East African Medical Journal Vol. 95 No. 11 November 2019
ANTI-MYCOBACTERIAL EFFECTS OF ASCORBIC ACID IN HUMAN MACROPHAGE-LYMPHOCYTE CO-CULTURE MODEL
Dr Mekonnen Teferi (DVM, MSc), Armauer Hansen Research Institute, Addis Ababa, Ethiopia, mekonnenteferi@yahoo.com, P.O. Box 1005, Dr Markos Abebe (PhD), Armauer Hansen Research Institute, Addis Ababa, Ethiopia, markosabebe@yahoo.com, P.O. Box 1005, Dr Abraham Aseffa (MD, PhD), Armauer Hansen Research Institute, Addis Ababa, Ethiopia, aseffaa@gmail.com, P.O. Box 1005, Dr Dawit Asmamaw Ejigu (MD, MSc), Department of Pharmacology, St Paul's Hospital Millennium Medical College, Addis Ababa, Ethiopia.

Corresponding author: Dr Dawit Asmamaw Ejigu (MD, MSc), Department of Pharmacology, St Paul's Hospital Millennium Medical College, Addis Ababa, Ethiopia, daejigu@gmail.com, P.O. Box 1271

ANTI-MYCOBACTERIAL EFFECTS OF ASCORBIC ACID IN HUMAN MACROPHAGE-LYMPHOCYTE CO-CULTURE MODEL

M. Teferi, M. Abebe, A. Aseffa and D. A. Ejigu

ABSTRACT

***Background*: Tuberculosis (TB) causes oxidative stress in patients contracting it while antioxidant vitamins such as ascorbic acid (AA) demonstrated anti-mycobacterial (anti-MTB) effects. Ascorbate's anti-MTB effect in 7H9 media depended on high iron concentration. However, high iron concentration *in vivo* rather favored mycobacterial growth. Iron dynamics in TB infected macrophages is also complex making it difficult to extrapolate ascorbate's *in vivo* effect based on observations in 7H9 media. To address this issue, we assessed ascorbate's anti-MTB effect in human macrophage cell culture model.**

***Methods*: We collected 20 ml blood from HIV negative subjects, isolated Peripheral Blood Mononuclear Cells (PBMCs) and separated monocytes from the PBMCs. Monocytes were allowed to mature, were infected with Mycobacterium TB (MTB) and were incubated in RPMI plus autologous serum after adding different concentrations of ascorbate. Colony Forming Units (CFU) were counted and compared among cultures incubated with different ascorbate concentrations. Ascorbate's anti-MTB effect was also assessed in 7H9 media for comparison.**

***Results*: At concentrations of 10^{-1} mM, 1 mM and 10 mM, ascorbate reduced MTB CFU in cell culture model by 13%, 53% and 71% respectively while in 7H9 medium CFU reductions were 2.8%, 58.7% and 99.3% respectively.**

Conclusions: Despite low iron concentration in human cell culture, ascorbate showed dose dependent anti-MTB effect in the cell culture and hence ascorbate can be further investigated in clinical trials to assess its use as an adjunct to anti-TB drugs

INTRODUCTION

Tuberculosis (TB) is an infectious disease caused by *Mycobacterium Tuberculosis* (MTB) complex with annual global incidence of 10 million and mortality of 1.6 million. There are 30 high TB burden countries in the world which report 87 % of the disease worldwide and Ethiopia is one of them (1). Success of TB treatment is low due to various factors including poor adherence to treatment (2), lack of effective immunity against the disease and malnutrition (3). Adjunct interventions against TB could have a potential to shorten TB treatment and improve treatment success.

Antioxidant vitamins such as ascorbate could possibly be used as potential adjunct in TB treatment possibly by quenching oxidative stress commonly seen in TB patients. The oxidative stress in TB patients is evidenced by high circulating free radicals and low antioxidants levels (4). Low ascorbate dietary intake and plasma concentration were also associated with increased TB incidence and dietary supplementation of ascorbate to people at risk of tuberculosis curtailed TB incidence by 60% (7). Vilchèze and colleagues demonstrated ascorbate's direct anti-mycobacterial effect in 7H9 media and elaborated its mechanism to be through free radicals generation (8) which depended on high iron concentration.

