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BEHAVIOURAL AND HISTOPATHOLOGICAL ALTERATIONS - INDUCED BY DIFFERENT DOSES OF NON STEROIDAL ANTI-INFLAMMATORY DRUG (INDOMETHACIN) IN RATS

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SUMMARY

A total of eighty albino adult rats (64 females and 16 males) were divided into four groups each contain 16 females and 4 males. The first group (I) was served as control untreated group; the other three groups (II, III and IV) were daily intramuscularly injected with different doses of indomethacin (0.5, 1 and 2 mg/kg body weight) respectively for two weeks after one-week post service. The animals subjected to behavioural, haematological and histopathological studies. Behavioural measurements revealed statistical differences among the groups for the maternal behaviour and the body weight of pups, however, there are no statistical differences between the groups for the physical performance of the pups. Haematological examination revealed significant changes in the measured haematological parameters of rats treated with high dose of indomethacine (group IV). Microscopical examination of liver revealed prominent hepatocellular vacuolations; portal infiltration with mononuclear leucocytic cells as well as multifocal areas of hepatic necrosis associated with mononuclear cells infiltration. The kidney showed different necrobiotic changes, focal interstitial nephritis as well as marked shrunken and atrophied glomeruli. Moreover, the brain showed marked meningeal hemorrhage and congestion. Examined testis revealed congestion of intertubular blood vessels together with marked destruction and necrosis of spermatogoneal and sertoli cells lining seminiferous tubules. In conclusion, This study claimed that the usage of indomethacin could alter the expression and delays the rapid onset of maternal behaviour as well as induced deleterious histopathological changes .

Key words: Non Steroid anti-inflammatory drugs - indomethacin ñ behaviour- haematology histopathology - rats.

INTRODUCTION

Non-steroid anti-inflammatory drugs (NSAIDs) are amongst the most commonly prescribed medications in the world attesting to their efficacy as anti-inflammatory, anti-thrombotic, antipyretic and analgesic agents (Davies, 1999). During the past 30 years, there has been a substantial increase in the number of clinically available NSAIDs. However, their use was associated with extensive side effects. The wide range of therapeutic applications for NSAIDs from the simple sprain to the management of chronic inflammatory conditions like Rheumatoid arthritis and joint inflammatory conditions extend the use of NSAIDs from one day to few months or even years (Sachin Manocha and Venkatoraman, 2000).

NSAIDs can generally prevent prostaglandin synthesis from arachidonic acid by inhibiting the activity of prostaglandin synthesizing enzyme, cyclooxygenase. These products have potent biological activities affecting cell function in every organ. The high levels of NSAIDs also inhibit the activities of various enzymes, the proteoglycan synthesis from chondrocytes, the ionic exchange rate and the processes depending on prostaglandins. (Gulsen et al, 2003).

Indomethacin belongs to a chemical subgroup of NSAIDs and is currently the most common non-steroidal anti-inflammatory drug used in treatment of preterm labour. As the process of labour resembles inflammation with prostaglandin and cytokine production both before and during labour, anti- inflammatory drugs therefore have the potential to prevent preterm labour. Indomethacin is the only tocolytic drug proven to delay delivary beyond 37 weeks and reduce the incidence of low birth weight infants (< 2500 g) (Loudon etal, 2003). Patent ductus arteriouses (PDA) is a frequent complication in preterm infants so far intravenous indomethacin is standard mode of medical therapy in such patients (Sharma et al., 2003). However, the usage of indomethacin could induce variable dose and time dependent deleterious effects in many different tissues include liver, kidney and testis (Hemieda and Abdel- Hady (1998) and Rahmy and Ramadan (1998)).

Although the efficiency of indomethacin in prevention of preterm labour is documented, few are known about its effect on maternal behaviour and its side effects.

The aim of this study was to investigate the effect of different doses of indomethacin (NSAIDs) on maternal behaviour as well as histopathological alterations induced in different organs of rats.

MATERIAL AND METHODS

Animals:

A number of eighty albino adult rats (64 females and 16 males), weighing 200- 250 gm were used

in this study. Placing one male in a cage with four females over night for 3 consecutive days was used for bred female rats. Mating was confirmed by presence of vaginal plugs on the following morning (day 1 of pregnancy). Pregnant rats were then selected and placed in individual cages. Wood shaving was provided as nesting material. After parturition pups were weighed on days 0, 7, 14, 21 and 28.

Housing:

Females were housed in cages (40x20x18cm). All rats were maintained under 12:12 hr reversed day/ night cycle and relative ambient temperature of 20- 25c. relative humidity was between 50- 70%. Commercial rat diet and water were available ad libitum.

