 0.05$) increase (515.58±43.86) as compared to groups 1 and 2.The activity of serum ALT (IU/L) in group 1 pregnant dams at 19^{th} day was 22.27±0.73, which was significantly ($P < 0.05$) increased in group 2 (28.70±0.81). The group 3 showed a significant ($P < 0.05$) increase (43.07±1.14) as compared to group 1 and 2.The activity of serum AST (IU/L) in group 1 pregnant dams at 19^{th} day was 223.50±7.64, which was significantly ($P < 0.05$) increased in group 2 (296.41±6.23). Group 3 showed a significant ($P < 0.05$) increa se (408.41±4.75) as compared to groups 1 and 2.The activity

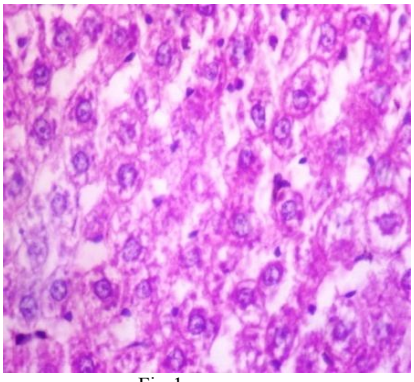
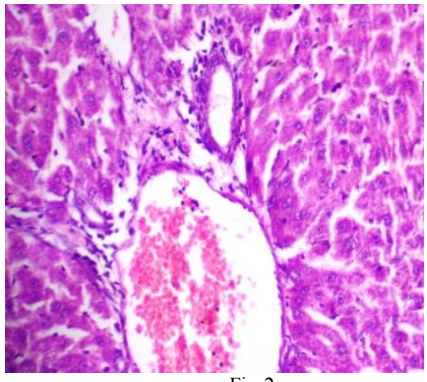


Fig-1



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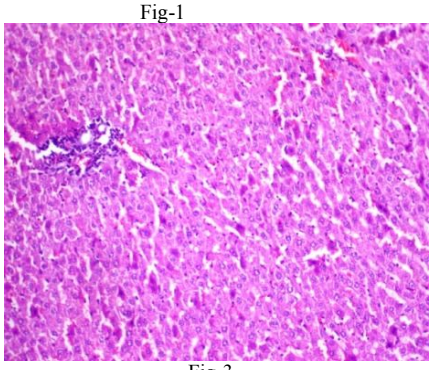

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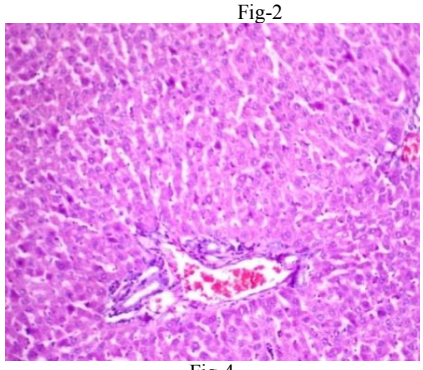


Fig-4

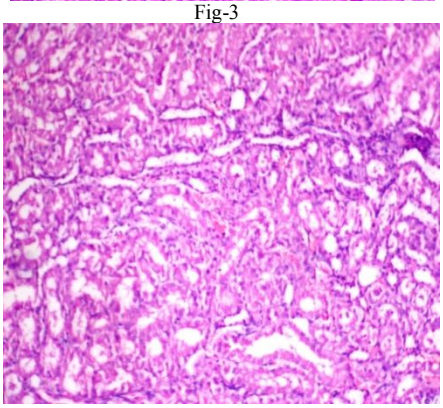
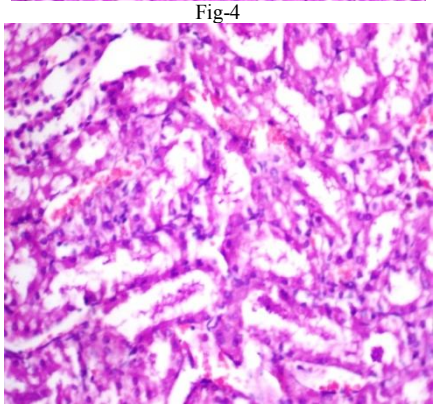