However, high iron concentrations was seen to activate latent TB and caused flaring up of TB (9). Moreover, macrophages attempt to deprive MTB of iron by sequestering iron in phagolysosomes making us uncertain whether ascorbate will get access to iron stores inside macrophages for its anti-MTB effect (10). Because of these complex dynamics of iron in macrophages, it would be unreasonable to expect ascorbate to behave *in vivo* and in 7H9 media alike. To address this issue, we assessed the anti-mycobacterial activity of ascorbate in Monocyte Derived Macrophages–Peripheral Blood Lymphocytes (MDM-PBL) co-culture model without additional iron supplementation. We also compared ascorbate's mycobacterial clearance against cytokine and Nitric Oxide (NO) production to further assess if ascorbate's immune modulatory effects contribute to its anti-TB effect.

This paper describes comparison between ascorbate's anti-mycobacterial effect in cell culture model and in 7H9 media as well as the role of ascorbate's immune modulatory effect in its anti-mycobacterial activity.

MATERIALS AND METHODS

Study design: This study is an *in vitro* human macrophage-lymphocyte co-culture experiment. We infected macrophages with MTB H37Rv strain in complete RPMI (R10) media [RPMI 1640, autologous serum, 1% glutamine]. The infected cultures were treated with different concentrations of ascorbate in duplicates and at the same time controls, i.e. infected macrophages with no ascorbate, were run in parallel. Rifampicin (RIF) at 1mg/L concentration was used as positive control. Experiments were also done in 7H9 media for comparison of direct anti-MTB activity. Additionally, we used THP-1 cell culture to assess ascorbates cytotoxicity and interaction between ascorbate and anti-TB drugs.

Blood collection and Peripheral Blood Mononuclear Cells (PBMC) isolation: We

collected 20 ml of blood from 8 (2 females and 6 males) apparently healthy volunteers aged 21 to 35 years and isolated PBMCs. Participants were negative for Quantiferon TB-Gold assay (Cellestis, Denmark) and HIV test (HIV 1/2 STAT-PAK® Assay). PBMCs were isolated by Ficoll-Hypaque gradient method as described by the manufacturer (Ficoll-Paque™, Methodology & applications, GE Healthcare). PBMCs were counted in methylene blue stain.

MACS separation and Human monocyte culture: We selected monocytes from the PBMC according to the manufacturer's instructions using anti-CD14+ microbeads (BD Biosciences, USA) and magnetic cell sorter (MACS) (Vario MACS, Milteny Biotech). These cells were incubated at 37°C and 5% CO_2 for 7 days to obtain matured monocyte derived macrophages (MDM). R10 media with 1% penicillin-streptomycin (Gibco Invitrogen, USA) was used to culture the cells and the media was changed on day 3 and day 5.

Growing and preparing MTB H37Rv bacilli for macrophage infection: We cultured MTB H37Rv (ATCC no 27294, Manassas, VA) strain in sterile Middlebrook 7H9 medium with 10% Middlebrook albumin-dextrose-catalase enrichment and 0.2% glycerol (all are products of Difco, Detroit, MI). After growth the MTB was stored at -20^{0}C using freezing media (50% 7H9 broth + 25% glycerol + 25% distilled water) in cryotubes. On the day of infection, the mycobacteria were prepared for macrophage infection. The number of bacteria in the suspension was calculated based on CFU estimation made at freezing while considering 10% loss due to storage.

Infection of MDM with MTB H37Rv: We resuspended macrophages in RPMI with 10% foetal calf serum, 1% glutamine and 5mg/l Amphotericin B. Macrophages (10^6 per ml) were infected using MTB H37Rv suspension at 1:1 bacilli to macrophage ratio. The infected macrophages were then incubated at 37^{0}C for 4hrs.

Addition of PBL to infected MDM culture: We thawed and resuspended PBL in R10, washed and incubated in a 37^{0}C CO_2 incubator a day before infection of MDM with MTB H37Rv. At the day of infection, the PBL was washed and resuspended at a density of $5x10^6$ ml^{-1}. It was then added to autologous infected MDM cultures immediately following the rinsing of non-ingested bacteria. A 10:1 PBL to MDM ratio was used in order to reconstitute the approximate composition of the PBMC.

