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Evaluation of Proconvulsive Potential of Levofloxacin

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ABSTRACT

The present investigation was under taken to study the evaluation of proconvulsivepotentials of levofloxacin a fluoroquinolonein epilepsy induced models like PTZ, Theophylline at sub convulsive dose and by electroconvulsometer; MES.Convulsions can be induced by electric shock of 150mA for 0.2 sec using ear clip electrodes. The duration in seconds of tonic flexion, tonic extension, clonic convulsion and post titanic depression can be noted. The increase in the duration of tonic extensor phase was considered as epileptic index. The convulsion can also be induced by giving sub convulsive dose i.e. 40mg/kg of PTZ intra-peritonealy. The animals were kept under observation for 15mins for the development of jerky movements and clonic seizures. Each mouse under the test received the test drug intraperitoneally 30 mins before i.p administration of subconvulsive dose of theophylline (125mg/kg). The animals were kept under observation for the onset of maximal seizures which was evidenced by tonic flexion of fore limbs, tonic extensor of hind limbs and clonic convulsions for 2 hours. The pro-convulsive potentials of Levofloxacin at two different doses were studied using MES, PTZ methods and co administered with theophylline and the results were compared with the control group and standard group. The results of the present study showed that levofloxacin demonstrated a significant proconvulsive profile in both MES and PTZ induced seizures and thus should be prescribed with caution. The % decrease in the level of GABA treated with levofloxacin at 25 mg/kg bodyweight in mice brain is 10.92 as compared to control was 16.14 in MES induced seizures.The %decrease in the level of GABA treated with levofloxacin at 25 mg/kg bodyweight in mice brain was 7.85 as compared to control was 16.14 on PTZ induced seizures. Similarly the GABA level was also decreased to 8.88% on treatment with levofloxacin at 25mg/kg bodyweight as compared to control was 16.14% on co administration withtheophylline.

Keywords: Levofloxacin, PTZ, MES, Theophylline, proconvulsive activity, GABA.

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INTRODUCTION

Epilepsy is a common chronic neurological disorders characterized by seizures [1.2]. These seizures may be the signs of the hyper synchronous neuronal activity in the brain. About 50 million people worldwide suffers from epilepsy and nearly 2 out of every 3 new cases are discovered in developing countries. It is more common in old age peoples. Onsets of new cases occur most frequently in infants and elderly.[3]

Epilepsy is usually controlled but not cured with medication. However over 30% of people with epilepsy do not have seizure control even with the best available medications. Surgery may be considered in difficult cases. Not all epilepsy syndromes are lifelong.[(4)] Epilepsy should not be understood as a single disorder but rather as syndrome with vastly divergent syndromes, or involving episodic abnormal electrical activity in the brain and numerous seizures. The various causes of epilepsy include hypoxic-ischemic encephalopathy, CNS infections, trauma, congenital CNS abnormalities, brain tumors, illicit drug use, alcohol withdrawal, and dementia.[5]

The fluoroquinolone arethe synthetic broad spectrum antibiotics and most commonly prescribed antimicrobial agent in current medical practice. In general, the common side effects are mild to moderate and self limiting. On occasion serious adverse effects can occur, some of ADR's which occur more commonly than with other classes of antibiotic drug include CNS toxicity, photo toxicity, cardiotoxicity, arthropathy, and tendon toxicity. Children and the elderly are at a greater risk.[6]

Levofloxacin:[7]

Levofloxacin is a 9-fluoro-2,3-dihydro-3-methyl-10-(4-methyl piperizin-1-yl)-7-oxo-7H-pyrido(1,2,3-de)-1,4 benzoxazine-6-carboxylic acid. Levofloxacin is a broad spectrum antibiotic which is active against gram +ve and gram –ve bacteria. It functions by inhibiting DNA Gyrase, a type II topoisomerase, and topoisomerase IV, which is an enzyme necessary to separate replicated DNA, thereby inhibiting cell wall division. The fluoroquinolone interferes with DNA replication by inhibiting an enzyme complex called DNA-Gyrase. This can also effect mammalian cell replication. In particular, some conveners of this drug family display activity not only against bacterial topoisomerases but also against eukaryotic topoisomerases, and are toxic to cultures mammalian cells and *in vivo* tumors models.

Levofloxacinis generally well tolerated but in rare instances produce serious and life threatening adverse reactions as well as spontaneous tendon ruptures, tendonitis, QTcprolongation/ torsades de pointes, irreversible peripheral neuropathy, toxic epidermal necrolysis (TEN) and Stevens-Johnson Syndrome, erythema multiforme, severe CNS disorder including seizures and clostridium difficult associated disease

(CDAD:Pseudomembranous colitis), photosensitivity, fatal hypoglycemia, kidney damage, rhabdomyolysis (muscle vesting), as well as anaphylactoid reactions and mysthenic crisis.