Experimental design:

The animals were divided into four equal groups each contain 16 females and 4 males. The first group (I) was served as control untreated group; the last three groups (II, III and IV) were daily intramuscularly injected with different doses of indomethacin (Sigma- Aldrich, Milan, Italy) (0.5, 1 and 2 mg/kg body weight) respectively for two weeks after one week from service (Tawfek, et al., 1996)

Animals' groupings, dose of indomethacin and route of administration are summarized in table 1.

Table (1) showing animal groups, the number of females and males in each group, dose of indomethacin, rout and time of administration:

Groups	Number of animals.	Number of females	Number of males	Dose of indomethacin. Mg/kg	Route and time of administration
I	21	16	4	Control	Daily Intramuscular Injection for 2 weeks after 1 week post service
II	21	16	4	0.5	
III	21	16	4	1	
IV	21	16	4	2	

Behavioural measurements:

Females in each cage were observed daily just before parturition and then for 15 days following parturition. Two times of observation were chosen at 8 A.M. and 2P.M. for each period 15 minutes were taken in observation (30 minutes for each dam daily), each observation was instantaneous and a note was made, either whether dams were in or out of the

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Nest building:

Nest building behaviour, resulting in an organized nest arises shortly before parturition and continuous for the first two weeks of litter care, then declines and disappears. Nest buildings were classified into four grades, excellent (+3), good (+2), fair (+1) and poor (0).

2- Physical contact:

Based on five scales, four points if the dam was in contact with all pups (excellent), three points if the dam was in contact was in contact with all pups except one or two (good), two points if the dam was in contact with three pups (fair), one point if the dam was in contact with only one pup or two pups (poor) and no points if she was in contact with any of them.

3- Retrieving:

After delivery the female pick up and carries to the general zone of parturition the newly born fetuses that rolled to a distance from it. Points were given as follows, four points if the pups were arranged in one pile (excellent), three points if the pups were arranged in a pile except for a single stray (good), two points if the young were grouped into two piles (fair), one point if there was one pile and two or three strays (poor) and no point if the pups were scattered.

4- Nursing behaviour (posture and time):

Nursing behaviour was recorded on bases of four scores. Score four (excellent), the dam is in nursing posture, score three (good), the dam was in partial nursing posture, score two (fair) the dam was not in nursing posture, but was relaxed or sleep on her side or she was in partial nursing posture, but actively eat or drink and score one (poor or non), the dam was not in nursing posture and moving about the cage. Nursing time was also recorded in minutes.

Performance of newly born pups:

Growth rate during the first 4 weeks of life.

Development of pups (fair growth, teeth eruption and opening of ear and eye).

Hematological studies:

Blood samples were collected from the dam short-

ly after parturition on anticoagulant for hematological profile, determining total erythrocytic (RBCs) count, total leucocytic count, differential leucocytic count, packed cell volume (PCV) and hemoglobin levels (Hb%) according to methods described by Natt and Herrick (1952).

Histopathological studies:

Specimens from liver, kidney, brain and testis were taken directly after scarification of examined animals (parturated females as well as males). The samples were fixed in 10% neutral buffered formalin, dehydrated in alcohol, cleared in xylene and embedded in paraffin. 4-6u thick sections were prepared and stained with Hematoxylin and Eosin according to Bancroft et al (1996).

Statistical analysis:

Data obtained from the determined behavioural and hematological parameters are presented as mean ± standard deviation. Significance of the results was evaluated by using student's test between control and treated groups according to Parker, (1979).

RESULTS

1- Behavioural measurements:-

Table (2): Incidence of maternal parameters in parturient female rats

Gp.	Physical contact		Nest building		Retrieving		Nursing post.		Nursing time		Cannibalism
	1st.wk	2nd. wk	1st. wk	2nd. wk	1st. wk	2nd wk	1st. wk	2nd wk	1st. wk	2nd.wk	0
I	77 ±1.8	65 ±3.1	75[a] ±4.3	67 ±3.2	80 ±3.8	70 ±2.9	78 3.6±	67[a] ±4.1	9.3 ±1.7	5.3[a] ±1.3	0
II	68 ±2.4	53 ±3.2	67[a] ±2.3	58 ±2.6	68 ±3.2	58 1.5±	66 ±3.1	54[a] 3.7±	7.7 ±1.4	3.4[b] ±1.2	0
III	57 2.1±	46 ±2.5	17[b] ±1.4	52 ±2.2	60 ±2.7	52 ±1.8	50 ±2.4	29[b] ±2.9	5.3 ±1.1	2.9[c] 0.8±	0
IV	0	0	0	0	0	0	0	0	0	0	100

Means ± (SEM) with different letters within each column are significantly different at ($p<0.05$).

