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Behavioural responses in rats; modulation with beta-lactam antibiotics and antioxidants

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ABSTRACT

N-Acetylcysteine (NAC) has been in clinical practice for several decades as a mucolytic agent and has been used also for the treatment of paracetamol intoxication, doxorubicin-induced cardiotoxicity, stable angina pectoris, ischemia-reperfusion cardiac injury, acute respiratory distress syndrome bronchitis, chemotherapy-induced toxicity, HIV/AIDS, radio-contrast induced nephropathy, heavymetal toxicity and psychiatric disorders including schizophrenia, bipolar disorder and addiction. It has been recently shown that NAC modulates the glutamatergic system through the system xc (Cystine-Glutamate Antiporter): Antiporter cysteine/glutamate. Ceftriaxone (CTX), a β-lactam antibiotic, is also shown to led to an increase of excitatory amino-acid transporter 2 (EAAT2) expression and glutamate transport activity in the brain in animal studies. It has been demonstrated that CTX has neuroprotective effects in both in vitro and in vivo models based on its ability to inhibit neuronal cell death by preventing glutamate excitotoxicity. The aim of the present study was to investigate the neurobehavioural effects of acute administration of NAC and CTX alone and in combination in open field and elevated plus maze tests. For this aim, three different doses (50, 100 and 200 mg/kg, i.p.) of CTX and NAC alone in the first part and their combination in the second part of the experiments and two different doses of diazepam were evaluated in open field and elevated plus maze tests. 200 mg/kg of NAC revealed anxiolytic-like behaviours in both tests while CTX 200 mg/kg failed to produce. Further investigations need to be conducted to rule out the involvement of system xc- on anxiety related behaviours. Increased system xc may represent an effective therapeutic endpoint.

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1. Introduction

Anxiety disorders (ADs) are the most common psychiatric conditions encountered by doctors in general. The range of lifetime-prevalence data of different ADs are as follows: specific phobia 1.5-12%; social phobia 0.2-9.4%; obsessive-compulsive disorder (OCD) 0.1-3%; generalized anxiety disorder (GAD) 0.1-6.9%; panic disorder 0.2-5%; post-traumatic stress disorder (PTSD) from 1 to over 10% (the wide range of prevalence data mirrors that these are derived from populations of different countries). Despite the variety of anxiolytic agents available over the last decades, not only the adverse effects causing poor treatment or complete abandonment of therapy or insufficient targeting the pathophysiology of the disease and patients characterizing resistance to the treatment, requires new anxiolytic agents, not only to reduce acute symptoms but also to prevent relapses in the long term. These unmet needs have stimulated the introduction of new pharmacological approaches for the treatment of anxiety in monotherapy or in augmentation to standard treatments (Faludi et al., 2012). The alteration of the relation between oxidative stress and neurotransmission in several psychiatric conditions has been previously demonstrated. N-acetylcysteine (NAC), the acetylated precursor of the amino acid L-cysteine, is a cysteine prodrug. It has been used as a mucolytic agent and for the treatment of paracetamol intoxication, doxorubicin-induced cardiotoxicity, stable angina pectoris, ischemia-reperfusion cardiac injury, acute respiratory distress syndrome bronchitis, chemotherapy-induced toxicity, HIV/AIDS, radio-contrast induced nephropathy, heavymetal toxicity and psychiatric disorders including schizophrenia, bipolar disorder and addiction. (Samuni et al., 2013). NAC

Table 1. Statistical analysis of behavioural performance of first and second part in elevated plus maze (EPM)

Treatment (mg/kg)	Open arm enteries (%) med (min-max)	Open arm time (%) med (min-max)	Closed arm enteries (%) med (minmax)	Total arm enteries (n) med (minmax)
1st Part				
Control(saline)	13.8 (11.1-20)	5.1 (1.5-15.7)	8 (4-13)	9 (5-15)
CTX 50	17.1 (17.1-33.3)	3.3 (2.5-7.7)	6 (2-13)	7 (3-14)
CTX 100	20 (10-33.3)	9.4 (3.3-15.9)	8 (4-11)	10 (6-13)
CTX 200	23.6 (20-33.3)*1	12.5 (8.8-18.4)	7 (2-9)	9 (3-13)
NAC 50	11.8 (10-40)	4.2 (1.3-16.6)	8 (3-9)	9 (5-12)
NAC 100	17.1 (11.1-20)	2.7 (2.2-6.9)	6 (4-8)	7 (5-10)
NAC 200	26.8 (12.5-37.5)*2	15.9 (15.2-17.1)*1	6 (5-11)	8 (7-14)
Diazepam 1	26.4 (11.1-42.8)	14.2 (9.2-24.7)	6 (4-8)	7 (7-13)
Diazepam 2	48.1 (41.7-80)*1	48.8 (20.6-82.89)*1	5 (1-8)	9 (5-16)
2nd Part				
Control (saline+saline)	11.1 (8.3-14.3)	7.5 (2.5-19.5)	8 (6-11)	9 (7-12)
Diazepam 2+saline	40.8 (33.3-45.5)*1	67.9 (45.6-82.8)*1	6 (6-8)	11 (10-12)
CTX200+saline	26.1 (11.1-50)*2	13.0 (5.1-47.9)	6 (1-8)	8.5(2-10)
NAC200+saline	22.2 (16.7-25)*1	19.0 (15.5-22.3)*3	7 (3-10)	9 (4-12)
NAC200+CTX200	15.5 (10-30)	19.5 (4.1-45.0)	7 (5-10)	9.5 (6-12)