Addition of AA and anti-TB drugs to infected cell cultures: We prepared various concentrations of ascorbate and added them into the test wells containing the infected MDM-culture. AA (A4403, Sigma-Aldrich Chemie GmbH, St. Louis, Missouri, USA) was dissolved in PBS at 10^{-1}M as a stock concentration. The test concentrations of AA were prepared as serial dilutions of 10, 1 and 10^{-1} mM using appropriate final media. No AA was added in the negative control wells while RIF at a concentration of 1 mg/L was added in the positive control wells instead of AA. Moreover, 5 mM of AA, 0.5mg of RIF and 0.25mg of Isoniazid (INH) were used to test mycobacterial clearance interaction between AA and anti-TB drugs.

Viable bacilli count: After incubating ascorbate treated MDM culture for different durations, we lysed the macrophages and counted the viable MTB bacilli. Bacilli count was done 4hrs, day 4 and day 7 after addition of AA into the cell culture. Samples for bacilli count were taken from duplicate wells. Cell pellets were lysed using 0.067% sodium dodecyl

sulphate (SDS) and a 10-fold serial dilution was made in PBS for plating on to 7H10 agar in duplicates for each dilution as described by Alfred (1990). The culture plates were incubated at 37^{o}C and colonies were counted on 28th day after inoculation on 7H10 medium. The results are expressed as CFU per milliliters of lysate, which corresponds to CFU/10^{5} MDM lysate.

Assessment of direct anti-TB effects in 7H9: We assessed direct anti-TB effect of ascorbate by first inoculating MTB H37Rv in Middlebrook 7H9 medium and then adding different concentrations of ascorbate in the medium. The 7H9 medium was enriched with 10% Middlebrook albumin-dextrose-catalase and 0.2% glycerol. The 7H9 medium inoculated with MTB and containing ascorbate was incubated for 7 days. To determine degree of inhibition by ascorbate, we plated the MTB from the 7H9 medium on to 7H10 medium and incubated it for 28 days to count the CFU.

Cytokines ELISA assay and Total Nitric Oxide determination: We measured TNF-α, IFN-γ, and interleukin IL-10 from culture supernatant on day 4 using ELISA. We also determined NO concentration on day 4 using nitric oxide assay kit. Both cytokine and NO kits are products of Endogen-Pierce Biotechnology, USA and used according to the manufacturer's instruction. Absorbance was measured using spectrophotometer (MultiscanEx) The concentrations were expressed in pg/ml and μM/ml for cytokines and NO respectively.

Statistical analysis: We presented CFU count, cytokine and NO measurement results as the standard errors of the means. Differences among treatment groups were analyzed using *t* test in Graph Pad Prism and if $p < 0.05$ statistical tests were considered significant. Additionally, data from CFU count was log transformed for its comparison between treatment groups and the result is presented as percent reduction in CFU. To calculate percent reduction in CFU we first calculated percent survival by dividing the CFU in ascorbate treated by the CFU in non-treated infected negative controls (CFU in ascorbate treated/ CFU in non-treated infected negative controls). Then the percent reduction in CFU was calculated by subtracting percent survival from hundred (100-percent survival).

RESULTS

Cytotoxicity of ascorbate to THP-1 cells: We assessed possible cytotoxicity of ascorbate to THP-1 cells and found out that ascorbate at concentrations used in these experiments were safe. PMA (Phorbol 12-myristate 13-acetate) stimulated THP-1 cells, but not infected with MTB H37Rv, were incubated together with ascorbate and monitored for 2 days. Using methylene blue exclusion staining, percent survival (viability) was determined where all live and dead cells were counted and the proportion of viable to dead cells was computed as percentage. All ascorbate concentrations used in this experiment conferred at least 95% viability surpassing the minimum viability requirement of 90% to consider a treatment safe

Reproducibility of the model: We tested different multiplicity of infection (MOI), and we found consistent multiplication pattern in MDM-PBL and THP-1 cell cultures at 1:1 MOI. Initial multiplication was achieved after 24hrs, reached peak at day 4, plateau at day 5 and started to decline at day 7. The viability of the macrophages was not compromised as they

were able to contain the multiplication of the bacilli later in culture (data not shown). Based on this observation, appropriate time points for CFU plating were selected to be Day 0 (4hrs), day 4 and day 7 to detect possible changes in CFU due to ascorbate treatment.

