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ANXIOGENIC ACTIVITY OF SOME FLUOROQUINOLONES IN ELEVATED PLUS MAZE IN RATS

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ABSTRACT
Background: Fluoroquinolones are commonly utilised antibiotics linked to harmful effects on the central nervous system, such as anxiety and various neuropsychiatric disorders.
Method: Thirty-six albino rats were allocated into six groups (n=6). Ciprofloxacin (100 mg/kg), pefloxacin (60 mg/kg), sparfloxacin (60 mg/kg), and lomefloxacin (60 mg/kg) were delivered via intraperitoneal injection. Anxiety-related behaviour was evaluated by quantifying arm entrances and the duration spent in both the open and closed arms of the "Elevated Plus Maze (EPM)".
Result: All fluoroquinolones markedly diminished open-arm entries, open-arm duration, and total arm entries, while augmenting time allocated to closed arms, signifying anxiogenic effects. Pefloxacin exhibited the most significant effect, succeeded by ciprofloxacin, while sparfloxacin and lomefloxacin demonstrated moderate effects.
Conclusion: Fluoroquinolones demonstrate considerable anxiogenic effects in rats, with pefloxacin exhibiting the highest anxiogenic potential.

KEYWORDS: Fluoroquinolones: Anxiety: Anxiogenic Activity: Elevated Plus Maze: Ciprofloxacin

1. INTRODUCTION

The pharmacotherapy of infections has been dominated by fluoroquinolones since 1990. Ciprofloxacin is considered as prototype for comparing the efficacy of never fluoroquinolones[1]. The quinolone structure proved to be boon in disguise and with further alteration of structure resulted in development of no of fluoroquinolones such as pefloxacin sparfloxacin and lomefloxacin with high potency wider spectrum of activity slower development of resistance better tissue penetrability[2].While simple quinolone structure had nothing complicated to offer nalidixic acid was considered a safe drug. But playing with simple structure not only gave better compounds but their adverse effect profile is also novel[3]A big jolt to the development of new fluoroquinolones was temafloxacin which resulted in serious and at times lethal toxicity with incidence so alarming as to withdraw it from market it is therefore warning bell for those involved in developing newer fluoroquinolones and those who like to go in for latest fluoroquinolones as part of fashionable prescribing[4]. The overall incidence of adverse reactions reported for fluoroquinolones "ranges from 4.4 to 16.5 % and that of CNS and peripheral nervous system from 0.9 to 2.1 %" .adverse reactions to fluoroquinolones are well known complication of fluoroquinolones[5]. Mild neurotoxic effects associated with fluoroquinolones encompass headache, dizziness, and anxiety. Restlessness and nightmares, along with significant neurotoxic consequences, including psychotic responses and hallucinations. Depression and convulsions are infrequent[6].
Fluoroquinolones have reported to cause anxiogenic effect in elevated plus maze, shorten pentobarbitone induced sleeping time depress locomotor activity[7]. Fluroquinolones have reported to cause anxiety dizziness and insomnia.[6] These effects of fluoroquinolones in the elevated plus maze in rat and other clinical reports suggest that fluoroquinolones may cause anxiety[8].Therefore the present study was undertaken to compare the anxiogenic potential of four fluoroquinolones used in India ciprofloxacin, pefloxacin, sparfloxacin and lomefloxacin in elevated plus maze in rats[9].
Fluoroquinolone treatment's neuropsychiatric side effects have garnered a lot of interest because of the possible influence may have on patients' well-being and safety[10]. Fluoroquinolones have the ability to cross the blood-brain barrier and disrupt the central nervous system's neurotransmitter systems, according to both experimental and clinical testing[11].Anxiety is a complicated emotional condition marked by abnormalities in behaviour, excessive worry, and terror[12].Anxiety that is brought on by drugs is a serious side effect of medication that is not always acknowledged. The possible anxiogenic effects of fluoroquinolones must be understood in order to optimise therapeutic outcomes and minimise adverse responses, as these drugs are widely prescribed around the world[13].

1.1. Research gap

Although fluoroquinolones are well known to exert a variety of negative effects on the central nervous system, including anxiety, insomnia and convulsions, the comparative evidence for the anxiogenic properties of different fluoroquinolones is limited. Most previous studies have focused on single antibiotics such as ciprofloxacin and norfloxacin, while few studies have compared commonly used fluoroquinolones (ciprofloxacin, pefloxacin, sparfloxacin, and lomefloxacin).