n=8-8 per group; **CTX50:** Ceftriaxone 50 mg/kg; **CTX 100:** Ceftriaxone 100 mg/kg; **CTX 200:** Ceftriaxone 200 mg/kg; **NAC 50:** N-acetylcysteine 50 mg/kg; **NAC 100:** N-acetylcysteine 100 mg/kg; **NAC 200:** N-acetylcysteine 200 mg/kg; Diazepam 1 mg/kg, Diazepam 2 mg/kg. Comparisons were done between control and other groups. (Mann-Whitney U test, The Bonferroni correction was used to adjust the P value for each hypothesis Values of p<0.006 (for first part) and p<0.010 (for second part) were considered statistically.)
*1: p<0.001 *2: p=0.003 *3: p=0.007 *4: p=0.021

is widely used antioxidant that acts not only as a direct free radical scavenger, but also promotes production of glutathione (GSH) by furnishing its limiting precursor l-cysteine (Chakraborti et al., 2008). It has been also shown in the recent literature that NAC modulates the glutamatergic system through the astrocytic antiporter cysteine/glutamate (system xc) that results in the activation of NMDA receptors and in the stimulation of metabotropic glutamate receptors, reducing synaptic glutamate release. The potential usefulness of NAC in the treatment of various psychiatric disorders is due to its antioxidant effect and glutamate modulation. In recent literature, NAC has been demostrated as an useful add-on medication for treating Parkinson's disease, schizophrenia and the depressive symptoms in bipolar patients in several clinical trials and to decrease the immobility time in the forced swimming test (a well accepted model with a predictive value for antidepressants) in animal studies (Linck et al., 2012). Glutamate is the major excitatory neurotransmitter responsible for excitatory neurotransmission in the brain, as well as a potent neurotoxin that may lead to excitotoxicity of nerves. Glutamate released from glutamatergic nerve endings participates in the signaling process through different types of glutamatergic receptors and then must be cleared from the synaptic cleft by glutamate transporters. Five subtypes of glutamate transporters have been identified to date. Three of these glutamate transporters were identified in rat brain: Named as GLAST, GLT-1 and EAAC1 with their human homologues which are: EAAT1, EAAT2 and EAAT3, respectively. The two remaining human and rodent subtypes, EAAT4 and EAAT5 share common nomenclature. It is noteworthy to highlight that the impaired glutamate transport by EAATs is common in many central nervous system (CNS) disorders. In the literature it has been shown that many β-lactam antibiotics are transcriptional activators of EAAT2 resulting in increased EAAT2 protein levels. Ceftriaxone (CTX), a β-lactam antibiotic, is also known to led to an increase of EAAT2 expression and glutamate transport activity in the brain in animal studies. It has been demonstrated that CTX has neuroprotective effects in both in vitro and in vivo models based on its ability to inhibit neuronal cell death by preventing glutamate excitotoxicity (Kim et al., 2011).

The elevated plus-maze (EPM) test, which is rapid and sensitive to the effects of both anxiolytic and anxiogenic agent, is based on the natural aversion of rodents from open spaces (Holmes et al., 2000). Percent time spent on open arms is a classical parameter usually measured for evaluating changes in the anxiety state. The EPM test has become a convenient procedure to measure not only the anxiety like behaviours but also sedation and activity. Total arms entry, it is mostly an indicator of motor activity, closed arm entries has already been defined as a pure index of locomotor activity. Open arm entries, considered as a motor activity pattern by some authors, was not found to reflect general activity of the mouse, in accordance to (File, 1992; Cruz et al., 1994; Hogg, 1996; Fernandes and File, 1996; Espejo, 1997). Open field test's (OFT) procedure is based on the involvement of forced confrontation of a rodent with the situation. The animal is placed in the center or close to the walls of the apparatus and the following behavioural items are recorded for a period ranging from 2 to 20 min (usually 5 min): Horizontal locomotion (number of squares passed on the marked flor), number of rearings or leaning (sometimes termed as vertical activity) (Prut and Belzung, 2003). The open field test provides to assess novel environment exploration, general locomotor activity and anxiety-related behaviour in rodents. Rodents tend to spent a significantly greater amount of time exploring the periphery of the arena, usually in contact with the walls (that is called thigmotaxis), than the center area. (Bailey and Crawley, 2009). Increase of time spent in the central part as well as of the ratio central/total locomotion or decrease of the latency to enter the central part are indications of anxiolysis (Walsh and Cummins, 1976; Prut and Belzung, 2003). The aim of the present study was to evaluate the neurobehavioural

Table 2. Statistical analysis of behavioural performance of first and second part in OFT

Treatment (mg/kg)	Time spent in center(s) med (min-max)	Squares passed (n) med (min-max)	Center crossings (n) med (min-max)	Rearings (n) med (minmax)
1st Part				
Control(saline)	5 (1-17)	35 (7-84)	2 (2-6)	21 (13-35)
CTX 50	1 (1-29)	12 (3-40)	2 (2-4)	14 (10-19)
CTX 100	5 (1-11)	51 (6-78)	2 (2-4)	18 (9-30)
CTX 200	1 (1-23)	46 (36-78)	2 (2-12)	23 (13-32)
NAC 50	4 (1-10)	57 (29-90)	4 (2-10)	23 (17-31)
NAC 100	4 (1-13)	37 (3-61)	2 (2-4)	19 (5-24)
NAC 200	16 (15-19)*1	37 (16-51)	6 (4-8)*1	34 (33-37)*1
Diazepam 1	7 (1-16)	27 (31-67)	4 (2-6)	14 (12-21)
Diazepam 2	19 (17-35)*2	26 (17-79)	6 (4-8)*1	13 (11-28)
2nd Part				
Control(saline+saline)	4 (1-6)	57.5 (45-75)	3 (2-4)	20.5 (12-30)
Diazepam 2+saline	9 (7-15)*2	43.5 (42-60)	6 (4-6) *4	14.5 (12-20)
CTX200+saline	4 (1-13)	49.5 (20-90)	2 (2-4)	20 (15-30)
NAC200+saline	5.5 (5-7)*3	45.5 (6-68)	6 (4-6) *5	28 (27-34)*4
NAC200+CTX200	3.5 (1-13)	47.5 (26-68)	2 (2-6)	17.5 (12-22)

n=8-8 per group; **CTX50:** Ceftriaxone 50 mg/kg; **CTX 100:** Ceftriaxone 100 mg/kg; **CTX 200:** Ceftriaxone 200 mg/kg; **NAC 50:** N-acetylcysteine 50 mg/kg; **NAC 100:** N-acetylcysteine 100 mg/kg; **NAC 200:** N-acetylcysteine 200 mg/kg, Diazepam 1mg/kg, Diazepam 2 mg/kg. Comparisons were done between control and other groups. (Mann-Whitney U test, The Bonferroni correction was used to adjust the P value for each hypothesis Values of p<0.006 (for first part) and p<0.010 (for second part) were considered statistically.)
*1p=0.003, *2p<0.001, *3 p=0.007, *4p=0.005, *5p=0.002

effects of acute administration of NAC and CTX over anxiety using open field and elevated plus maze tests.

