www.elsevier.com/locate/psychres

L-carnosine as an add-on to risperidone for treatment of negative symptoms in patients with stable schizophrenia: a double-blind, randomized placebo-controlled trial

Alireza Ghajar, Mohammad-Reza Khoaie-Ardakani, Zahara Shahmoradi, Amir-Reza Alavi, Mohsen Afarideh, Mohammad-Reza Shalbafan, Maryam Ghazizadeh-Hashemi, Shahin Akhondzadeh

PII: S0165-1781(17)31801-2
DOI: https://doi.org/10.1016/j.psychres.2018.02.012
Reference: PSY11186

To appear in: *Psychiatry Research*

Received date: 30 September 2017
Revised date: 8 January 2018
Accepted date: 2 February 2018

Cite this article as: Alireza Ghajar, Mohammad-Reza Khoaie-Ardakani, Zahara Shahmoradi, Amir-Reza Alavi, Mohsen Afarideh, Mohammad-Reza Shalbafan, Maryam Ghazizadeh-Hashemi and Shahin Akhondzadeh, L-carnosine as an add-on to risperidone for treatment of negative symptoms in patients with stable schizophrenia: a double-blind, randomized placebo-controlled trial, *Psychiatry Research,* https://doi.org/10.1016/j.psychres.2018.02.012

L-carnosine as an add-on to risperidone for treatment of negative symptoms in patients with stable schizophrenia: a double-blind, randomized placebo-controlled trial

Alireza Ghajar[a1] MD., Mohammad-Reza Khoaie-Ardakani[b1] MD., Zahara Shahmoradi[a] MD, Amir-Reza Alavi[b] MD., Mohsen Afarideh[a] MD., Mohammad-Reza Shalbafan[c] MD., Maryam Ghazizadeh-Hashemi[c] MD., Shahin Akhondzadeh[a,*] Ph.D.

[a] Psychiatric Research Center, Roozbeh Hospital, Tehran University of Medical Sciences, Tehran, Iran

[b] Razi Hospital, University of Social Welfare and Rehabilitation, Tehran, Iran.

[c] Mental Health Research Center, School of Medicine, Iran University of Medical Sciences, Tehran, Iran.

s.akhond@neda.net

[*] Corresponding author: Psychiatric Research Center, Roozbeh Hospital, Tehran University of Medical Sciences, South Kargar Street, Tehran 13337, Iran. Tel: (+98, 21)-55419113, fax: (+98, 21)-55412222

Abstract

Since l-carnosine has shown effectiveness in improvement of cognition in patients with schizophrenia, this 8-week, randomized, double-blind, placebo-controlled pilot study was conducted. Sixty-three patients with chronic schizophrenia, who were clinically stable on a stable

[1] The first two authors contributed equally to this study.

dose of risperidone, entered the study. The patients were randomly assigned to l-carnosine (2 gr/day in two divided doses) or placebo for eight weeks. The patients were assessed using the positive and negative syndrome scale (PANSS), extrapyramidal symptom rating scale (ESRS), and Hamilton depression rating scale (HDRS) during the study course. Sixty patients completed the trial. L-carnosine resulted in greater improvement of negative scores as well as total PANSS scores but not positive subscale scores compared to placebo. HDRS scores and its changes did not differ between the two groups. Both groups demonstrated a constant ESRS score during the trial course. Frequency of other side effects was not significantly different between the two groups. In a multiple regression analysis model (controlled for positive, general psychopathology, depressive and extrapyramidal symptoms, as well as other variables), the treatment group significantly predicted changes in primary negative symptoms. In conclusion, l-carnosine add-on therapy can safely and effectively reduce the primary negative symptoms of patients with schizophrenia.

Keywords

Add-on therapy; L-carnosine; NMDA receptor; Primary negative symptoms; Schizophrenia

1. Introduction

Negative symptoms of schizophrenia, with about 23% to 26% prevalence, are the most challenging aspects of this disease with high burden in regard to inconsistent effectiveness of the

current antipsychotic drugs (McGlashan, 1991; Akhondzadeh, 2001; Chue and Lalonde, 2014; Fenton and Murphy et al., 2006; Millan, 2014). Several underlying mechanisms are postulated to be responsible for the negative symptoms. However, there is no common approved medication for treatment of either the negative symptoms or cognitive dysfunction in schizophrenia (Buchanan, 2007; Ibrahim and Tamminga, 2011). Glutamate dysfunction and N-methyl-D-aspartate (NMDA) receptor (a glutamatergic receptor) dysregulation are believed to be associated with the pathophysiology of cognitive deficits and negative symptoms of schizophrenia (Beck *et al*., 2016; Field *et al*., 2011; Moghaddam and Javitt, 2012). Decrease in NMDA receptor (NMDAR) function contributes to decrease in gamma-aminobutyric acidergic (GABAergic) activity which is linked to hyperactive glutamatergic activity in several brain regions including the prefrontal cortex (Cohen *et al*., 2015; Krystal *et al*., 2003; Paz *et al*., 2008). Enhancing NMDA transmission and increasing NMDA receptor activation through glycine site agonists are drug target mechanisms indicated to improve the primary negative symptoms of schizophrenia (Evins *et al*., 2002; Heresco-Levy and Javitt, 2004).

There is an emerging role for nutritional supplements in different neuropsychiatric disorders (Ghajar *et al*., 2016; Hajizadeh-Zaker *et al*., 2017; Matsukura and Tanaka; 2000). Carnosine (a dipeptide of β-alanine and L-histidine amino acids), a widely consumed dietary supplement (Hipkiss, 2011), has high concentrations in excitable tissues such as muscle and nervous tissue (Prokopieva *et al*., 2016). Carnosine is a known antioxidant, co-localized at glutamatergic synapses (Sassoè-Pognetto *et al*., 1993), which assists with the impaired cellular antioxidant defenses in patients with schizophrenia (Karton *et al*., 2012; Kulebyakin *et al*., 2012). Furthermore, carnosine is indicated to regulate NMDAR activity, decrease glutamate levels, inhibit glutamate release (Shen et al., 2010; Shen et al., 2007), and also reverse glutamate

increase induced by morphine in the ventral tegmental area (Gong et al., 2007). In a study conducted by Hatano *et al*., serum alanine levels (a component of l-carnosine) of patients with schizophrenia were associated with their symptomatic improvement (Hatano et al., 2010), and investigation of the postmortem schizophrenic brains showed altered aspartate–alanine metabolism in the prefrontal cortex (Middleton *et al*., 2002).

To the best of our knowledge, there is only one randomized clinical trial investigating l-carnosine in patients with schizophrenia with promising beneficial effects on cognition in these patients (Chengappa *et al*., 2012). We were interested in studying l-carnosine effectiveness because of its wide use, high bioavailability, easy penetration of the blood-brain barrier (BBB), lack of danger in overdose, few side-effects, and long-term administration with no accumulation in tissues (Matsukura and Tanaka, 2000; Prokopieva *et al*., 2016). The present study aimed to assess the safety and efficacy of l-carnosine add-on to risperidone on negative symptoms of patients with stable schizophrenia in an 8-week treatment course.

2. Methods

2.1 Study design and setting

This study was conducted as an 8-week, randomized, double-blind, placebo-controlled, parallel group study of outpatients with chronic stable schizophrenia at the outpatient general psychiatry clinics of Roozbeh Psychiatric Hospital (Tehran University of Medical Sciences, Tehran, Iran) from May 2015 to February 2017. The trial was registered in the Iranian registry of clinical trials (www.irct.ir; trial identifier with the IRCT database: IRCT201504211556N75).