I= control II= 0.5-mg/ kg bw III=1 mg/kg bw IV= 2mg/kg bw

Table (3): Physical performance of pups

Group	Opening ear (day)	Fur growth (day)	Teeth erup. (Day)	Opening eye (day)
I	2 ± 0	3 ±0.4	8 ±0.7	12 ±0.9
II	2 ± 0	5 ±0.5	9 ± 0.5	13 ±0.7
III	2 ± 0	5 ± 0.4	10 ±0.8	14± 0.5
IV	-	-	-	-

Means ± (SEM) there are no statistically differences between the groups for the physical performance of the pups.

Table (4): Body weight of pups.

Body weight / Group	At birth	1st week	2nd week	3rd week	4th week
I	5.9± 0.5	8.1 ± 1.1	16.8±2.9 [a]	18.3±2.2 [a]	23.8±1.6 [a]
II	5.9 ± 0.4	7.1± 0.9	9.5±1.8 [b]	12.4±1.3 [b]	20.5±1.4 [b]
III	4.6 ± 0.2	5.3±0.7 [b]	8.8±2.1 [b]	11.7±1.5 [b]	19.3±1.3 [b]
IV	-	-	-	-	-

Means ± (SEM) with different letters within each column are significant at ($p<0.05$).

Table (5): The effect of indomethacine on haematological parameters of rats:

Group	Hb (g%)	PCV (%)	RBCs (cell/ul)	WBCs (cell/ul)	Differential leucocytic count (%)		
					Lymphocyte	Neutrophil	Monocyte
I	12.1 ±1.5 [a]	42 ± 0.7	18.300 ±2.4	10640 ±0.3 [a]	64 ±3.2 [a]	32 ±2.1 [a]	4 ± 0.6
II	11.3 ±1.3	44 ± 1.3	16.200 ± 2.1	11400± 1.5	62 ±3.1	32 ± 2.6	6± 0.8
III	10.6± 1.5	46 ± 1.1	15.600 ± 1.8	12000± 1.7	58 ±2.4	36 ±3.4	6± 0.8
IV	7.9 ±0.4 [b]	46± 1.5	12.350 ±1.2 [b]	12110 ±1.9 [b]	54 ±2.1 [d]	38 ±3.6 [c]	8± 0.7

Means ± (SEM) with different letters within each column are significant at ($p<0.05$).

2- Haematological results:-

The measured haematological parameters including Hb%, RBCs count and lymphocytes% of rats belong to group IV were significantly decreased whereas the total WBCs count as well as neutrophils % were significantly increased. While, groups II and III showed no significant changes in all measured haematological parameters as presented in table (5)

Histopathological results:-

The most prominent histopathological alterations were observed in liver, kidney, brain and testis.

Liver:-

This study revealed dose- dependant histopathological changes in the liver of treated rats when compared to control. Liver of rats from group II (0.5 mg/kg body weight) showed mild changes confined as granular degeneration of hepatocytes,