2. MATERIAL AND METHOD

2.1. Study design

Elevated Plus Maze (EPM) model was used in an experimental, randomized, controlled animal study to assess and compare the anxiogenic effects of certain fluoroquinolones in rats.

2.2. Experimental Animals

The study used albino rats of either sex weighing between 100 and 300 grams. Animals were housed in groups of five or six in standard polypropylene laboratory cages and maintained under typical laboratory conditions. All studies were carried out between 10:00 and 16:00 hours.

2.3. Drugs and Chemical

Ciprofloxacin (Zim Laboratories, Nagpur), pefloxacin and sparfloxacin (Wockhardt, Aurangabad), and lomefloxacin (IPCA Laboratories, Mumbai) were sourced from their respective manufacturers. Ciprofloxacin and pefloxacin were dissolved in distilled water, while sparfloxacin and lomefloxacin were dissolved in distilled water with 0.1 N NaOH.

2.4. Drugs administration and grouping

The concentration of the medication was changed to enable it to be administered at the dose rate of 0.1 mL/100 g of body weight. All drugs were injected into the abdomen. The doses selected were based on the therapeutic range of one clinical dose in humans. 36 rats were randomly divided into 6 groups (6 rats in each). The distilled water (1 mL/kg) was given to Group I while distilled water containing 0.1 N NaOH (1 mL/kg) was given to Group II and served as controls. The ciprofloxacin (100 mg/kg), pefloxacin (60 mg/kg), sparfloxacin (60 mg/kg) and lomefloxacin (60 mg/kg) group types received ciprofloxacin, pefloxacin, sparfloxacin and lomefloxacin, respectively.

2.5. Elevated Plus Maze Procedure

The "Elevated Plus Maze" was employed to evaluate anxiety-related behaviour. The apparatus comprised two open arms "(50 x 10 cm) and two closed arms (50 x 10 x 40 cm)", along with a central square platform, all elevated 50 cm above the ground. Each rat was subsequently positioned in the centre of the maze, orientated towards a closed arm, and monitored for duration of 5 minutes. An arm entry was recorded only when all four paws were within the arm.

2.6. Outcomes Measure

The quantity of open-arm entries, closed-arm entries, total arm entries, duration spent in open arms, and duration spent in closed arms was meticulously documented during the observation period. A reduction in the quantity of open-arm entries and the duration spent in open arms was construed as an indication of anxiogenic activity.

2.7. Statistical analysis

The relevance of Groups III and IV was evaluated in relation to Group I, whereas Groups V and VI were compared to Group II. "A p-value of <0.05" was judged statistically significant.

3. OBSERVATION AND RESULT

3.1. Effects of Fluoroquinolones on Anxiety Status of Rats in Elevated Plus Maze

The "Elevated Plus Maze (EPM)" was utilised to evaluate the anxiogenic effects of fluoroquinolones in rats. Anxiety was assessed by measuring the frequency of entries and the time spent in both the open and closed arms of the maze. (Given in Table 1)

Table 1: Effect of fluoroquinolones on Anxiety Related behaviour in Rats assessed by the Elevated Plus Maze (Mean ± SEM,) n = 6

Group N=6	Pre-treatment	Dose mg/Kg IP	"No Of Entries in Open arm Mean ±SEM"	No of entries in Closed arm Mean ±SEM	"Total no of entries Mean ±SEM"
I	Distilled water	1ml/Kg	3.66 ±0.38	6.94±0.34	10.60±0.76
II	Distilled Water + Drop Of 0.1N NaOH	1ml/Kg	3.72 ±0.38	7.05±0.29	10.77±0.56
III	Ciprofloxacin	100mg/Kg	2.11 ±016**	5.44±0.39*	7.55±0.51**
IV	Pefloxacin	60mg/Kg	1.11 ±0.24***	4.83±0.40**	5.94±0.45***
V	Sparfloxacin	60mg/Kg	2.33 ±0.15**	5.67±0.42*	8.00±0.40**
VI	Lomefloxacin	60mg/Kg	2.33 ±0.09**	5.72±0.41*	8.05±0.43**

