MAK HILL Publications Research Journal of Medical Sciences

Research Article
doi: 10.59218/makrjms.2023.620.625

Efficacy of N-Acetyl Cysteine in COVID-19 Patients: A Randomised Double Blind Placebo Control Clinical Trial in Central India

[1]N. Jaiswal, [2]K.J. Patel, [2]S. Saxena, [3]P. Nyati, [2]S. Maurya and [4]H. Shah

[1]Index Medical College Hospital and Research Centre, Indore, Madhya Pradesh, India
[2]Department of Medicine, Index Medical College Hospital and Research Centre, Indore, Madhya Pradesh, India
[3]Department of Pharmacology, Index Medical College Hospital and Research Centre, Indore, Madhya Pradesh, India
[4]Department of Microbiology, Madhya Pradesh, India

ABSTRACT

COVID 19 has emerged as one of the worst pandemics that have distressed the global. Being highly infectious in mild and moderate cases it is likely to cause fever, headache, myalgia, throat irritation and dry cough whereas delayed or insufficient immune reaction leads to a pulmonary phase which manifests as viral pneumonia with hypoxia and in worsens condition typically labelled as 'cytokine storm' with excess level of pro-inflammatory cytokine protein. In order to investigate the efficacy of N-Acetyl cysteine as a potent nutraceutical in early reversal of RTPCR test in mild and moderate patients of COVID-19, the present clinical trial was conducted. Patients were divided into two groups' placebo control and NAC treated and received the experimental drug along with the conventional treatment of COVID (as per Govt. guidelines). Nasopharyngeal samples were collected and analysed for reversal of RTPCR test on day 5th and 7th. Additional number of patients with Negative RTPCR test on day 5th and 7th in NAC treated group is observed as marker of early recovery due to NAC administration in COVID-19 patients. Therefore we requisite intense research inputs with larger sample size to elucidate the role of NAC in critical and non-critical COVID-19 patients.

OPEN ACCESS

Key Words
COVID-19, N-acetyl cysteine, glutathione, cytokine storm nutraceutical, antioxidant

Corresponding Author
Neha Jaiswal,
Index Medical College Hospital and Research Centre, Indore, Madhya Pradesh, India Tel: 8949988510

Received: 2 July 2023
Accepted: 31 July 2023
Published: 1 August 2023

Citation: N. Jaiswal, K.J. Patel, S. Saxena, P. Nyati, S. Maurya and H. Shah, 2023. Efficacy of N-Acetyl Cysteine in COVID-19 Patients: A Randomised Double Blind Placebo Control Clinical Trial in Central India. Res. J. Med. Sci., 17: 620-625, doi: 10.59218/makrjms.2023.620.625

INTRODUCTION

COVID-19, highly infectious disease caused by SARS CoV-2, has affected almost the whole world and is spreading rapidly. The pandemic not only had an immense health risk but also shattered the economy of many countries. With everyday growing number of COVID-19 cases, most cases reported are mild and asymptomatic. Moderate cases report for pneumonia whereas serious cases are characterized by ARDS, sepsis and multiple organ failure. According to WHO reports till February 2021, there have been 105, 394, 301 confirmed cases of COVID-19, including 2, 302, 302 deaths across the world.

SARS CoV-2 and SARS CoV share many genetic characters like angiotensin-converting enzyme-2 (ACE2) as receptor for entry and the cellular protease TMPRSS2 as primer[1]. Histopathological assessment of SARS-CoV, MERS-CoV and influenza virus shared some similar symptoms as diffuse alveolar damage, edematous lung lesions and pneumonia with high morbidity and mortality rates which are also confirmed in case of SARS CoV-2[2-4]. Studies highlight the role of potent antioxidant system in providing defence against SARS and MERS[5,6]. Lung inflammation being the major cause of ARDS and consecutive mortality is being characterised by "Cytokine Released Syndrome" (CRS). The release of cytokine and further end result can be inhibited either by blocking the replication a viral RNA or by holding back the inflammatory pathways involved. Administration of a potent antioxidant which is a safe and well-tolerated supplementary drug without any considerable side effects might be effective. N-Acetylcysteine, the cellular precursor of glutathione, is one such antioxidant. In SARS CoV-2 infection both Th1 and Th2 responses are seen which activate an inflammatory cascade with a remarkable increase of cytokines IL-1, IL-2, IL-4, TNF and INFs[7,8]. This creates an oxidative stress environment, produces cytokine storms which are primary reason for mortality. NAC in COVID patient might upsurge the cellular glutathione levels and replenish glutathione pool thus maintain the oxidant/antioxidant levels.NAC interrupts the NLRP3 inflasome pathway (mediates host immune responses through the activation of caspase-1 and IL-1), lowers IL1β, IL8, IL6 and TNF-α[9,10].