MATERIALS AND METHOD

List of instruments used during the experiment:

Digital Balance, pH meter, Analytical Digital Balance, Homogenizer, Cooling centrifuge, centrifuge, Spectrofluorimeter, MES.

List of the chemicals used:

Pentylentetrazole, Theophylline, Levofloxacin, GABA, Tri chloroacetic acid, Ninhydrin, Sodium carbonate, Sodium Bicarbonate, Di-sodium carbonate, copper sulphate, tartaric acid, Glutamic acid.

Method

Swiss albino mice of either sex (25-35gm)bred in the animal house of Dayananda Sagar College of Pharmacy, Bangalore were used to induce convulsions by electroshock method, PTZ and by theophylline. These animals were divided into three groups; one such group was subjected to 150mA of electroshock for 0.2 secs, through auricular electrodes, majority of the mice showed tonic flexion of fore and hind limbs with tail erection, and stupor followed by post tetanus depression and recovery. The animals showing convulsions were used in the experiment and divided into 6 groups having six animals each for MES, PTZ induced seizures and co administered with theophylline.

All the test animals were allowed for food and water *add libitium*both being withdrawn 24hrs prior to experimentation. All the preparations were administered intraperitonealy. The experiment was conducted as per guidelines of CPCSEA, Chennai, INDIA (Approval no: DSCP/M.Pharmacol/IAEC/82/12-13).

Table 1: Groups of Animals

Group	For MES method	For PTZ method	For theophylline method
Group 1	Negative control (normal saline)	Negative control (normal saline)	Negative control (normal saline)
Group 2	Positive control (diazepam in normal saline)	Positive control (diazepam in normal saline)	Positive control (diazepam in normal saline)
Group 3	Test drug (12.5 mg)	Test drug (12.5 mg)	Test drug (12.5 mg)
Group 4	Diazepam + test drug (12.5 mg)	Diazepam + test drug (12.5 mg)	Diazepam + test drug (12.5 mg)
Group 5	Test drug (25 mg)	Test drug (25 mg)	Test drug (25 mg)
Group 6	Diazepam + test drug (25 mg)	Diazepam + test drug (25 mg)	Diazepam + test drug (25 mg)

Model 1: Maximal Electroshock Seizure (MES)[8]

Convulsion was induced by giving an electric shock of 150 mA for 0.2 sec using ear clip electrodes. The duration in seconds of tonic flexion, tonic extension, clonic convulsion and

post titanic depression were noted. The increase in the duration of tonic extension is considered as the index of epileptic activity and vice versa.

Model 2: Pentylentetrazole (PTZ) induced seizures: [8, 9]

Each mice under the test received a test drug intraperitonealy 30 mins before administration of sub convulsive dose of PTZ (40 mg/kg body weight) i.p. The animals were kept under observation for the onset of maximal seizures which is evidenced by tonic flexion of forelimbs, tonic extension of hind limbs and clonic convulsions for 15 mins.

Model 3: Co- administration with sub convulsive dose of theophylline [8, 9]

Each mice received the test drug intraperitoneally, 5 mins before intraperitoneal administration of sub convulsive dose of theophylline (125 mg/kg body weight) i.p. They were observed for the onset of maximal seizures which was evidenced by tonic flexion of fore limbs, tonic extension of hind limbs and clonic convulsions for 2 hrs.

Model 4: Brain GABA estimation :[10]

Reagent preparation:

1. 10% (w/v) trichloro acetic acid buffer.
2. 0.14M Ninhydrin solution in 0.5 M carbonate bicarbonate buffer pH- 9.9
3. Copper tartrate reagent: dissolve 0.16% Disodium carbonate, 0.3% Copper sulphate, 0.0329% tartaric acid in100ml of distilled water.
4. 1.5µM Glutamic acid.
5. Prepare 20, 40, 60, 80,100 µg of GABA standard in distilled water.

Procedure:

Animals were killed by decapitation at predetermined intervals after the administration of test drug, diazepam and saline followed by subjecting the animals to Electro convulsion/Chemo convulsions .the brain were rapidly removed, blotted, weighed and taken in the ice cold 5ml trichloro acetic acid (10 % w/v), homogenized and centrifuged at 1000rpm for 10 min at 0℃. A sample of 0.1 ml tissue extract was taken in 0.2 ml of 0.14 M Ninhydrin solution in 0.5 M carbonate- bicarbonate buffer pH (9.9), was kept in water bath at 60℃ for 30min, cooled and treated with 5 ml of Copper tartrate reagent. After 10 min, the fluorescence reading was taken at 377/ 451 nm in a spectrofluorimeter.

