

Effects of ciprofloxacin or co-amoxicillin with and without antidepressants on behavioral deficit in rats induced with chronic suppurative otitis media

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Abstract: Chronic suppurative otitis media (CSOM) is chronic infection of middle ear which is usually managed with antibiotic therapy. This infection may cause the depression and cognitive changes in patients. The aim of present study was to evaluate the effect of antibiotic (ciprofloxacin and co-amoxicillin) with antidepressant or without antidepressants (bromazepam and imipramine) at low doses on rats with induced with CSOM. *Pseudomonas aeruginosa* was used to induce CSOM by in rat ear (tympanic bulla). The rats were divided into eight groups having six animals in each group. Neuropharmacological activities and gross behavior were observed in open field activity, force swimming cage, maze test, light and dark activity box and traction test. Observations were noted weekly after the administration of ciprofloxacin (15.3mg/kg), co-amoxicillin (15.3mg/kg), imipramine (1.15mg/kg) and bromazepam (0.09mg/kg) intraperitoneally. The altered behavior and depression was observed in control positive but reverted back in groups maintained on antidepressants with antibiotics with significant improved locomotor activity, memory in memory cage, muscular co-ordination and body balance and decreased anxiety. On the other hand, groups treated with only antibiotics showed significant improvement only in force swimming and traction test at day 14. Therefore, the antidepressant effects of the drugs can be employed to attenuate stress and depression in patients with CSOM.

Keywords: Chronic suppurative otitis media, depression, antibiotics, antidepressants.

INTRODUCTION

Chronic suppurative otitis media (CSOM) is a middle ear chronic infection which caused tympanic membrane perforation and hearing loss in patients of CSOM (Aubert *et al.*, 2010). This infection induced sensory modification and altered hearing threshold which contribute conductive or sensorineural auditory loss in patients. Different studies showed the co-relation of hearing loss with depression along with risk factors of socio-economical problems and co-morbidities. However, one of the studies conducted in local population of Pakistan in CSOM patients showed association of stress, anxiety and depression with hearing loss induced by CSOM (Mehboob *et al.*, 2019).

Different organisms are responsible for CSOM; therefore, resistance sensitivity test is usually employed to find out the responsible micro-organism and sensitive antibiotics against it which may vary from individual to individual. Studies showed that different antibiotics such as ofloxacin, ciprofloxacin, amoxicillin-clavulanic acid in

combination with dexamethasone and quinolones were proved very effective in chronic otorrhoea (O'Connor *et al.*, 2009; Verhoeff *et al.*, 2006). It is also reported in different studies that amoxicillin and ciprofloxacin have anti-inflammatory properties (Sachsel *et al.*, 2008, Casellas *et al.*, 1998) which may be an additional benefit in CSOM with inflammation of middle ear. It was reported that antidepressants use in depression in adults suffering from HIV infection was more beneficial as compared with placebo (Wilson *et al.*, 2018).

Ciprofloxacin is the most effective antibiotic in CSOM while co-amoxicillin is most commonly used particularly in pediatric population. Imipramine and bromazepam produced anti-depressant effects especially at low doses and for short period withdrawal symptoms are negligible (Gillman PK; 2007, Amphoux *et al.*, 1982).

The aim of the study is to find out the effects of selected antibiotics (amoxicillin and ciprofloxacin) with or without selected antidepressants (bromazepam and imipramine) at low doses on rats with induced with CSOM on behavioral deficits induced by CSOM.

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MATERIALS AND METHODS

Animals

Male Sprague-dawley rats (N=48) were selected and kept under NIH recommendations to ensure healthy and controlled conditions (Saleem *et al.*, 2018).

Animal's protocol

Animals were divided into 8 groups randomly (six rats in each group) as G1; Control negative (healthy rats maintained on normal saline), G2; control positive (diseased without any treatment), G3 (ciprofloxacin 15.3 mg/kg), G4 (ciprofloxacin 15.3mg/kg with imipramine 1.15mg/kg), G5 (ciprofloxacin 15.3mg/kg with bromazepam 0.09mg/kg), G6 (co-amoxicillin 15.3mg/kg), G7 (co-amoxicillin 15.3mg/kg with imipramine 1.15mg/kg) and G8 (co-amoxicillin 15.3mg/kg with bromazepam 0.09mg/kg). All the drugs were administered intraperitoneally for seven days (Mehboob *et al.*, 2020) as per recommended doses.