2. Material and methods

Animals

Experimentally naive, one hundred and twelve, adult, male, Wistar albino rats (290-320 g each), obtained from Kobay Laboratories (No. 003679) were allocated to fourteen groups (n=8 per group). Rats were maintained for at least 2 weeks before the experiments. The rats were housed in standard plastic cages (4 animals per cage), maintained under standardized conditions of light 12-h light/dark cycle, room temperature (22±2°C) and humidity (60%), with free access to food (standard chowpellets) and tap water in Dollvet Laboratories animal facility.

Ethics

This study was approved by the Animal Experiments Local Ethics Committee of Dollvet Laboratories (Approval number:13-08). All the experiments were carried out in accordance with the Declaration of Helsinki and with the Guide for the Care and Use of Laboratory Animals (USA, NIH).

Behavioural tests

Elevated plus-maze test

The elevated plus maze is a pharmacologically validated model for assessment of anxiety state in rodents. The plus-maze consisted of two open arms (50x10cm), facing each other, and two closed arms (50x50x40 cm) with an open roof and was elevated to a height of 50cm and placed in a quiet dimly lit room. The following measures were taken by an observer during a 5 min test period after the rat had been placed on the centre of the maze facing an open arm: The number of entries into (with the criterion for an arm entry defined as, all four paws in an arm of the maze) and the time spent in each of the two types of arm. Distribution of behaviour (arm entries and time spent) on the maze was additionally calculated as 'percent total' both for frequency and duration measures. After each subject completes its test session, fecal boli and urine are removed, surfaces are wiped with 70% ethanol and the test chamber is allowed to dry completely before starting another subject and rats were returned to their home cages.

Open field test

The open field apparatus consisted of a square arena 100cm × 100cm with 40cm walls. The floor was subdivided in a centre and margin compartment with 16 squares. Rats were placed singly in one corner of open field, following measures were observed during a 5-min exposure period; rearings (vertical locomotion), center crossings, squares passed (horizontal locomotion) and time spent in the periphery and center of the arena. At the end of each trial, mice were returned to their home cages, and test box was carefully cleaned with %70 ethyl alcohol and permitted to dry between tests. It is a validated test that benzodiazepines increase the amount of time a rodent will spend in the center of the arena (Bailey and Crawley, 2009).

Drugs and experimental groups

The drugs administered were; CTX sodium was a gift from Deva Pharmaceuticals (50, 100 and 200 mg/kg, i.p.), NAC was a gift from Bilim Pharmaceuticals (50, 100 and 200 mg/kg, i.p.) and diazepam (1 and 2 mg/kg, i.p.) (Sigma-Aldrich). All drugs were dissolved in physiological saline, freshly prepared on the days of the test and administered i.p. in a volume of 1 ml/kg. Control animals received physiological saline. Testing was conducted between 13.00 pm and 18.00 pm. and rats acclimated to the testing room for a minimum of 45 min prior to testing for all tests. First, open field (OF) behaviour was measured as part of a locomotor testing paradigm. The plus-maze procedure was validated pharmacologically in rats by systemic treatment with a benzodiazepine, diazepam.

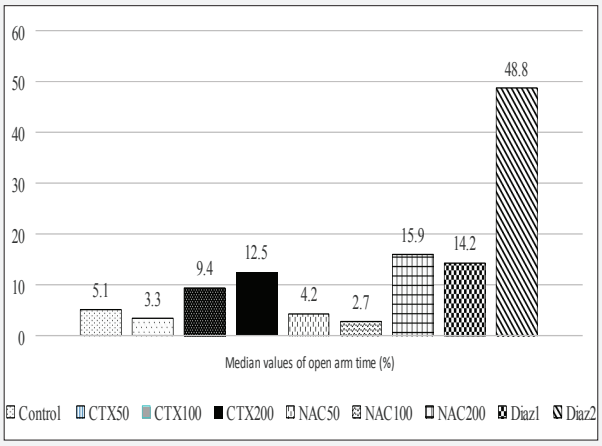

Fig. 1. EPM-1st Part

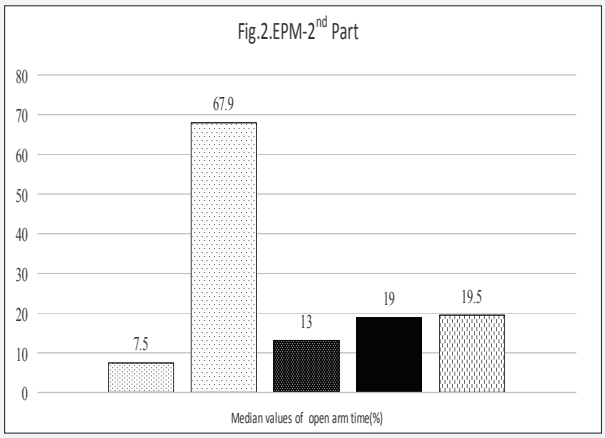

Fig. 2. EPM-2nd Part

Diazepam (2 mg/kg) and in the second part diazepam 2 mg/kg+saline caused a significant increase in the percentage of open arm entries and percentage of open arm time when compared with controls, applied 30 min before testing without significantly changing locomotor activity. Treatments were given 65 min before the elevated plus maze testing because of the pharmacokinetics of NAC and CTX (Rebuelto et al., 2003, Linck et al., 2012). The doses of diazepam, NAC and CTX were selected on the basis of those reported in the literature (Kurt et al., 2003; Chakraborti et al., 2008; Karaman et al., 2013). In the first part of the experiments, three different doses of each drugs were administered and in the second part, the combination of statistically significant doses of NAC and CTX were evaluated.