Fig-5



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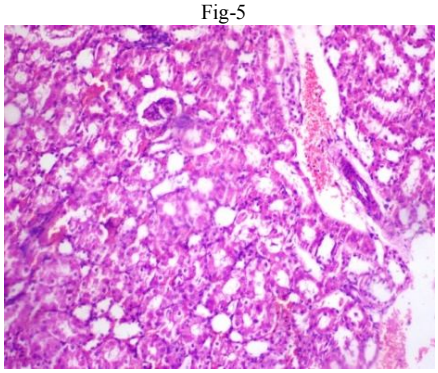

Fig-7

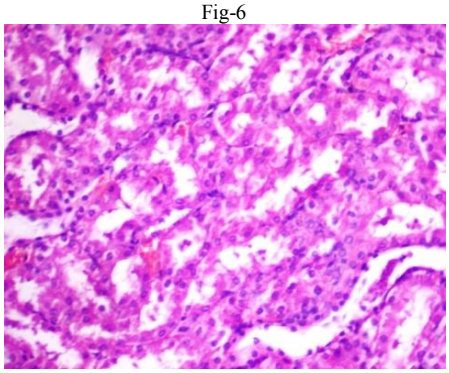

Fig-8

Fig-1 Photomicrograph of liver showing mild hydropic degeneration of hepatocytes. H&E X 400 (Group 2)
Fig-2 Photomicrograph of liver showing central vein congestion and bile duct hyperplasia H&E X 200 (Group 2)
Fig-3 Photomicrograph of liver showing focal areas of lymphoid aggregates H&E X 200 (Group 3)
Fig-4 Photomicrograph of liver showing moderate central vein congestion and bile duct hyperplasia H&E X 200 (Group 3)
Fig-5 Photomicrograph of kidney showing mild intertubular congestion and hemorrhages. H&E X 400 (Group 2)
Fig-6 Photomicrograph of kidney showing mild degenerative changes, intertubular haemorrhages and hyaline casts. H&E X 200 (Group 2)
Fig-7 Photomicrograph of kidney showing moderate intertubular haemorrhages H&E X 100 (Group 3)
Fig-8 Photomicrograph of kidney showing moderate degenerative changes in tubular epithelium and congestion. H&E X 200 (Group 3)

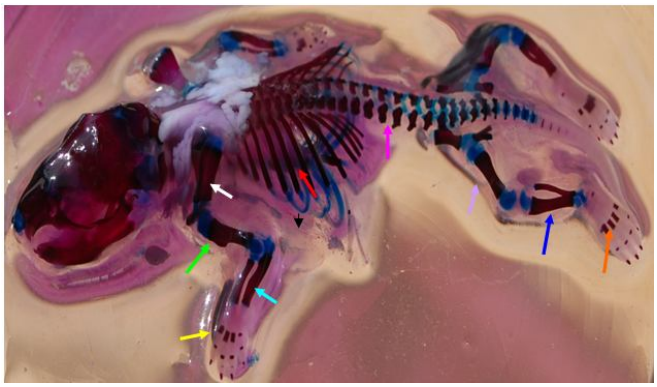

Fig-9 Photograph of fetal skeleton from group 1 stained with Alcian Blue and Alizarin Red-S, showing bony skeleton of normal architecture viz. Scapula (white arrow)
Humerus (green arrow), Radius & ulna (turquoise arrow), Metacarpals (yellow arrow), Ribs (red arrow), Vertebral column (Pink arrow), Femur (lavender arrow), Tibia & fibula (Blue arrow) and Metatarsals (orange arrow).