Spike protein present on the surface of SARS CoV-2 mediates its entry into the cell by binding to human receptor ACE-2. The counteracting enzyme ACE2 and ACE are present in balanced amount in every individual but causes inflammation if ACE protein increases and levels of ACE2 decreases which may be in case when ACE2 are occupied by Spikes of SARS CoV2[11]. ACE2 convert Ang II (Angiotensin II) to Ang (1-7) (Angiotensin heptamer) that mediate vasodilatation, anti-inflammatory, antifibrotic, antiproliferative and anti-oxidative effects. Studies revealed that NAC lower Ang II receptor binding and Ang II-stimulated signal transduction[12]. NAC as well as Glutathione in cells displays an inhibitory effect on ACE thus NAC administration may lessen the oxidative stress caused due to ACE and ACE2 disproportion by increasing cellular glutathione[13,14].

With a historical worth, NAC has been widely used as mucolytic medicine treating bronchitis and other pulmonary diseases[15]. This mucolytic effect of NAC might be due to its free sulfhydryl group which reduces disulphide bonds in the cross-linked mucus glycoproteins matrix thus dilute the mucus.

Although, hypothesis explains the therapeutic efficacy of NAC in SARS CoV-2 infection still very few studies are present in support[16-19]. In this pandemic's of COVID-19, with high health risk and mortality, quite a few drugs are recommended without much clinical evidence, NAC being one of them[20]. Although, NAC has already approved by FDA for hepatic toxicity by acetaminophen[21]. Keeping this in mind we aim to evaluatethe crucial roles and therapeutic efficacy of NAC in COVID-19 infection and accessing the feasibility of using NAC as a means of the treatment for COVID patients.

The trial was approved by IEC (IMCHRC/IEC/2020/114) and was registered in CTRI (COVID-19 Trial REF/2020/06034062) before proceeding.

MATERIALS METHODS

This study was a randomized, double blind, placebo-controlled, clinical trial conducted in patients admitted in COVID isolation wards of Index hospital, Indore which was already designated as Red Zone hospital for treatment of the COVID patients. The trial was conducted in the COVID 19 pandemic.

Random selection of patients was done from the admitted patients with positive RTPCR test and undergoing COVID-19 treatment in corona ward after signing the written informed consent. Total 101 patients were selected for this study. Amongst this 1 patient dropped out on 2nd day and 100 patients continued for the trial. Further enrolled 100 patients were divided into two groups: Study group (NAC (n = 50)) and placebo control group (n = 50).

Inclusion criteria: Asymptomatic patients or patients of both the genders with mild to moderate symptoms, aged between 10 years to 80 years with laboratory confirmed COVID-19 infection.

Exclusion criteria:

- Patients with co-morbidity such as diabetes, hypertension, cardiac disease, COPD, Bronchial asthma
- Seriously ill patients or Patients requiring ventilators support
- Children below 10 years

- Adults above 80 years of age
- Pregnant females

All patients received the standard treatment for COVID-19 infection (as per the protocol in study period):

- Hydroxychloroquine (HCQ) 200 mg twice a day
- Azithromycine (Azid) 500 mg once a day
- Paracetamol (Pcm) 500 mg as and when required to control fever
- Pantaprazole (Pantop) 40 mg once a day
- Levocetirizine (5 mg) and montelukast (10 mg) (Levocet M) once a day

The study group additionally received N-acetyl cysteine (NAC) in dose of 600 mg daily twice a day for 10 days whereas the control group received similar capsule containing glucose 600 mg daily twice a day for 10 days.

Diary record of all the medications given was maintained. Patients were monitored for the vital and incidence of any adverse event if occurred.

Nasopharyngeal samples were collected in VTM tube and transported to lab in ice box within 20 min of collection as per ICMR protocol. RT-PCR was done using Q_line kit to check the presence of *E, N, Orf* and *Cy5* gene on 5th and 7th day to check the response to treatment and recovery from COVID-19 infection. Data was recorded and analysed for primary and secondary outcomes and interpretations were made to compare the effectiveness of NAC in COVID treatment.