Statistical analysis

Statistical analysis was performed using SPSS 13.0 software. All numerical data are expressed as median values (minimum-maximum). For each continuous variable, normality was checked by Kolmogorov Smirnov and Shapiro-Wilk tests and by histograms. Comparisons between groups were applied using Kruskal Wallis test were used for the data not normally distrubuted. Since analysis of variance was significant, comparisons were applied using Mann-Whitney U test. Pre-post measures data were analysed using Wilcoxon test. The Bonferroni correction to adjust the P value for each hypothesis to 0.0055 and 0.010. Values of $p<0.006$ and $p<0.010$ were considered statistically.

3. Results

There were no significant differences in the body weights when compared before and after the injections in the statistical analysis ($p>0.050$).

Elevated plus-maze test

The results of the first part of EPM test are presented in Table 1. The analysis of elevated plus maze test revealed significant differences in the percentage of open arm entries and time spent in open arms ($H=52.38$, $H=34.33$ respectively, $p<0.050$) but there were no significant difference between groups for the number of total arm and close arm entries ($H=11.35$, $H=8.53$, $p>0.050$) those can be implemented as predictors of locomotor activity. The post-hoc test showed that the percentage of time spent in the open arms was increased only by diazepam 2 mg/kg and NAC 200 mg/kg ($p<0.0055$, $U=0.00$, $U=2.00$ respectively) versus control and no significant differences were detected among remaining groups when compared to the control (Fig. 1). The analysis of the percentage of open arm entries revealed a significant effect of treatment with 2 mg/kg of diazepam ($p<0.0055$ $U=0.00$) and 200 mg/kg CTX ($p<0.0055$, $U=1.50$) 200 mg/kg NAC ($p<0.0055$, $U=5.00$) groups when compared to the control group (Table1). No significant differences were found in the remaining groups. It should be mentioned that determined elevation in the percentages of time spent in open arm and open arm entries of aforementioned drugs doses were not due to an increase in locomotor activity because the analysis of total arms and close arms entries data of these groups, did not revealed a significant difference when compared to control group. In EPM, a slight elevation was evaluated with CTX200 mg/kg that were not significant but a significant difference is determined with CTX 200mg/kg in the percentage of open arm enteries that may be implemented in favour of anxiolytic-like behaviour. 200 mg/kg of CTX and NAC, 2 mg/kg of diazepam is selected for the second part of experiments.

The data of the EPM test in the second part of the experiments, is shown in Table 1. The non-parametric-Kruskal Wallis test showed significant differences in the percentage of time spent in the open arms ($H=23.36$, $p<0.050$). Intergroup post-hoc comparisons showed that this parameter was significantly increased by the groups treated with 2 mg/kg of diazepam+saline ($U=0.00$, $p<0.010$) and 200 mg/kg NAC+saline ($U=7.00$, $p<0.010$) versus control group (saline+saline) (Fig. 2). The analysis of the percentage of open arm entries showed significant difference among treatments ($H=25.85$, $p<0.050$), this effect is augmented in 2 mg/kg of diazepam +saline ($U=0.00$, $p<0.010$), 200 mg/kg NAC+saline ($U=0.00$, $p<0.010$) and 200 mg/kg CTX+saline groups($U=5.00$, $p<0.010$) respect to control group (Table 1). The anxiolytic-like behavioural change seen with these drugs may be implemented as their changes were not due to a non-specific affect of these drugs on locomotor behaviour because the data analysis of these treatments, number of closed arm entries and total arm entries, did not revealed a significant difference when they were both compared to control. In addition to this, in Kruskal Wallis analysis, the number of closed arm entries showed no significant differences among groups ($H=9.06$, $p>0.050$), but there were significant

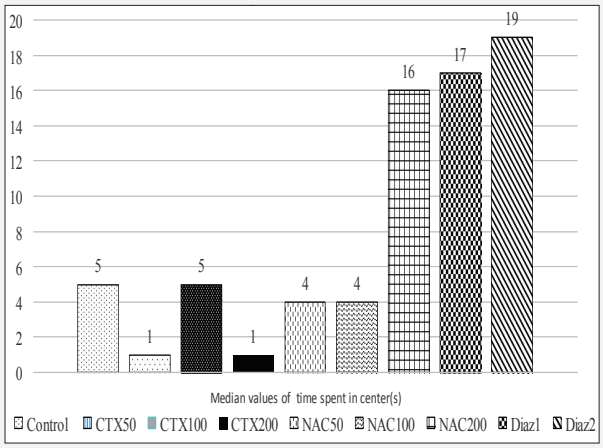

Fig. 3. OFT-1st Part

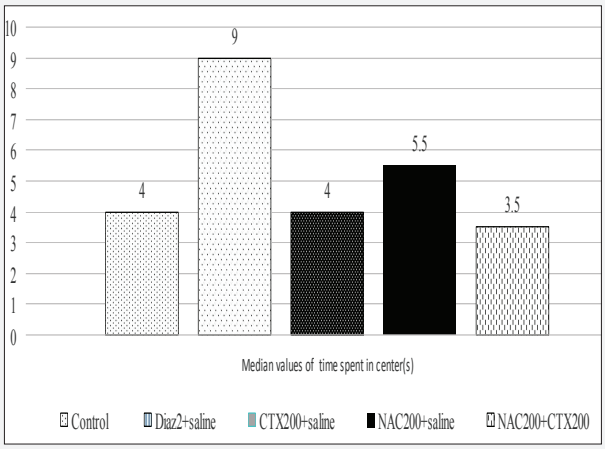

Fig. 4. OFT-2nd Part

difference in the number of total arm enteries among groups (H=11.04, p<0.050), intergroup post-hoc analysis is made to show the differential groups, there were no significant difference between all groups when compared to control but only significant difference was determined between 2 mg/kg Diazepam+saline group other and 200 mg/kg CTX+saline group when they were compared to each other. The post-hoc comparision of CTX 200 mg/kg+saline and CTX combination with NAC did not revealed any significant difference in EPM when compared to each other. Post-hoc comparision of NAC 200 mg/kg+saline and NAC combination with CTX did not presented statistically significant difference in EPM when compared to each other.

