

## Antidepressant, anxiogenic, and antinociceptive properties of levofloxacin in rats and mice

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### Abstract

Levofloxacin, an optically active isomer of ofloxacin, is a fluorinated quinolone with a broad spectrum of antibacterial activity. Fluoroquinolones have been used for the treatment of bacterial infections for many years. Although they were considered as relatively safe drugs, various adverse effects have recently been reported along with increase in the usage of new-generation fluoroquinolones. In the present study, some of the central nervous system (CNS)-related side effects of levofloxacin were clarified in animals. Our results suggested that: levofloxacin (10–20–40 mg/kg ip) had no depression-like effect in the forced swimming test (FST) in rats; exerted anxiety-like effect in the elevated plus maze test in rats; did not alter the locomotor activity in rats; had no apparent effect on sleep latency but shortened the sleeping time on pentobarbital sleeping time in mice; and showed analgesic activity in acetic acid writhing and hot plate test in mice. © 2001 Elsevier Science Inc. All rights reserved.

*Keywords:* Levofloxacin; Forced swimming test; Plus-maze test; Pentobarbital sleep; Writhing test; Hot-plate test

The widespread use of fluorinated quinolone antimicrobials in the treatment of various bacterial infections has proven to be very effective. Levofloxacin is an optically active isomer of ofloxacin, having a broad spectrum of antibacterial activity against both Gram-positive and Gram-negative bacteria, and is generally twice as potent as ofloxacin (Eliopoulos et al., 1996; Une et al., 1988). Levofloxacin has a tolerability profile similar to that of other fluoroquinolones. The most frequently reported adverse effects are related with central nervous system (CNS) stimulation and gastrointestinal disturbances (Lode, 1999; Shimada, 1995). Some of the CNS-related side effects of fluoroquinolones are headache, dizziness, restlessness, tremor, insomnia, hallucinations, convulsions, anxiety, and depression. Akahane et al. (1989) and Tsutomi et al. (1994) suggested that quinolone-induced convulsion in mice is a result of gamma-aminobutyric acid (GABA<sub>A</sub>) receptor inhibition. In addition, levofloxacin inhibited the GABA response in a competitive and voltage-independent manner in the dissociated rat hippocampal neurons (North et al.,

1998). In further studies, levofloxacin exerted depressant activity on the CNS in mice, decreased spontaneous motor activity in mice, and produced hypothermia in mice and rabbits. It had both stimulant and depressant effects in cats (Takasuna et al., 1992). Levofloxacin was approved for use in Turkey in September 1999. Since CNS reactions are commonly encountered in patients receiving ofloxacin, this article examines whether levofloxacin, a second-generation fluoroquinolone (North et al., 1998), has similar CNS-related side effects in rats and mice.

### 1. Materials and methods

#### 1.1. Animals

Adult male Wistar rats weighing 220–250 g and Balb/c albino mice weighing 25–30 g (Animal Research Center, Kocaeli, Turkey) were used in this study. The animals were maintained in constant room temperature (21 ± 2°C) under a 12-h light/dark cycle (light onset at 0800 h). Tap water and food pellets were available ad libitum. Ethical approval was granted by the Kocaeli University of Ethics Committee (Kocaeli, Turkey).

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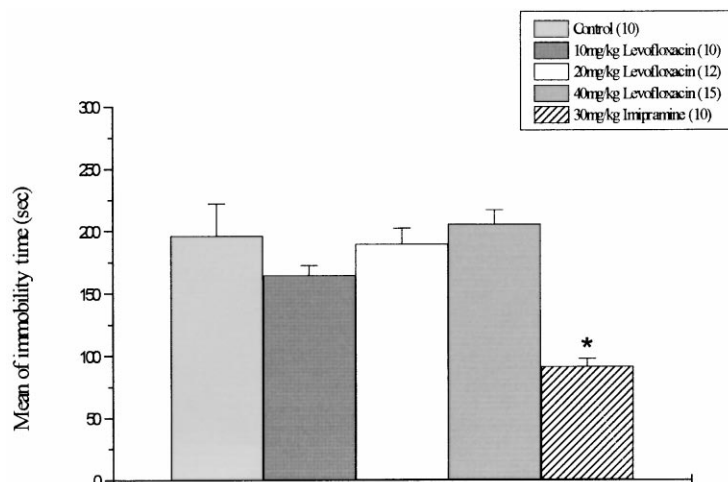