For GABA standards, different amounts of (20, 40, 60, 80, 100 µg) were mixed with 1.5 µM Glutamic acid were dissolved in 0.1 ml of 10% trichloro acetic acid. GABA was determined by the measurement of the formed fluorescent product resulting from the reaction of GABA with ninhydrin in an alkaline medium in the presence of glutamate. The GABA content in the brain was expressed in µg g^{-1} of the wet brain tissue.

RESULTS AND DISCUSSION

Model 1: MES (Electroshock of 150 mA for 0.2 secs), DZPM- 2mg/kg

Table 2: Effect of Levofloxacin (LF) on MES induced shock in Mice

Group	Tonic flexion(secs)	Tonic extension (secs)	Clonic convulsion (secs)	Post titanic depression (secs)	Recovery/death (mins)
Normal Saline + Shock	2.66±0.33	14.5±0.56	40.5±4.98	91.83±12.66	7±0.36
DZPM+ Shock	2.83±0.54	9.83±0.60	34.33±3.23	117.16±6.17	10±0.36
LF (12.5) +Shock	2.66±0.33	17.83±0.74	122.5±5.88	97.14±3.6	10.83±0.60
DZPM+ LF (12.5) + Shock	3.16±0.47	14.66±0.42	114.16±4.36	84±4.16	9.83±0.30
LF (25) +Shock	2.5±0.22	20.33±1.02	134.16±3.76	134.16±4.11	14.33±0.42
DZPM + LF (25) + Shock	2.16±0.16	14.33±0.42	133±4.81	108±5.32	15.33±0.42

All values are expressed as mean ± SEM, n=6, one way analysis of variance (ANOVA) followed by Dun net's Multiple Comparison test. The minimum of $p<0.0001$ was considered as significant; if the value of q is greater than 2.70 then the p value is less than 0.05.

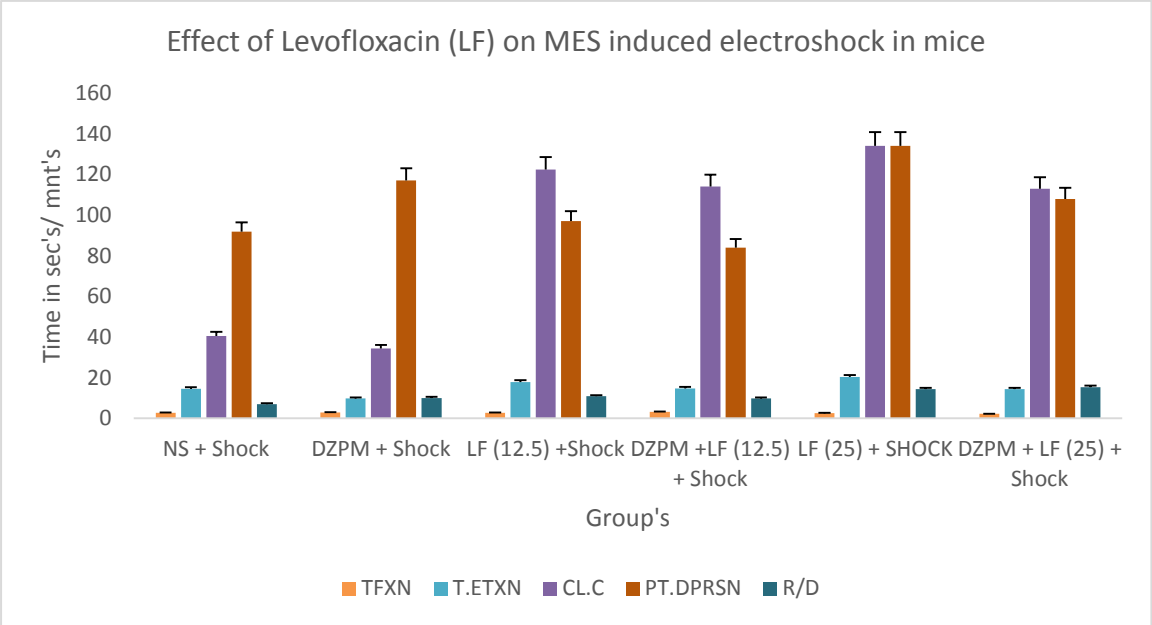

Figure 1: Effect of LF on MES induced electroshock in mice

The animals were observed for tonic flexion, tonic extension, clonic convulsion, post titanic depression and recovery/ death. The drug was administered intraperitonialy (i.p) and the shock treatment was given after 30 min of administration of the drug.