Interpretation: table 1 show that in the "Elevated Plus Maze", all fluoroquinolones reduced the number of open arm, closed arm and total arm entries in comparison to the control groups, indicating increased anxiety-like behavior and decreased exploratory activity of rats. It is concluded that pefloxacin had the most significant reduction in open arm entries (1.11 ± 0.24), closed arm entries (4.83 ± 0.40) and total arm entries (5.94 ± 0.45), followed by ciprofloxacin, while sparfloxacin and lomefloxacin showed relatively weak but still significant effects. The results suggest that the fluoroquinolones have anxiogenic activity, with Pefloxacin and Sparfloxacin having the same potencies and Lomefloxacin being less potent than Ciprofloxacin.

Significance of group III & IV calculated in comparison with Group I, Significance of group V &VI calculated in comparison with Group II "*P <0.05,**p<0.01, ***P<0.001"

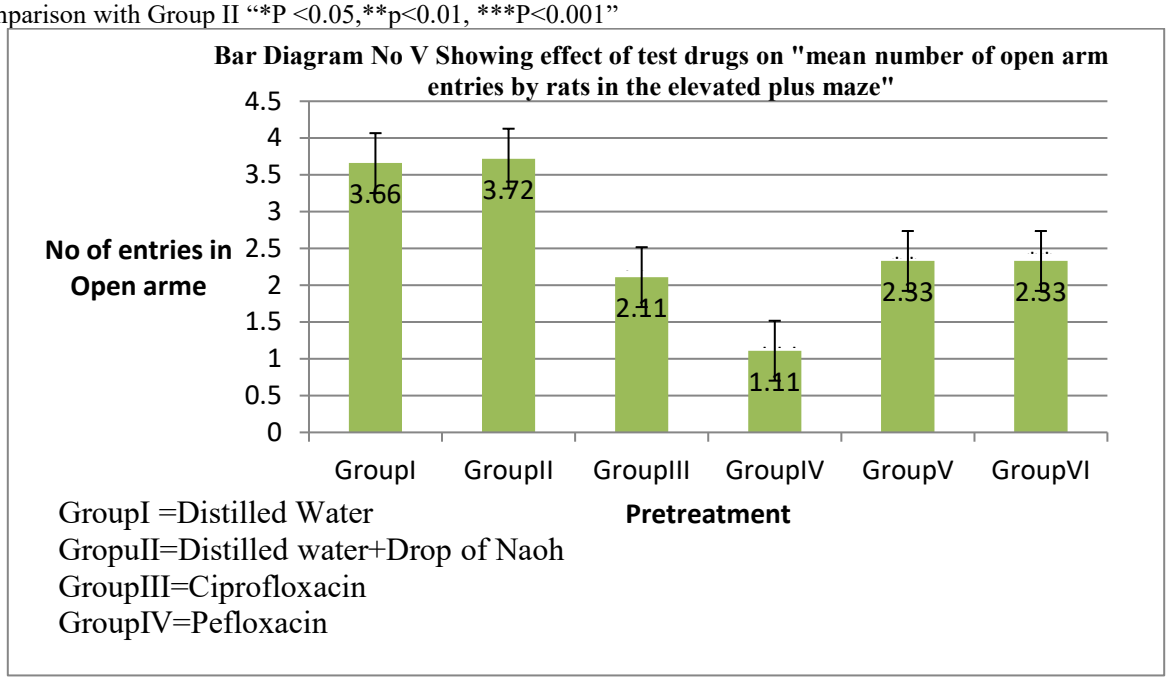

Figure 1: Effects of fluoroquinolones on the number of open Arm- Entries in the Elevated Plus Maze in Rats

Interpretation: Pefloxacin (Group IV) showed the greatest reduction (1.11; ***p < 0.001) followed by ciprofloxacin (2.11; **p < 0.01) but there were also moderate reductions for sparfloxacin and lomefloxacin (2.33 each; **p < 0.01). Pefloxacin showed the greatest anxiogenic effect