2.2 Participants

Male and female outpatients with schizophrenia (based on Diagnosis and Statistical Manual (DSM-5) aged 18–60 years and a minimum disease duration of 2 years were recruited to the study. The diagnosis was based on the Structured Clinical Interview and was confirmed by chart review and interview of a senior psychiatrist. To be eligible, clinical stability on a stable dose of risperidone was required for at least eight weeks before entering the study. Clinical stability was defined as total score change ≤20 % on two sequential ratings (every two weeks for a month) on the positive and negative syndrome scale (PANSS) (Kay *et al*., 1987). Exclusion criteria were existence of serious medical, neurological or any other psychiatric disorder, being subject to electroconvulsive therapy (ECT) in the past 6 months, a score of ≥14 on the 17-item Hamilton Depression Rating Scale (HDRS) (Hamilton, 1960), score ≥4 on the depression item of PANSS, alcohol or substance (with the exception of nicotine) dependence, intelligence quotient <70 (based on clinical judgment), history of neurosurgery or head trauma, low blood pressure, pregnancy and breastfeeding. Patients were not allowed to use antidepressants, mood stabilizers, sedating antihistamines or a second antipsychotic (as an augmentative strategy) during the course of the trial. None of the patients were addicted to caffeine, and no other extra dietary supplements were used by the participants. All participants were informed that they are free to withdraw from the trial at any time. The institutional review board (IRB) of Tehran University of Medical Sciences approved the trial protocol (Reference number: 27749). All patients and their legally authorized representatives provided written informed consent in accordance with the procedures defined by the local IRB. The trial was performed in accordance with the Declaration of Helsinki and its latest revisions.

2.3 Dosing and treatment regimens

Patients were randomly allocated to receive either 2 gr/day l-carnosine (ACER, Iran) or placebo. L-carnosine titration was conducted for ten days. Participants received 500 mg/day of l-carnosine for five days; on day six, the dose of l-carnosine was increased to 1,000 mg/day in two divided doses for five days. On day eleven, patients received the maximum fixed dose of 2 gr/day in two divided doses throughout the trial. The schedule of dose titration for placebo followed the schedule of the active medication. All participants received risperidone (Risperdal, Janssen Pharmaceuticals) up to 6 mg/day during the course of the trial. Patients with sleep problems received 1 mg lorazepam every night for the first week of the trial. Medication adherence was measured using weekly capsule counts justified against participant reports of medication intake to calculate the proportion of dispensed medication doses that were actually ingested.

2.4 Assessments (outcomes, side effects):

Patients were rated using the PANSS based on a structured clinical interview at weeks 0, 2, 4, 6, and 8 following baseline. PANSS has been applied in several studies of our center (Kashani *et al*., 2017; Rezaei *et al*., 2017; Tajik-Esmaeeli *et al*., 2017; Nikbakhat *et al*., 2016) Extrapyramidal Symptom Rating Scale (ESRS) (Chouinard et al., 1980) was used to assess the extrapyramidal symptoms at baseline and every 2 weeks. The primary outcome measure was the difference in PANSS negative subscale score reduction from baseline to week 8 between the l-carnosine and the placebo groups. Secondary outcome measures were the difference of change in the PANSS subscale scores and the PANSS and ESRS total scores (at each visit) between the two groups. In addition, HDRS was administered at baseline and week 8 in order to assess changes in depressive symptoms (Hamilton, 1960). HDRS has also been applied in previous studies in Iran (Jafarinia *et al*., 2016). In addition to ESRS, adverse events were systematically evaluated at each time point using a checklist. Patients were first asked an open-ended question

about any adverse event that was not mentioned on the checklist (Noorbala *et al*., 1999; Akhondzadeh et al., 2000; Modabbernia et al., 2012).

2.5 Sample size

Based on our previous studies on augmentative therapy in stable schizophrenia (Noroozian *et al*., 2013); assuming a difference of 3 on the PANSS negative scores between the two groups of the trial, a standard deviation (SD) of 3, a two-tailed significance of 5%, and a power of 95%, a sample size of 52 was calculated. Considering a 20% dropout rate, sixty-three patients were required.

2.6 Randomization, allocation concealment, and blinding

A computerized random number generator was used to randomly assign the participants to either the l-carnosine or the placebo group (blocks of 4, allocation ratio 1:1). The patients, the psychiatrists who referred them, the rater and the person who administered the medications and the statistician were all blinded to the allocation. Placebo was not distinguishable in appearance (shape, size, color, and taste) from l-carnosine and was dispensed by the investigational drug pharmacist.

2.7 Statistical analysis

Statistical analysis was conducted using SPSS 20.0 (IBM Corporations). Categorical variables and continuous variables were reported as frequency (percentage) and mean ± standard deviation (SD), respectively. Mean differences between the groups were reported as the mean (95 % confidence intervals, 95% CI). All analyses were based on the intention-to-treat sample and were performed using the last observation carried forward (LOCF) procedure. To compare the score

and the behavior of the two treatment groups over the course of the trial, a two-factor repeated measures analysis of variance (ANOVA) with Greenhouse–Geisser correction was used. To compare score changes from baseline to each time interval between the two study groups, the independent t-test and Cohen's d effect size were used. A p value of <0.05 was considered statistically significant. Multivariate linear regression analysis of study variables was conducted to predict changes in negative scores as the primary outcome. Utilizing the Bonferroni adjusting method, p value of <0.012 was considered statistically significant in the primary outcome analysis (adjustment for comparisons of PANSS total and three subscales between the two groups). When comparing adverse events, a p value <0.01 (adjustment for comparing five side effects), and a p value <0.05 was considered meaningful in all other analyses.

3. RESULTS

3.1 Baseline Patient Characteristics

Of 101 patients screened for the trial, 63 patients were randomly assigned to either the L-carnosine or the placebo trial arms (more than the assumed sample size). All patients had at least one post-baseline measurement and 60 patients completed the study. Mean dose of risperidone (mg/day) did not differ significantly between the two groups [MD (95 % CI) = -0.02 (-0.24 to 0.20), t (58) = 0.18, p = 0.858]. Anticholinergic medications were allowed to treat severe extrapyramidal symptoms (EPS). However, severe EPS was not reported by any of the patients requiring this type of medication. One patient in the L-carnosine and two patients in the placebo group dropped out before the end of the trial (Fig.1) (None of the withdrawn consents were due to side effects). Negative, positive, general psychopathology, and total PANSS symptom scores,

as well as ESRS and HDRS values were not significantly different between the two groups at baseline. Table 1 summarizes the baseline data of the participants.

3.2 Negative Symptoms

In two-factor repeated measure ANOVA, effect of time [Greenhouse-Geisser corrected: F (1.821, 105.618) =19.373, p<0.001] was significant. The effect of time × treatment interaction [Greenhouse- Geisser corrected: F (1.821, 105.618) = 6.891, p = 0.002] was also significant (Fig. 2) showing that the l-carnosine treatment group behavior was different from the placebo group across time. At the end of the trial, patients in the l-carnosine group experienced significantly greater reduction in their negative subscale score than patients in the placebo group [MD: (95 %CI) = 1.466 (0.50–2.42), t (42.57) = 3.071, p=0.004] (Table 2).

3.3 Positive Symptoms

In repeated measure analysis, effect of time [Greenhouse-Geisser: F (1.111, 64.418) = 6.424, p =0.011] was significant (Fig. 3). In repeated measure analysis of ANOVA, time × treatment interaction [Greenhouse-Geisser corrected: F (1.111, 64.418) = 1.224, P=0.278] did not show significant effect on positive score reduction, showing that behavior of the two treatment groups was similar across time. By week 8, patients in the l-carnosine group did not differ significantly in reduction of their positive subscale score than patients in the placebo group [MD: (95 %CI) =0.16 (−0.21 to 0.54), t (58) =−0.885, p=0.380] (Table 2 and Fig. 3).

3.4 General Psychopathology

Two-factor repeated measure ANOVA demonstrated significant effect of time [Greenhouse-Geisser corrected: F (1.252, 72.616) = 9.595, p = 0.001] but found no significant time × treatment interaction [Greenhouse-Geisser corrected: F (1.252, 72.616) =3.385, p= 0.061]. At week 6, the L-carnosine group showed significantly greater improvement in general psychopathology subscale score than the placebo group (p=0.03), which returned to a nonsignificant difference by week 8 post-intervention (p=0.07) (Table 2). At the end of the trial, changes of 0.63 (3.3 % reduction from baseline) and 0.17 (0.8 % reduction from baseline) in general psychopathology subscale score were observed in the L-carnosine and the placebo groups, respectively (Table 2 and Fig. 4).