Normal saline showed 2.66±0.33, 14.5±4.98, 91.83±12.66 and 7.0±0.36 secs, tonic flexion, tonic extension, clonic convulsion, post titanic depression and recovery respectively in secs.

After diazepam administration post titanic depression showed an increased value of 117±6.71.

LF showed clonic convulsion of 122.5±5.88 secs, DZPM + LF (12.5 mg) shows a clonic convulsion of 114.16±4.36 secs

LF 25 mg showed clonic convulsion of 134.16±3.76 and post titanic depression of 134.16±4.11 secs and recovery was observed after 14.33±0.42 mins. DZPM + LF showed clonic convulsion of 113±4.81 secs, post titanic depression 108±5.32 secs and recovery after 15.33±0.42 mins.

Model 2: Chemoconvulsion- Petylenetetrazole, PTZ- 40 mg/kg/(sub convulsive dose), DZPM-2mg/kg

Table 3: Effect of LF on experimental seizures induced by PTZ in mice

Groups	Jerky movements (secs)	Clonic convulsions (secs)	Recovery/ death (mins)
NS+ PTZ	14.5±0.42	15.16±1.13	12±0.36
DZPM + PTZ	5.83±0.54	12.33±1.22	10.83±0.30
LF (12.5) +PTZ	21.83±0.98	25.83±2.57	13.66±0.49
DZPM+ LF (12.5) + PTZ	---	6.5±0.56	5.33±0.42
LF (25) + PTZ	28.66±2.17	37±3.24	15±0.36
DZPM + LF (25) + PTZ	13.16±0.79	23.5±0.71	8.66±0.42

All values are expressed as mean ± SEM, n=6, one way analysis of variance (ANOVA) followed by Dun net's Multiple Comparison test. The minimum of $p<0.0001$ was considered as significant; if the value of q is greater than 2.70 then the p value is less than 0.05.

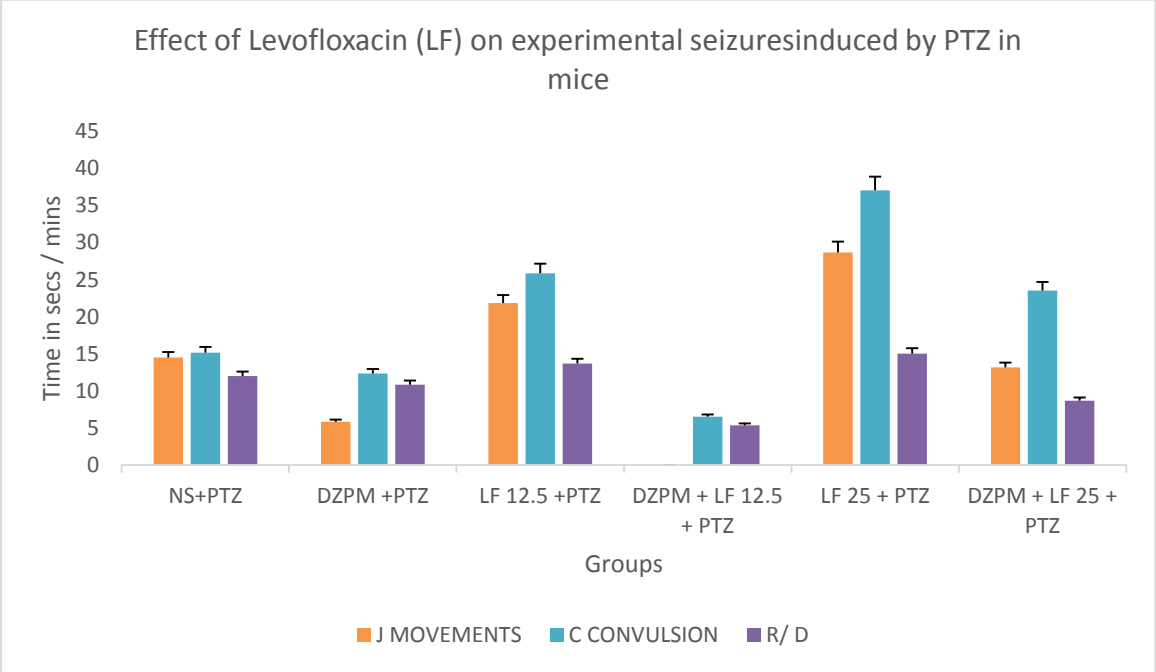

Figure 2: Effect of LF on experimental seizures induced by PTZ in mice

The animals were observed for jerky movements, clonic convulsions, and recovery or death. The drug was administered through i.p., PTZ was given after 30mins of administration of test drug. Normal saline with PTZ showed 14.5±0.42 secs, 15.16±1.13 secs, 12.0±0.36 mins, jerky movements, clonic convulsions and recovery respectively. After diazepam administration mice showed decreased values 5.83±0.54 jerky movements, 12.33±1.22 secs of clonic convulsions and 10.83±0.30 mins of recovery.