Induction of CSOM; Animals were given anaesthesia intraperitoneally through ketamine (100mg/kg) and diazepam (0.1mg/kg) and induced with 0.04 ml of 6.4×10^7 CFU of *P.aerugenosa* into tympanic bulla. All the animals were kept under the guidelines of NIH for two weeks (Bhutta *et al.*, 2012).

STATISTICAL ANALYSIS

Results are expressed as mean+ S.D and the degree of freedom was 7 between groups and 40 within the subjects and 47 in total. One way ANOVA was followed by Tukey's test for group comparisons (Post Hoc) taking $p > 0.01$ as significant (Tamara, 2008).

Experimental protocol

Locomotor activities, muscular co-ordination and depression was monitored in open field for 15 minutes, anxiety and depression in light and dark cage for 6 minutes (Haleem, 2009), memory in maze test for 15 minutes were considered as cut off time (Stemmelin *et al.*, 2008). and for physical response and depression in forced swimming test for 6 minutes (Porsolt *et al.*, 1977). Body balance and co-ordination effects in stress were monitored in traction test with 120 taking seconds as cut off (Bogo *et al.*, 1981).

RESULTS

Open Field Test

The behavioral activity monitored by open field test is shown in fig. 1. The value of F was 123.476 at day-1, at day-7 F=1198.024 and highest at day-14 which is 2251.637.

When Post Hoc test was applied, the control negative (group 1) showed no significant differences in numbers of squares covered in given time (between subjects) from

day1 to day 14. Similarly, control positive (group 2) presented no significant difference in open field test from day 1 to day14.

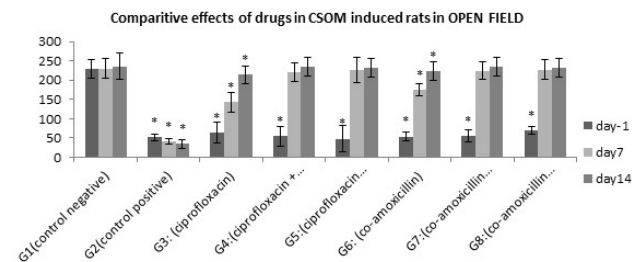


Fig. 1: activity expressed in mean with \pm S.D in open field. Significant difference $*(p < 0.05)$ by different groups is evaluated by Tukey's test as compare to control group.

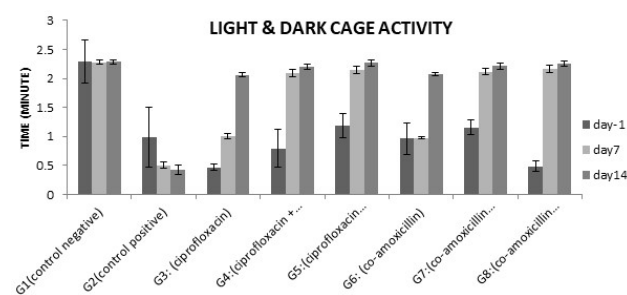


Fig. 2: activity expressed in mean with \pm S.D in light and dark cage. Significant difference $*(p < 0.05)$ by different groups is evaluated by Tukey's test as compare to control group.

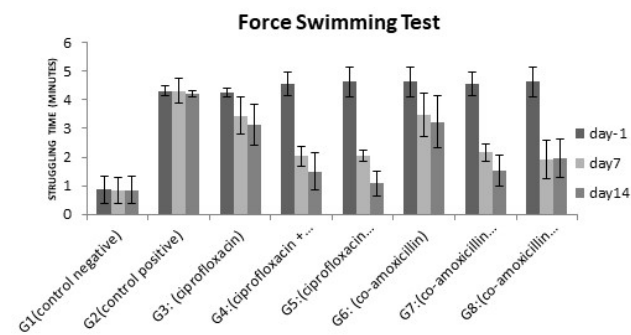


Fig. 3: Activity expressed in mean with \pm S.D in swimming cage. Significant difference $*(p < 0.01)$ by different groups is evaluated by Tukey's test as compare to control group.

However, for day 1, control group showed significant difference ($p < 0.01$) from all groups (group 2 to group8) and there was no significant differences among group2, 3, 4, 5, 6, 7 and 8.

At day 7, (after the completion of treatment from group-3 to 8), control negative showed significant higher readings in open field ($p < 0.05$) as compare to control positive and group 3 and 6 but not with groups 4,5,7 and 8.

When the results of the day 14 were evaluated, control negative group reflected significant difference from not

only control positive but also with group 3 and 6 which were maintained on antibiotics (without antidepressant).