Anti-MTB activity of AA: Ascorbate showed a dose dependent inhibition of MTB in MDM-PBL culture model, but this effect was generally of lower magnitude compared to its effect in 7H9 medium. High degree of anti-MTB activity was obtained as early as day 4 (Figure 1). Mycobacterial clearance by ascorbate was positively correlated with increasing concentrations of ascorbate (Table 1). The highest level of anti-TB effect in the MDM-PBL model (71.5% CFU reduction) was achieved at 10mM of ascorbate on day 7 ($p=0.001$). Anti-MTB activity of ascorbate in 7H9 medium was even more prominent and showed a dose dependent inhibition. Ten millimole of ascorbate in 7H9 media cleared 99.3% of MTB and this anti-mycobacterial effect was comparable to that of RIF, which was used as a positive control in the experiment. Despite superior effect of ascorbate in 7H9 medium at 1- and 10-mM concentrations, at a concentration of 10^{-1} mM ascorbate's anti-mycobacterial effect was better in MDM-PBL cell culture than in 7H9.

Table 1

Reduction in CFU by different concentrations of ascorbate in MDM-PBL and 7H9 media at day 7 of culture

Experimental Model	**Ascorbate conc. (*mM*)**	**10**	**1**	**10^{-1}**	**Controls**	
					RIF (1mg/L)	**Negative**
MDM-PBL	**Mean CFU x10^4 (n=8)**	2.04*	3.36*	6.25	0.22	7.15
	% CFU reduction	*71.5%*	*53.0%*	*12.6%*	*96.9%*	-
7H9	**Mean CFU x10^4**	0.46*	25.2*	59.27	0.29	61
	% CFU reduction	*99.3%*	*58.7%*	*2.8%*	*99.5%*	**-**

* *$p<0.01$*

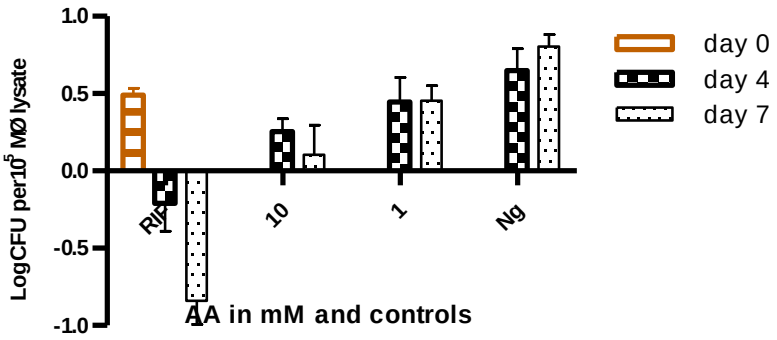
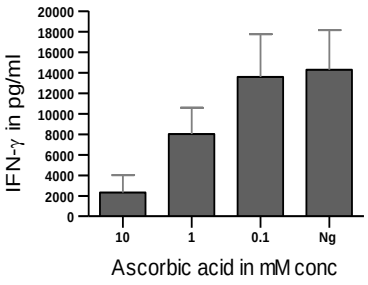
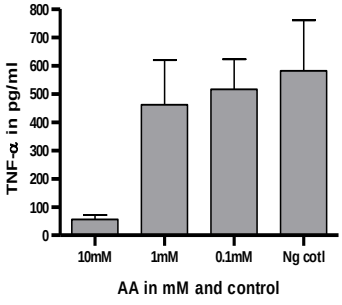


Figure 1: Log Mean ± SEM CFU of MTB H37Rv from infected MDM-PBL co-culture under 10- and 1-mM AA treatment at day 4 and 7, n=8.

Cytokine and NO response profile to AA treatment: The anti-MTB activity of ascorbate might not be due to neither enhanced production of Th_1 cytokines nor NO. Ascorbate reduced concentrations of TNF-α, IFN-γ, and NO in a dose dependent manner (figure 2) whereas increasing concentrations of ascorbates resulted in progressively increasing anti-TB effects. At 10mM concentration, statistically significant ($p < 0.05$) reduction of MTB-induced IFN-γ and TNF-α responses were observed. Accordingly, 71.5% CFU reduction by ascorbate was associated with greater than seven-fold and six-fold reduction in IFN-γ and TNF-α concentrations respectively when compared to controls not treated with ascorbate. However, the cytokine suppression effect of ascorbate is less remarkable at lower concentrations. The measurement of NO was also reduced in ascorbate treated MDM-PBL cultures in a dose dependent manner. Ten millimole of ascorbate reduced NO level by up to nine-fold relative to the non-treated negative control ($p=0.001$) (figure 3b) while10^{-1} and 1 mM of ascorbate reduced NO amount to a lesser extent.