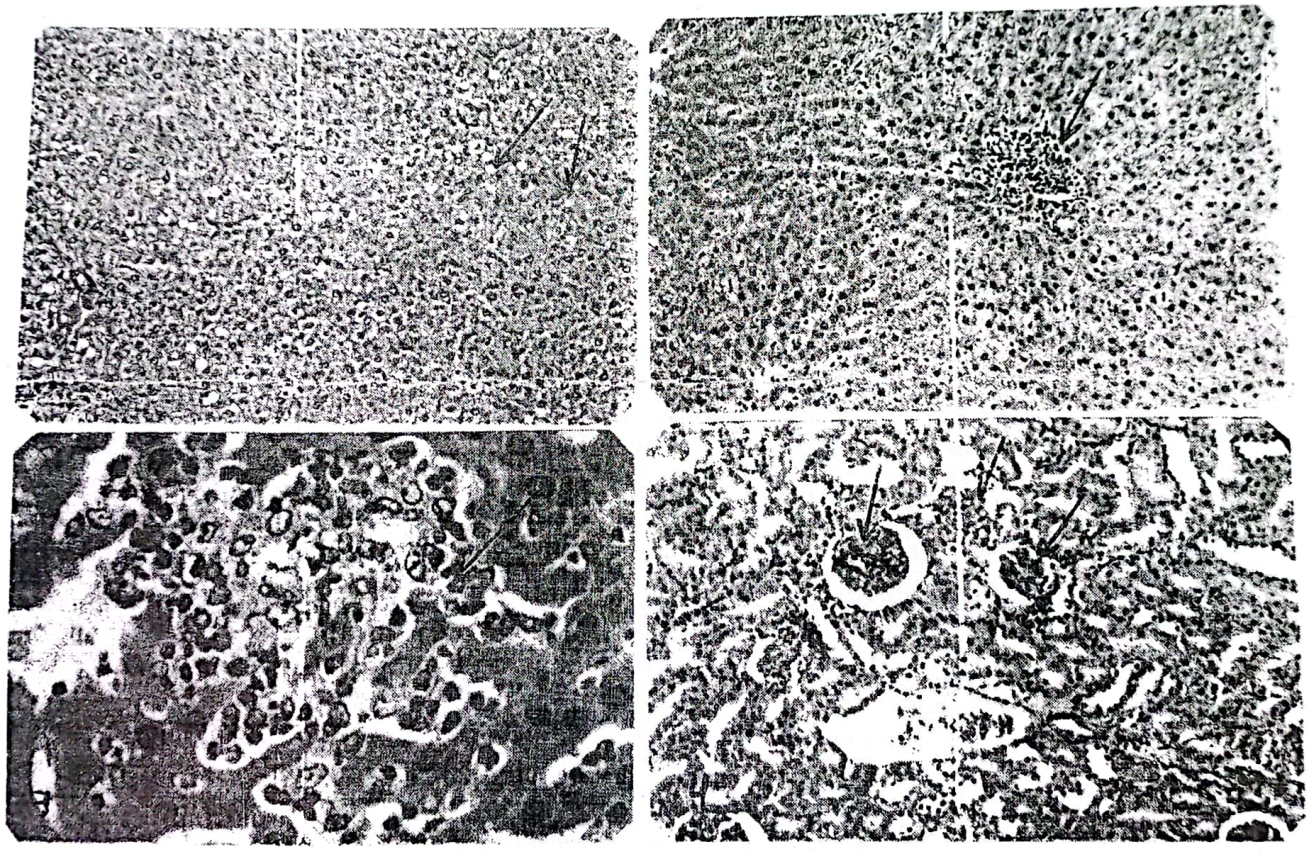

Fig. (1): Liver of indomethacin treated rat from group II showing congested hepatic sinusoids as well as vacuolar degeneration of hepatocytes (arrows) (H&E X100).
Fig. (2): Liver of indomethacin treated rat from group IV showing Kupffer cell activation and portal infiltration with mononuclear cells (arrow) (H & E X 100).
Fig. (3) Liver of indomethacin treated rat from group IV showing focal area of hepatic necrosis associated with mononuclear cells infiltration (arrow) (H& E X400).
Fig. (4): Kidney of indomethacin treated rat from group II showing marked congestion of the glomerular tufts (arrows) (H & E X 100).

congested central veins and hepatic sinusoids as well as Kupffer cell activation. In addition, prominent hepatocellular vacuolations (Fig. 1) was observed in liver of animals from group III (1.0 mg/kg b w).

Examination of liver from group IV (2.0 mg/kg b w) revealed Kupffer cell activation, hepatocellular vacuolation and portal infiltration with mononuclear leucocytic cells (Fig. 2) as well as multifocal areas of hepatic necrosis associated with mononuclear cells infiltration (Fig. 3).