3.5 Total Score

Repeated measure ANOVA showed significant effect for time [Greenhouse-Geisser: F (1.732, 100.438) = 35.343, p <0.001] and time × treatment interaction [Greenhouse-Geisser: F (1.732, 100.438) = 11.124, p <0.001] (Fig. 5). Until week 4, there was still no significant difference between the two groups. By week 6, patients in the L-carnosine group showed significantly greater improvement in total PANSS score than the placebo group. A similar effect for L-carnosine was observed at week 8 post-intervention (Table 2). At the end of the trial, statistically meaningful changes of 2.97 (6.3 % reduction from the baseline) and 0.83 (1.7 % reduction from the baseline) in the total score were observed in the L-carnosine and the placebo groups, respectively [MD: (95 %CI)= 2.13 (0.96 to 3.31), t(39.89)= 3.663, p=0.001].

3.6 Extrapyramidal Symptom Rating Scale (ESRS)

ESRS scores remained constant in both treatment groups (L-carnosine and placebo) at various checkpoints from baseline to week 8 post-intervention. Mean ± SD of the ESRS scores for

citicoline and placebo groups were 1.56 ± 2.78 and 1.53 ± 2.11, respectively from the beginning to the end of the study.

3.7 Hamilton Depression Rating Scale (HDRS)

Baseline HDRS did not differ between the two groups significantly [MD: (95 %CI) = 0.46 (-0.07 to 1.00), t (58) =1.74, p=0.087]. At the end of the study, change in the HDRS score in the two groups was not significantly different [MD: (95%CI) = 0.17(-0.23 to 0.56), t (58) = -0.846, p=0.401].

3.8 Effect of other variables on changes in negative symptoms

To predict score change in negative symptoms, we performed a multivariate linear regression analysis of study variables (method: Enter). Changes in the negative score were only associated with the treatment group (standardized β= -0.328, p= 0.016). It was not associated with change in positive (standardized β= -0.141, p= 0.293), general psychopathology (standardized β= 0.083, p= 0.523), ESRS (no change was seen in ESRS scores), and Hamilton scores (standardized β= -0.176, p= 0.171). It was also not associated with age (standardized β= -0.331, p= 0.115), schizophrenia subtype (standardized β= - 0.152, p= 0.261), duration of disease (standardized β= -0.374, p= 0.067), smoking (standardized β= 0.016, p= 0.906), and education (standardized β= 0.183, p= 0.323).

3.9 Adverse Events

Other than extrapyramidal symptoms, 16 adverse effects were recorded during the course of the study. The frequency of side effects did not differ significantly between the two groups (Table 3). Adverse effects were all mild to moderate and none of the dropouts were due to side effects.

4. Discussion

In line with our hypothesis, we showed that l-carnosine add-on to risperidone has considerable beneficial effects in treatment of primary negative symptoms in patients with schizophrenia. Improvements that occur in negative symptoms might be related to alterations in positive, extrapyramidal, and depressive symptoms (Buchanan, 2007; Kirkpatrick *et al*., 2006; Murphy *et al*., 2006). In order to minimize effects of the aforementioned confounding factors and show pure effect of treatment on primary negative symptoms, patients were stabilized adequately before the trial and changes in positive, depressive, and extrapyramidal symptoms were minimal during the trial course. The observed Cohen's d were 0.95 and 0.79 in total PANSS score and negative subscale scores, respectively, which showed a relatively large effect size for the primary negative score. It is noteworthy that changes of about 2 (10.5 % reduction from the baseline) and 0.50 (2.7 % reduction from the baseline) in the negative subscale scores were observed in the L-carnosine and the placebo groups at the end of the trial, respectively. More complicated and longer average illness duration (21 years) of patients referred to our tertiary care center, more resistant nature of negative and cognitive symptoms, patient stabilization prior to entry and low baseline PANSS subscale scores should be considered when interpreting the total treatment effect.

There is no unifying concept about the mechanism of action and functions of carnosine in the brain (Shen *et al*., 2007); however, anti-oxidant, NMDA modulatory, glutamatergic, and histaminergic effects are proposed. Anti-inflammatory mechanisms are becoming of interest in treating neuropsychiatric disorders including schizophrenia (Alamdarsaravi *et al*., 2017; Akhondzadeh *et al*., 2007). However, anti-inflammatory effects of l-carnosine might not completely explain our findings because of the controversial results gained from different

randomized controlled trials of aspirin and celecoxib administration in schizophrenia (Andrade, 2015).

NMDAR modulatory activity of l-carnosine might be associated with significant improvement observed in negative symptoms (Balu and Coyle, 2015; Murphy *et al.*, 2006). Glycine and D-cycloserine (enhancers of the NMDAR neurotransmission) are reported to improve the primary negative symptoms of schizophrenia in some (but not all) studies (Buchanan, 2007; Goff *et al*., 1999). In addition, applying NMDA receptor antagonists to induce negative and cognitive symptoms in experimental models lend further support to the NMDAR hypothesis (Neill *et al*., 2010). D-serine, an NMDAR modulator that reverses the effects of NMDAR antagonists, is indicated to induce significant improvement in negative symptoms (Kantrowitz *et al*., 2015), cognitive symptoms and total psychopathology compared with placebo (Coyle and Tsai, 2004). Furthermore, D-alanine, which is an endogenous NMDA receptor agonist at the glycine site, (McBain *et al*., 1989) improved positive and cognitive symptoms in patients with schizophrenia (Tsai *et al*., 2006). Moreover, N-acetylcysteine (NAC), which increases the brain glutathione, is indicated to significantly improve PANSS total, negative and PANSS general psychopathology scores as an add-on therapy in chronic schizophrenia patients (Berk *et al*., 2008).

It should be noted that memantine (an NMDA receptor antagonist) adjunctive therapy, has been demonstrated to have beneficial effects on overall symptoms and negative symptoms compared to placebo (De Lucena *et al*., 2009; Rezaei *et al*., 2013); however, this finding is controversial (Lee *et al*., 2012; Lieberman *et al*., 2009) and memantine has several other mechanisms of action including its action as an antagonist for serotonin-3 and a-7 nicotinic acetylcholine receptors as well as an agonist for dopamine D2 receptor which should be considered. Nevertheless, in a meta-analysis, NMDA receptor antagonist adjunctive therapy was not superior to placebo in

overall, positive, and negative symptoms (Matsuda *et al*., 2013). Progressive separation of the l-carnosine and the placebo arms in terms of primary negative symptom score improvement during the study course should be noted because it is suggestive of the more pronounced beneficial effect of l-carnosine if the drug is administered for a more extended period of time.

In the only similar study comparing l-carnosine (same dose as our study) with placebo add-on to risperidone in patients with schizophrenia (Chengappa et al., 2012), the l-carnosine group performed significantly faster on non-reversal condition trials of the set-shifting test, displayed significant improvement on the strategic target detection test, and made fewer perseverative errors compared with placebo. Both studies used similar doses of l-carnosine. No significant improvement in positive, negative or depressive symptoms was reported. Comparing study participants, baseline negative symptom score was significantly lower than the present study (14 ± 4.3 vs. 18.70 ± 3.80; $p<0.001$). Moreover, baseline positive symptom score was significantly higher (14.8 ± 3.8 vs. 9.3 ± 2.54; $p<0.001$). However, it should be noted that the two studies differed in their objectives. We stabilized our patients to measure the pure effect of l-carnosine on negative symptoms. In our study, all patients were on a stable dose of risperidone; however, in the study conducted by Chengappa et al., patients were stable on different antipsychotic drugs. In contrast to this study, they reported some treatment-emergent adverse events.