Open field test

The effect of the treatments administered in the first part of the study on time spent in the centre, number of squares passed, center crossing and rearings in the OFT is presented in Table 2. The analysis of non-parametric-Kruskal Wallis test showed significant differences in those aforementioned measures (H=34.81, H=17.17, H=28.59, H=35.21, p<0.050, respectively). Post hoc analysis of the time spent in the centre presented that doses of NAC and Diazepam increased dose-dependently, but it was significantly increased with only diazepam 2 mg/kg (p<0.0055, U=1.50) and NAC 200 mg/kg (p<0.0055, U=5.00) versus control and no significant differences were found in the remaining groups when compared to control (Fig. 3). Number of squares passed, did not show any significant difference between groups (with 200 mg/kg NAC, p>0.0055, U=31.00 and 2 mg/kg Diazepam, p>0.0055 U=29.50) when they were compared to control, so the anxiolytic like effets of NAC and Diazepam may be implemented as they were not due to an increase in locomotor activity. Post-hoc analysis for center crossing showed significant increase with only diazepam 2 mg/kg (p<0.0055, U=5.00) and NAC 200 mg/kg (p<0.0055 U=5.50) versus control and no significant differences were found in the remaining groups when compared to control (Table 2). For rearings measurements, post-hoc analysis presented significant difference only for 200mg/kg NAC dose when compared to control (p<0.005, U=5.00), there were no differences in other groups when they compared to control group(Table2). The CTX 200 mg/kg presented a slight elevation in time spent in center parameter when CTX 100 mg/kg slightly decreased but those were not significant. The elevation seen in squares passed with CTX100 mg/kg was not significant, either. 2 mg/kg diazepam, 200mg/kg CTX and NAC were selected for the second part of experiments.

The effect of administered treatments on time spent in the centre, number of squares passed, center crossings and rearings in the open field in the second part of the study is presented in Table 2. The analysis of the non-parametric test revealed significant differences in time spent in the center, center crossings and rearings (H=14.44, H=25.12, H=21.44 respectively, p<0.050). Post-hoc comparisons showed that time spent in centre and centre crossings significantly augmented in 2 mg/kg Diazepam+saline (U=0.00, U=6.00, p<0.010) and 200 mg/kg NAC+saline(U=7.50, U=4.00, U=6.50, p<0.010) (Fig. 4). In addition to these, rearing was also augmented significantly with 200 mg/kg NAC+saline group (U=6.5, p<0.010) when compared with control group (saline+saline) (Table2). Post-hoc comparision of NAC 200 mg/kg+saline and NAC combination with CTX revealed that there were significant decrease in center crossings and rearings in combination (U=6.00, U=0.00, respectively, p<0.010). The post-hoc comparision of CTX 200 mg/kg+saline and CTX combination with NAC did not revealed any statistically significant difference in OFT when compared to each other.

4. Discussion

Stress is considered as the response of an organism to environmental stimuli (stressors) that threaten its internal equilibrium, also called homeostasis (Ramos and Mormede, 1998). Stress causes changes in anxiety-like states by effecting emotional behaviour in regulation of stress responses and the central nervous system plays a crucial role. Stressor factors effects the neurobehavioural profile of an organism and can cause an anxiety-like syndrome and behavioural factors such as emotionality that are the valid predictors of stress susceptibility. This roles consist of complex neurochemical pathways (Chakraborti et al., 2008). The anxiolytic-like baseline behaviour is determined in the open field test if a rodent spends significantly more time exploring the center area. The two factors have been suggested to trigger the anxiety-like behaviour in this test: individual testing (Social isolation, a result of the physical separation from cage mates when performing the test) and the second one is: the stress created by the brightly lit, unprotected, novel test