Levofloxacin (12.5 mg) showed 21.83±0.98 secs, 25.83±2.57 sec, jerky movements and clonic convulsions respectively and recovery was increased to13.66±0.42 min. Levofloxacin (25 mg) showed increased value of 28.66±2.17 sec, 37.0±3.24 sec, jerky movements, clonic convulsions respectively and recovery time was increased to 15±0.36mins. Diazepam with LF (25mg) showed 13.16±0.79 sec jerky movements, 23.5±0.71 sec of clonic convulsions and 8.66±0.42 mins of recovery.

Model 3: Chemo convulsion – theophylline, theophylline 125 mg/kg (sub convulsive dose), DZPM-2mg/kg.

Table 4: Effect of LF on experimental seizures induced by theophylline in mice

Groups	Tonic flexion of fore limbs (secs)	Tonic extension of hind limbs(secs)	Clonic convulsions(secs)
NS + TP	37.33±5.18	29.66±2.34	7.8±1.20
DZPM+TP	24.66±1.74	23.5±1.40	4.5±0.56
LF 12.5+TP	55.16±5.51	35.87±2.01	18.33±1.33
DZPM+LF 12.5+TP	26±4.54	29.16±2.88	---
LF 25+TP	240±22.91	150±8.26	164.5±5.88
DZPM+LF 25+TP	31.16±2.63	43.33±1.02	11.16±0.98

All values are expressed as mean ± SEM, n=6, one way analysis of variance (ANOVA) followed by Dun net's Multiple Comparison test. The minimum of $p<0.0001$ was considered as significant; if the value of q is greater than 2.70 then the p value is less than 0.05.

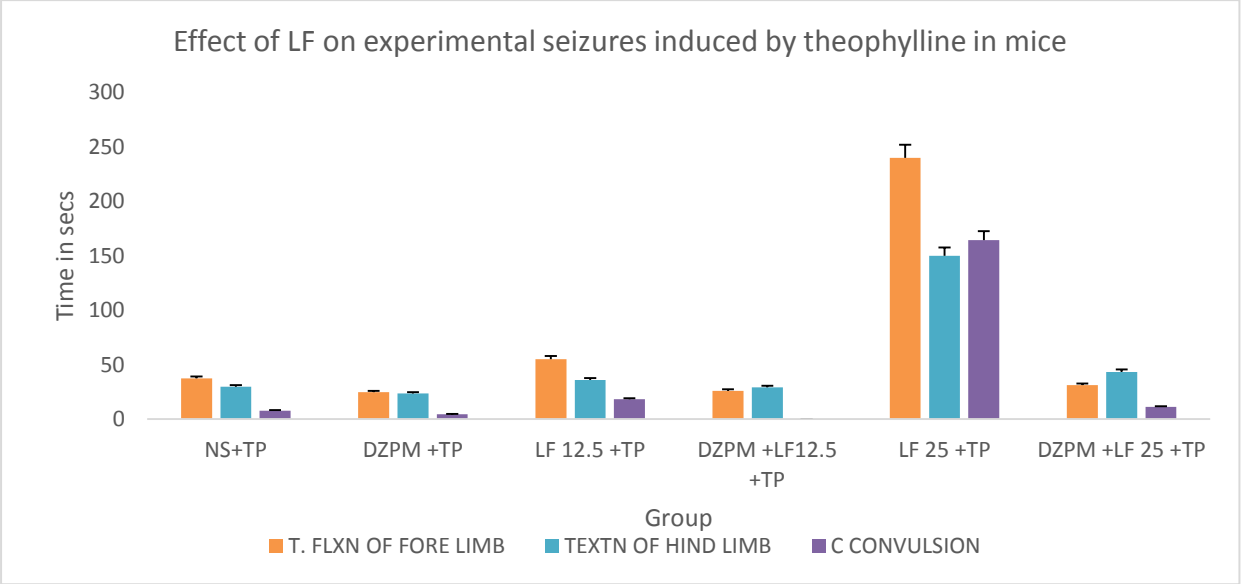

Figure 3: Effect of LF on experimental seizures induced by theophylline in mice

The animals were observed for tonic flexion of fore limbs, tonic extension of hind limbs and clonic convulsions upto 2 hrs.

The drug was administered to i.p., and theophylline was given after 5 mins of test drug administration.