Even though the present study has several advantages, such as the double-blind, placebo-controlled design and the rigorous adjustment for baseline clinical variables, various limitations should be addressed to prevent over-generalization of the findings. Population size was relatively small and the follow-up period was of relatively short duration. Measurement of serum or CSF concentrations of alanine and/or glutamate could be suggestive of possible causative associations. Male predominance might have limited interpretation of our findings to male-

predominant populations although in another similar study male to female ratio was about 1:7 (Chengappa *et al*., 2012). We also analyzed the male-only participants and achieved similar results. Finally, absence of functional and cognitive assessments (e.g. clinical global impression) are also among other limitations of the present study.

Conclusion

Eight weeks of treatment with l-carnosine as an add-on to risperidone showed good tolerability and significant beneficial effects on negative symptoms of patients with chronic stable schizophrenia.

Acknowledgments of funding and grants

This study was supported by a grant from Tehran University of Medical Sciences (Grant No: 27749). The funding organization had no role in the design and conduct of the study; in the collection, analysis, and interpretation of the data; or in the preparation, review, or approval of the manuscript and the decision to submit the paper for publication. The current study complies with contemporary laws and regulations in Iran.

Conflict of Interest The authors declare no conflict of interest.

References:

Akhondzadeh, S., 2001. The 5-HT hypothesis of schizophrenia. IDrugs. 4(3):295-300.

Akhondzadeh, S., Ahmadi-Abhari, S.A., Assadi, S.M., Shabestari, O.L., Kashani, A.R., Farzanehgan, Z.M., 2000. Double-blind randomized controlled trial of baclofen vs. clonidine in the treatment of opiates withdrawal. J Clin Pharm Ther. 25(5):347-53.

Akhondzadeh, S., Tabatabaee, M., Amini, H., Abhari, S.A.A., Abbasi, S.H., Behnam, B., 2007. Celecoxib as adjunctive therapy in schizophrenia: a double-blind, randomized and placebo-controlled trial. Schizophr Res. 90 (1), 179-185.

Alamdarsaravi, M., Ghajar, A., Noorbala, A.-A., Arbabi, M., Emami, A., Shahei, F., Mirzania, M., Jafarinia, M., Afarideh, M., Akhondzadeh, S., 2017. Efficacy and safety of celecoxib monotherapy for mild to moderate depression in patients with colorectal cancer: A randomized double-blind, placebo controlled trial. Psychiatry Res. 255, 59-65.

Andrade, C., 2015. Anti-inflammatory strategies in the treatment of schizophrenia. Expert Rev Clin Pharmacol., 9(2):161-3.

Balu, D.T., Coyle, J.T., 2015. The NMDA receptor 'glycine modulatory site'in schizophrenia: d-serine, glycine, and beyond. Curr Opin Pharmacol. 20, 109-115.

Beck, K., Javitt, D.C., Howes, O.D., 2016. Targeting glutamate to treat schizophrenia: lessons from recent clinical studies. Psychopharmacology, 233(13), 2425-8.

Berk, M., Copolov, D., Dean, O., Lu, K., Jeavons, S., Schapkaitz, I., Anderson-Hunt, M., Judd, F., Katz, F., Katz, P., 2008. N-acetyl cysteine as a glutathione precursor for schizophrenia—a double-blind, randomized, placebo-controlled trial. Biol. Psychiatry. 64 (5), 361-368.

Buchanan, R.W., 2007. Persistent negative symptoms in schizophrenia: an overview. Schizophr. Bull. 33 (4), 1013-1022.

Chengappa, K.R., Turkin, S.R., DeSanti, S., Bowie, C.R., Brar, J.S., Schlicht, P.J., Murphy, S.L., Hetrick, M.L., Bilder, R., Fleet, D., 2012. A preliminary, randomized, double-blind, placebo-controlled trial of L-carnosine to improve cognition in schizophrenia. Schizophr Res. 142 (1), 145-152.

Chouinard, G., RossChouinard, A., Annable, L., & Jones, B., 1980. Extrapyramidal symptom rating-scale. Paper presented at the Canadian Journal of Neurological Sciences.

Chue, P., Lalonde, J.K., 2014. Addressing the unmet needs of patients with persistent negative symptoms of schizophrenia: emerging pharmacological treatment options. Neuropsychiatr Dis Treat. 10:777-89.

Cohen, S.M., Tsien, R.W., Goff, D.C., Halassa, M.M., 2015. The impact of NMDA receptor hypofunction on GABAergic neurons in the pathophysiology of schizophrenia. Schizophr Res. 167 (1), 98-107.

Coyle, J.T., Tsai, G., 2004. The NMDA receptor glycine modulatory site: a therapeutic target for improving cognition and reducing negative symptoms in schizophrenia. Psychopharmacology 174 (1), 32-38.

De Lucena, D., Fernandes, B.S., Berk, M., Dodd, S., Medeiros, D.W., Pedrini, M., Kunz, M., Gomes, F.A., Giglio, L.F., Lobato, M.I., 2009. Improvement of negative and positive symptoms in treatment-refractory schizophrenia: a double-blind, randomized, placebo-controlled trial with memantine as add-on therapy to clozapine. J Clin Psychiatry. 70 (10), 1-478.

Evins, A.E., Amico, E., Posever, T.A., Toker, R., Goff, D.C., 2002. D-Cycloserine added to risperidone in patients with primary negative symptoms of schizophrenia. Schizophr Res. 56 (1), 19-23.

Fenton, W.S., McGlashan, T.H., 1991. Natural history of schizophrenia subtypes: II. Positive and negative symptoms and long-term course. Arch Gen Psychiatry. 48 (11), 978-986.

Field, J.R., Walker, A.G., Conn, P.J., 2011. Targeting glutamate synapses in schizophrenia. Trends Mol Med. 17 (12), 689-698.

Ghajar, A., Neishabouri, S., Velayati, N., Jahangard, L., Matinnia, N., Haghighi, M., Ghaleiha, A., Afarideh, M., Salimi, S., Meysamie, A., 2016. Crocus sativus L. versus Citalopram in the treatment of major depressive disorder with anxious distress: A double-blind, controlled clinical trial. Pharmacopsychiatry. 50(04), 152-160.

Goff, D.C., Tsai, G., Levitt, J., Amico, E., Manoach, D., Schoenfeld, D.A., Hayden, D.L., McCarley, R., Coyle, J.T., 1999. A placebo-controlled trial of D-cycloserine added to conventional neuroleptics in patients with schizophrenia. Arch Gen Psychiatry. 56 (1), 21-27.

Gong, Y.-x., Wang, H.-j., Zhu, Y.-p., Zhang, W.-p., Dai, H.-b., Zhang, S.-h., Wei, E.-q., Chen, Z., 2007. Carnosine ameliorates morphine-induced conditioned place preference in rats. Neurosci. Lett. 422 (1), 34-38.

Hajizadeh-Zaker R, Ghajar A, Mesgarpour B, Afarideh M, Mohammadi MR, Akhondzadeh S, 2017. l-Carnosine As an Adjunctive Therapy to Risperidone in Children with Autistic Disorder: A Randomized, Double-Blind, Placebo-Controlled Trial. J Child Adolesc Psychopharmacol. doi: 10.1089/cap.2017.0026. [Epub ahead of print].

Hamilton, M., 1960. A rating scale for depression. J. Neurol. Neurosurg. Psychiatry. 23 (1), 56-62.

Hatano, T., Ohnuma, T., Sakai, Y., Shibata, N., Maeshima, H., Hanzawa, R., Suzuki, T., Arai, H., 2010. Plasma alanine levels increase in patients with schizophrenia as their clinical symptoms improve—Results from the Juntendo University Schizophrenia Projects (JUSP). *Psychiatry* Res. 177 (1), 27-31.

Heresco-Levy, U., Javitt, D.C., 2004. Comparative effects of glycine and D-cycloserine on persistent negative symptoms in schizophrenia: a retrospective analysis. Schizophr Res. 66 (2), 89-96.