environment, agoraphobia as the arena is very large relative to the animal's breeding or natural environment. The elevated plus maze test that also takes the same advantage of the natural tendency of rodents to explore novel environments. Rodents tend to avoid the open areas, favoring darker, more enclosed spaces (Hogg,1996; Bailey and Crawley, 2009). In the present study, we explored the locomotor activity and anxiolytic-like effects of three different doses (50, 100 and 200 mg/kg, i.p.) of NAC and CTX, and also their combination in open field and elevated plus maze tests, respectively. The results of the study showed that highest dose of NAC produced anxiolytic-like effects without changing the locomotor activity in both open field and elevated plus maze tests. Our results are in agreement with Chakraborti et al. (2008), who has evaluated acute administration of the doses of 100 and 200 mg/kg NAC on restraint wistar rats and reported the anxiolytic like effects with 200 mg/kg NAC in elevated plus maze and open field tests. In mice study of Bernabucci et al. (2012), study, it is shown that a single injection of NAC (100 mg/kg, i.p.; 30 min before the test) causes analgesia in the second phase of the formalin test by the activation of system xc. The System xc-is a membrane antiporter that mediates the chloride-dependent, sodium-independent, 1:1 exchange of extracellular L-cystine and intracellular L-glutamate, and provides the intracellular L-cysteine required for the synthesis of glutathioe (GSH) and oxidative protection. The exchange-mediated export of L-glutamate represents a non-vesicular route of glutamate release through which it can participate in either neuronal signalling or excitotoxicity pathology. In the present study, behavioural changes produced by the highest dose of NAC may be a result of increased GSH and glutamate. Free radicals play important role in health and disease because of their being highly reactive moieties. The central nervous system is especially vulnerable to free radical damage because of brain's high oxygen consumption, abundant lipid content and relative paucity of antioxidant enzymes. For this reason cell damage formed by the oxidative stress has been suggested to lead to several CNS disorders (Chakraborti et al., 2008). In the study of Chakraborti et al. (2008), it is suggested that therapeutic effects shown with NAC may be provided as a result of elevation of GSH levels as NAC is likely to stimulate the L-cystine/L-glutamate membrane exchanger System xc that mediates non-vesicular release of glutamate from astrocytes and microglia, thereby also promotes glutamate release into the extrasynaptic compartment, resulting in the stimulation of presynaptic mGlu2/3 receptors. Lutgen (2009), have reported that acute inhibition of system xc with sulphasalazine was anxiogenic in both elevated plus maze and open field paradigms. Additionally, the effects of sulphasalazine have been completely reversed with administration of NAC after sulphasalazine treatment in the EPM and OFT. Nonvesicular glutamate released during cystine-glutamate exchange activates extrasynaptic glutamate receptors by stimulating extrasynaptic group II mGluRs without exerting postsynaptic effects. This extrasynaptic glutamate seems to inhibit synaptic glutamate release, resulting a protection from excitotoxicity (Bridges et al., 2012). This factors may have limited the extreme behaviour responses in ambulation and helps the interpretation of anxiolytic like behaviour without any change in locomotion. The stimulated system xc may have given rise to an apparent hyperglutamatergic state largely involving either increased glutamate release into the extrasynaptic compartment or enhanced GSH levels after increased cystine transport. In contrast to the extrasynaptic compartment, stimulated system xc activity may lead to a hypoglutamatergic state within the synapse. This may have been occured as a result of increased activation of group II mGlu Rs which have been shown (Bridges et al., 2012) to inhibit synaptic release of glutamate and dopamine. In Baumann et al. (2008) study, it has been shown that dopamine (DA) nerve terminals in the nucleus accumbens are important mediators of amphetamine-induced locomotor activity, especially ambulation (horizontal locomotion). Destruction of DA nerve terminals in the nucleus accumbens markedly inhibited ambulation produced by systemically injected 3,4-methylenedioxy-N-methylamphetamine (MDMA). Moreover, microinjection of (+)- MDMA into the accumbens stimulated ambulation, and this effect has been shown to involve DA but not 5-hydroxytryptamine (5-HT). According to Baumann et al. (2008), study such findings implicate n.accumbens DA in the mechanism of MDMA's locomotor actions. Additionally, pretreatment with D1 or D2 receptor antagonists can reduce ambulation produced by i.p. administered MDMA, suggesting both receptor subtypes are involved (Baumann et al., 2008). In conclusion, while the beneficial effects of mGluR2/3 agonists in clinical trials are inconclusive, the results in preclinical and clinical studies suggest that stimulation of the mGluR2/3 receptor may lead to improvements in schizophrenic symptoms as would increased extrasynaptic glutamate potentially through system xc (Bridges et al., 2012). There is a growing amount of preclinical and clinical reports representing therapeutic potential of administration of NAC for psychiatric diseases. Clinically, NAC has been used as an adjunct therapy in the treatment of schizophrenia and shown beneficial effects and improvements in overall symptom severity, mismatch negativity and improved prefrontal cortex synchronization using EEG recordings (Chakraborti et al., 2008). Multiple nonvesicular release mechanisms may contribute to extracellular levels of glutamate, nonvesicular release of glutamate into the extrasynaptic compartment is incapable of stimulating high-affinity glutamate receptors in the synapse unless EAAT function is compromised. EAATs have been shown to clear glutamate released by system xc, supporting the idea that these transporters function to compartmentalize extracellular glutamate into multiple domains (e.g., synaptic, extrasynaptic) (Bridges et al., 2012). In addition to these, it has been also shown to be a potent activator of system xc. The beta-lactam family of antibiotics are shown to increase the expression and function of GLT-1 in vitro and in vivo. GLT-1 upregulation is attributable to an increase in gene transcription through the nuclear factor signaling cascade. Genetic deletion of glial GLT-1 produced elevated extracellular glutamate levels (Trantham-Davidson et al., 2012). In the rat study of Verma et al., 2010, presented that a single dose of 100 mg/kg i.v CTX was given 2 h after the reperfusion in cerebral ischemia/reperfusion injury also resulted in upregulation of GLT-1 protein. And also in the study of Maculoso et al. (2013), it is shown that a single preoperative dose of ceftriaxone (200 mg/kg) caused analgesia in humans and also performed animal