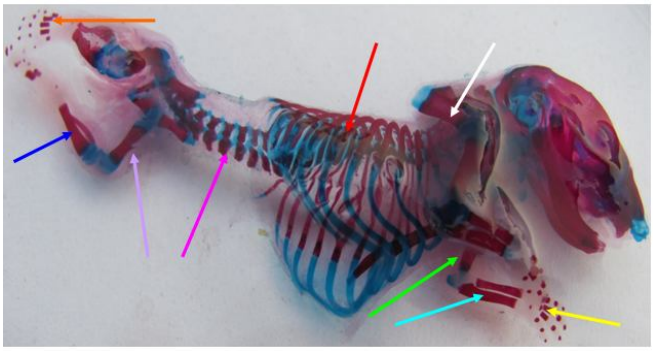

Fig-10 Photograph of fetal skeleton from group 2 stained with Alcian Blue Alizarin Red-S, showing bony skeleton of normal architecture viz. Scapula (white arrow),
Humerus (green arrow), Radius & ulna (turquoise arrow), Metacarpals (yell row), Ribs (red arrow), Vertebral column(Pink arrow), Femur (lavender arrow), Tibia & fibula (Blue arrow) and Metatarsa (orange arrow).

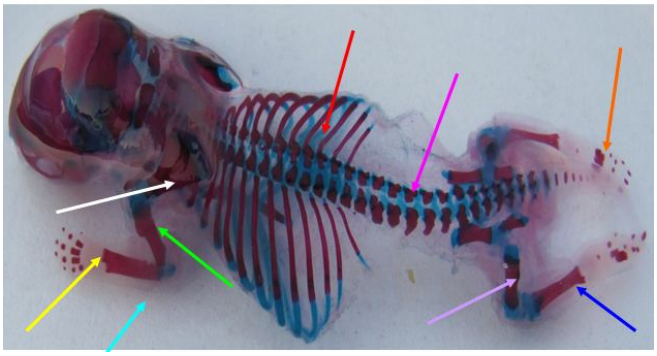

Fig-11 Photograph of fetal skeleton from group 3 stained with Alcian Blue a Alizarin Red-S, showing bony skeleton of normal architecture viz. Scapula (white arrow),
Humerus (green arrow), Radius & ulna (turquoise arrow), Metacarpals (yellc arrow), Ribs (red arrow), Vertebral column(Pink arrow), Femur (lavender arrow), Tibia & fibula (Blue arrow) and Metatarsals (orange arrow).

of serum GGT (IU/L) in group 1 pregnant dams at 19th day was 1.32±0.03, which was significantly ($P < 0.05$) increased in group 2 (1.77±0.39). Group 3 showed a significant ($P < 0.05$) increase (3.70±0.24) as compared to groups 1 and 2. The concentration of serum total protein (g/dl) in group 1 pregnant dams at 19th day was 6.86±0.12, which was non-significantly decreased in group 2 (6.67±0.09).

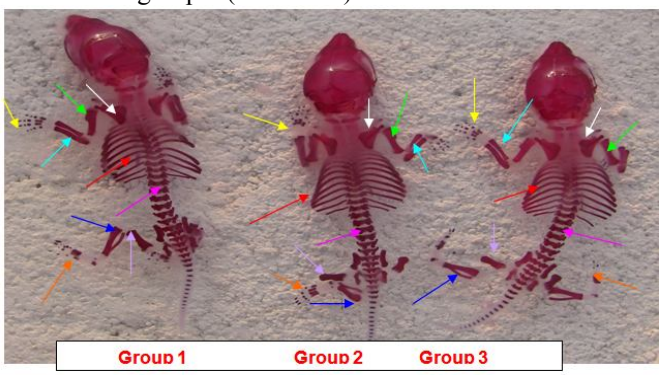

Fig-12 Photograph of fetal skeleton from groups 1, 2 & 3 stained with Alizarin Red-S, showing bony skeleton of normal architecture viz. Scapula (white arrow), Humerus (green arrow), Radius & ulna turquoise arrow), Metacarpals(yellow arrow), Ribs (red arrow), Vertebral column (Pink arrow), Femur (lavender arrow), Tibia & fibula (Blue arrow) and Metatarsals (orange arrow).

The group 3 showed a significant ($P < 0.05$) decrease (6.16±0.07) as compared to groups 1 and 2.