Hipkiss, A.R., 2011. Energy metabolism, proteotoxic stress and age-related dysfunction–Protection by carnosine. Mol. Aspects Med. 32 (4), 267-278.

Ibrahim, H.M., Tamminga, C.A., 2011. Schizophrenia: treatment targets beyond monoamine systems. Annu Rev Pharmacol Toxicol. 51, 189-209.

Jafarinia, M., Afarideh, M., Tafakhori, A., Arbabi, M., Ghajar, A., Noorbala, A.A., Saravi, M.A., Agah, E., Akhondzadeh, S., 2016. Efficacy and Safety of Oral Ketamine versus Diclofenac to Alleviate Mild to Moderate Depression in Chronic Pain Patients: A Double-Blind, Randomized, Controlled Trial. J Affect Disord. 204:1-8.

Kantrowitz, J.T., Woods, S.W., Petkova, E., Cornblatt, B., Corcoran, C.M., Chen, H., Silipo, G., Javitt, D.C., 2015. D-serine for the treatment of negative symptoms in individuals at clinical high risk of schizophrenia: a pilot, double-blind, placebo-controlled, randomised parallel group mechanistic proof-of-concept trial. Lancet Psychiatry. 2 (5), 403-412.

Kashani, L., Shams, N., Moazen-Zadeh, E., Karkhaneh-Yousefi, M.A., Sadighi, G., Khodaie-Ardakani, M.R., Rezaei, F., Rahiminejad, F., Akhondzadeh, S., 2017. Pregnenolone as an

adjunct to risperidone for treatment of women with schizophrenia: A randomized double-blind placebo-controlled clinical trial. J Psychiatr Res. 94: 70-77.

Karton, A., O'Reilly, R.J., Pattison, D.I., Davies, M.J., Radom, L., 2012. Computational Design of Effective, Bioinspired HOCl Antioxidants: The Role of Intramolecular Cl+ and H+ Shifts. J. Am. Chem. Soc. 134 (46), 19240-19245.

Kay, S.R., Flszbein, A., Opfer, L.A., 1987. The positive and negative syndrome scale (PANSS) for schizophrenia. Schizophr. Bull. 13 (2), 261.

Kirkpatrick, B., Fenton, W.S., Carpenter, W.T., Marder, S.R., 2006. The NIMH-MATRICS consensus statement on negative symptoms. Schizophr. Bull. 32 (2), 214-219.

Krystal, J.H., D'Souza, D.C., Mathalon, D., Perry, E., Belger, A., Hoffman, R., 2003. NMDA receptor antagonist effects, cortical glutamatergic function, and schizophrenia: toward a paradigm shift in medication development. Psychopharmacology 169 (3-4), 215-233.

Kulebyakin, K., Karpova, L., Lakonsteva, E., Krasavin, M., Boldyrev, A., 2012. Carnosine protects neurons against oxidative stress and modulates the time profile of MAPK cascade signaling. Amino acids. 43 (1), 91-96.

Lee, J.G., Lee, S.W., Lee, B.J., Park, S.W., Kim, G.M., Kim, Y.H., 2012. Adjunctive memantine therapy for cognitive impairment in chronic schizophrenia: a placebo-controlled pilot study. Psychiatry Investig. 9 (2), 166-173.

Lieberman, J.A., Papadakis, K., Csernansky, J., Litman, R., Volavka, J., Jia, X.D., Gage, A., 2009. A randomized, placebo-controlled study of memantine as adjunctive treatment in patients with schizophrenia. Neuropsychopharmacology 34 (5), 1322-1329.

Matsuda, Y., Kishi, T., Iwata, N., 2013. Efficacy and safety of NMDA receptor antagonists augmentation therapy for schizophrenia: An updated meta-analysis of randomized placebo-controlled trials. J Psychiatr Res. 47 (12), 2018-2020.

Matsukura, T., Tanaka, H., 2000. Applicability of zinc complex of L-carnosine for medical use. Biochemistry (Mosc). 65 (7), 817-823.

McBain, C., Kleckner, N., Wyrick, S., Dingledine, R., 1989. Structural requirements for activation of the glycine coagonist site of N-methyl-D-aspartate receptors expressed in Xenopus oocytes. Mol. Pharmacol. 36 (4), 556-565.

Middleton, F.A., Mirnics, K., Pierri, J.N., Lewis, D.A., Levitt, P., 2002. Gene expression profiling reveals alterations of specific metabolic pathways in schizophrenia. J. Neurosci. 22 (7), 2718-2729.

Millan, M.J., Fone, K., Steckler, T., Horan, W.P., 2014. Negative symptoms of schizophrenia: clinical characteristics, pathophysiological substrates, experimental models and prospects for improved treatment. Eur. Neuropsychopharmacol. 24 (5), 645-692.

Moghaddam, B., Javitt, D., 2012. From revolution to evolution: the glutamate hypothesis of schizophrenia and its implication for treatment. Neuropsychopharmacology. 37 (1), 4-15.

Murphy, B.P., Chung, Y.-C., Park, T.-W., McGorry, P.D., 2006. Pharmacological treatment of primary negative symptoms in schizophrenia: a systematic review. Schizophr Res. 88 (1), 5-25.

Modabbernia, A., Sohrabi, H., Nasehi, A.A,, Raisi, F., Saroukhani, S., Jamshidi, A., Tabrizi, M., Ashrafi, M., Akhondzadeh, S., 2012. Effect of saffron on fluoxetine-induced sexual impairment in men: randomized double-blind placebo-controlled trial. Psychopharmacology (Berl). 223(4):381-8.

Neill, J.C., Barnes, S., Cook, S., Grayson, B., Idris, N.F., McLean, S.L., Snigdha, S., Rajagopal, L., Harte, M.K., 2010. Animal models of cognitive dysfunction and negative symptoms of schizophrenia: focus on NMDA receptor antagonism. Pharmacol. Ther. 128 (3), 419-432.

Nikbakhat, M.R., Arabzadeh, S., Zeinoddini, A., Khalili, Z., Rezaei, F., Mohammadinejad, P., Ghaleiha, A., Akhondzadeh, S., 2016. Duloxetine Add-On to Risperidone for Treatment of Negative Symptoms in Patients with Stable Schizophrenia: Randomized Double-Blind Placebo-Controlled Study. Pharmacopsychiatry 49(4):162-9.

Noroozian M, Ghasemi S, Hosseini SMR., Modabbernia A, Khodaie-Ardakani MR, Mirshafiee O, Farokhnia M, Tajdini M, Rezaei F, Salehi B, Ashrafi M, Yekehtaz H. Tabrizi M, Akhondzadeh S. 2013. A placebo-controlled study of tropisetron added to risperidone for the treatment of negative symptoms in chronic and stable schizophrenia. Psychopharmacology. 228(4):595-602.

Noorbala, A.A., Akhondzadeh, S., Davari-Ashtiani, R., Amini-Nooshabadi, H., 1999. Piracetam in the treatment of schizophrenia: implications for the glutamate hypothesis of schizophrenia. J Clin Pharm Ther. 24(5):369-74.

Paz, R.D., Tardito, S., Atzori, M., Tseng, K.Y., 2008. Glutamatergic dysfunction in schizophrenia: from basic neuroscience to clinical psychopharmacology. Eur. Neuropsychopharmacol. 18 (11), 773-786.

Prokopieva, V., Yarygina, E., Bokhan, N., Ivanova, S., 2016. Use of Carnosine for Oxidative Stress Reduction in Different Pathologies. *Oxid* Med Cell Longev. 2016:2939087

Rezaei, F., Mohammad-karimi, M., Seddighi, S., Modabbernia, A., Ashrafi, M., Salehi, B., Hammidi, S., Motasami, H., Hajiaghaee, R., Tabrizi, M., 2013. Memantine add-on to

risperidone for treatment of negative symptoms in patients with stable schizophrenia: randomized, double-blind, placebo-controlled study. J Clin Psychopharmacol. 33 (3), 336-342.