studies to examine whether a single dose of ceftriaxone (200 mg/kg) was sufficient to induce analgesia. A single intraperitoneal injection of ceftriaxone could also caused analgesia in mouse models of inflammatory or postsurgical pain, and upregulated GLT-1 in the spinal cord. Ceftriaxone is also a transcriptional regulator of xCT, Nrf2, and thereby increases system xc activity (Lewerenz et al., 2009). In the present study, acute administration of three different doses of CTX was insufficient to produce anxiolytic like effects. The dose 200 mg/kg CTX increased only the percentage of open arm entries in EPM. The combination of acute administration of NAC 200 mg/kg and CTX 200 mg/kg fail to produce anxiolytic like effects when they were administered in combination in both tests in the second part of the experiments. Post-hoc comparision of NAC 200 mg/kg+saline and NAC 200 mg/kg combination with CTX 200 mg/kg revealed a significant decrease in center crossings and rearings in OFT. A decrease is also seen in the compared varibles of EPM in the analysis of post-hoc comparision of NAC and its combination with CTX but it was not significant. It should be kept in mind that system xc and EAATs are different from each other. The system xc activity was chloride-dependent, sodium-independent, and electroneutral, properties that clearly differentiate it from the sodium-dependent, electrogenic EAATs. It is noteworthy that the EAATs have been implicated in the transport of both cystine and cysteine GSH, also serves to modulate the synaptic transmission. System xc is capable of contributing to the antioxidant capacity of a cell through the maintenance of glutathione levels and to glutamate homeostasis. The dual nature of system xc may be advantageous because it permits increased antioxidant capacity via cystine uptake to buffer the potential toxic effects of glutamate release (Bridges et al., 2012). This effect may have served as an advantage of NAC when used alone. The results produced by CTX in two parts of the study may indicate that anxiolytic-like behavioural changes is not directly modulated by GLT-1 or perhaps a higher dose or more likely a sub or chronic treatment regimen of CTX could produce alterations in these tests. When they are used in combination NAC and CTX, glutamate changes may not be stabile enough to produce significant changes or both activators may be insufficient to balance the homestasis. It is noteworthy that in general, a neuroprotective role has been clearly established for glutathione and NAC. The precise role of glutathione balance in anxiolytic-like behavioural changes needs to be conducted with further studies. In clinical approach for the bacterial infections, 1 or 2 g for adult and 50 to 100 mg/kg for pediatric patients for CTX administrations is given once daily. The dose used in this study (200 mg/kg per day) is relatively high as compared with clinical practice but normal antimicrobial CTX doses are likely to be sufficient to induce GLT-1 upregulation in humans (Chu et al., 2007). However, chronic use of CTX, a parenteral antibiotic, is not practical for routine treatment of behavioural disorders and also may change the microflora of the gut in favour of a superinfection and in addition to this, concern of antibiotic resistance with the repeated doses of the drug should be kept in mind. Given the role of EAATs to compartmentalize glutamate into functionally distinct pools (e.g., synaptic, extrasynaptic), it is possible that receptors located in the synapse or in the extrasynaptic compartment are being stimulated by glutamate diffusing across microdomains (Bridges et al., 2012). It may be a further investigation area for us to demonstrate whether the possible interaction of glutamate regulation and anxiety behaviour may arise from a loss of signal integrity instead of, or in addition to, abnormal levels of receptor activation. Glutamate homeostatic control mechanism, includes two main pathways: the cystine/glutamate exchanger system xc and the glial glutamate transporter EAAT2/GLT-1. Changes in the balance between synaptic and extrasynaptic glutamate levels in turn influence signaling through pre and postsynaptic glutamate receptors, and thus affect synaptic plasticity and circuit-level activity. Synchronization of cortical activity is regulated by complex inter-neuronal connections (Reissner and Kalivas, 2010).

Research literature indicating the role of glutamate pathways of these drugs is becoming increasingly important to reveal the relation between glutamate and psychiatric conditions. There is a growing body of literature of potential benefit of NAC and CTX in a wide range of neuropsychiatric disorders (Linck et al., 2012; Alajaji et al., 2013). Further preclinical efforts to define their neuropsychopharmacological activity profile could be useful for more precisely defining and understanding the entire therapeutic potential of these drugs either used as monotherapy or in combination with presently used drugs. The results of the present study suggest that system xc- contributes to states of anxiety, increased system xc - may represent an effective therapeutic endpoint; however the specific brain region and neurotransmitter system mediating these behaviours will require further investigations.

REFERENCES

Alajaji, M., Bowers, M.S., Knackstedt, L., Damaj, M.I., 2013. Effects of the beta-lactam antibiotic ceftriaxone on nicotine withdrawal and nicotine-induced reinstatement of preference in mice. Psychol. 228, 419-426. doi:10.1007/s00213-013-3047-3043.

Bailey, K.R., Crawley, J.N., 2009. Anxiety-Related Behaviours in Mice. In: Buccafusco, J.J., editor. Methods of behaviour analysis in neuroscience. 2nd edition. Boca Raton (FL): CRC Press; Chapter 5, 3-27.

Baumann, M.H., Clark, R.D., Rothman, R.B., 2008. Locomotor stimulation produced by 3,4-methylenedioxymethamphetamine (MDMA) is correlated with dialysate levels of serotonin and dopamine in rat brain. Pharmacol. Biochem. Behav. 90, 208-217. doi:10.1016/ j.pbb.2008.02.018.

Bernabucci, M., Notartomaso, S., Zappulla, C., Fazio, F., Cannella, M., Motolese, M., Battaglia, G., Bruno, V., Gradini, R., Nicoletti, F., 2012. N-Acetyl-cysteine causes analgesia by reinforcing the endogenous activation of type-2 metabotropic glutamate receptors. Mol. Pain. 23, 77. doi: 10.1186/1744-8069-8-77.

Bridges, R., Lutgen, V., Lobner, D., Baker, D.A., 2012. Thinking outside the cleft to understand synaptic activity: Contribution of the cystine-glutamate antiporter (System xc-) to normal and pathological glutamatergic signaling. Pharmacol. Rev. 64, 780-802. doi:10.1124 / pr.110.003889.

Chakraborti, A., Gulati, K., Banerjee, B.D., Ray A. 2007. Possible involvement of free radicals in the differential neurobehavioural responses to stress in male and female rats. Behav. Brain Res. 179, 321-325.

Chakraborti, A., Gulati, K., Ray, A., 2008. Age related differences in stress-induced neurobehavioural responses in rats: Modulation by antioxidants and nitrergic agents. Behav. Brain Res. Dec. 194, 86-91.

Chu, K., Lee, S.T., Sinn, D.I., Ko, S.Y., Kim, E.H., Kim, J.M., Kim, S.J., Park, D.K., Jung, K.H., Song, E.C., Lee, S.K., Kim, M., Roh, J.K., 2007. Pharmacological induction of ischemic tolerance by glutamate transporter-1 (EAAT2) upregulation. Stroke. 38, 177-182.