Data was recorded and analyzed for the following primary and secondary outcomes and interpretations were made to compare the effectiveness of NAC in COVID-19 treatment.

Primary outcome:

- Effect of NAC in the study group on alleviating patient's signs and Symptoms of COVID-19 in forms of severity and duration
- Rate of cure from the disease as measured by negative results of RT-PCR test on nasopharyngeal swabs

Secondary outcome:

- Incidence of the adverse effects in both the group
- Association of age and gender with the clinical outcome in COVID-19 patients
- Progression of the patient towards severity of disease

RESULTS

In total 101 patients were recruited. 100 patients completed the study. About 1 patient dropped out due to anxiety and vomiting (Table 1).

DISCUSSIONS

In the present study we evaluated the activity of N-acetylcysteine on mild to moderate COVID-19 patient and compared it with the control group. NAC is known for its antioxidant property by scavenging certain reactive species directly and indirectly being the precursor to glutathione, restoring the cellular thiol pools, governing the redox state respectively[22]. The antioxidant therapy using NAC demonstrated beneficial effects which might be due to elevation of glutathione[23]. The "master antioxidant" glutathione displays antiviral defence and immune response by overriding replication and translation of viral genome[24]. Review by Flora *et al*.[25] revealed the protective activity of NAC influenza and other viral diseases.

do Nascimento *et al*.[26] highlighted the sexual dimorphism in COVID-19 mortality rates and susceptibility to sever illness, presenting men to be more prone to suffer severe effects of COVID-19 infections which might be due to lower GSH levels in males[27]. The results obtained are in concern with sexual dimorphism in COVID 19 infection and role of NAC administration on it, are in accordance with the

Table 1: Detailed results of the patients enrolled for the trial

		Placebo control			NAC treated	
Age	Total patients enrolled	Males	Females	Total patients enrolled	Males	Females
10-20	4	4	0	5	3	2
21-30	17	11	6	15	6	9
31-40	16	10	6	10	8	2
41-50	5	3	2	7	5	2
51-60	7	3	4	9	4	5
61-70	0	0	0	3	1	2
71-80	1	0	1	1	1	0

Table 2: Reversal of RT-PCR to negative status at 5th day

Row labels	(No. of patients) negative	(No. of patients) positive	Grand total	Reversal of RT-PCR to negative status at 5th day (%)
Placebo control	28	22	50	56
NAC treated	38	12	50	76
Grand total	66	34	100	

Table 3: Reversal of RT-PCR to negative status at 7th day

	Negative	Positive	Grand total	Reversal of RT-PCR to negative status at 7th day (%)
Placebo control	33	17	50	66
NAC treated	43	7	50	86
Grand total	76	24	100	

Table 4: Reversal of RT-PCR to negative status at 5th day in males and females

	5th day	
	Males	Females
Placebo control	16 (55.17%)	12 (57.14%)
NAC	25 (83.33%)	13 (65%)

Table 5: Reversal of RT-PCR to negative status at 7th day in males and females

	7th day	
	Males	Females
Placebo Control	18 (62.00%)	14 (66.66%)
NAC	28 (93.33%)	15 (75%)

Table 6: Comparative reversal of RTPCR done on 5th day in young (10-18), adult (19-65) and elderly (>65) patients after NAC administration in NAC treated and control group (children below 10 years were excluded for this trial)

	5th day			
	PLACEBO control		NAC treated	
Age group	Positive	Negative	Positive	Negative
Young (10-18)	2 (50%)	2 (50%)	2 (50%)	2 (50%)
Adult (19-65)	14 (31.11%)	31 (68.88%)	13 (30.95%)	29 (69.05%)
Elderly (>65)	1 (100%)	0 (0%)	1 (25%)	3 (75%)

Table 7: Comparative reversal of RTPCR done on 7th day in young (10-18), adult (19-65) and elderly (>65) patients after NAC administration in NAC treated and control group (Children below 10 years were excluded for this trial)