Fig. 1. The effects of levofloxacin (10, 20, 40 mg/kg) administered intraperitoneally 60 min prior to 5-min test on immobility time in the FST in rats. Each column represents the mean  $\pm$  S.E.M. for the number of animals given in parentheses. \* $P < .01$  significantly different from saline.

## 1.2. Drugs

Levofloxacin was a gift from Hoechst Marion Roussel in Turkey. Imipramine hydrochloride was purchased from Sigma (St. Louis, MO, USA) and dissolved in 0.9% physiological saline (PS). All drugs were freshly prepared and given intraperitoneally in a volume of 0.2 ml/100 g or 0.1 ml/25 g body weight of rats and mi-ce, respectively.

## 1.3. Treatments

### 1.3.1. Forced swimming test (FST)

The FST, a currently used behavioral test for the detection of antidepressants, was performed following the procedure described by Porsolt et al., 1977, 1978. The rats were placed individually in Plexiglass cylinders (40 cm in

height, 18 cm in diameter) filled with water (25°C) up to 15 cm. A 15-min preswimming period was followed 24 h later by a 5-min test period during which the total immobility time was recorded. Rats were considered immobile when they made no further attempts to escape except making only necessary movements to keep their heads above water. The absence of hindleg movement was recorded as immobility by stopwatch cumulation by a constant observer during the exposures. The water in the cylinders was changed before every trial.

### 1.3.2. Elevated plus-maze test

Anxiety-related behavior was measured by the elevated plus-maze test. The apparatus was made of wood according to the specifications reported by Pellow et al. (1985). It consisted of two open arms (50  $\times$  10 cm<sup>2</sup>) surrounded by a

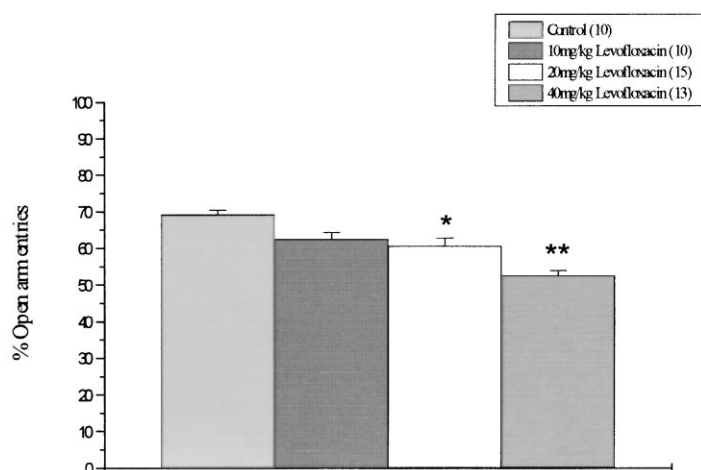


Fig. 2. The effects of levofloxacin (10, 20, 40 mg/kg) administered intraperitoneally 60 min prior to 5-min test on the number of open arm entries in the elevated plus-maze test. Each column represents the mean  $\pm$  S.E.M. for the number of animals given in parentheses. \* $P < .05$ , \*\* $P < .01$  significantly different from saline.