The concentration of blood urea nitrogen (mg/dl) in group 1 pregnant dams at 19th day was 20.87±0.79, which was significantly ($P < 0.05$) increased in group 2 (27.48±1.52). The group 3 showed a significant ($P < 0.05$) increase (42.36±1.48) as compared to groups 1 and 2.The concentration of serum creatinine (mg/dl) in group 1 pregnant dams at 19th day was 0.625±0.01, which was non-significantly increased in group 2 (0.710±0.01). Group 3 showed a significant ($P < 0.05$) incre ase (0.955±0.06) as compared to groups 1 and 2.

The hepatotoxicity of nimesulide has been linked to oxidative stress, generation of ROS caused damage to hepatocytes (Sarkar *et al*., 2005), and immunological and metabolic idiosyncratic reactions induced pathogenetic mechanism (Thawani *et al*., 2003), which was responsible for alteration in serum biochemical profile (Ramesh *et al*., 2001; Chatterjee *et al*., 2008 and Sozer *et al*., 2011). The mean concentration of GSH (uM/mg protein) in kidney and liver revealed a significant ($P < 0.05$) decrease in group 2 (21.34±2.54 and 25.39±3.25, respectively) as compared to group 1 (27.95±4.69 and 30.76±3.61, respectively) pregnant dams at 19th day. The group 3 showed a significant ($P < 0.05$) decrease in GSH as compared to groups 1 and 2.The mean concentration of TBARS (nM of MDA/mg protein) in kidney and liver revealed a significant ($P < 0.05$) increase in group 2 (1.96±0.06 and 0.41±0.01, respectively) as compared to group 1 (1.77±0.04 and 0.33±0.03, respectively) pregnant dams at 19th day. Group 3 showed a significant ($P < 0.05$) increase in TBARS as compared to groups 1 and 2 (Table 2). These results suggest peroxidative stress and compromised antioxidant defense mechanisms. Sarkar *et al*. (2005) reported that nimesulide has the ability to provoke massive oxidative stress *in vivo*. Liver has the unique property of regeneration by itself after any drug and toxins injury. Nimesulide is exclusively metabolized and cleared by the liver (Davis *et al*., 1994). Hence, its injury can be induced by the exposure to various toxicants and by a number of drugs when taken very frequently or beyond therapeutic doses. These toxicants mainly damage liver by producing reactive oxygen species (Slater, 1995). These reactive oxygen species (ROS) have been

implicated in nimesulide-induced adverse effects including hepatotoxicity (Sozer *et al*., 2011). The mechanism of nimesulide-induced hepatic injury seems to involve generation of ROS, causing oxidative stress to hepatocytes as proposed by Sozer *et al*. (2011). Results of the present study indicated that nimesulide induced oxidative insult and suggested that the oxidant defense of the body has been suppressed effectively by nimesulide. The mean litter size (number) in group 1 pregnant dams at 19th day of gestation was 10.50±1.05, which was non-significantly decreased in group 2 (10.00±1.06). The group 3 showed a non-significant decrease in litter size number (8.83±0.60) as compared to groups 1 and 2.The mean live and dead number in group 1 pregnant dams at 19th day of gestation was 10.00±0.85 and 0.50±0.34, respectively, which was non-significantly decreased in group 2 (9.50±0.88 and 0.50±0.34, respectively). Group 3 showed a non-significant decrease in live and dead number (8.33±0.33 and 0.50±0.34, respectively) as compared to groups 1 and 2.

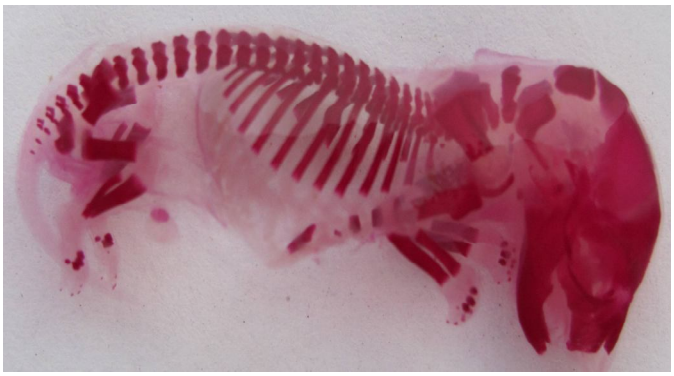

Fig-13 Photograph of fetal skeleton from group 1 stained with Alizarin Red S showing normal skeleton without any external variation.