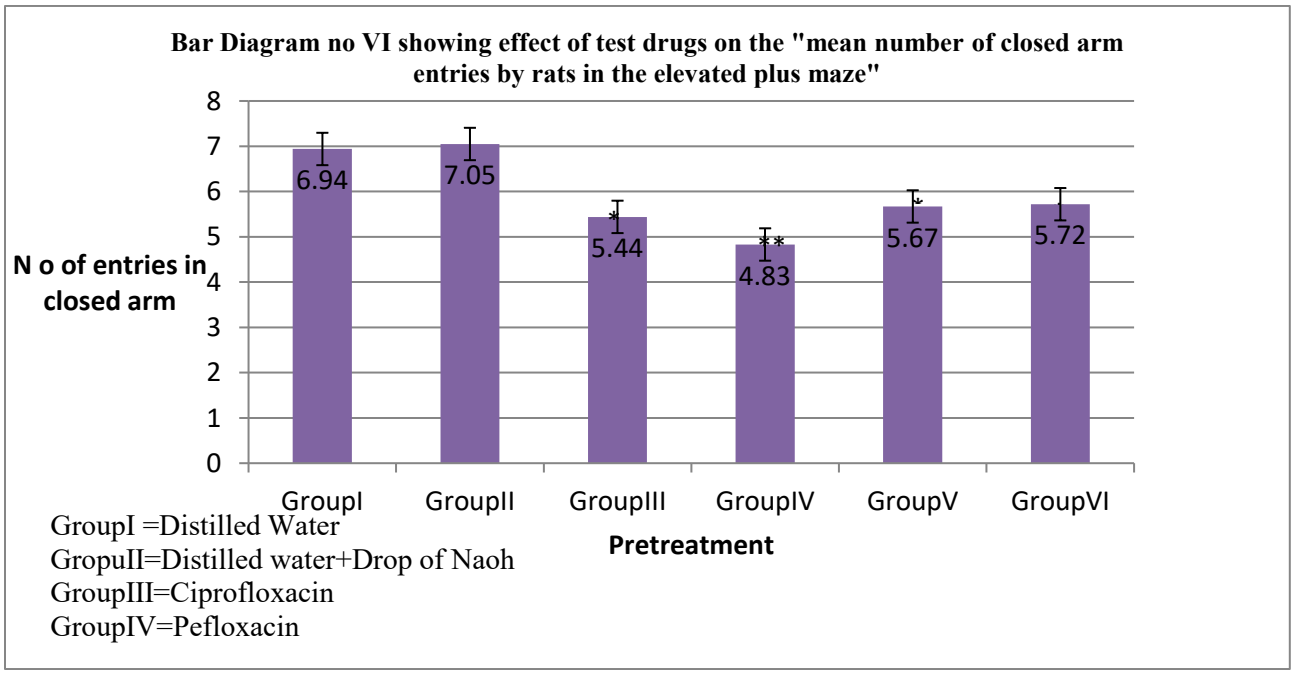

Figure 2 Effect of Fluoroquinolones on the number of closed – Arm Entries in the "Elevated Plus Maze" in Rats

Interpretation: The bar figure shows that all of the fluoroquinolones significantly reduced (P<0.05) the number of closed-arm entries compared with the control groups, which is indicative of reduced exploratory and locomotor activity in rats. Pefloxacin exhibited the most significant decrease in closed-arm entries (4.83 ± 0.40, P < 0.01), succeeded by ciprofloxacin (5.44 ± 0.39, P < 0.05), whereas sparfloxacin (5.67 ± 0.42, P < 0.05) and lomefloxacin (5.72 ± 0.41, P < 0.05) yielded very minor reductions. The order of efficacy of reducing closed arm entry was Pefloxacin > Ciprofloxacin > Sparfloxacin > Lomefloxacin.