	7th day			
	PLACEBO control		NAC treated	
Age group	Positive	Negative	Positive	Negative
Young	2 (50%)	2 (50%)	1 (25%)	3 (75%)
Adult	9 (20%)	36 (80%)	11 (26.19%)	31 (73.80%)
Elderly	1 (100%)	0 (0%)	0 (0%)	4 (100%)

previous findings highlighting the role of glutathione in recovery rates from COVID-19 infection. The perceptible increase in 5th day negative RT-PCR number in male patients treated with NAC was observed in this study (Table 2). Males have lower plasma levels of reduced glutathione (GSH) as compared to females[28]. In thepresent study, administration of NAC in male patient demonstrated higher rates of recovery on day 5th as well as 7th when compared with male patients of control group (Table 3-5). The consequent reason behind this might be the elevation of cellular GSH levels after NAC administration. A remarkable difference in recovery rates in males and females patient of NAC treated group was also observed which propose a new theory dictating that in COVID patients NAC administration in males provides speedy recovery in comparison with female patients (Table 4). No scientific argument can be given for it, as no known studies have been documented till date highlighting comparative results of NAC administration in different sexes. As well, this study is a pilot study with lower sample size therefore further clinical trials are needed to objectively assess this statement.

Studies on humans and animal models publicised progressive decline in GSH levels with aging[29]. Clinical studies have demonstrated that elderly people and people with chronic illness are more susceptible to progress to severity[30]. Administration of N-Acetylcysteine to the elderly people (>65 years) demonstrated better recovery rates (Table 6 and 7). Although, in these trials as the number of total patients enrolled was lower the results obtained could not be considered as the final results and further trials using NAC as nutraceutical and including elderly patients with COVID-19 infection are requisite.

The response of NAC administration in COVID-19 was favourable in most of the patients with minimum adverse effects (Table 8). Few patients in the control group complaint of headache, nausea and constipation. One dropout was also documented in control group reporting anxiety and vomiting which was excluded from study. None of patients in either of trial group progress towards severity. Nonetheless this trial shows that NAC administration in COVID-19 mild and moderate patients is potentially beneficial with no side effects or leading patient towards severity.

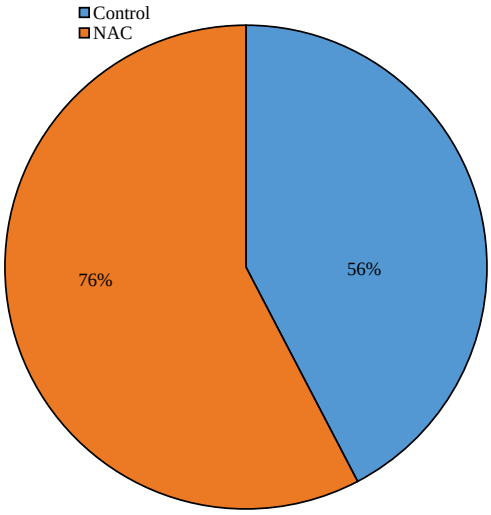

Fig. 1: Proportions of negative test for 5th day

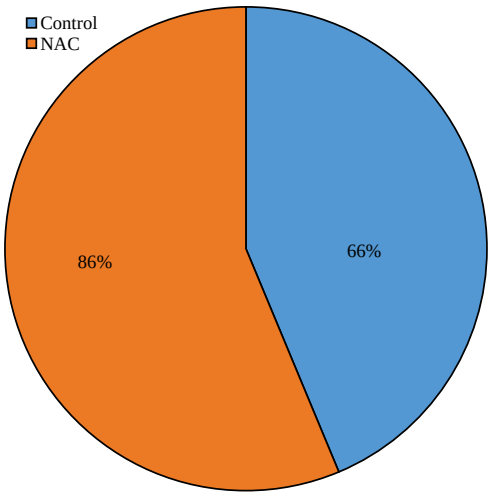

Fig. 2: Proportions of negative test for 7th day

Table 8: Number of patients with adverse effects

Adverse effects	Placebo control	NAC treated
Breathing difficulty	0	0
Anxiety	6	0
Nausea/vomiting	8	1
G.I upsets	3	1
Headache	2	1
Skin rashes	0	0

If we compare the data as a whole (Fig. 1 and 2), clinically there is a little enhancement in the reversal rate of RT-PCR test on 5th day in patients in the NAC treated Group (76% reversal in comparison to 56% in controlled group). However, being a pilot study of a small group it is not statistically significant. Similarly on 7th day the difference is almost similar (86% in NAC treated group and 66% in controlled group). The negative RT-PCR is considered as nil or negligible viral load (SARS CoV-2).