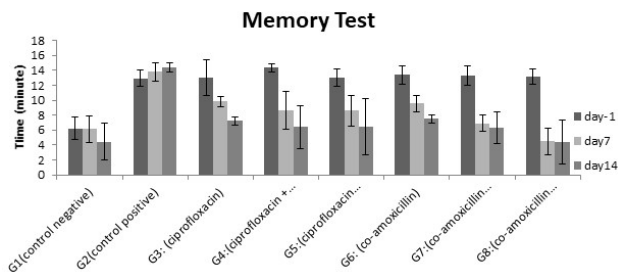


Table 4: activity expressed in mean with \pm S.D in memory test. Significant difference $^*(p<0.01)$ by different groups is evaluated by Tukey's test as compare to control group.

Light activity

The light and dark cage activity were evaluated in terms of time spend in the lighter part of the cage by the subject to be monitored (white albino rats). The F-value for day-1 is 30.413, day-7 is 99.499 and day 14 is 906.374, giving very low p value 0.00 which is significant ($p<0.05$).

There was no significant differences in time for control negative group (group) from day 1 to day14 so as for control positive (group 2).

After the induction of CSOM in rats (day 1), the healthy groups (control negative) showed significant difference from all the groups (group 2 to group 8).

At day-7 and 14, control positive (group 2), group 3 and 6 (maintained on ciprofloxacin and co-amoxicillin respectively), showed significant difference from control negative (group 1), on the other hand, groups maintained on (4, 5, 7 & 8) anti-depressants with antibiotics were insignificant different from control group.

Force swimming test

The interpretation was based on the results obtained when one way ANOVA was applied and significant differences were observed in struggling time passed in swimming cage among the groups in force swimming test for day-1, 7 and 14 with the respective F-values 55.88, 19.48 and 18.68 with significant differences ($p<0.05$). Post Hoc test for multiple group comparison evaluation is shown in fig. 2.

Day 1 presented the significant difference of control negative from control positive and group 3, 4, 5, 6, 7 and 8.

At day 7, control negative (group 1) was significantly different from the control positive (group 2) and those which were maintained only on antibiotics (group 3 & group 6).

After the one week of the treatment, control negative (group 1) showed significant differences from the same groups as mentioned at day-14 (group 3 & group 6).

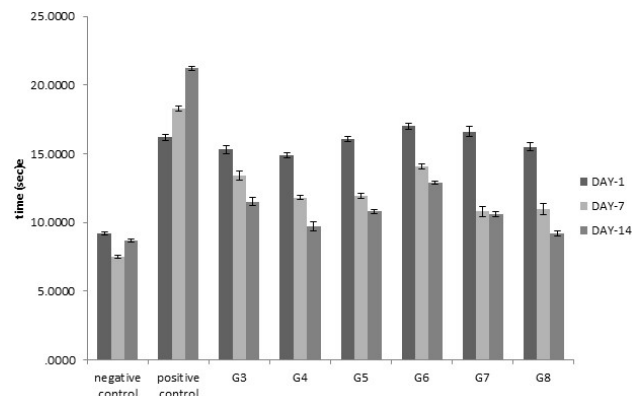


Table 5: activity expressed in mean with \pm S.D in traction test. Significant difference $^*(p<0.05)$ by different groups is evaluated by Tukey's test as compare to control group.

All the groups treated with antibiotics with anti-depressants (group 4 group 5, group 7 & group 8) showed insignificant difference in force swimming test in contrast with control negative (group 1).

Memory

The effects of CSOM on activity of rats in memory cage is evaluated by one way ANOVA in which $F= 21.01$ for day-1, $F=17.66$ for day-7 and $F= 11.15$ for day 14 with $p< 0.01$ was obtained, therefore, Post hoc was applied to evaluate the comparative effects of responses among groups as shown in fig. 4.

From the day 1 to day 14, control negative (group 1) were significantly different from control positive (group 2). After the treatment at day 7, all groups showed insignificant different behavior from the control negative (group 1) except control positive (group 2) and group-3 (maintained on ciprofloxacin) but at the last day all the groups showed insignificant difference from control group.

Traction test

The comparative behavior effects of CSOM on different groups are tested by one way ANOVA by traction test giving p value less than 0.05 which is considered significant in all three days i-e, day1, 7 and 14 with F-values 11.388, 18.167 and 285.13 respectively. The post hoc test was done for multi group comparison as shown in fig. 4.

Traction results were very interested that showed significant difference of control negative (group 1) from the all other groups at day-1, but at day-7, all the groups were insignificant different from control negative (group 1) except the untreated rats (group-2), and the groups maintained on antibiotics (group 3& 6) but after one week

of the treatment all groups except group-2 showed insignificant difference from control negative (group 1).