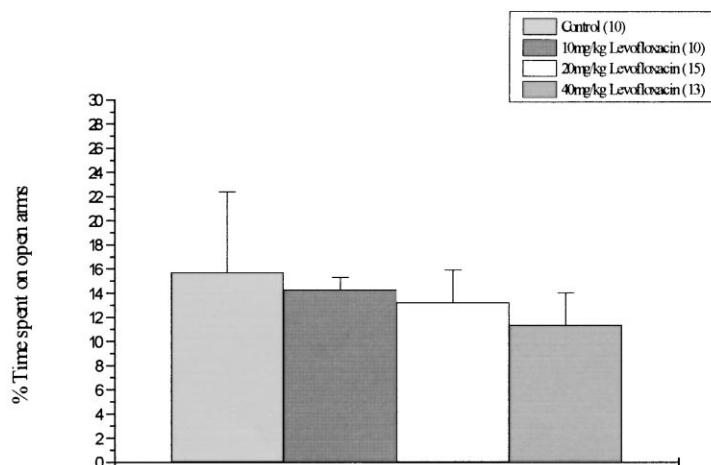


Fig. 3. The effects of levofloxacin (10, 20, 40 mg/kg) administered intraperitoneally 60 min prior to 5-min test on the time spent in the open arms in the elevated plus-maze test. Each column represents the mean  $\pm$  S.E.M. for the number of animals given in parentheses.

short (1 cm) edge to avoid falls and two enclosed arms ( $50 \times 10 \times 40 \text{ cm}^3$ ) arranged such that two open arms were opposite to each other. The maze was elevated to a height of 50 cm above the floor.

Each rat was placed at the center of the maze, facing one of the open arms, and was allowed to explore the maze. During a 5-min test period, the number of entries into either open or enclosed arms of the maze (defined as the entry of all four limbs into arms) and the time spent on open arms were recorded. The open arm activity was evaluated as: (1) time spent in the open arms relative to the total time spent in the plus maze (300 s), expressed as a percentage; (2) number of entries into the open arms relative to the total number of entries into both open and closed arms, expressed as a percentage. These values were accepted as indexes of anxiety in rats (File, 1981; Handley and Mithanis, 1984; Pellow et al., 1985). Any animal that fell off the maze was excluded from the experiment.

If values for both of the measured parameters were changed in the same direction compared to control values (i.e., if both the time spent in open arms and the number of open arm entries were increased, or if both decreased) and the change in one of the parameters was statistically significant, then an effect on anxiety was considered to have occurred (File, 1981; Handley and Mithanis, 1984; Pellow et al., 1985).

### 1.3.3. Locomotor activity

We measured locomotor activity automatically with a computerized on-line open-field test for 4 min ( $40 \times 40 \times 35 \text{ cm}^3$  box; May, Commat, Ankara, Turkey). Open field was equipped with a  $15 \times 15$  matrix of infrared sensors. Rats were put in the open-field arena 30 s before starting the experiment. A printout for each session showed the ambulatory movements of the animals in the open-field box. The distance traveled in centimeters by the rats in the

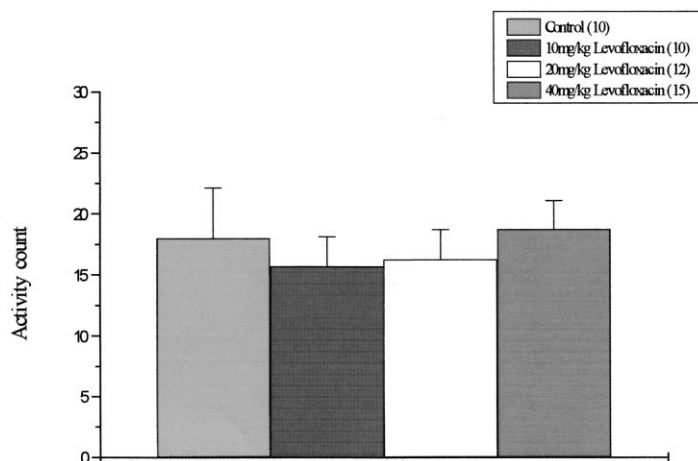


Fig. 4. The effects of levofloxacin (10, 20, 40 mg/kg) administered intraperitoneally 60 min prior to 4-min test on the locomotor activity number in rats. Each column represents the mean  $\pm$  S.E.M. for the number of animals given in parentheses.