CONCLUSION

Oral administration of NAC in COVID-19 patients could promote the onset of recovery as demonstrated by Negative RTPCR test on day 5th and 7th, a significant marker of recovery from COVID-19. NAC as an adjuvant treatment is effective with higher retrieval rates in males as compared to females. A noteworthy gender wise association is observed between rate of cure in COVID patients and NAC administration. No significant adverse effect has been observed after NAC administration in COVID patients. In the meantime, based on mucolytic effects, NAC was included in the protocol of COVID management. Our observation also suggests it beneficial in early reversal of RT-PCR. Hence it could be recommended for the routine management not only for mucolytic effect but also to get early reversal of the test, which is indicative of high viral load. However, additional studies are required to confirm the therapeutic efficacy of N-Acetylcysteine in COVID-19 treatment and prevention of complication after COVID infection. To demonstrated significant efficacy of NAC clinical trial should be conducted on larger sample size on serious patients with and without co-morbidities. It could also be assessed for early reversal of moderate to severe patient of COVID-19.

REFERENCES

1. Hoffmann, M., H.K.Weber, S. Schroeder, N. Krüger, T. Herrler *et al*., 2020. The novel coronavirus 2019 (2019-nCoV) uses the SARS-coronavirus receptor ACE2 and the cellular protease TMPRSS2 for entry into target cells. BioRxiv, 181: 271-280.
2. Munster, V.J., E.d. Wit, J.M.A. van den Brand, S. Herfst and E.J.A. Schrauwen *et al*., 2009. Pathogenesis and transmission of swine-origin 2009 A(H1N1) influenza virus in ferrets. Science, 24: 481-483.
3. Shieh, W.J., D.M. Blau, A.M. Denison, M. DeLeon-Carnes and P. Adem *et al*., 2010. 2009 pandemic influenza a (H1N1): Pathology and pathogenesis of 100 fatal cases in the United States. Am. J. Pathol., 177: 166-175.
4. de Wit, E., A.L. Rasmussen, D. Falzarano, T. Bushmaker and F. Feldmann *et al*., 2013. Middle east respiratory syndrome coronavirus (MERS-CoV) causes transient lower respiratory tract infection in rhesus macaques. Proc. Nat. Acad. Sci., 110: 16598-16603
5. Ko, J.H., G.E. Park, J.Y. Lee, J.Y. Lee and S.Y. Cho *et al*., 2016. Predictive factors for pneumonia development and progression to respiratory failure in MERS-CoV infected patients. J. Infec., 73: 468-475.