Normal saline with theophylline showed 37.33±5.18 sec, 29.66±2.34, 7.8±1.20 tonic flexion of fore limbs, tonic extension of hind limbs and clonic convulsions respectively. LF 12.5 mg

showed slight increase value of55.16±5.51 sec, 35.87±2.01 sec of tonic flexion of fore limbs, tonic extension of hind limbs respectively and no clonic convulsions. Diazepam with levofloxacin 12.5mg showed similar observations as that of normal. LF 25 mg with theophylline showed increased value of 240±22.91 sec, 150±8.26 sec, 164.5±5.88 sec of tonic flexion of fore limbs, tonic extension of hind limbs and clonic convulsions respectively.

Model 4: Brain GABA estimation

The brain GABA level was estimated in groups of mice. The measurement of GABA based on the method of Lowe was carried out.

Table 5: Comparison of GABA- concentration between groups (LF) with MES induced seizures

Groups	GABA-concentration (µg) mean ±sem	% of GABA
Control	563.06±5.89	16.41%
NS+ Shock	506.41±7.65	14.50%
DZPM+ Shock	534.95±9.09	15.33%
LF 12.5 +Shock	466.84±10.59	13.38%
DZPM+ LF 12.5 + Shock	564.10 ±5.70	16.19%
LF 25 +SHOCK	381.02±10.72	10.92%
DZPM+ LF 25 +Shock	471.25±13.57	13.50%

All values are expressed as mean ± SEM, n=6, one way analysis of variance (ANOVA) followed by Dun net's Multiple Comparison test. The minimum of $p<0.0001$ was considered as significant; if the value of q is greater than 2.70 then the p value is less than 0.05.

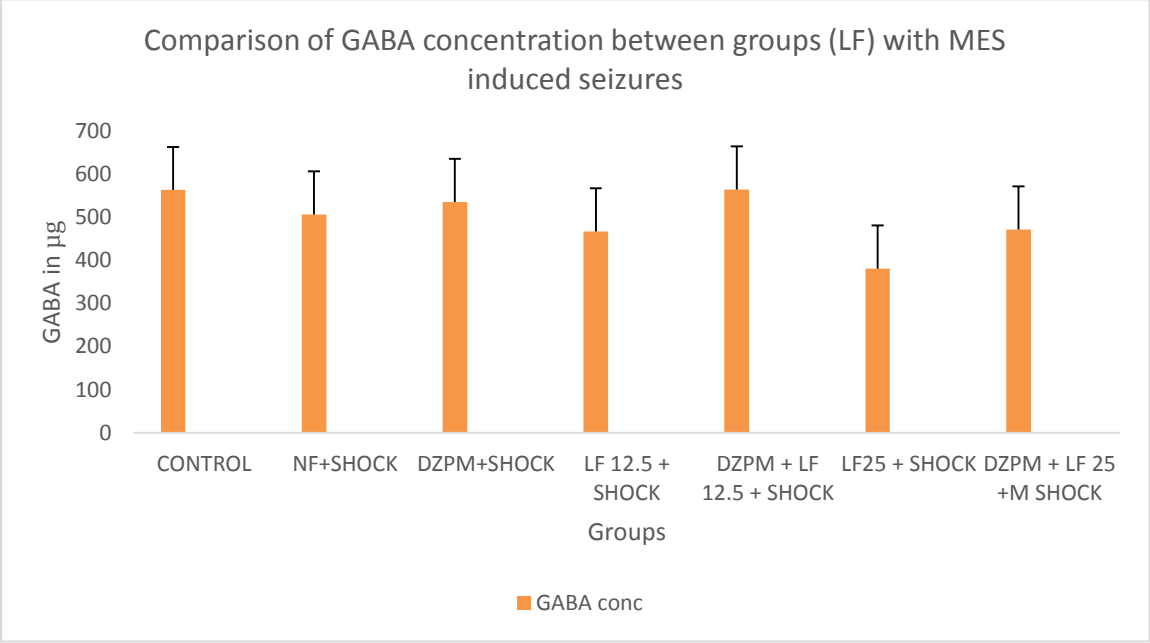

Figure 4: Comparison of GABA concentration between groups (LF) with MES induced seizures

In normal mice the GABA was 563.66µg, for other group of mice were subjected to normal saline with electric shock then the concentration of GABA was found to be 506.421µg. this showed that the electric shock reduced the GABA level in mice.

Levofloxacin of 2 doses (12.5mg/kg, 25 mg/kg) was treated with electric shock, the concentration of GABA was slightly reduced in low dose of levofloxacin and in high dose it was still reduced. In the presence of diazepam the GABA level was increased when treated with both electric shock treatment and test drug levofloxacin.

Table 6: Comparison of GABA- concentration between groups (LF) with PTZ (sub convulsive doses) induced seizures.