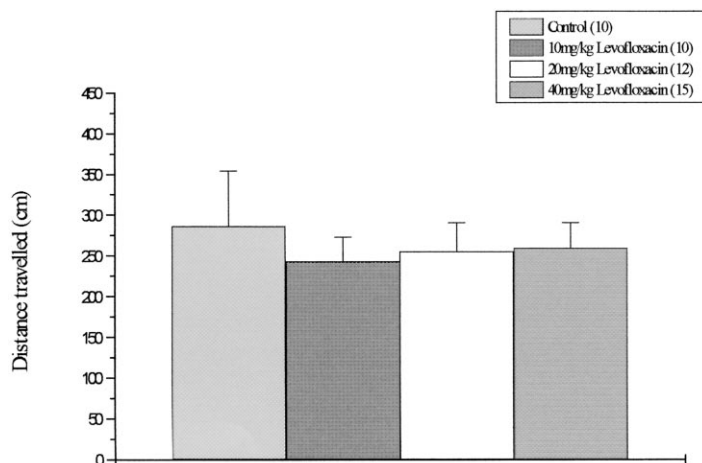


Fig. 5. The effects of levofloxacin (10, 20, 40 mg/kg) administered intraperitoneally 60 min prior to 4-min test on the distance travelled. Each column represents the mean  $\pm$  S.E.M. for the number of animals given in parentheses.

horizontal locomotor activity was analysed. In all behavioral experiments, in order to remove odors, the apparatus was cleaned with 5% ethanol solution after each test. Each rat was tested individually and only once.

#### 1.3.4. Pentobarbital sleeping time

It is suggested that the righting reflex is a useful method in assessing whether or not the animals are 'asleep' (Enginar et al., 1991; Matsumoto et al., 1996). Following the pentobarbital (35 mg/kg) injection, the time passed for the mouse to be placed on its back was recorded as the sleep latency. Sleeping time was taken as the period between the loss of the righting reflex and its return. The experiments were carried out in a quiet room in which temperature was maintained at 22–24°C during sleeping time.

#### 1.3.5. Analgesic activity

Analgesia was evaluated by using writhing movements and hot-plate test in mice.

Acetic acid was injected at 1% in a volume of 10 ml/kg ip to produce the typical writhing reaction, which is characterized by a wave of contraction of the abdominal musculature followed by extension of hindlimbs. Writhing movements were counted after 5 min of acetic acid administration and during a 15-min period. The reduction of the writhing count in relation to control is an index of antinociceptive action.

In hot-plate test, the plate temperature was  $55 \pm 0.5^\circ\text{C}$  and the maximal cutoff time was 60 s. The latency time for hindpaw-licking after exposure to the hot-plate surface was evaluated. The increase of the indicated latency time in relation to control is an index of the antinociceptive effect.

Levofloxacin (10, 20, 40 mg/kg) or PS was administered intraperitoneally 60 min prior to testing and each experimental group consisted of 10–15 animals (Fish and Chow, 1997). All experiments were performed between

1000 and 1200 h. The animals were used only once in these tests.

#### 1.4. Statistics

The statistical significance of differences between control and treatment group was determined using one-way analyses of variance (ANOVA). Post-hoc comparisons between individual groups were performed by means of the Tukey HSD test. Data are expressed as the mean  $\pm$  S.E.M. Probabilities of less than 5% ( $P < .05$ ) were considered significant.

## 2. Results

### 2.1. FST

Levofloxacin (10, 20, 40 mg/kg ip), given 60 min before testing, changed the immobility time from  $196.2 \pm 25.6$  s to  $164.6 \pm 7.7$ ,  $189.8 \pm 12.1$ , and  $205.5 \pm 11.3$  s, respectively, which were statistically insignificant (Fig. 1). However, imipramine (30 mg/kg) significantly reduced the immobility time.