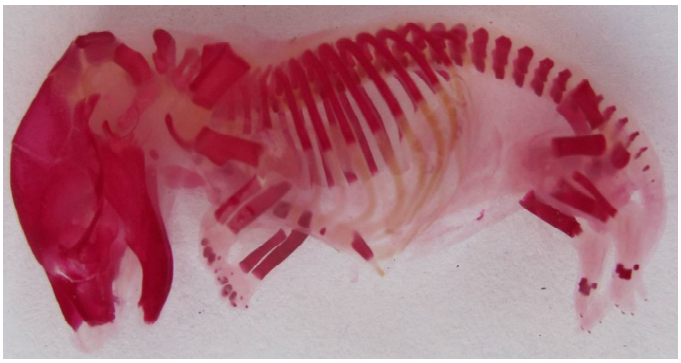

Fig-14 Photograph of fetal skeleton from group 2 stained with Alizarin Red S showing normal skeleton without any external variation.

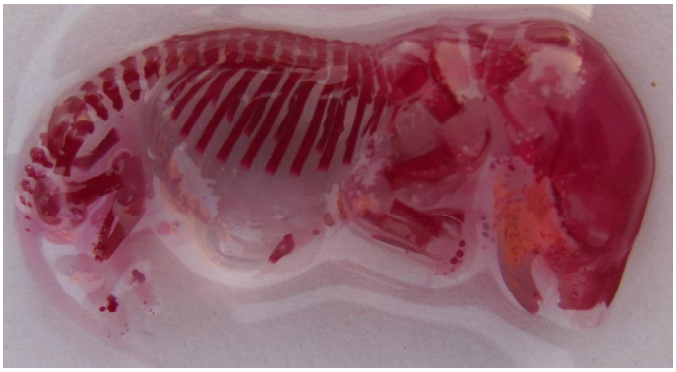

Fig-15 Photograph of fetal skeleton from group 3 stained with Alizarin Red S showing normal skeleton without any external variation.

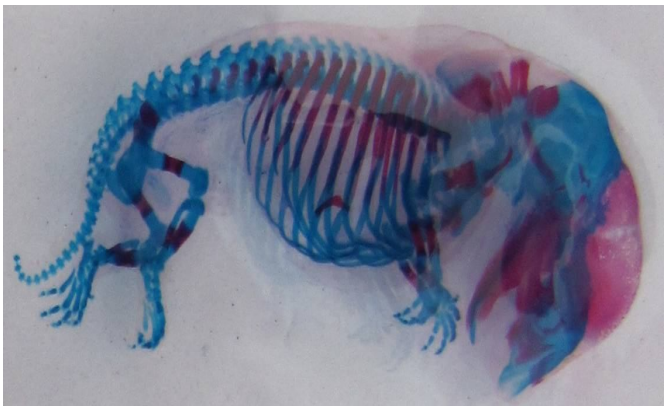

Fig-16 Photograph of fetal skeleton from group 1 stained with Alcian Blue with Alizarin Red S showing normal skeleton without any external variation

Fig-17 Photograph of fetal skeleton from group 2 stained with Alcian Blue with Alizarin Red S showing normal skeleton without any external variation

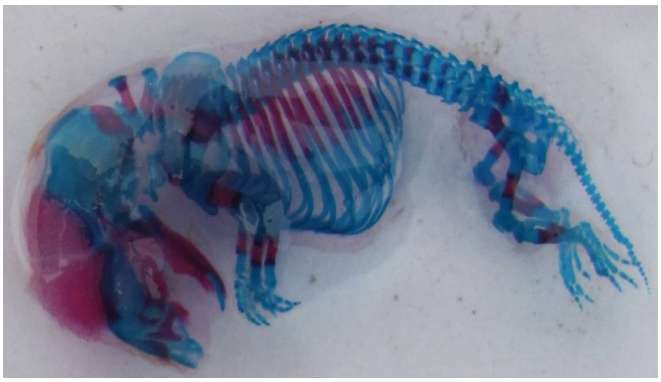

Fig-18 Photograph of fetal skeleton from group 3 stained with Alcian Blue with Alizarin Red S showing normal skeleton without any external variation