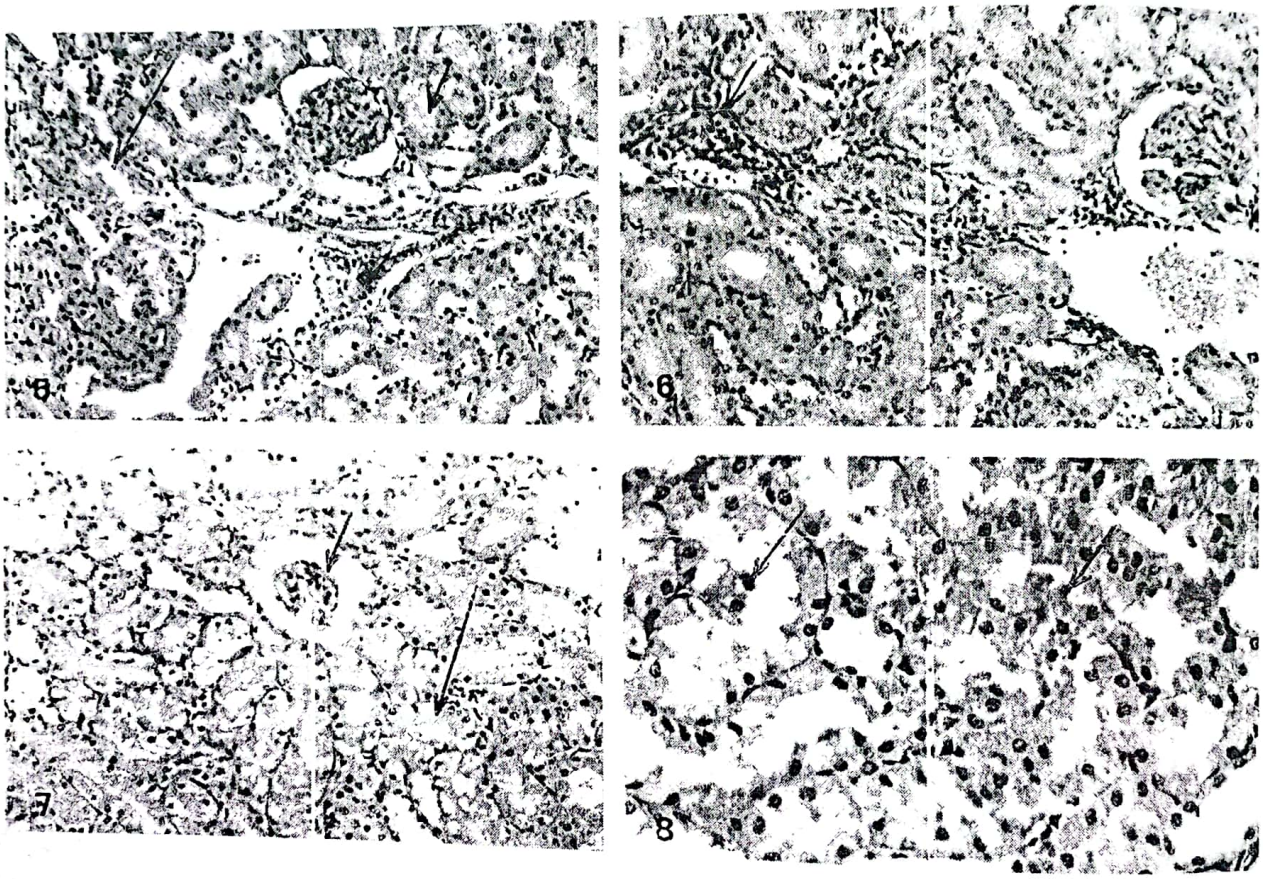

Fig. (5): Kidney of indomethacin treated rat from group III showing eosinophilic-flocculated renal casts in some renal tubules (small arrow) as well as focal area of renal hemorrhage (large arrow) (H & E X 200).
Fig. (6): kidney of indomethacin treated rat from group III showing Focal interstitial nephritis (arrow) (H & E X 200).
Fig. (7): Kidney of indomethacin treated rat from group IV showing shrunken and atrophied glomeruli, (small arrow) necrobiotic changes of renal tubular epithelium with marked vacuolation of their cytoplasm (large arrow) (H & E X 200).
Fig. (8): Kidney of indomethacin treated rat from group IV showing marked necrosis and destruction of the renal tubular epithelium as well as deposition of golden brown pigments (arrows) (H & E X 400).