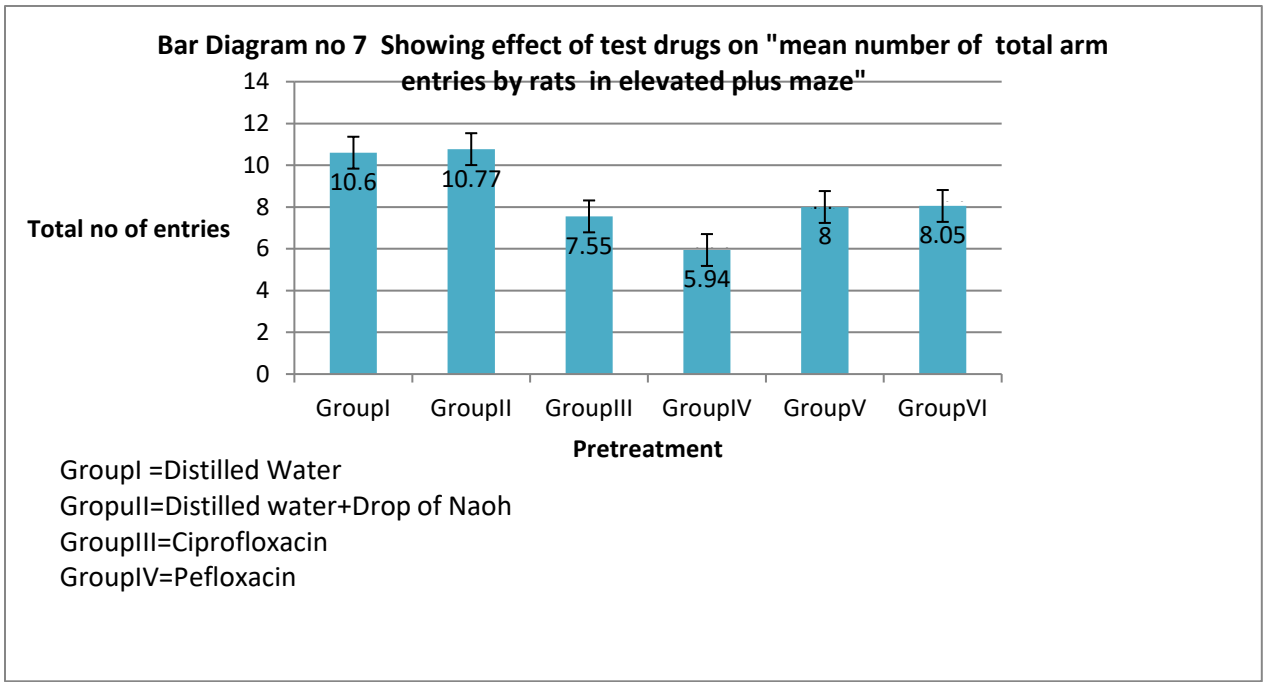

Figure 3: Effects of Fluoroquinolones on the total Arms Entries in the Elevated Plus Maze in Rats

Interpretation: The results are displayed as a bar chart showing the effects of fluoroquinolones on the total number of arm entries made in the

"Elevated Plus Maze". The highest number of total arm entries (10.60 ± 0.76 and 10.77 ± 0.56) were seen in the control groups (Group I and Group II), indicating normal exploratory movements. When treated with ciprofloxacin, the total entries were significantly reduced to 7.55 ± 0.51 (P < 0.01) and with pefloxacin, the total entries were found to be the most significantly reduced (P < 0.001) to 5.94 ± 0.45. Both sparfloxacin (8.00 ± 0.40) and lomefloxacin (8.05 ± 0.43) significantly reduced the total arm entries (P < 0.01). The rats' exploratory behaviour was reduced by all the fluoroquinolones, and the following order of efficacy was obtained: Pefloxacin > Ciprofloxacin > Sparfloxacin > Lomefloxacin.

3.2. Effects of fluoroquinolones on time spent in open and closed Arms

Table 2: Effects of fluoroquinolones on time Spent in Open and Closed Arms of Elevated Plus Maze (mean ± SEM)

Group	Pre-treatment	Dose (mg/kg, IP)	"Time in Open Arm (sec) Mean ± SEM"	"Time in Closed Arm (sec) Mean ± SEM"
I	Distilled Water	1 ml/kg	46.17 ± 3.31	207.17 ± 6.78
II	Distilled Water + 0.1N NaOH	1 ml/kg	45.72 ± 3.33	208.00 ± 6.77
III	Ciprofloxacin	100 mg/kg	30.67 ± 4.05*	245.17 ± 6.31**
IV	Pefloxacin	60 mg/kg	23.00 ± 4.09**	249.67 ± 7.14**
V	Sparfloxacin	60 mg/kg	33.50 ± 4.98	240.00 ± 6.61**
VI	Lomefloxacin	60 mg/kg	33.67 ± 4.98	242.00 ± 6.94**

Interpretation: Table 2 illustrates The time spent in the open arm was reduced and the time in the closed arm of the Elevated Plus Maze was increased during fluoroquinolone treatment, indicating increases in anxiety-like behaviour of rats. Pefloxacin had the greatest effect in reducing open arm duration (23.00 ± 4.09 sec, P < 0.01) and increasing

closed arm duration (249.67 ± 7.14 sec, P < 0.01) followed by ciprofloxacin. Both reduced the time spent in the open arm, although the changes were not significant, but both significantly increased the time spent in the closed arm (P < 0.01).