The mean male and female number in group 1 pregnant dams at 19th day of gestation was 4.83±0.70 and 5.16±0.70, respectively, which was non- significantly decreased in group 2 (4.66±0.49 and 5.33±1.25, respectively). Group 3 showed a non-significant decrease in male and female progeny number (4.66±0.95 and 5.00±0.73, respectively) as compared to groups 1 and 2. The mean gravid uterine body weight (g) in group 1 pregnant dams at 19th day of gestation was 42.30±1.66, which was non-significantly decreased in group 2 (41.70±2.33). Group 3 showed a non-significant decrease in uterine body weight with progeny (37.75±0.98) as compared to groups 1 and 2 (Table 3).

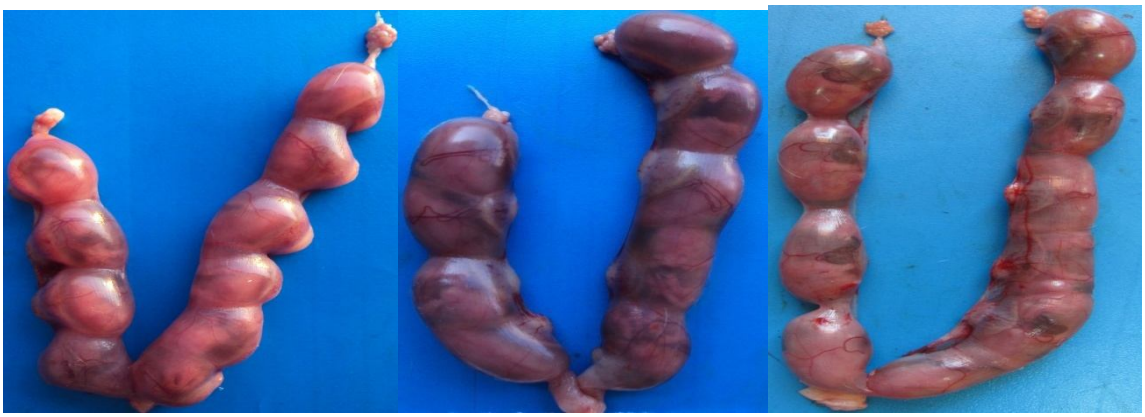

Fig-19 Fig-20 Fig-21

Fig-19 Photographs of rat uterus with ovary sacrificed on 19th day of gestation showing 10 number of fetus with no resorption sites in group 1.
Fig-20 Photographs of rat uterus with ovary sacrificed on 19th day of gestation showing 9 number of fetus with no resorption sites in group 2.
Fig-21 Photographs of rat uterus with ovary sacrificed on 19th day of gestation showing 10 number of fetus with no resorption sites in group 3

Table1 Sero-biochemical parameters in different groups of rats

Group	ALP (IU/L)	AST (IU/L)	ALT (IU/L)	GGT (IU/L)	BUN (mg/dl)	Creatinine (mg/dl)	Total Protein (mg/dl)
Control	239.36±10.75[a]	223.50±7.64[a]	22.27±0.73[a]	1.32±0.03[a]	20.87±0.79[a]	0.625±0.01[a]	6.86±0.12[a]
Nimesulide @ 20mg/kg body weight	382.30±10.10[b]	296.41±6.23[b]	28.70±0.81[b]	1.77±0.39[b]	27.48±1.52[b]	0.710±0.01[a]	6.67±0.09[a]
Nimesulide @ 60mg/kg body weight	515.58±43.86[c]	408.41±4.75[c]	43.07±1.14[c]	3.70±0.24[c]	42.36±1.48[c]	0.955±0.06[b]	6.16±0.07[b]

Values are Mean±SE (n=12); One way ANOVA (SPSS); Means with different alphabets as superscripts differ significantly (P<0.05)