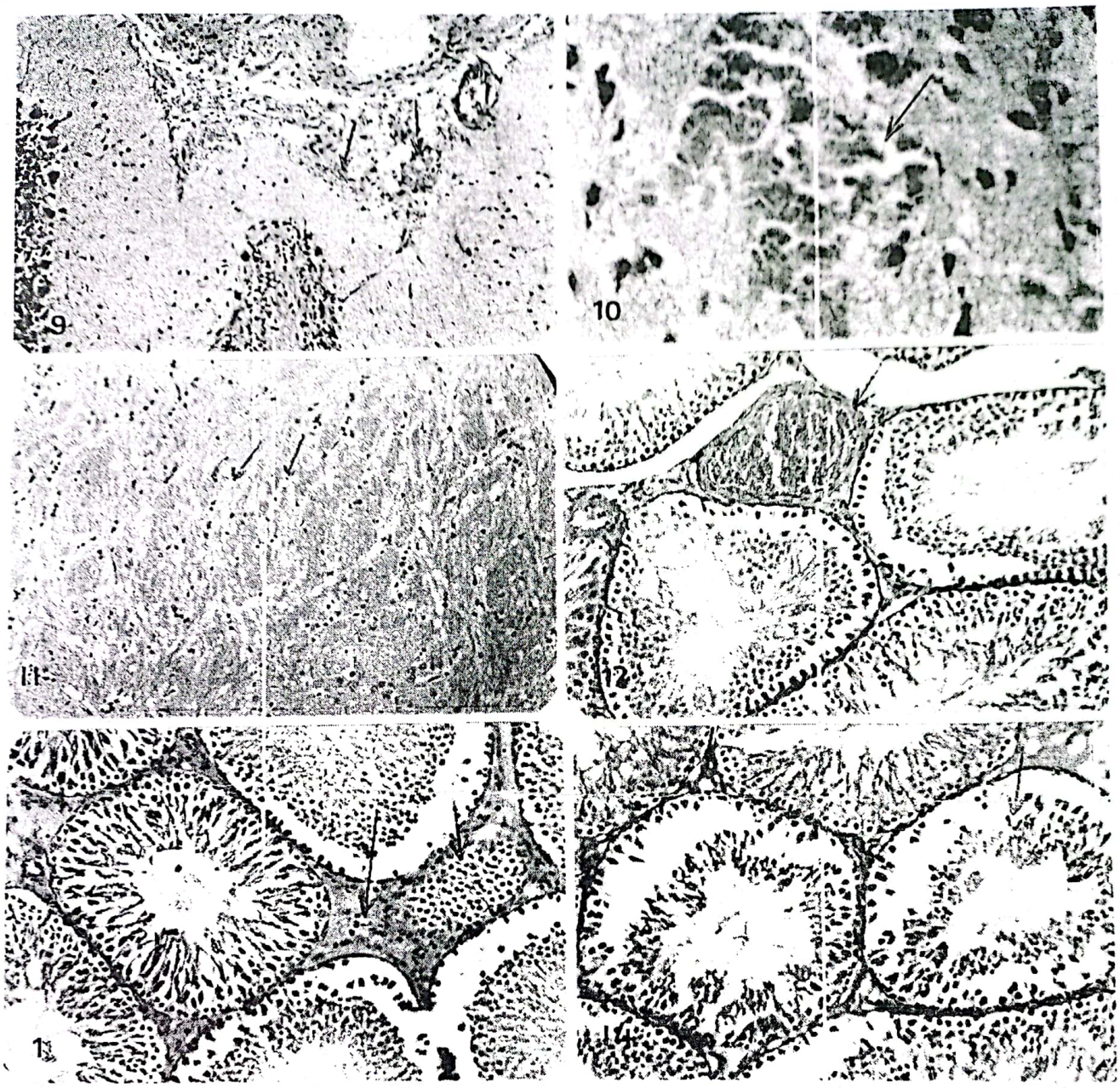

Fig. (9): Brain (cerebellum) of indomethacin treated rat from group IV showing marked meningeal hemorrhage associated with dilatation and congestion of the meningeal blood vessels (arrows) (H & E stain X 100).
Fig. (10): Brain (cerebrum) of indomethacin treated rat from group IV showing focal hemorrhage (arrow) (H & E X400).
Fig. (11): Brain (cerebrum) of indomethacin treated rat from group IV showing demyelination of some nerve fibers (arrows) (H & E X100).
Fig. (12): Testis of indomethacin treated rat from group II showing congestion of intertubular blood vessels (arrow) (H & E X 200).
Fig. (13): Testis of indomethacin treated rat from group III showing focal interstitial hyperplasia (small arrow) as well as edema (large arrow) (H & E X 200).
Fig. (14): Testis of indomethacin treated rat from group IV showing marked destruction and necrosis of spermatogoneal and sertoli cells lining the seminiferous tubules (arrow) (H & E X200).

Kidney:-

Kidney of rats treated with low doses of indomethacin (groups II and III) showed severe congestion of renal blood vessels and glomerular tufts (Fig. 4). The renal tubular epithelium showed different necrobiotic changes with dark pyknotic nuclei accompanied with eosinophilic-flocculated renal casts in some renal tubules as well as focal area of renal hemorrhage were also observed (Fig. 5). Focal interstitial nephritis was noticed in some examined cases (Fig. 6). Kidney of animals treated with high dose of indomethacin (group IV) showed marked shrunken and atrophied glomeruli, which associated with widening and dilatation of Bowmanís space (Fig. 7). The renal tubules suffered from necrobiotic changes with marked vacuolation of their cytoplasm and karyopyknosis or even karyolysis of their nuclei, some renal tubules showed marked necrosis and destruction as well as deposition of golden brown pigments (Fig. 8).

Brain:-

Brain of rats from group II & III showed no histopathological alterations. Meanwhile, the brain of animals from group IV showed marked meningeal hemorrhage associated with dilatation and congestion of the meningeal blood vessels covering the cerebellum (Fig. 9). The cerebrum showed focal hemorrhages (Fig. 10) and neuronal degeneration. Demylination of some nerve fibers was noticed in some examined animals (Fig. 11).