6. MohnK, K.G.I., R.J. Cox, J.E. Berdal, G. Tunheim and A.G Hauge *et al* ., 2015. Immune responses in acute and convalescent patients with mild, moderate and severe disease during the 2009 influenza pandemic in Norway. PLOS ONE, Vol. 2015. 10.1371/journal.pone.0143281.
7. Huang, C., Y. Wang, X. Li, L. Ren and J. Zhao *et al*., 2020. Clinical features of patients infected with 2019 novel coronavirus in Wuhan, China. Lancet, 395: 497-506.
8. Liu, P., J.Z. Jiang, X.F. Wan, Y. Hua and L. Li *et al*., 2020. Are pangolins the intermediate host of the 2019 novel coronavirus (SARS-CoV-2)? Plos Pathogens, Vol. 17, No. 6. 10.1371/journal.ppat.1008421
9. Liu, Y., W. Yao, J. Xu, Y. Qiu and F. Cao *et al*., 2015. The anti-inflammatory effects of acetaminophen and N-acetylcysteine through suppression of the NLRP3 inflammasome pathway in LPS-challenged piglet mononuclear phagocytes. Innate Immun.., 21: 587-597.
10. Mata, M., E. Morcillo, C. Gimeno and J. Cortijo, 2011. N-acetyl-l-cysteine (NAC) inhibit mucin synthesis and pro-inflammatory mediators in alveolar type ii epithelial cells infected with influenza virus a and b and with respiratory syncytial virus (RSV). Biochem. Pharmacol., 82: 548-555.
11. Kuba, K., Y. Imai, S. Rao, H. Gao and F. Guo *et al*., 2005. A crucial role of angiotensin converting enzyme 2 (ACE2) in SARS coronavirus-induced lung injury. Nat. Med., 11: 875-879.
12. Ullian, M.E., A.K. Gelasco, W.R. Fitzgibbon, C.N. Beck and T.A. Morinelli, 2005. N-acetylcysteine decreases angiotensin II receptor binding in vascular smooth muscle cells. J. Am. Soc. Nephrol, 16: 2346-2353.
13. Boesgaard, S., J. Aldershvile, H.E. Poulsen, S. Christensen, H. Dige-Petersen and J Giese. 1993. N-acetylcysteine inhibits angiotensin converting enzyme *in vivo*. J. Pharmacol. Exp. Ther, 265: 1239-1244.
14. Basi, Z. and V. Turkoglu, 2019. In vitro effect of oxidized and reduced glutathione peptides on angiotensin converting enzyme purified from human plasma. J. Chromatogr. B, 1104: 190-195.
15. Sanguinetti, C.M., 2015. N-Acetylcysteine in Copd: Why, How and When? Multidiscip Respir. Med., Vol. 11. 10.1186/s40248-016-0039-2
16. Jaiswal, N., M. Bhatnagar and H. Shah, 2020. N-acetycysteine: A potential therapeutic agent in COVID-19 infection. Med. Hypotheses, Vol. 144. 10.1016/j.mehy.2020.110133
17. Jorge-Aarón, R.M. and M.P. Rosa-Ester, 2020. N-acetylcysteine as a potential treatment for COVID-19. Future Microbiol., 15: 959-962.
18. Poe, F.L. and J. Corn, 2020. N-acetylcysteine: A potential therapeutic agent for SARS-CoV-2. Med. Hypotheses, Vol. 143. 10.1016/j. mehy.2020.109862
19. Ibrahim, H., A. Perl, D. Smith, T. Lewis and Z. Kon *et al*., 2020. Therapeutic blockade of inflammation in severe COVID-19 infection with intravenous N-acetylcysteine. Clin Immunol, Vol. 219. 10.1016/j.clim.2020.108544.
20. Fajgenbaum, D.C., J.S. Khor, A. Gorzewski, M.A. Tamakloe and V. Powers *et al*., 2020. Treatments administered to the first 9152 reported cases of COVID-19: A systematic review. Infect. Dis. Ther., 9: 435-449.
21. Heard, K.J., 2008. Acetylcysteine for acetaminophen poisoning. N Engl. J. Med., 359: 285-292
22. Aldini, G., A. Altomare, G. Baron, G. Vistoli, M. Carini, L. Borsani and F. Sergio, 2018. N-acetylcysteine as an antioxidant and disulphide breaking agent: The reasons why. Free Radical Res., 52: 751-762.
23. Silvagno, F., A. Vernone and G.P Pescarmona, 2020. The role of glutathione in protecting against the severe inflammatory response triggered by COVID-19. Antioxidants, Vol. 9, No. 7. 10.3390/antiox9070624
24. Forman, H.J., H. Zhang and A. Rinna, 2009. Glutathione: Overview of its protective roles, measurement and biosynthesis. Mol. Aspects Med., 30: 1-12.
25. Flora, S.D., R. Balansky and S.L. Maestra, 2020. Rationale for the use of N acetylcysteine in both prevention and adjuvant therapy of COVID 19. FASEB J., 34: 13185-13193.
26. do Nascimento, I. J. B., N. Cacic, H.M Abdulazeem, T.C. von Groote, U. Jayarajah *et al*., 2020.Novel coronavirus infection (COVID-19) in humans: A scoping review and meta-analysis. J. Clin. Med., Vol. 9, No. 4. 10.3390/jcm9040941
27. Wang, L., Y.J. Ahn and R. Asmis, 2020. Sexual dimorphism in glutathione metabolism and glutathione-dependent responses. Redox Biol., Vol. 31. 10.1016/j.redox.2019.101410
28. Polonikov, A., 2020. Endogenous deficiency of glutathione as the most likely cause of serious manifestations and death in COVID-19 patients. ACS Infect. Dis., 6: 1558-1562.
29. Zhu, Y., P.M. Carvey and Z. Ling, 2006. Age-related changes in glutathione and glutathione-related enzymes in rat brain. Brain Res., 1090: 35-44.
30. Liu, K., Y. Chen, R. Lin and K. Han, 2020. Clinical features of COVID-19 in elderly patients: A comparison with young and middle-aged patients. J. Infec., 80: E14-E18.