Table 2 Concentration of TBARS and GSH in liver and kidney in different groups

Group	Liver		Kidney	
	TBARS (nM of MDA/mg protein)	GSH(µM /mg protein)	TBARS (nM of MDA/mg protein)	GSH (µM /mg protein)
Control	0.33±0.03[a]	30.76±3.61[a]	1.77±0.04[a]	27.95±4.69[a]
Nimesulide @ 20mg/kg body weight	0.41±0.01[b]	25.39±3.25[b]	1.96±0.06[b]	21.34±2.54[b]
Nimesulide @ 60mg/kg body weight	0.51±0.03[c]	15.27±2.98[c]	2.40±0.07[c]	20.78±1.27[b]

Values are Mean±SE (n=12); One way ANOVA (SPSS); Means with different alphabets as superscripts differ significantly (P<0.05)

Table 3 Parameters pertaining to progeny of different groups of dams

Group	Inborn progeny body weight (g)	Litter size number	Live and dead number		Male and female progeny number		Uterine weights with progeny (g)
			Live	Dead	Male	Female	
Control	3.06±0.32[a]	10.50±1.05[a]	10.00±0.85[a]	0.50±0.34[a]	4.83±0.70[a]	5.16±0.70[a]	42.30±1.66[a]
Nimesulide @ 20mg/kg body weight	2.82±0.33[a]	10.00±1.06[a]	9.50±0.88[a]	0.50±0.34[a]	4.66±0.49[a]	5.33±1.25[a]	41.70±2.33[a]
Nimesulide @ 60mg/kg body weight	2.51±0.10[a]	8.83±0.60[a]	8.33±0.33[a]	0.50±0.34[a]	4.66±0.95[a]	5.00±0.73[a]	37.75±0.98[a]

Values are Mean±SE (n=12); One way ANOVA (SPSS); Means with different alphabets as superscripts differ significantly (P<0.05)

The histological sections of the liver from group 2 revealed mild hydropic degeneration of hepatocytes (Fig-1) with mild central vein congestion and bile duct hyperplasia (Fig-2) and group 3 revealed focal areas of lymphoid aggregates (Fig-3), marked central vein congestion and bile duct hyperplasia (Fig-4), whereas sections from group 1 did not reveal any lesions of pathological significance. The histological sections of the kidney from group 2 revealed mild inter tubular congestion and haemorrhages (Fig-5), hyaline casts and few tubules showing mild degenerative changes (Fig-6), and group 3 revealed moderate inter tubular haemorrhages (Fig-7) and moderatedegenerative changes in tubular epithelium (Fig-8), whereas sections from group 1 did not reveal any lesions of pa thological significance.Gross photographs of progeny in Alcian Blue + Alizarin Red S skeleton staining showed no skeletal abnormalities in scapula, humerus, radius , ulna, metacarpals, ribs, vertebral column, femur, tibia , fibula and metatarsals in groups 2 and 3 (Fig-10,11) as compared to group 1 (Fig-9). Gross photographs of progeny in Alizarin Red S skeleton staining showed no skeletal abnormalities in scapula, humerus, radius, ulna, metacarpals, ribs, vertebral column, femur, tibia, fibula and metatarsals in groups 1, 2 and 3 (Fig-12).Photographs of fetal skeleton stained with Alizarin Red S (Fig-13, 14, 15) and Alcian Blue + Alizarin Red S from groups 1, 2 and 3 (Fig-16, 17, 18) showed normal skeleton without any external variation.

The number of resorption sites was evaluated insacrificed rats (Fig-19, 20, 21). There were no resportion sites in groups 1, 2 and 3, which showed 10, 9 and 10 viable foeti, respectively.

SUMMARY

In conclusion, the study revealed that treatment with nimesulide @ 20 and 60 mg/kg body weight showed no teratogenicity. There was a significant difference in sero-biochemical profile of dams. Treatment with nimesulide at high dose induced oxidative stress and tissue damage of liver and kidney as evident from increased levels of MDA and decreased levels of GSH, histopathology of liver and kidney.

References

Balasubramaniam, J. 2000. Nimesulide and neonatal renal failure. Lancet. 355, 575.