DISCUSSION

Several studies showed that in chronic infections accompanied by inflammation causative micro-organisms promotes catabolism of tryptophan which results in decreased serotonin levels ultimately causes depression in patients (Khan *et al.*, 1996).

In all the activity tests, the significant difference between positive (G1) and negative control (G2) showed that CSOM may cause depression and behavioral deficit in rats. In open field test, significant decreased in activity was observed in groups maintained on antibiotics alone but insignificant decreased in number of squares were obtained in the groups treated with antibiotics and antidepressants. Similarly, in light and dark cage activity box, only the groups maintained antidepressants with antibiotics (G4, G5, G7 & G8) showed close reading to the healthy rats (G1; negative control) in spending time in lighter part with insignificant difference. These results showed that imipramine and bromazepam were responsible to recover the condition upto the normal condition.

As studies showed their role to normalize behavior and to restore normal serum serotonin (Tatsumi *et al.*, 1997).

Interesting and a little different result was obtained in force swimming test where G4 (maintained on ciprofloxacin with imipramine), G5 (maintained on ciprofloxacin with bromazepam) and G7 (maintained on co-amoxicillin with imipramine) reflected insignificant difference at day 7 ($p < 0.05$ after treatment) but not G8 (maintained on co-amoxicillin with bromazepam). At day 14 (after one week of treatment) all the group treated with antidepressants with antibiotics showed insignificant decreased than negative control (G1).

In memory test, all treated groups (G3, G4, G5, G6, G7 & G8) showed significant increased to reach the goal at day 7 but after one week of treatment (day 14) all the groups showed insignificant difference from G1. Hence, took one week to reflect anxiety free attitude in force swimming cage. In the case of traction test, G 3 and G6 (treated with only antibiotics) took one week after the treatment to show insignificant difference from healthy group (G1) but the groups maintained antidepressants with antibiotics (G4, G5, G7 & G8) showed insignificant increase in time as compare to negative control (G1).

Several studies suggested that inflammatory markers increased in chronic infection such as CSOM may affect the metabolism of precursor of serotonin, leading to the decrease level of neurotransmitters and presented the

cognitive and behavioral disorders (Smiałowski, 1991, Saleem *et al.*, 2017).

Several studies suggested that altered serotonin concentration may lead to the depression which can be evaluated by significant changes in open field activities and transition period to the light in light /dark box activity in the case of animal studies or serum concentration of neurotransmitters such as serotonin in clinical study (Saleem *et al.*, 2018). Both the drugs bromazepam and imipramine are capable to attenuate depression and anxiety and increase serotonin levels (Dikeos *et al.*, 2008, Oelschläger, 1989, Amphoux, 1982).

Therefore, in the present pre-clinical study, combined effects of antibiotics with antidepressants successfully recovered the condition of stress and depression in most of the cases.

CONCLUSION

The current study provide evidences that chronic infection of suppurative otitis media induces depression which may be due to decreased level of serotonin which couldn't revert back by only antibiotic therapy but regain normal physical and psychological status when treated with antidepressant (in combination with antibiotics).

In future, we plan to extend this work at clinical level and will model our data on blockchain technology based decentralized platform. This emerging technology will help us maintain integrity of our data and draw an improved scientific solution in health care system.

REFERENCES

- Mehboob S, Rafi SMT, Mehjabeen, Saleem D, Mehboob M and Ahmed H (2020). Effects of ceftazidime with and without imipramine and bromazepam on behavior and neuro-inflammatory parameters in rats with chronic suppurative otitis. *Pak. J. Pharm. Sci.*, **33**(3): 1271-1276.
- Amphoux G, Agussol P and Girard J (1982). The action of bromazepam on anxiety. *La Nouvelle pressemédicale.*, **11**(22): 1738-1740.
- Aubert-Khalfa S, Granier JP, Reynaud E, Khoury E M, Grosse EM, Samuelian JC and Blin O (2010). Pure-tone auditory thresholds are decreased in depressed people with post-traumatic stress disorder. *J. Affect Disord.*, **127**(1-3): 169-176.
- Bhutta MF, Thornton RB, Kirkham LAS, Joseph EK and Cheeseman MT (2017). Understanding the aetiology and resolution of chronic otitis media from animal and human studies. *Disease Models and Mechanisms*, **10**(11): 1289-1300.
- Bogo V, Hill TA and Young RW (1981). Comparison of accelerod and rotarod sensitivity in detecting ethanol-