(a)



(b)

Fig 2: Mean ± SEM IFN-γ (a) and TNF-α (b) production in MDM-PBL co-culture under AA treatment at day 4, n=8.

On the other hand, ascorbate reduced IL-10 amount in a dose dependent manner (figure 3a). Ascorbate at a concentration of 10mM reduced the amount of IL-10 by six-fold (p=0.006). Moreover, all ascorbate concentrations reduced IL-10 amount consistently in all experiments (n=8).

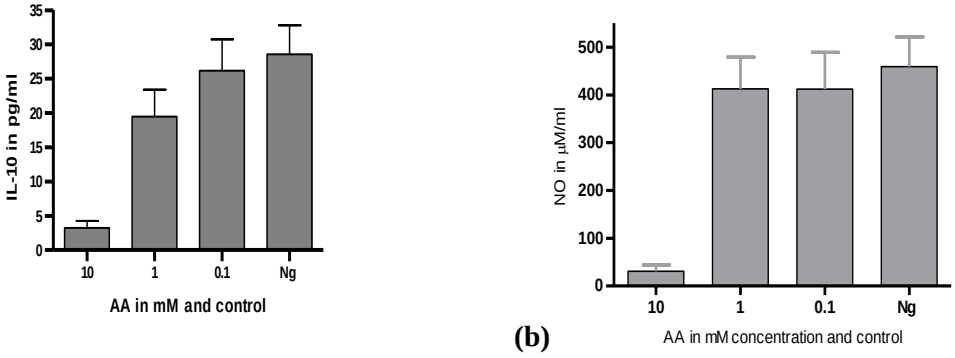


Figure 3 Mean ± SEM IL-10 in pg/ml (a) and NO in *mM* (b) production response from MDM-PBL co-culture under AA treatment at day 4, n=8.

Interaction of Ascorbate with INH and RIF: Ascorbate augmented anti-MTB effects of INH as well as RIF in THP-1 cell culture model. INH alone reduced the CFU by 29% whereas combining INH with ascorbate increased the clearance to 50% (Table 2). However, the combined CFU reduction by ascorbate and INH was lower than the one by ascorbate alone which was 63%.

Table 2

Percent reduction in CFU by INH0.25mg/L and RIF0.5mg/L combined with AA in THP-1 cells at day 7 of culture

AA drug combination.	**INH+ AA**	**RIF+AA**	**AA**	**Controls**		
				INH(0.25mg/L)	**RIF(0.5 mg/L)**	**Negative**
Mean CFU x10⁴	44.7	6.54	32.9	63.5	40.98	90
% CFU reduction	50.3	92.8	63.4	29.4	54.5	-

DISCUSSION

Ascorbate at 10mM concentration showed higher anti-mycobacterial clearance in 7H9 medium as compared to in the MDM-PBL cell culture (71.3% vs 99.3%). However, at 1 mM concentration ascorbate's effect was more or less similar in the two systems (53.1% vs

58.7%) and 0.1mM concentration ascorbate's effect was even better in the cell culture model (12.6% vs 2.8%). This could be explained by the enhanced ascorbate transport into macrophages in the cell culture model at low extracellular ascorbate concentrations against concentration gradient by sodium dependent vitamin C transport 2 (SVCT 2) (11, 12). Based on previous studies, SVCT 2 has a capacity to accumulate ascorbate 80-100 times more in macrophages than the extracellular environment (11).

Ascorbate showed anti-mycobacterial effect in cell culture model despite lower iron concentration and this may suggest different mechanism other than free radical generation through Fenton's reactions. Shukla and colleagues also recently suggested another possible anti-mycobacterial mechanism through MTB isocitrate lyase (MTBIL) inhibition and this could happen even in a low iron concentration environment (13). Apart from these mechanisms elucidated in culture media by Vilchèze *et al* as and Shukla *et al*, interaction of ascorbate with immune cells could have also enhanced GSH level in the leukocytes (14). Based on a report by Lenton and colleagues, ascorbate enhanced GSH level in lymphocytes (15) and in turn GSH showed anti-mycobacterial effects (16).

