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EFFECT OF FLUNIXIN MEGLUMINE ON FERTIILITY OF MALE RATS

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SUMMARY

The effect of flunixin meglumine (FM) in doses of 4 and 8 mg/kg. b. wt. on reproduction in rats were studied. The tested doses were given daily intramuscularly to male rats for 65 days. Sex organs weights anal semen picture, testosterone, FSH and LH hormone levels as well as mating performance were the criteria used to evaluate the reporductive efficiency of treated rats.

Prolonged i. m. administration of FM for 65 days to male rats, significantly decreased the weights of most genital organs, sperm cell concentration, sperm motility and live sperm percent associated with an increase in the percentages of dead and morphological abnormal spermatozoa of treated rats. A decrease in plasma testosterone level and increase in plasma LH and FSH levles was osberved in the treated groups.

Administration of the tested drug to male rats decreased their ability to mate females. Moreover, the number of viable feti in some pregnant rats was significantly decreased as compared with that of controls.

INTRODUCTION

Flunixin meglumine is a potent anti-inflammatory drug used in many species (Houdeshel and Hennessey, 1977 and Kopcha and Ahl, 1989). It has been shown that this drug is an efficient inhibitor of the enzyme prostaglandin cyclooxygenase, resulting in decreased formation of prostaglandins and related substances (Odensvik et al., 1989). It is also a valuable tool in the studies of prostaglandin synthesis in different experimental animals (Odensvik et al., 1991). Prostaglandins have a wide range of physiological activities. The most important property of the day is that concerned with animal reporduction, in which the ability of prostaglandin-F2a cause functional and morphological regression of the corpus luteum (Morrow, 1980). Moreover, prostaglandins increased conception rate and improved the male fertility (Dimov and Georgiev, 1977 and Gustafsson et al., 1975). FM is used in the horse, cow, dog and cat and in these species detailed pharmacokinetics studies have been undertaken following intramuscular, oral and/or intravenous administration (Lees and Taylor, 1991). The present investigation is an attempt to elucidate the effects of FM on fertility of Wistar rats, since no available literature could be obtained on its reproductive efficiency

MATERIALS AND METHODS

Drug:

Flunixin meglumine (Finadyne®, Schering-Plough), was available as a sterile injectable solution containing 50 mg flunixin meglumine and 5 mg phenol as a preservative, per 1ml. Flunixin meglumine (FM) was administered via intramuscular route at 4 and 8 mg/kg. b. wt.

Animals:

1- Effect on male fertility:

Seventy five, two months old male Wistar rats weighing between 175-185g. were obtained from the Laboratory Animal Colonies, Ministry of Public Health, Helwan, Egypt. Animals were kept under hygienic conditions and fed on bread, barley, milk and water *ad libitum*. The rats were divided into three groups of 25 rats each. The first group served as a control and were treated with sterilized distilled water intramuscualr (0.5ml/kg/day), while the second and third groups were dosed intramuscularly with FM in a dose of 4 and 8 mg/kg b. wt. day, respectively. The treated groups wer dosed daily for 65 days to cover a complete spermatogenic cycle which range from 56-60 days in rats (Hershberger et al., 1969).

Rats were sacrificed by decapitation on day 65. The testes, epididymides, seminal vesicles and prostate glands were dissected out and weighed (5 rats from each group). Weights of testes and accessory glands were calculated in relation to body weight.

Blood samples were obtained from the orbital plexuses of 5 rats from each group before and at 15, 30, 45 and 65 days after administration and 21 days after stopping of FM administration. Plasma was separated by centrifugation at 3500 rpm for 15 min. The obtained plasma was assayed for testosterone, FSH and LH hormone using radioimmunoassay method by using kits of DPC (Diagnostic product corporation, LA, USA).

The epididymal content of each rat (10 rats from eah group on day 65 after administration and 21 days after stopping of FM administration) was obtained by cutting the tail of the epididymis and squeezing its contents in a sterile clean watch glass, then diluted 10 times with isotonic sodium citrate solution (2.9%) and used for determining sperm cell concentrations, percentages of live sperms, progressive motility and total sperm abnormalities as proceeded by Miller and Rass (1952).