Table 1  
Effect of levofloxacin on the sleep latency and sleeping time in mice

Groups	Sleep latency (min)	Sleeping time (min)
Control, saline (12)	$6 \pm 0.5$	$39 \pm 2.0$
Levofloxacin, 10 mg/kg (10)	$5 \pm 0.2$	$38 \pm 2.0$
Levofloxacin, 20 mg/kg (17)	$5 \pm 0.3$	$30 \pm 1.9$
Levofloxacin, 40 mg/kg (16)	$6 \pm 0.4$	$29 \pm 2.6^*$

The values are means  $\pm$  S.E.M. with the number of animals in parentheses.

All mice were injected sodium pentobarbital (intraperitoneal) 60 min after levofloxacin (intraperitoneal).

\*  $p < .05$  significantly different from saline.

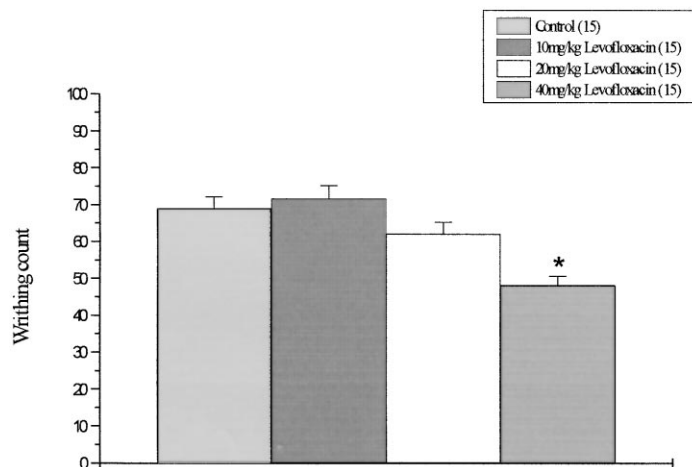


Fig. 6. The effects of levofloxacin (10, 20, 40 mg/kg) administered intraperitoneally 60 min prior to 15-min test on the abdominal writhing count in mice. Each column represents the mean  $\pm$  S.E.M. for the number of animals given in parentheses. \* $P$  < .01 significantly different from saline.

## 2.2. Elevated plus-maze test

Levofloxacin exerted anxiety-like effect in the elevated plus-maze test. Levofloxacin decreased the open arms entries, being statistically significant at 20–40 mg/kg doses. But the decrease of time spent on the open arms was statistically insignificant (Figs. 2, 3).

## 2.3. Locomotor activity

It is well known that an antidepressant-like effect in the FST and anxiety test can be also evoked by drugs which induce hyperactivity (Borsini and Meli, 1988; Maj et al., 1992); hence, the influence of levofloxacin on the locomotor activity was concurrently evaluated. Levofloxacin did not alter the locomotor activity in the

computerized on-line open field test at the doses tested (Figs. 4, 5).

## 2.4. Pentobarbital sleeping time

Levofloxacin had no apparent effect on sleep latency. However, it significantly shortened the sleeping time only at 40 mg/kg dose (Table 1).

## 2.5. Analgesic activity

In the acetic acid test, the number of writhes was  $68.8 \pm 3.2$  for a 15-min period in saline-treated mice. Levofloxacin significantly reduced this number only at 40 mg/kg dose (Fig. 6).

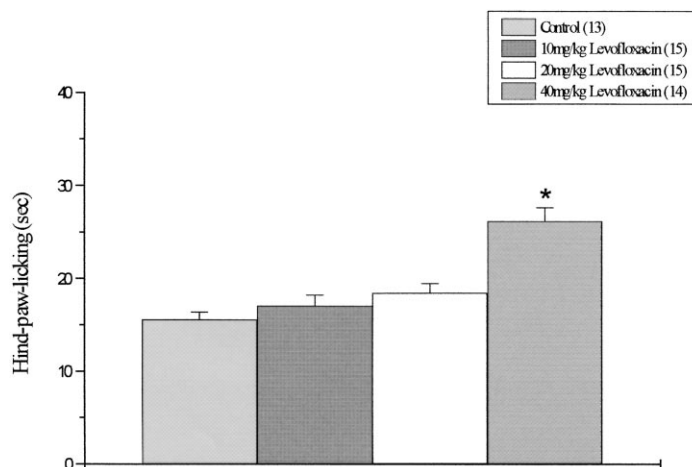


Fig. 7. The effects of levofloxacin (10, 20, 40 mg/kg) administered intraperitoneally 60 min prior to hot-plate test on the latency time in mice. Each column represents the mean  $\pm$  S.E.M. for the number of animals given in parentheses. \* $P$  < .01 significantly different from saline.