Significance of group III & IV calculated in comparison with Group I, Significance of group V &VI calculated in comparison with Group II*P <0.05, **p<0.01,

Statistical analysis by Students Unpaired t test

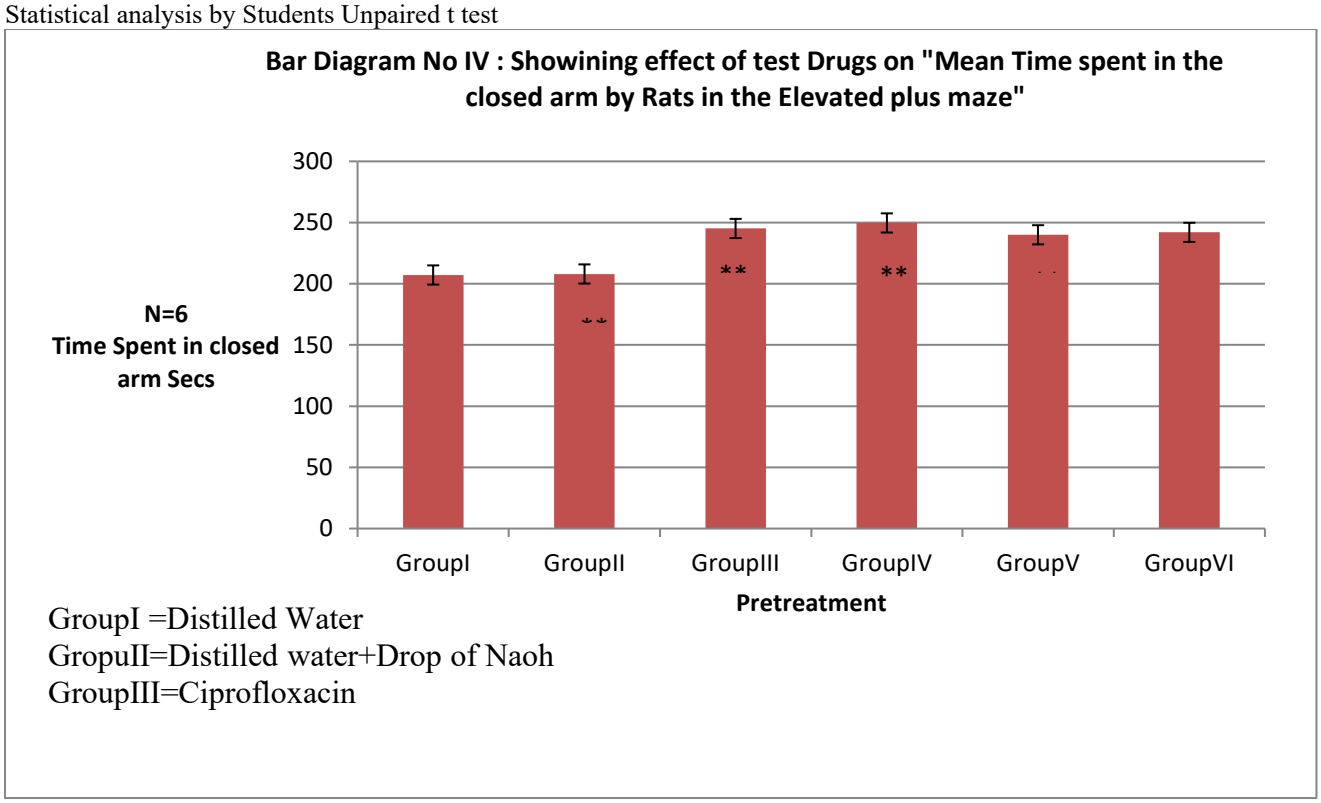

Figure 4: Effects of Fluoroquinolones on mean time spent in the Closed Arms of the Elevated Plus Maze in Rats