Testis:-

Testis of animals from group II showed congestion of intertubular blood vessels (Fig. 12) as well as slight degeneration of spermatogenic epithelium lining some seminiferous tubules. Moreover, examined sections from group III revealed intertubular edema with homogenous eosinophilic edematous fluid between the seminiferous tubules accompanied with focal interstitial hyperplasia (Fig. 13). In addition, marked destruction and necrosis of spermatogoneal cells and sertoli cells lining seminiferous tubules was observed in examined animals from group IV (Fig. 14).

DISCUSSION

The nest has functional value in that young were packed together in the nest, thus helping to maintain body warmth and enhancing survival value of the species (Deneberg et al., 1969). These artrical species require more protection since they are born relatively naked, and mother sitting above the young to form heated roof for them (Payne, 1976).

Table (2) showed that dams of group (I) (control group) had the best nest building among groups. The percentages of nest building for group I, II, III during 1st and 2nd weeks were 75, 67, 17% respectively for 1st week and were 67, 58, 52% respectively for 2nd week. The statistical analysis

indicated a significant difference between groups I, II and III at (P< 0.05). These results were in agreement with the findings of Gilbert et al., 2002 who recorded that indomethacin treated pigs showed less nest building than controls between 1 and 5 hrs after injection, during which time they were mostly inactive and lay down for longer than controls. He suggested that indomethacin treatment reversibly and specifically inhibits procine pre-partum nest building by mechanism that may involve endogenous prostaglandin F2 alpha synthesis inhibition but is independent of circulating oxytocin, cortisol and progesterone concentrations.

Physical contact is needed for direct contact with young establishment of post-partum maternal responsiveness and survival of neonate (Findley, 1966, Jakubowski and Terket, 1986 and Mervat and Jakeen, 1993). From the results in table (2), it is found that the control group had the highest score percent of physical contact during 1st and 2nd weeks (77, 65%) respectively. The statistical analysis manifested no significant difference between groups. These findings were in agreement with those of Bridges, 1984 and Robert et al., 2001.

Pup retrieval behaviour is regulated by multisensory processes and can be considered as a chain of motor response elicited by a variety of stimuli emanating from female and/ or pups which promotes orientation, attention and arousal (Stern, 1990). The initial stimuli induce proximity of dam and pups and perioral trigeminal stimulation elicits the retrieval and grouping of the pups in the nest (Stern and Kolunie, 1889 and Stern, 1990). A variety of factors can affect pup retrieval behaviour, including pharmacological and environmental manipulation of mothers or pups (Giodano et al., 1990 and Mamm et al., 1990). The present findings clearly demonstrate that, the dams of group I showed also the highest score percent of retrieving behaviour during 1st and 2nd weeks (80, 70%) respectively, while statistical analysis showed no significant difference among groups during 1st and 2nd weeks. It has been suggested that time spent in nest by dams is mainly regulated by heat production by the dams as she crouches over pups (Lean et al., 1978). In addition, as cited by Stern and Lonstein 1996, the heat flow between dam and litter occurs only from dam to pups and not vice versa as proposed by Lean et al., 1978.

The results indicated that the dams of control group showed the highest nursing behaviour (posture and time) toward their pups during 1st and 2nd week and statistical analysis revealed that there was a significant difference among group I and groups II and III (at P < 0.05) during 2nd week. It was also found that group I consume a longest time for nursing their pups than other

groups during 1st and 2nd week. Mervat and Jakeen, 1993 indicated that dams that consume long time for suckling their neonate with adequate crouching posture, to facilitate the process of suckling, would give healthy and strong pups. Moltz 1975 described the mother crouching over young in such way to expose her mammary region and adjusts her posture allowing attachment with nipples and remains entirely passive to sustain attachment. Concerning cannibalism, this behaviour is not known with certainty but maternal hormones imbalance may play an important role (Schardein, et al., 1978). Group IV that was injected with 2mg/ kg body weight showed 100% of cannibalism to their pups shortly after birth and didnít perform any maternal parameters. This confirmed by the histopathological findings in the brain of those animals (group IV), which showed marked meningeal hemorrhage, associated with dilatation and congestion of the meningeal blood vessels. The cerebrum showed focal hemorrhages and neuronal degeneration as well as demylination of some nerve fibers was noticed.