In hot-plate test, levofloxacin significantly prolonged the latency time for the licking of hindpaws at all doses used, being significant at 40 mg/kg dose (Fig. 7).

### 3. Discussion

The search for enhancing the activity of fluoroquinolones against Gram-positive and Gram-negative pathogens has led to the development of a number of new fluorinated quinolones. Although fluoroquinolones are considered as relatively safe drugs, limited adverse reaction profile has been defined during their clinical usage. It is suggested that all quinolones have CNS stimulant activity although there are interspecies differences and interquinolone variations in the intensity of CNS stimulant activity (Takasuna et al., 1992). In the present study, we have attempted to clarify some of the CNS-related side effects of levofloxacin, a new fluoroquinolone currently available commercially in Turkey.

The FST is widely used in testing depression-like activity of new compounds. Levofloxacin neither significantly changed the locomotor activity nor affected the immobility time in the FST in rats. It is stated that in the mouse behavior experiment, levofloxacin had no influence at doses up to 200 mg/kg (Takasuna et al., 1992). But a dose of 600 mg/kg caused behavioral depressant signs. Moreover, at this high dose, levofloxacin was also demonstrated to decrease spontaneous motor activity. In addition, it showed signs of behaviour stimulation at 30 mg/kg ip and both stimulant and depressant effects at doses up to 100 mg/kg ip in the cat behaviour experiments (Takasuna et al., 1992). In general, levofloxacin is shown to be devoid of depression-like side effects.

The elevated plus maze, an animal model of anxiety, is widely used as a pharmacological and physiological test (Pellow et al., 1985). Our results indicated that levofloxacin exerted anxiogenic-like effect in rats. In addition, levofloxacin shortened the sleeping time on pentobarbital-induced sleep in mice as examined in a previous study with ofloxacin (Enginar et al., 1991). Takasuna et al. (1992) stated that levofloxacin had little or no effect on hexobarbital-induced sleeping time after oral administration of 60–600 mg/kg in mice. Fluoroquinolones have an inhibitory effect on the receptor binding of GABA<sub>A</sub> and thus exert a CNS stimulant action (Akahane et al., 1994; Imanishi et al., 1995). It has been shown that ofloxacin and the L-isomer, levofloxacin, each hardly inhibited GABA response in the rat hippocampal neurons (Imanishi et al., 1995). Moreover, levofloxacin was less effective than ofloxacin in inhibition of GABA response. This may be attributed to the finding that levofloxacin binding affinity to the GABA receptors is weaker than ofloxacin (Imanishi et al., 1995). Clinical studies also reveal less frequent CNS side effects with levofloxacin compared to ofloxacin (Lode, 1999).

Although inhibition of GABA receptor binding is strongly believed to be a possible explanation for the observed CNS effects including sleep disorders and anxiety with fluoroquinolones, further studies will be needed confirming that GABA receptor mechanism is involved.

Treatment with certain antibiotics can decrease pain sensation. Levofloxacin produced analgesic activity at 40 mg/kg. Takasuna et al. (1992) also showed that levofloxacin significantly decreased the number of writhes in mice. Several mechanisms may exist in the analgesic effects of antibiotics: (1) a depressive action on calcium currents; (2) blocking an activity related to pain sensation at some central level; or (3) producing a stress effect responsible for the analgesic effect (Yildiran et al., 1997). The detailed mechanisms underlying the antinociceptive effect of levofloxacin need to be clearly determined. The knowledge that levofloxacin has analgesic properties could be useful in the choice of this drug for the treatment of patients with painful infections.

### 4. Conclusion

The authors presume that these CNS-related adverse effects of levofloxacin may be evaluated by physicians.

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