Interpretation: All fluoroquinolones significantly increased the time spent in the closed arm of EPM compared to control groups, indicating increased anxiety-like behaviour in rats (see bar diagram). The control groups (Group I and Group II) spent roughly 207–208 seconds in the closed arm, while ciprofloxacin, pefloxacin, sparfloxacin, and lomefloxacin extended the duration to 245.17 ± 6.31, 249.67 ± 7.14, 240.00 ± 6.61, and 242.00 ± 6.94 seconds, respectively (P < 0.01). Pefloxacin caused the greatest increase followed by ciprofloxacin, lomefloxacin and sparfloxacin.

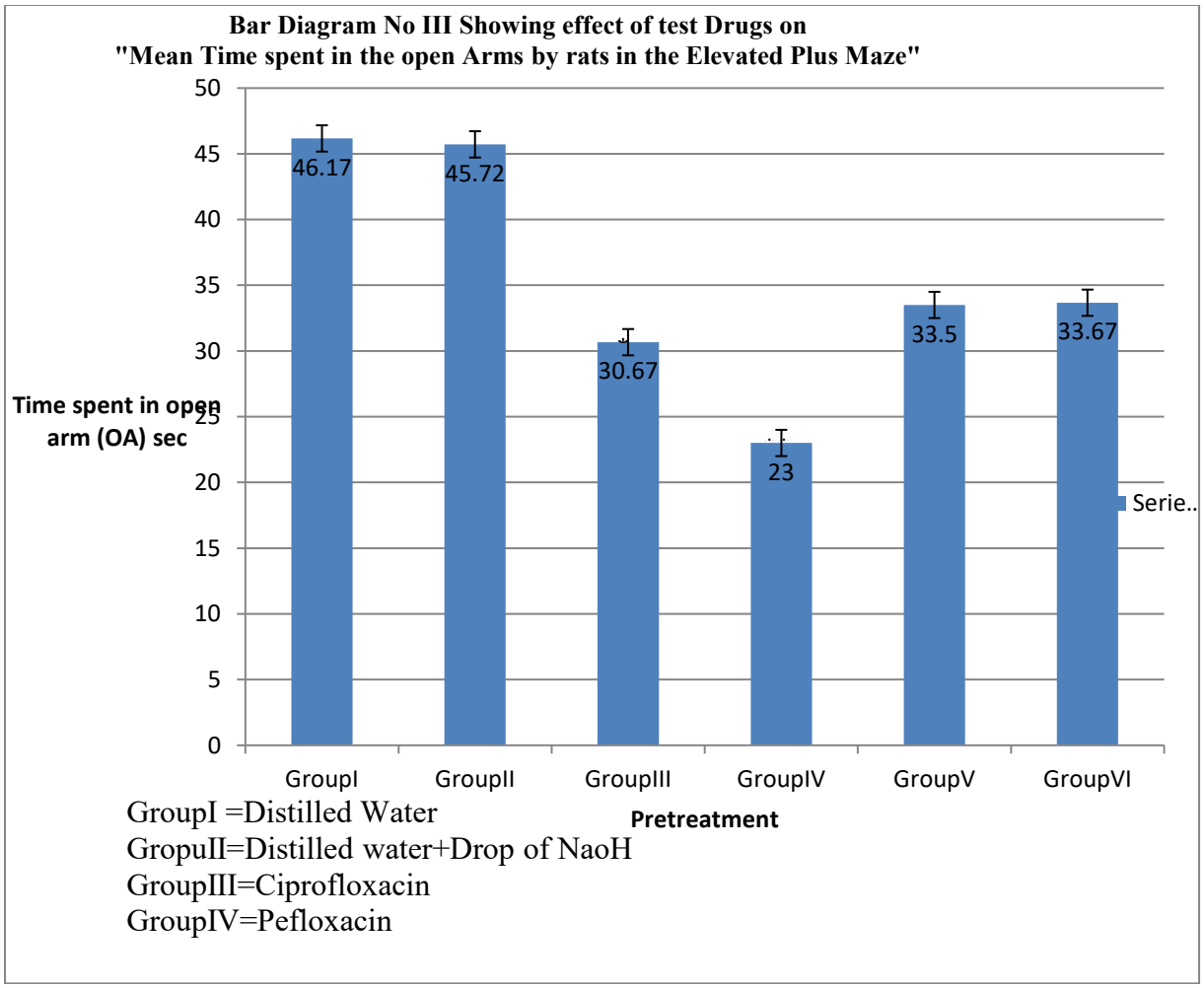

Figure 5: Effects of Fluoroquinolones on mean time spent in the open arms of the "Elevated Plus Maze" in Rats