The most important physical parameters were demonstrated in tables (3 & 4), table (3) showed no statistically difference between the groups for physical performance of the pups. It was found that the average body weight of pups from group I from birth till 4th week of age was the highest and there was significant difference among groups during 1st; 2nd and 3rd weeks of life. It was suggested that higher body weight of pups might be due to more time spent nursing by dams of group I compared to other dams of groups II and III. This findings was disagree with that of Loudon et al., (2003) who suggested that indomethacin is only tocolytic drug proven to delay delivery beyond 37 weeks and to reduce the incidence of low birth weight. The rapid onset of maternal behaviour in newly parturient rat is stimulated in parturation, by the hormonal changes accompanying pregnancy. The parturient female displays full complement of maternal behaviour at birth and in some cases before parturation (Rosenblatt 1967 and Slotnick et al., 1973). Bridges and Ronsheim 1990 demonstrated that the rapid onset of maternal behaviour induced by steroid treatment in virgin rats could be delayed by the systemic administration of non-steroid treatment, which suppress endogenous prolactin receptor secretion.

In the present study, groups II and III showed no significant changes in all measured haematological parameters. These findings agreed with Andonova, et al., (1998) in that indomethacin did not significantly influence the haematological parameters. Also with Villegas, et al., (2002) who concluded that all the haematological parameters obtained after administration of NSAIDs for 14 and 28 days were in the range of normal values, meanwhile, in this study the animal group treated with high dose of indomethacin showed significant variation in their hematological parameters.

This may be attributed to the usage of high dose of the drug.

From histopathological point of view, in this investigation, the liver showed dose- dependent histopathological changes. Group II showed mild changes confined as granular degeneration of hepatocytes, congested central veins and hepatic sinusoids as well as Kupffer cell activation. In addition, prominent hepatocellular vacuolations was observed in liver of animals from group III. In the fourth group the changes seen were more severe: there were portal infiltration with mononuclear leucocytic cells as well as multifocal areas of hepatic necrosis associated with mononuclear cells infiltration. Those changes were similar to that described by Tawfek, et al., (1996) and with Hassounah et al., (1995) who described that indomethacin treatment led to distortion of hepatic cell arrangement as well as marked cellular infiltration and congestion. They claimed that those changes could be attributed to the toxic effect of indomethacin, inhibition of prostaglandins and weakness of the immunological cells of the liver. Although, the association of NSAIDs with liver disease is poorly documented and reports on hepatic injury have ranged from insignificant and transient liver enzyme elevation to severe and fluminant hepatitis (Manoukian and Carson 1996). The toxic effect of anti-inflammatory drugs on hepatocytes may be caused by drug- induced mitochondrial impairment together with a futile consumption of nicotinamide adenine dinucleotide phosphate (NADPH) (Bort, et al., 1999).

Kidney is another organ affected by the toxic effects of NSAIDs (Palmer, 1995 and Clive and Stoff 1984). It is known to be an important target organ for the untoward effects of NSAID, which can produce acute, reversible or permanent effects (Brune, and Lindner 1992; Abromson, and Weissmann 1988; and Kappus, 1986). The NSAIDs adversely change the kidney functions as during their metabolism the number of reactive oxygen species can be increased. These products induced prooxidative damage in renal tissue (Gokcimen, et al., 2001 and Gokcimen, et al., 2000).

In this investigation, the renal tubular epithelium showed different necrobiotic changes with dark pyknotic nuclei accompanied with eosinophilic-flocculated renal casts in some renal tubules as well as focal area of renal hemorrhage were observed. Marked shrunken and atrophied glomeruli were also noticed. This results were in agreement with Hemieda and Abdel- Hady (1998) who showed renopathy displayed shrunken and atrophied glomeruli and degeneration of the epithelial lining the convoluted tubules and with Hassounah et al., (1995) in that indomethacin caused marked congestion and cellular infiltration. Moreover, Mahmoud et al., (1994) investigated that indomethacin induced certain destructive alterations in

the fine structure of the kidney cells and tissues. They added that, in the renal corpuscles, the glomerular capillaries were dilated and their lumina were filled with flocculent materials.

In this study, testes of animals from group II showed congestion of intertubular blood vessels as well as slight degeneration of spermatogoneal cells. Sections from group III revealed intertubular edema with homogenous eosinophilic edematous fluid between the seminiferous tubules accompanied with focal interstitial hyperplasia In addition, marked destruction and necrosis of spermatogoneal cells was observed in animals from group IV. The present results were in agreement with Rahmy and Ramadan (1998) who concluded that the usage of indomethacin could induce variable dose and time dependant deleterious effects in the testicular tissues and consequently, it lead to suppression of spermatogenesis, thus affecting the fertility.

IN CONCLUSION

This study claimed that the usage of indomethacin could alter the expression and delays the rapid onset of maternal behaviour as well as induced deleterious histopathological changes in different organs (liver, kidney brain and testes) of both female and male rats and this indicated that caution must be taken before uses of this drug especially during pregnancy.

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