- and acrylamide-induced performance decrement in rats: review of experimental considerations of rotating rod systems. *Neurotoxicology*, **2**(4): 765-787.
- Casellas F, Borrueal N, Papo M, Guarner F, Antolín M, Videla S and Malagelada JR (1998). Anti-inflammatory effects of enterically coated amoxicillin-clavulanic acid in active ulcerative colitis. *Inflamm. Bowel. Dis.*, **4**(1): 1-5.
- Dikeos DG, Theleritis CG and Soldatos CR (2008). Benzodiazepines: An overview. Chapter 10. *In: Sleep Disorders: Diagnosis and Therapeutics*. Edited by Pandi-Perumal SR, Verster JC, Monti JM, Lader M and Langer SZ. 2008 Informa, London UK, pp.96-104.
- Gillman PK (2007). Tricyclic antidepressant pharmacology and therapeutic drug interactions updated. *Br. J. Pharmacol.*, **151**(6): 737-748.
- Haleem DJ (2009). Exaggerated feedback control decreases brain serotonin concentration and elicits hyperactivity in a rat model of diet-restriction-induced anorexia nervosa. *Appetite*, **52**: 44-50.
- Khan NA, Meyniel JP and Deschaux P (1996). Ca^{2+} /calmodulin and protein kinase C regulation of serotonin transport in human K562 lymphocytes. *Cell. Immunol.*, **172**(2): 269-274.
- Mehboob S, Rafi SMT, Ahmed N and Mehjabeen (2019). Association of hearing loss with depression, anxiety and stress in patients suffering from chronic suppurative otitis media. *Pak. J. Med. Sci.*, **35**(2): 510-514.
- O'Connor T, Perry C and Lannigan F (2009). Complications of otitis media in Indigenous and non-Indigenous children. *Med. J. Aust.*, **191**(9): S60-S64.
- Oelschlager H. (1989). Chemical and pharmacologic aspects of benzodiazepines. *Schweiz Rundsch Med Prax.*, **78**(28): 766-772.
- Pandi-Perumal SR, Verster JC, Monti JM, Lader M, Langer Eshun-Wilson SZI, Siegfried N, Akena DH, Stein DJ, Obuku EA and Joska JA (editors) (2018). Antidepressants for depression in adults with HIV infection. Chapter 21. Informa Healthcare, London UK. *CDSR* **1**(1): 31-39.
- Porsolt RD, Bertin A and Jalfre M. (1977). Behavioral despair in mice: A primary screening test for antidepressants. *Arch. Int. Pharmacodyn. Ther.* **229**(1): 327-336.
- Sachsel C, Eiff Von, Becker K and Rudack C (2008). Anti-inflammatory effects of ciprofloxacin in *S. aureus Newman* induced nasal inflammation *in vitro*. *J. Inflamm.* **5**(11): 1-6.
- Smiałowski A (1991). Dopamine D2 receptor blocking effect of imipramine in the rat hippocampus. *Pharmacol. Biochem. Behav.* **39**(1): 105-108.
- Saleem DM, Mehboob S, Khan MM, Samad N, Zafar A and Haleem DJ (2018). Inhibition of diet-restriction-induced behavioral deficits by tryptophan administration in rats. *Pak. J. Pharm. Sci.*, **31**(3): 1021-1029.
- Saleem DM, Khan MM, Aftab K., Mehboob S, Tabassum S and Haleem DJ (2017). Behavioral response to apomorphine in diet restriction-induced hyperactivity in rats. *J. Pharm. Pharmacol.* **1**(2): 1-9.
- Stemmelin J, Cohen C, Terranova JP, Lopez-Grancha M, Pichat P and Bergis *et al.*, (2008). Stimulation of the beta(3)-adrenoceptor as a novel treatment strategy for anxiety and depressive disorders. *Neuropsychopharmacology*, **33**(3): 574-587.
- Tamara H and John J Ryan (2008). Selective Serotonin Reuptake Inhibitors Suppress Mast Cell. *Function. J Immunol.*, **200**(1): 105-108.
- Tatsumi M, Groshan K, Blakely RD and Richelson E (1997). Pharmacological profile of antidepressants and related compounds at human monoamine transporters. *Eur. J. Pharmacol.*, **340** (2-3): 249-258.
- Verhoeff M, van der Veen EL, Rovers MM, Sanders EA, Schilder AG (2006). Chronic suppurative otitis media: A review. *Int. J. Pediatr. Otorhinolaryngol.*, **70**(1): 1-12.