Interpretation: The bar diagram shows that treatment with the fluoroquinolones reduced the time that rats spent in the open arms of the "Elevated Plus Maze", which appears to be anxiogenic. The mean open arm duration for control (Group I) and vehicle control (Group II) was similar (46.17 vs 45.72 sec), but was significantly shortened by ciprofloxacin (Group III) to 30.67 sec ($p < 0.05$) and by pefloxacin (Group IV) to 23.00 sec ($p < 0.01$). Moderate reductions (33.50 and 33.67 seconds) were achieved with sparfloxacin (Group V) and lomefloxacin (Group VI). Pefloxacin had the most significant anxiogenic effect, followed by ciprofloxacin, while sparfloxacin and lomefloxacin had more muted effects.

DISCUSSION

The study confirms that fluoroquinolones have considerable anxiogenic effects in rats. In comparison to the control group (3.66 open-arm entries), ciprofloxacin, pefloxacin, sparfloxacin, and lomefloxacin decreased open-arm entries to 2.11, 1.11, 2.33, and 2.33, respectively. Pefloxacin resulted in the most significant reduction (69.7% decrease) and the fewest total arm entries (5.94 compared to 10.60 in the control; $p < 0.001$), signifying the most pronounced anxiety-inducing impact. Horii et al., (2018) reported that the study raised plus maze test is a behavioral assessment for evaluating anxiety in rodents. To ascertain the reasons for the divergent outcomes, we previously concentrated on arm characteristics, which vary among laboratories, particularly the presence or lack of ledges on the sides of open arms and the transparency or opacity of closed arm walls. In summary, open arm ledges markedly heightened anxiety-like behaviour in rats, whereas translucent arms potentially mitigated this behaviour. We present our protocol for the "Eevated plus maze" test, accompanied by a discussion of the significant findings from the prior report and our experimental insights[14].

Fluoroquinolones elicited notable anxiogenic effects in rats, evidenced by a decrease in open-arm entries from 3.66 ± 0.38 (control) to 2.11 ± 0.16 (ciprofloxacin), 1.11 ± 0.24 (pefloxacin), 2.33 ± 0.15 (sparfloxacin), and 2.33 ± 0.09 (lomefloxacin). Pefloxacin exhibited the most pronounced anxiogenic action ($p < 0.001$).ilgin et al., (2015) provide

Ciprofloxacin (CPX) is a fluoroquinolone antibiotic employed in the treatment of respiratory, urinary tract, gastrointestinal, and stomach infections. CPX was delivered in single oral daily doses of 20 and 50 mg/kg for a period of 14 days in rats. The impact of oxidative stress on the alterations caused by CPX treatment was assessed by quantifying brain levels of catalase, superoxide dismutase, glutathione and malondialdehyde[15].

CONCLUSION

The study concluded that fluoroquinolones exhibit considerable anxiogenic effects in rats, as evaluated by the "Elevated Plus Maze" model. All evaluated fluoroquinolones—ciprofloxacin, pefloxacin, sparfloxacin, and lomefloxacin—significantly decreased open-arm entrances, time allocated to open arms, and total exploratory behaviour, while augmenting time spent in closed arms, so showing heightened anxiety-like behaviour. Pefloxacin demonstrated the most pronounced anxiogenic impact among the studied medications, succeeded by ciprofloxacin, whereas sparfloxacin and lomefloxacin revealed relatively smaller nevertheless significant effects. These findings align with prior publications indicating the role of GABAergic and NMDA receptor-mediated pathways in fluoroquinolone-induced neurotoxicity. Given the extensive therapeutic application of fluoroquinolones, it is crucial to recognise their possible neuropsychiatric side effects, especially in those with pre-existing anxiety disorders or other central nervous system susceptibilities. Additional research is necessary to clarify the underlying mechanisms and assess the anxiogenic potential of novel fluoroquinolones.

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