2- Effect on female fertility:

Fifty three weeks old mature female Wistar rats weighing between 200-225 were obtained from the Laboratory Animal Colonies, Helwan, Egypt. The rats were divided into groups (10, 20 and 20 rats each) and used for the mating method. At 65 days after administration and 21 days after stopping of administration the tested drug. Male rats proven to be ferile in the control group and other treated groups (5 rats from eahc group) were placed with the mature females (previously proved to be of regular osterus cycle) for 48 hours. The number of pregnant females among each group of 10 females was recorded and the conception rate was determined. The feti from each pregnant female were also exmained morphologically (mean weight and crown rump length).

The results obtained otatined were statistically analyzed using "t" test. as explained by Snedecor (1969).

RESUTLS

Intramuscular injectionof FM in doses of 4 and 8 mg/kg. b. wt. for 65 day to male rats revelaed a significnat ($P<0.001$) decrease in the weights of testes, epididymis, prostate glands and seminal vesicles as compared to those of controls (Table 1). Intramuscular dministration of FM for 65 days to male rats significantly reduced sperm cell concentrations, live sperm and increased the percentages of total sperm abnormalities (Table 2). After stopping of treatment with 21 days, semen picture measured in group previously treated with FM in dose of 4 mg/kg. b. wt. was

reverted to a value nearly to that of non-treated control, while sperm cell concentration and total sperm abnormalities were still significantly lower than that of control values in rats previously treated with FM in a dose of 4 mg/kg. b. wt. Moreover, sperm cell concentration, live sperm and total sperm abnormalities were still significantly lower than that of control values in rats previously given FM in a dose of 8 mg/kg. b. wt. Twenty one days afterstopping of FM administtration, morphological abnormalities seen in the spermatozoa of rats given FM were characterized by detached heads, cytoplasmic droplets as well as bent and/or coiled tails.

Prolonged intramuscular administration of FM in doses of 4 and 8 mg/kg. b. wt. for 65 days to male rats revelaed a significant ($P< 0.001$) decrease in plasma testosterone levels at 30th, 45th and 65th days of administration and 21 days after stopping of treatment as compared to with control with those of control rats (Table 3).

Concerning FSH level in treated rats, it was significantly increased at 30th, 45th and 65th days stopping of administration with 4mg/kg. b. wt. and at (15th,, 30th, 45th, and 65th) days with (8mg/kg. b. wt). At 21 days after stoppage of drug administration. FSH level was significantly increased in those given higher dose LH level of rats was significantly increased at given the smaller dose 45th and 65th days of administration and at 30th and 65th increased at days of administration. In high dosage treated rats a singificant decrease in LH level was recorded in high dosage treated rats at 15th and 45th days of drug administration. At 21 days after stoppage of drug administration, the LH level was significantly decreased in low and high dosage treated rats as compared to control untreated rats

Table (1): Effect of intramuscular injection of flunixine in a dose of 4 and 8 mg/kg. b.wt./day for 65 successive days on weights of sex organs of male rats(n =5).

Groups	Dose (mg/kg.b.wt.)	Weights of sex organs (g./100g.b.wt.)			
		Testicles	Epididymis	Prostate gland	Seminal vesicles
Control group (untreated)	-	1.78 ± 0.08	0.64 ± 0.04	0.52 ± 0.05	0.76 ± 0.04
Flunixine	4	(1.62 ± 0.03***	0.59 ± 0.02***	0.36 ± 0.02***	0.65 ± 0.05***
Flunixine	8	(1.58 ± 0.02***	0.54 ± 0.02***	0.33 ± 0.01***	0.60 ± 0.04***

*** Significant at($P < 0.001$)

(Table 3).

The present study showed that intramuscualr injection of FM for 65 successive days induced impairment of the reproductive functions in treated male rats, therefore it decreased their ability to impregnate females rats (Table 4). The number of viable feti was significantly decreased as compared to that of controls. Also there was a significant decrease in the numbers of feti in pregnant rats by male 3 weeks after stopping of FM administation. Resorbed or dead feti were recorded in the group of rats impregnated by ma rats administered FM 8 mg/kg. b. wt. for successive days. On the other han morphological examinaition of delivered feti fro females impregnated by males administered FM mg/kg b. wt. in a dose of for 65 successive da and 21 days after stopping of its administratio showed that the malformations were significantl reduced (Table 4). These fetal malformation were of lower type and were expressed a shortening of crown rumps only.