Rezaei, F., Mesgarpour, B., Jeddian, A., Zeionoddini, A., Mohammadinejad, P., Salardini,.E., Shahriari, M., Zeinoddini, A., Akhondzadeh, S., 2017. Cilostazol adjunctive therapy in treatment of negative symptoms in chronic schizophrenia: Randomized,.double-blind, placebo-controlled study. Hum Psychopharmacol. 32 (4). (doi: 10.1002/hup.2583).

Sassoè-Pognetto, M., Cantino, D., Panzanelli, P., di Cantogno, L.V., Giustetto, M., Margolis, F.L., De Biasi, S., Fasolo, A., 1993. Presynaptic colocalization of carnosine and glutamate in olfactory neurones. Neuroreport 5 (1), 7-10.

Shen, Y., He, P., Fan, Y.-y., Zhang, J.-x., Yan, H.-j., Hu, W.-w., Ohtsu, H., Chen, Z., 2010. Carnosine protects against permanent cerebral ischemia in histidine decarboxylase knockout mice by reducing glutamate excitotoxicity. Free Radic Biol Med. 48 (5), 727-735.

Shen, Y., Hu, W.-w., Fan, Y.-y., Dai, H.-b., Fu, Q.-l., Wei, E.-Q., Luo, J.-h., Chen, Z., 2007. Carnosine protects against NMDA-induced neurotoxicity in differentiated rat PC12 cells through carnosine-histidine-histamine pathway and H 1/H 3 receptors. Biochem Pharmacol. 73 (5), 709-717.

Tajik-Esmaeeli, S., Moazen-Zadeh, E., Abbasi, N., Shariat, S.V., Rezaei, F., Salehi, B., Akhondzadeh, S., 2017. Simvastatin adjunct therapy for negative symptoms of schizophrenia: a randomized double-blind placebo-controlled trial. Int Clin Psychopharmacol. 32(2):87-94.

Tsai, G.E., Yang, P., Chang, Y.-C., Chong, M.-Y., 2006. D-alanine added to antipsychotics for the treatment of schizophrenia. Biol. Psychiatry. 59 (3), 230-234.

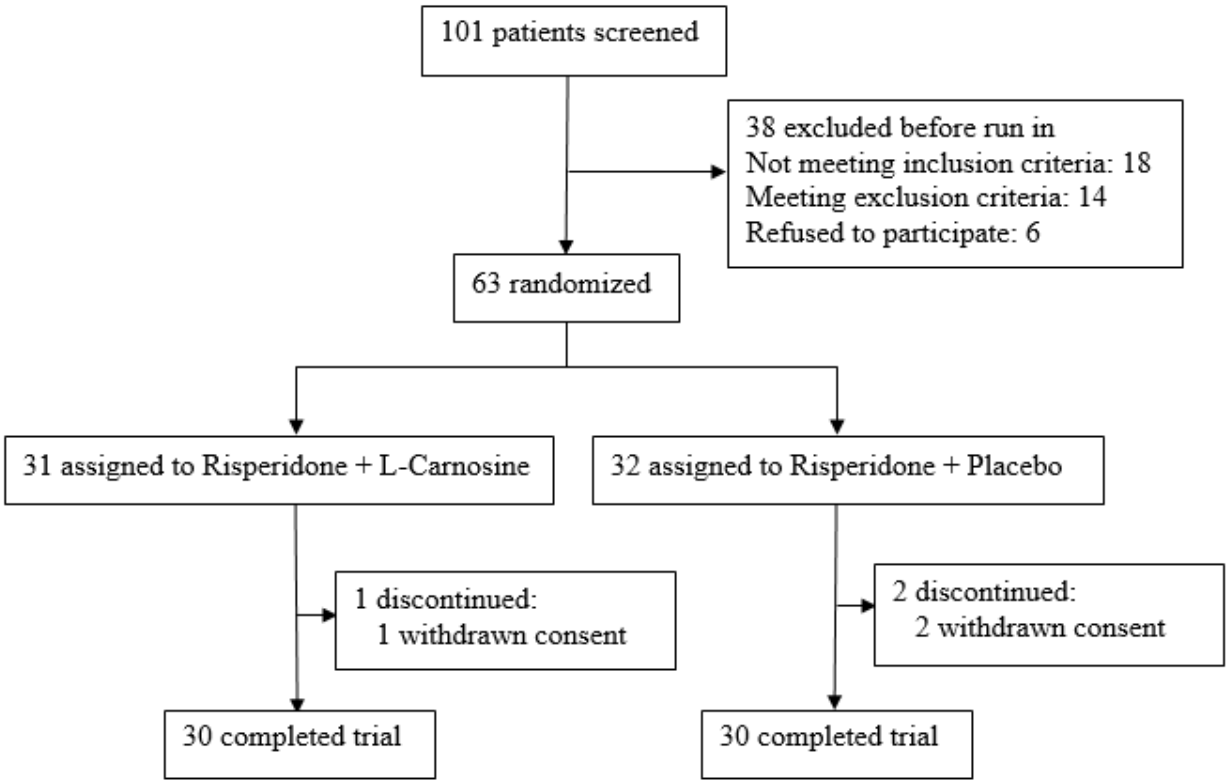
Fig. 1 Flow diagram representing case selection for the trial

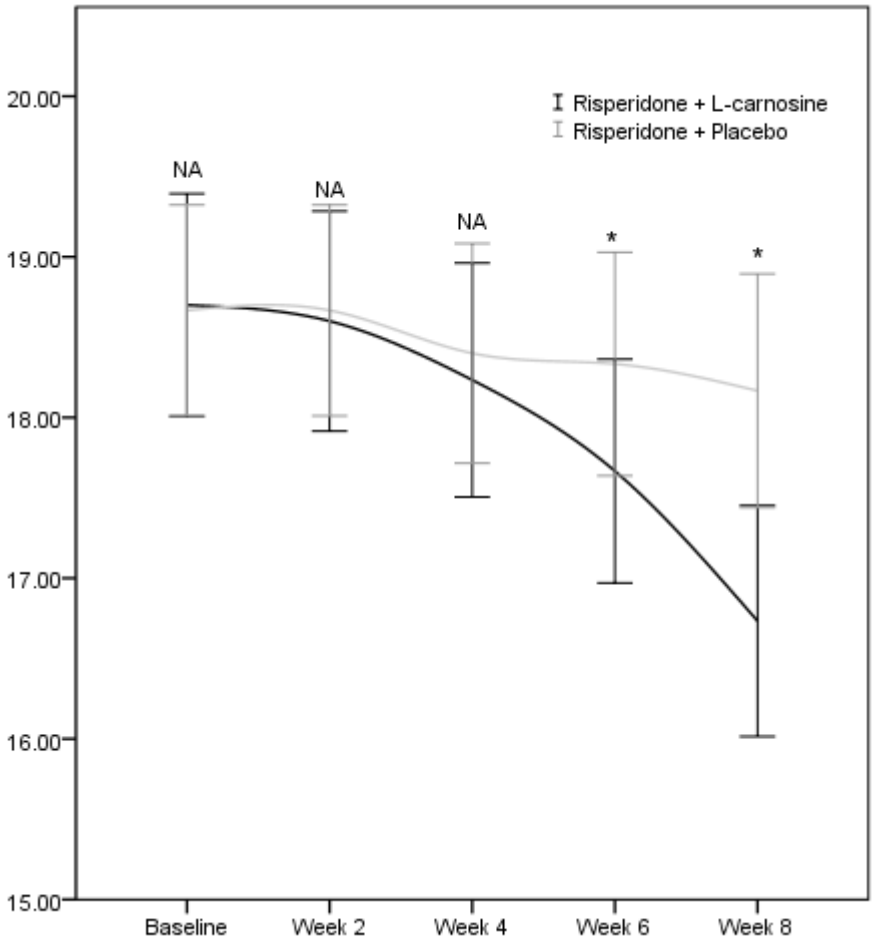
Fig. 2 Repeated measure analysis for comparison of the two treatments effects on negative subscale score of the Positive and Negative Syndrome Scale (PANSS). Values represent mean ± SEM (standard error of mean). P values show results of the independent sample t-test for comparison of score change from baseline between the two groups at each time point. NS: non-significant. *p≤0.05; ***p≤0.001.