Cruz, A.P., Frei, F., and Graeff, F.G., 1994. Ethopharmacological analysis of rat behaviour on the elevated plus-maze, Pharmacol. Biochem. Behav. 49, 171-176.

Espejo, E.F., 1997. Structure of the mouse behaviour on the elevated plus-maze test of anxiety Behav. Brain Res. 86. 105-112.

Faludi, G., Gonda, X., Bagdy, G., Dome, P., 2012. Pharmaco- and therapygenetic aspects in the treatment of anxiety disorders beyond the serotonergic system: A brief review. Neuropsychopharmacol. Hung. 14, 221-229.

Fernandes, C., File, S.E., 1996. The influence of open arm ledges and maze experience in the elevated plus-maze. Pharmacol. Biochem. Behav. 54, 31-40.

File, S.E., 1992. Behavioural detection of anxiolytic action. In J.M.Elliott, D.J. Heal and C.A. Marsden (Eds.) Experimental Approachesto Anxiety and Depression. Wiley, Chichester, pp. 25-44.

Hogg, S., 1996. A Review of the Validity and Variability of the Elevated Plus-Maze as an Animal Model of Anxiety. Pharmacol. Biochem. Behav. 54. 21-30.

Holmes, A., Parmigiani, S., Ferrari, P.F., Palanza, P., Rodgers, R.J., 2000. Behavioural profile of wild mice in the elevated plus-maze test for anxiety. Physiol. Behav. 71, 509-516.

Karaman, I., Kizilay-Ozfidan, G., Karadag, C.H., Ulugol, A., 2013. Lack of effect of ceftriaxone, a GLT-1 transporter activator, on spatial memory in mice. Pharmacol. Biochem. Behav. 108, 61-65. doi: 10.1016/j.pbb.2013.04.013.

Kim, K., Lee, S.G., Kegelman, T.P., Su, Z.Z., Das, S.K., Dash, R., Dasgupta, S., Barral, P.M., Hedvat, M., Diaz, P., Reed, J.C., Stebbins, J.L., Pellecchia, M., Sarkar, D., Fisher, P.B., 2011. Role of excitatory amino acid transporter-2 (EAAT2) and glutamate in neurodegeneration: Opportunities for developing novel therapeutics. J. Cell Physiol. 226, 2484-2493. doi:10.1002/jcp.22609.

Kurt, M., Bilge, S.S., Kukula, O., Celik, S., Kesim, Y., 2003. Anxiolytic-like profile of propofol, a general anesthetic, in the plus-maze test in mice. Pol. J. Pharmacol. 55, 973-977.

Lewerenz, J., Albrecht, P., Tien, M.L., Henke, N., Karumbayaram, S., Kornblum, H.I., Wiedau-Pazos, M., Schubert, D., Maher, P., Methner, A., 2009. Induction of Nrf2 and xCT are involved in the action of the neuroprotective antibiotic ceftriaxone in vitro. J. Neurochem. 111, 332-343. doi:10.1111/j.1471-4159.2009.06347.x.

Linck, V. M., Costa-Campos, L., Pilz, L.K., Garcia, C.R., Elisabetsky E. 2012. AMPA glutamate receptors mediate the antidepressant-like effects of N-acetylcysteine in the Mouse tail suspension test. Behav. Pharmacol. 23, 171-177. doi:10.1097/FBP.0b013e3283512c3a.

Lutgen, Victoria, The Contribution of System Xc-to Prefrontal Cortical Mediated Behaviours Associated with Schizophrenia (2012). Dissertations (2009 -). 103-130. http://epublications.marquette.edu/dissertations_mu/176ç.

Macaluso, A., Bernabucci,, M., Trabucco, A., Ciolli, L., Troisi, F, Baldini, R., Gradini, R., Battaglia, G., Nicoletti, F., Collini, S., 2013. Analgesic effect of a single preoperative dose of the antibiotic ceftriaxone in humans. Pain. 14, 604-612. doi: 10.1016/j.jpain.2013.01.774.

Prut, L., Belzung, C., 2003. The open field as a paradigm to measure the effects of drugs on anxiety-like behaviours: A review. Eur. J. Pharmacol. 463, 3-33.

Ramos, A, Mormède, P., 1998. Stress and emotionality: A multidimensional and genetic approach. Neurosci. Biobehav. Rev. 22, 33-57.

Rebuelto, M., Ambros, L., Rubio, M., 2003. Daily variations in ceftriaxone pharmacokinetics in rats. Antimicrob. Agents Chemother. 47, 809-812.

Reissner, K.J., Kalivas, P.W., 2010. Using glutamate homeostasis as a target for treating addictive disorders. Behav Pharmacol. 21, 514-522. doi: 10.1097/FBP.0b013e32833d41b2.

Samuni, Y., Goldstein, S., Dean, O.M., Berk, M., 2013. The chemistry and biological activities of N-acetylcysteine. Biochim Biophys Acta. Aug;1830, :4117-4129. doi:10.1016/j.bbagen.2013.04.016.

Trantham-Davidson, H., LaLumiere, R.T., Reissner, K.J., Kalivas, P.W., Knackstedt, L.A., 2012. Ceftriaxone normalizes nucleus accumbens synaptic transmission, glutamate transport, and export following cocaine self-administration and extinction training. J. Neurosci. 32, 12406-12410. doi: 10.1523/JNEUROSCI.1976-12.2012.

Verma, R., Mishra, V., Sasmal, D., Raghubir, R., 2010. Pharmacological evaluation of glutamate transporter 1 (GLT-1) mediated neuroprotection following cerebral ischemia/reperfusion injury. Eur. J. Pharmacol. 638, 65-71. doi:10.1016/j.ejphar.2010.04.021.

Walsh, R.N., Cummins, R.A., 1976. The Open-Field Test: A critical review. Psychol. Bull. 83, 482-504.