Table (2): Effect of intramuscular injection of flunixine in a dose of 4 and 8 mg/kg.b.wt./day for 65 successive days and 21 days after stopping of tested drug (A) on the semen picture of male rats(n =5).

Groups	Dose (mg/kg.b.wt.)	Semen picture			
		Sperm cell concentration (10^6/ml)	Live sperm (%)	Motility (%)	Total sperm abnormality (%)
Control group	-	1.67± 0.02	96 8 ± 0.66	93.0 ± 1.10	5.40 ± 0.67
(A)	-	1.68 ± 0.02	96.4 ± 0.46	92.0 ± 1.10	5.20 ± 0.52
Flunixine	4	1.61 ± 0.03***	94.4 ± 0.22 **	83.0 ± 1.79	14.0 ± 0.63***
(A)	4	1.64 ± 0.03***	95.6 ± 0.46	87.0 ± 1.10	10.6 ± 0.83***
Flunixine	8	1.51 ± 0.02***	93.2 ± 0.34***	80.0 ± 1.41**	15.6 ± 1.00***
(A)	8	1.55 ± 0.02***	94.6 ± 0.46**	84.0 ± 1.67	11.8 ± 0.96***

** Significant at (P < 0.01).
*** Significant a (P < 0.001).

Table (3): Mean ± S.E. plasma testosterone (mg/ml), FSH(miu/ml) and LH (miu/ml) levels of male rats administered flunixine for 65 successive days and 21 days after stopping of treatment (A) (n = 5).

Types of hormones	Time (days)	Control nontreated)	Flunixine (4 mg/kg)	Flunixine (8 mg/kg)
Testosterone (mg/ml)	Before	1.23 ± 0.01	1.23 ± 0.03	1.23 ± 0.03
	15	1.38 ± 0.03	1.30 ± 0.22	1.35 ± 0.03
	30	1.48 ± 0.03	0.17 ± 0.03***	0.27 ± 0.09***
	45	1.59 ± 0.03	0.20 ± 0.01***	1.16 ± 0.07***
	65	1.62 ± 0.01	0.26 ± 0.04***	1.52 ± 0.05***
	(A)	1.68 ± 0.03	1.18 ± 0.03***	1.58 ± 0.04***
FSH (Miu/ml)	Before	1.76 ± 0.05	1.76 ± 0.05	1.76 ± 0.07
	15	1.86 ± 0.05	1.62 ± 0.32	2.28 ± 0.09***
	30	2.08 ± 0.07	2.76 ± 0.05***	4.20 ± 0.16***
	45	2.36 ± 0.07	2.66 ± 0.05***	2.94 ± 0.30***
	65	2.54 ± 0.07	3.18 ± 0.28***	3.06 ± 0.21***
	(A)	2.64 ± 0.05	2.44 ± 0.16***	2.76 ± 0.20*
LH (miu/ml)	Before	0.50 ± 0.02	0.50 ± 0.01	0.50 ± 0.01
	15	0.52 ± 0.02	0.51 ± 0.01***	0.50 ± 0.01***
	30	0.55 ± 0.02	0.50 ± 0.01***	0.70 ± 0.08***
	45	0.80 ± 0.06	1.00 ± 0.03***	0.50 ± 0.01***
	65	0.86 ± 0.06	1.04 ± 0.05***	0.88 ± 0.06*
	(A)	0.92 ± 0.07	0.84 ± 0.05***	0.76 ± 0.05***

* Significant at ($P < 0.05$) *** Significant at ($P < 0.001$)

Table (4): Effect of daily administration of flunixine at doses of 4 and 8 mg/kg b.wt. for 65 successive days on the male fertility (reproductive function) expressed by percentage pregnancy after mating and change in fetal morphology of impregnanted female rats.