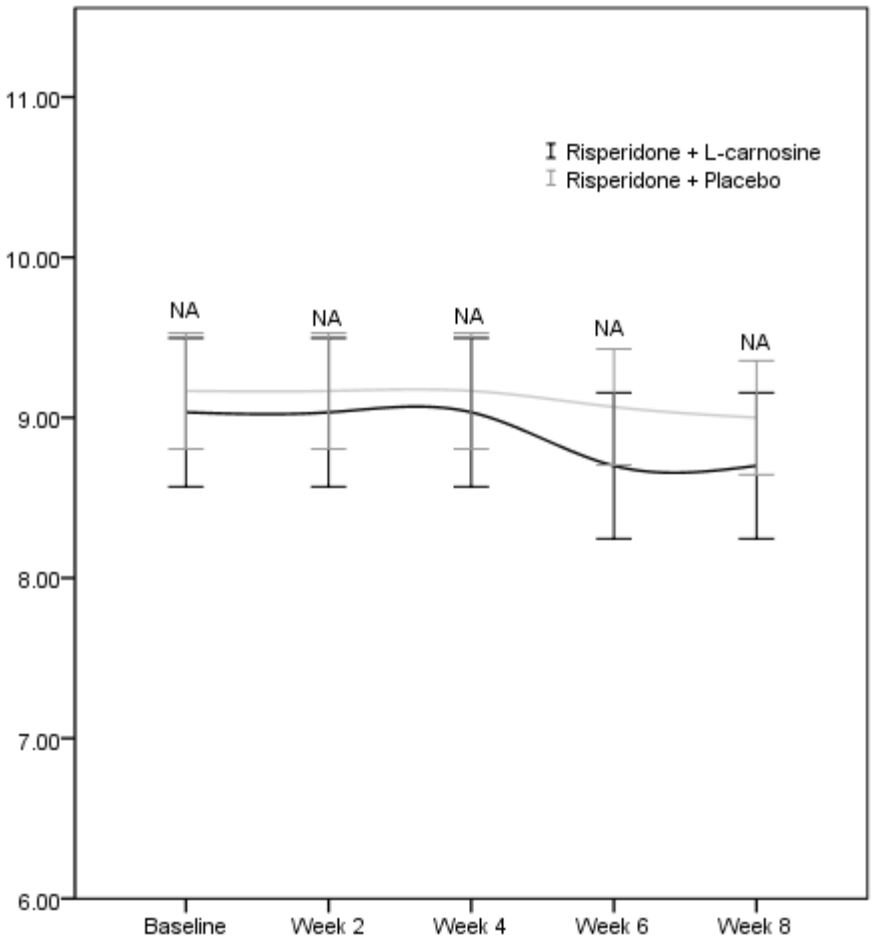
Fig. 3 Repeated measure analysis for comparison of the two treatment effects on positive subscale score on the Positive and Negative Syndrome Scale (PANSS). Values represent mean ± SEM. P values show results of the independent sample t-test for comparison of score change from baseline between the two groups at each time point. NS: non-significant. *p≤0.05; ***p≤0.001.

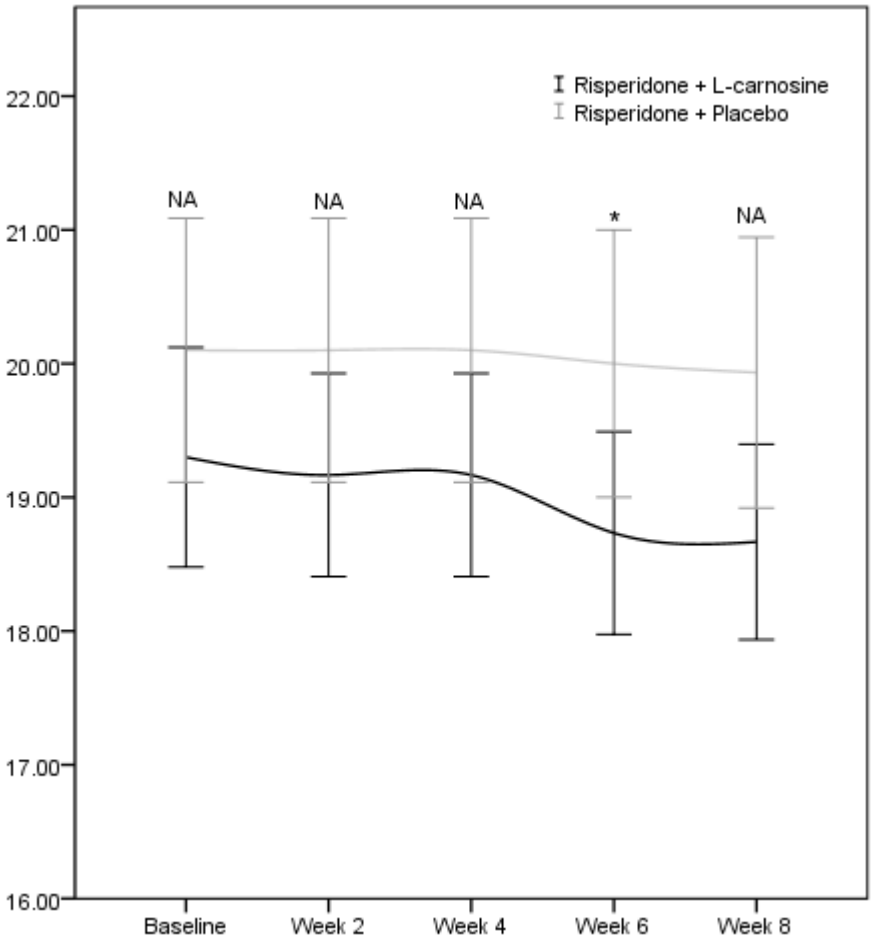
Fig. 4 Repeated measure analysis for comparison of the two treatments effects on general psychopathology subscale score on the Positive and Negative Syndrome Scale (PANSS). Values represent mean ± SEM. P values show the result of the independent sample t-test for comparison of the score change from baseline between the two groups at each time point. NS: non-significant. *p≤0.05; ***p≤0.001.

Fig. 5 Repeated measure analysis for comparison of the two treatment effects on total score on the Positive and Negative Syndrome Scale (PANSS). Values represent mean ± SEM. P values show the result of the independent sample t-test for comparison of the score change from baseline between the two groups at each time point. NS: non-significant. *p≤0.05; ***p≤0.001.


101 patients screened
38 excluded before run in
Not meeting inclusion criteria: 18
Meeting exclusion criteria: 14
Refused to participate: 6
63 randomized
31 assigned to Risperidone + L-Carnosine
32 assigned to Risperidone + Placebo
1 discontinued:
1 withdrawn consent
2 discontinued:
2 withdrawn consent
30 completed trial
30 completed trial