Burdan, F. 2005. Comparison of developmental toxicity of selective and non-selective cyclooxygenase-2 inhibitors in CRL :(WI)WUBR Wistar rats – DFU and piroxicam study. Toxicology. 211, 12-25.

Burke, A.; Smyth, E.; Fitzgerald, G. A. 2005. Analgesic-antipyretic agents: Pharmacotherapy of gout. *In* : Brunton LL, Lazo, JS, Parker KL, eds. Goodman and Gilman's The Pharmacological Basis of Therapeutics.

Chatterjee, S.; Pal, J.; Biswas, N. 2008. Nimesulide-induced hepatitis and toxic epidermal necrosis. Journal of Postgraduate Medicine. 54, 150-151.

Davis, R. ; Brogden, R. N. 1994. Nimesulide: An update of its pharmacodynamic and pharmacokinetic properties, and therapeutic efficacy. Drugs. 48, 431-54.

Dawson, A. B. 1926. A note on the staining of skeleton of clear specimens with Alizarin Red S. Stain Technology. 1, 123-124.

Farid, B.; Mariella, V.; Patricia, G. M.; Christophe, B.; Veronique, M.; Umberto, S. 2006. Effects of maternally administered drugs on the fetal and neonatal kidney. Drug Safety. 29, 397-419.

Goyal, P. K.; Chandra, J.; Unnikrishnan, G.; Sudarshan, K. ;Passah, S. M. 1998. Double blind randomized comparative evaluation of nimesulide and paracetamol as antipyretics. Indian Padiatrics. 35, 519-522.

Humason, G. L. 1967. Animal Tissue Techniques. W H Reeman and company, San Francisco pp: 179-181.

Ostensen, M. 2006. Antirheumatic therapy and reproduction. The influence on fertility, pregnancy and breast feeding. Rheumatol. 65, 217-220, 222-224.

Ramesh, N.; Jaykumar, K.; Honnegowda; Narayana, K.; Vijaysarathi, S. K. 2001. Nimesulide toxicity in dogs. Indian Journal of Pharmacology. 33, 217-218.

Saillant, G. Infocus. Nimesulide Gel. Information accessed from www.nimesulide.net/pdf/Nimesulide%20Gel%20In% Focus.pdf As on 11/08/09.

Sarkar, M. K.; Sarkar, K.; Bhattacharjee, R.; Chatterjee, M.; Sil, P. C. 2005. Curative role of the aqueous extract of the herb, *Phyllanthus niruri*, against nimesulide induced oxidative stress in murine liver. Biomedical Research. 16, 171-176.

Sbeit, W.; Krivoy, N.; Shiller, M.; Farah, R.; Cohen, H. I.; Struminger, L. 2001. Nimesulide-induced acute hepatitis. Annals of Pharmacotherapy. 35, 1049-1052.

Singh, U. B.; Sulochana, S. 1997. Handbook of Histological, Histochemical Techniques. 2nd Edn., Premier Publishing House, Hyderabad, India.

Slater, D.; Berger, L.; Moore, G. E.; Bennett, P. 1995. Changes in the expression of types 1 and 2 cyclo-oxygenase in human fetal membranes at term. American Journal of Obstetrics and Gynecology. 172, 77–82.

Sozer, S.; Ortac, R.; Lermioglu, F. 2011. An investigation of toxicity potential of nimesulide in juvenile rats. Turkish Journal of Pharmaceutical Sciences. 8, 147-158.

Suleyman, H. Demircan, B.; Karagoz, Y. 2007. Anti-inflammatory and side effects of cyclooxygenase inhibitors. Pharmacological Reports. 59, 247-258.

Tan, H. H.; Ong, W. H. C.; Lai, S. H.; Chow, W. C. 2007. Nimesulide-induced hepatotoxicity and fatal hepatic failure. Singapore Medical Journal. 48, 582.

Thawani, V.; Sontakke, S.; Gharpure, K.; Pimpalkhute, S. 2003. Nimesulide: The current controversy. Indian Journal of Pharmacology. 35, 121-122.

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