Items	Control rats (nontreated)	Flunixine (4 mg/kg) (A)	Flunixine (4 mg/kg) (B)	Flunixine (8 mg/kg) (A)	Flunixine (8 mg/kg) (B)
Number of female rats (a)	10	10	10	10	10
Number of pregnant rats	8	6	7	5	6
Percentage pregnant	80	60	70	50	60
Dames-					
Number of viable fetuses	6.6 ± 0.36	5.6 ± 0.22***	6.2 ± 0.18*	5.2 ± 0.35***	5.6 ± 0.22***
Number of resorbed fetuses/mother	-	-	-	1.2 ± 0.18	-
Number of dead fetuses/mother	-	-	-	0.4 ± 0.22	-
Fetuses					
Mean weight	4.8 ± 0.34	5.2 ± 0.18*	5.0 ± 0.28	5.4 ± 0.22**	5.6 ± 0.22***
Mean croun rump length	31.0 ± 0.40	29.2 ± 0.34*	28.6±0.46***	31.4 ± 0.22	30.6 ± 0.36

4 (A) After 65 days of flunixine administration.
(B) 3 weeks after stopping of fluinxine administration.
(a) Mating trials (2 females per male in each group).

* Significant at ($P < 0.05$)
** Significant at ($P < 0.01$)
*** Significant at ($P < 0.001$).

DISCUSSION

The present study was performed to explore the effect of FM a commonly used analgesic and anti-inflammatory cyclo-oxygenase inhibitor drug . Many endogenous mediators may be involved in the phenomena of peripheral and central sensitization. Postaglandins are important mediators in the development of peripheral sensitization (Nolan, 1994). Along with work of this proved strong evidence that prostaglandins play an important role in the development of hyperalgesia and that non-steroidal anti-inflammatory drugs (NSAIDs) have an analgesic action within the CNS, distinct from thier anti-inflammatory effects (Uda et al., 1990).

The present study showed that i. m. injection of FM had a dose-dependent adverse effect on male reproduction as evidenced by a marked decrease in the weights of genital organs and semen picture. There was also an increase in the percentagesof dead and morphologically abnormal spermatozoa. These effects may be due to the inhibition of prostaglandins synthesis by FM, because prostaglandins have a wide range of physiological activities such as animal reproduction (Morrow, 1980).

The results revelaed that, i. m. administration of FM for 65 days to male rats induced a significant decrease in plasma testosterone levels and increase in FSH and LH levels in most situations of the experiment was observed in the treated groups as compared to control rats. These effects were confirmed by Laing et al., (1988), who found that marked elevation in LH and FSH is an indication that testicular failure is present. Moreover, transitory increases in prolactin, growth hormone and glucocorticoid concentration have also been observed following prostaglandin-$F_2\alpha$ administration (Morrow, 1980).

The present study showed that i. m. administration of FM for 65 days induced impairment in the reproductive functions in treated male rats, therefore it decreased their a bility to impregnate female rats. These findings were consistent with those reported by Dimov and Geogiev (1977) and Gustafsson et al., (1975), who found that addition of prostaglandins to ram semen increased conception rate and improved the fertility due to improvements in sperm treansport and in the survival rate of the cells. Moreover, there is evidence in some mammals that sperm motility can be influenced by the presence of certain levels of prostaglandins and the indications are that prostaglandins exert a protective influence on sperm motility (Schleger et al., 1980). Other workers have also reported that prostaglandins at certain concentrations can reduce sperm motility (Schleger et al., 1981 and Cohen et al., 1976). Cordon (1983), stated that the use of prostaglandins as semen additives to improve the fertility after sheep artificial insemination. Laing et al., (1988)) found that , ejaculation difficulties have been treated with beta-blockers, oxytocin or prostaglandin-$F_2\alpha$ with some success and prostaglandin-$F_2\alpha$, 5-10 min . before mating or semen collection, might stimualte erection and ejaculation.

The results revealed that i. m. injection of FM for 65 succesive days induced impairment in the reporductive functions in treated male and female pregnant rats, therefore it decreased the number of viable feti but increased the those of resorbed or dead feti and fetal malformations. These findings were consistent with those reported by Laing et al., (1988), who found that, administration of FM can prevent postaglandin release if it is

administered shortly before a conceptus is crushed, this compound and other prostaglandin synthetase inhibitors have not been shown to improve the rate of success of the embryo crushing technique.

In conclusion, in view of these findings, special attention should be payed to farm animals administered FM as their prolonged administration would affect their reproduction efficiency.

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