Groups	GABA-Concentration (µg) Mean ±Sem	% Of GABA
CONTROL	563.06±5.89	14.01%
NS+ PTZ	517.55±11.74	12.88%
DZPM+PTZ	690.49±12.41	17.19%
LF 12.5 +PTZ	454.76±11.42	11.32%
DZPM+ LF 12.5 + PTZ	735.81±14.81	18.32%
LF 25 +PTZ	315.39±17.00	7.85%
DZPM+ LF 25 + PTZ	739.26±17.13	18.40%

All values are expressed as mean ± SEM, n=6, one way analysis of variance (ANOVA) followed by Dun net's Multiple Comparison test. The minimum of $p<0.0001$ was considered as significant; if the value of q is greater than 2.70 then the p value is less than 0.05.

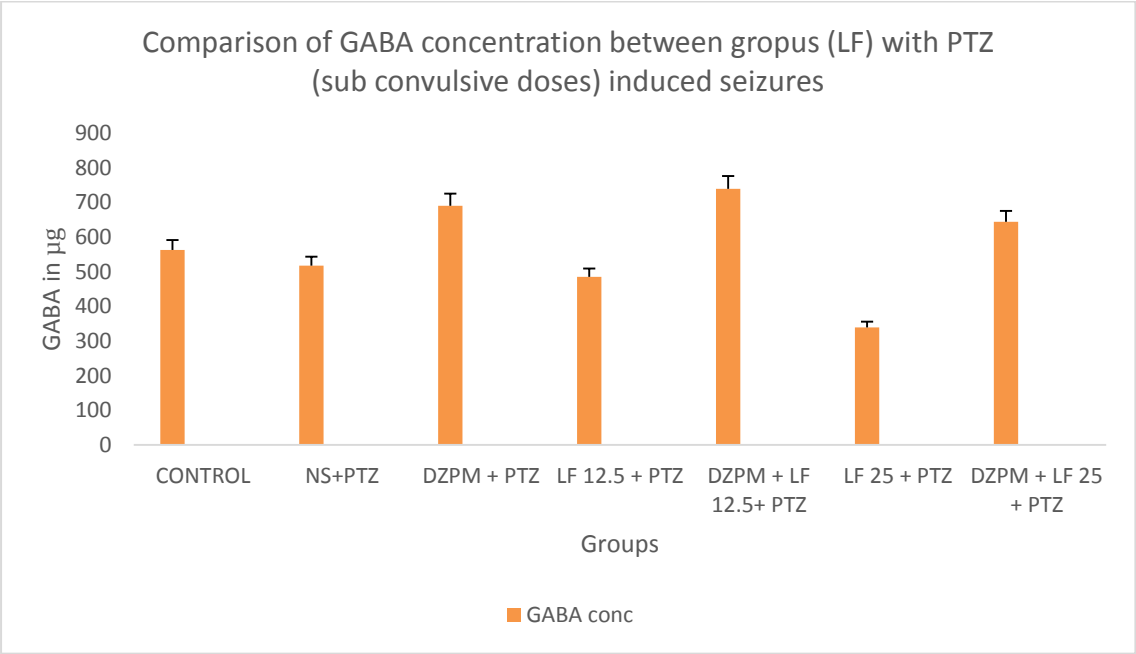

Figure 5: Comparison of GABA- concentration between groups (LF) with PTZ (sub convulsive doses) induced seizures.

The GABA level at normal saline with PTZ was 517.55µg.it was reduced when compared to normal mice (563.06µg) at both doses of LF, the GABA level reduced as 454.76 and 315.39 µg respectively. In the presence of diazepam GABA value was increased as 690.49, 739.26 µg with PTZ, LF 12.5, LF 25mg/kg respectively.

Table 7: Comparison of GABA- concentration between groups (LF) with Theophylline(sub convulsive doses) induced seizures.

Groups	GABA-Concentration (µg) Mean ±Sem	% Of GABA
Control	563.06±5.89	16.37%
NS+ TP	435.55±10.66	12.66%
DZPM+TP	734.44±18.45	21.35%
LF 12.5 +TP	361.92±9.88	10.52%
DZPM+ LF 12.5 + TP	563.55±16.37	16.38%
LF 25 +TP	305.38±11.76	8.88%
DZPM+ LF 25 + PTZ	475.05±12.76	13.81%

All values are expressed as mean ± SEM, n=6, one way analysis of variance (ANOVA) followed by Dun net's Multiple Comparison test. The minimum of $p<0.0001$ was considered as significant; if the value of q is greater than 2.70 then the p value is less than 0.05.