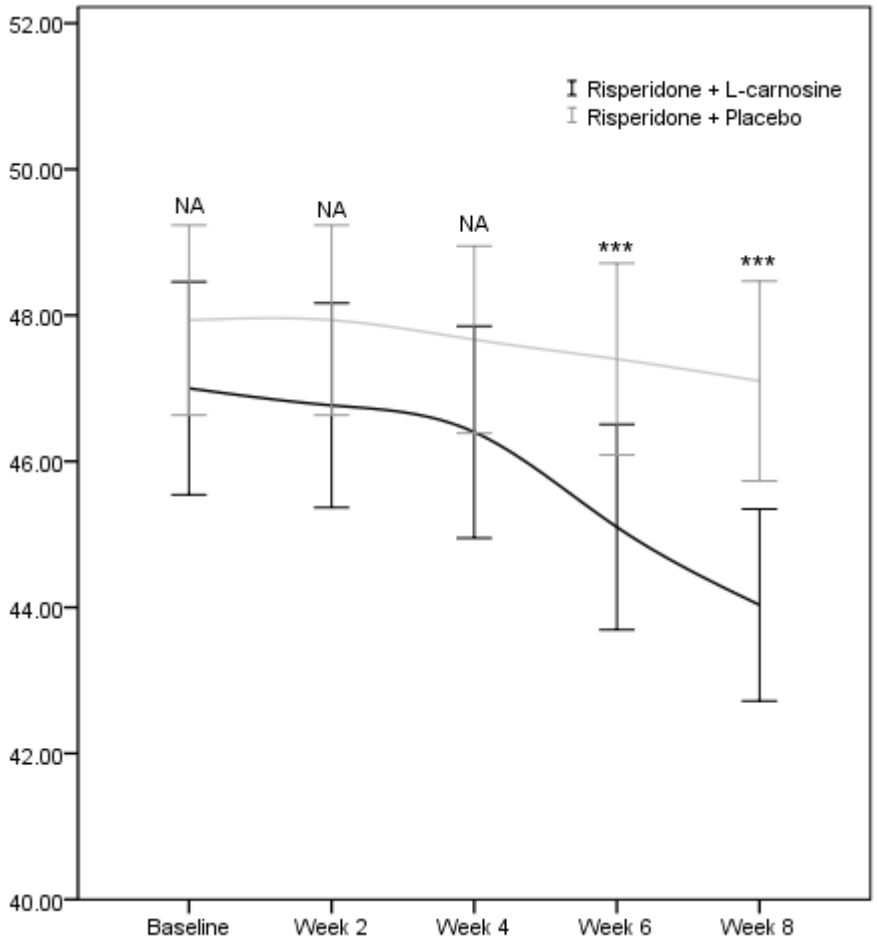


Table 1 Baseline patient characteristics.

Item	Risperidone + L-Carnosine (n = 30)	Risperidone + Placebo (n = 30)	p value
Male gender (%)	28 (93.3)	26 (86.7)	0.671[a]
Age at illness onset, years (mean ± SD)	22.00 ± 7.76	24.93 ± 6.78	0.125
Duration of illness, years (mean ± SD)	21.67 ± 11.35	21.03 ± 11.0	0.823
Age, years (mean ± SD)	43.67 ± 8.78	45.97 ± 9.30	0.329
Smoking, n (%)	19 (63.3)	14 (46.7)	0.194
Risperidone dose (mean ± SD)	4.38 ± 0.46	4.40±0.40	0.858
Educational status, n (%)			0.837
• Illiterate	9 (30)	12 (40)	
• Primary school	6 (20)	6 (20)	
• Secondary school	9 (30)	8 (26.7)	
• Diploma	6 (20)	4 (13.3)	
History of prior antipsychotic medications, n (%)			
• Risperidone	18(60)	16(53.3)	0.60
• haloperidol	6 (20.0)	6 (20)	1
• Fluphenazine	5 (16.6)	6 (20)	0.74
• Olanzapine	8 (26.6)	9 (30)	0.77
Schizophrenia subtype, n (%)			0.100 [a]
• Paranoid	27 (90)	26 (86.7)	
• Disorganized	2 (6.7)	2 (6.7)	
• Undifferentiated	1 (3.3)	2 (6.7)	
Baseline ESRS (mean ± SD)	1.57 ± 2.79	1.53 ± 2.11	0.959
Baseline HDRS (mean ± SD)	8.50 ± 1.14	8.03 ± 0.93	0.087
Baseline PANSS (mean ± SD)			
• Total score	47.0 ± 8.0	47.93 ± 7.12	0.634
• Negative symptoms	18.70 ± 3.80	18.67 ± 3.59	0.972
• Positive symptoms	9.03 ± 2.54	9.17 ± 2.0	0.822
• General psychopathology	19.30 ± 4.50	20.10 ± 5.40	0.536

PANSS positive and negative syndrome scale, *ESRS* extrapyramidal symptom rating scale, *HDRS* Hamilton depression rating scale, *SD* standard deviation
a indicates the *p* value reported by the Fischer exact test

Table 2 Comparison of Positive and Negative Syndrome Scale (PANSS) total and subscales score changes from baseline between two groups

Week	Risperidone + L-Carnosine (mean ± SD)	Risperidone + Placebo (mean ± SD)	Mean difference (95% CI)	*t* (58)	*p* value	Cohen's *d*
Total Score						
Week 2	0.23 ± 0.73	0	0.23 (−0.03 to 0.50)	1.756[a]	0.090	0.44
Week 4	0.60 ± 1.13	0.27 ± 0.83	0.33 (−0.18 to 0.85)	1.302[b]	0.199	0.33
Week 6	1.90 ± 1.90	0.53 ± 0.97	1.37 (0.58 to 2.15)	3.506[c]	0.001	0.91
Week 8	2.97 ± 2.92	0.83 ± 1.29	2.13 (0.96 to 3.31)	3.663[d]	0.001	0.95
Negative subscale score						
Week 2	0.10 ± 0.55	0	0.10 (−0.10 to 0.30)	1.000[a]	0.326	0.26
Week 4	0.47 ± 1.10	0.27 ± 0.83	0.20 (−0.29 to 0.69)	0.808	0.423	0.20
Week 6	1.03 ± 1.63	0.33 ± 0.88	0.70 (0.02 to 1.38)	2.068[e]	0.044	0.53
Week 8	1.97 ± 2.34	0.50 ± 1.17	1.47 (0.50 to 2.43)	3.071[f]	0.004	0.79
Positive subscale score						
Week 2	0	0	n/a	n/a	n/a	n/a
Week 4	0	0	n/a	n/a	n/a	n/a
Week 6	0.33 ± 0.88	0.10 ± 0.40	0.23 (−0.12 to 0.59)	1.316[g]	0.196	0.34
Week 8	0.33 ± 0.88	0.17 ± 0.53	0.17 (−0.21 to 0.54)	0.885	0.380	0.22
General psychopathology score						
Week 2	0.13 ± 0.51	0	0.13 (−0.05 to 0.32)	1.439[a]	0.161	0.36
Week 4	0.13 ± 0.51	0	0.13 (−0.05 to 0.32)	1.439[a]	0.161	0.36
Week 6	0.57 ± 1.10	0.10 ± 0.30	0.47 (0.04 to 0.89)	2.231[h]	0.033	0.58
Week 8	0.63 ± 1.27	0.17 ± 0.53	0.47 (−0.04 to 0.97)	1.854[i]	0.071	0.47

SD standard deviation *CI* confidence interval

[a] denotes the value of *t* (29), [b] denotes the value of *t* (53.101), [c] denotes the value of *t* (43.236), [d] denotes the value of *t* (39.897), [e] denotes the value of *t* (44.718), [f] denotes the value of *t* (42.574), [g] denotes the value of *t* (40.531), [h] denotes the value of *t* (33.402), [i] denotes the value of *t* (38.789)

Table 3 Frequency of adverse events in the study groups

Adverse events	L-Carnosine + Risperidone (n = 30)	Placebo + Risperidone (n = 30)	p value
Headache, n (%)	1 (3.3%)	3 (6.6%)	0.6120[a]
Dry Mouth, n (%)	2 (6.6%)	2 (6.6%)	1.000[a]
Nausea, n (%)	1 (3.3%)	1 (3.3%)	1.000[a]
Daytime Drowsiness, n (%)	1 (3.3%)	2 (6.6%)	1.000[a]
Sweating, n (%)	1 (3.3%)	2 (6.6%)	1.000[a]

a p value is reported using the Fischer exact test

Highlights:

- We assessed the safety and efficacy of L-carnosine as an adjuvant to risperidone for treatment of negative symptoms in patients with stable schizophrenia.
- Patients who received L-carnosine showed significant improvement in negative subscale scores of the positive and negative syndrome scale (PANSS) over the course of eight weeks
- Treatment with L-carnosine seems to be well tolerated with no serious adverse events.