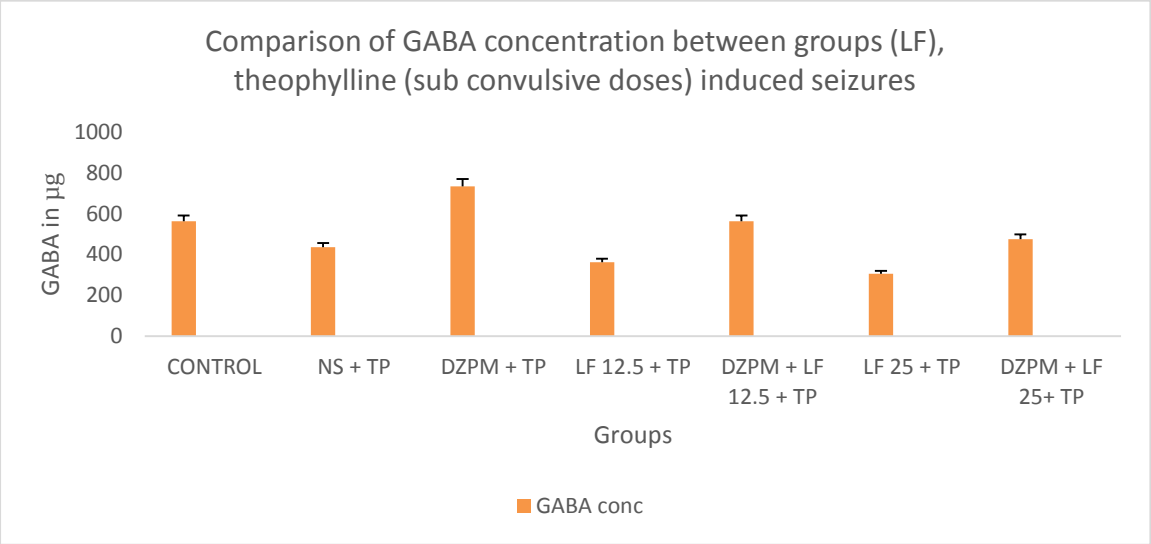

Figure 6: Comparison of GABA- concentration between groups (LF) with Theophylline (sub convulsive doses) induced seizures.

The GABA level at normal saline with theophylline was 435.55 µg. it was reduced in concentration when compared to normal mice (563.06µg) and also it was less when compared to electric shock and PTZ treated mice, at both doses of LF (12.5mg/kg, 25mg/kg), the GABA level was reduced to 361.92 and 305.38 µg respectively. In presence of diazepam it was increased to 734.44, 563.55, 475.05 µg with theophylline, levofloxacin 12.5mg/kg and 25 mg/kg respectively but at 25 mg it increased less in GABA level.

DISCUSSION:

Levofloxacin was subjected to electroshock model, it was observed that the values are increased in all the phases of seizures but in presence of diazepam seizure was controlled but recovery was delayed.

Animals at lower dose of levofloxacin(12.5mg/ kg) and sub convulsive doses of PTZ (40 mg/kg) showed increased values of jerky movements and clonic convulsions as compared to

control and the seizure values werefurthur increased as the dose of levofloxacin 25 mg/kg but in presence of diazepam the seizure values were decreased.

At low doses of levofloxacin(12.5 mg/kg) when co-administered with theophylline (125 mg/kg) showed increased values of seizures at all phases which demonstrate that levofloxacin has a pro convulsant activity when given with theophylline. With diazepam the seizure values were decreased which shows the protective actions of diazepam.

Effect of fluroquinolone on GABA level on Maximal electroshock induced seizures:

Levofloxacin at both the doses (12.5 and 25 mg/kg) showed decreased concentration of GABA but with diazepam GABA level was increased.

Effect of fluroquinolone on GABA level on PTZ induced seizures:

GABA level ws still decreased with fluoroquinolones treated with PTZ.

Effect of fluroquinolone on GABA level with co-administration of Theophylline induced seizures:

Here also GABA level was decreased with co administration of theophylline.

CONCLUSION:

In the present study Levofloxacin produced a dose dependent pro- convulsive effect. It is found that levofloxacin has pro-convulsing activity in both *in vivo* (PTZ) and *in vitro* (MES) induced seizures and co-administered with theophylline. The pro- convulsing activity is more with PTZ and with theophylline induced seizures as compared to MES model. Thus it's concluded that Levofloxacin should be used with caution in patients with predisposing epileptogenic factors. The GABA level in Levofloxacin treated animals was low compared to control and diazepam treated animals. This indicates that levofloxacin may interfere with the synthesis of GABA or may decrease the affinity of GABA towards GABA receptors. Thus the study provides information about the protective activity of diazepam against pro convulsive activity of levofloxacin treated animals.

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