Ascorbate reduced mycobacterial CFU despite reduced amount of TNF-α, IFN-γ and NO in MDM-PBL culture. Ten millimole of ascorbate that resulted in 71.5% CFU clearance caused statistically significant IFN-γ (>7-fold), TNF- α (>6 fold) and NO (9-fold) reduction when compared to the non-treated control (figures 2ab, 3b). Similarly other studies also reported that ascorbate reduced TNF-α level in TB patients (17) as well as in animal models (18). The latter study further reported the inhibitory effect of ascorbate on IFN-γ and NO. A recent study on N-acetyl cysteine also showed reduction of TNF-α by the antioxidant in an *in vitro* culture model (19). Moreover, ascorbate reduced the number of lipopolysaccharide (LPS)-induced TNF-α and IFN-γ producing monocytes in a flow cytometry study on human whole blood cells (20). Therefore, our results suggested that the anti-MTB effect of ascorbate may involve pathways other than Th_1 cytokine induction and NO modulation. We also observed that ascorbate suppressed the level of IL-10 production. The expression of IL-10 in TB may not be beneficial to the host since it down-regulates macrophage function and interferes with efficient intracellular killing (21). Therefore, ascorbate may have additional role of suppressing MTB-induced Th_2 responses.

Reduced NO measurement may hint reduced production of NO or its enhanced conversion to other forms. As we have seen in the previous paragraphs, ascorbate increases the amount of GSH in macrophages (15). NO and GSH are known to combine to each other and form S-nitrosoglutathione (GSSNO), a chemical with enhanced anti-mycobacterial effect. Hence, increased production of GSSNO may be the reason why the level of NO reduced.

Ascorbate did not show antagonist effect to both INH and RIF. INH is a prodrug and needs activation with catalase peroxidase and this activation is enhanced in situations where there is increased oxidative stress (23). Hence, it may be logically expected that enhancing antioxidant system by administration of drugs such as ascorbate may reduce the anti-mycobacterial effects of INH. Such antagonist effects are not observed in our experiments and rather ascorbate increased CFU clearance

by INH from 29.4% to 50.3% and clearance by RIF from 54.5% to 92.8%. In agreement with our report, Valchèze *et. al.* also reported that there is additive interaction between anti-TB drugs and ascorbate (8).

CONCLUSION

Ascorbate showed dose dependent inhibition of MTB in MDM PBL cell culture model which has relatively lower iron concentration and the inhibition was independent of Th_1 cytokines. Hence, ascorbate can be further investigated in clinical trials as an adjunct to anti-TB drugs for treatment of tuberculosis. In the future we will investigate possible roles of NO and GSH in ascorbate's anti-MTB effect.

REFERENCES

1. Global tuberculosis report 2018. Geneva: World Health Organization 2018.
2. Burman WJ, Cohn DL, Rietmeijer CA, Judson FN, Sbarbaro JA, Reves RR. Noncompliance with directly observed therapy for tuberculosis. Epidemiology and effect on the outcome of treatment. Chest. 1997 May;111(5):1168-73.
3. Gupta KB, Gupta R, Atreja A, Verma M, Vishvkarma S. Tuberculosis and nutrition. Lung India : official organ of Indian Chest Society. 2009 Jan;26(1):9-16.
4. Madebo T, Lindtjorn B, Aukrust P, Berge RK. Circulating antioxidants and lipid peroxidation products in untreated tuberculosis patients in Ethiopia. The American journal of clinical nutrition. 2003 Jul;78(1):117-22.
7. Hemila H, Kaprio J. Vitamin E supplementation may transiently increase tuberculosis risk in males who smoke heavily and have high dietary vitamin C intake. The British journal of nutrition. 2008 Oct;100(4):896-902.
8. Vilcheze C, Hartman T, Weinrick B, Jacobs WR, Jr. Mycobacterium tuberculosis is extraordinarily sensitive to killing by a vitamin C-induced Fenton reaction. Nature communications. 2013;4:1881. PubMed PMID: 23695675.
9. Boelaert JR, Vandecasteele SJ, Appelberg R, Gordeuk VR. The effect of the host's iron status on tuberculosis. The Journal of infectious diseases. 2007 Jun 15;195(12):1745-53.
10. Weiss G, Schaible UE. Macrophage defense mechanisms against intracellular bacteria. Immunological reviews. 2015 Mar;264(1):182-203. PubMed PMID: 25703560.
11. Bergsten P, Amitai G, Kehrl J, Dhariwal KR, Klein HG, Levine M. Millimolar concentrations of ascorbic acid in purified human mononuclear leukocytes. Depletion and reaccumulation. The Journal of biological chemistry. 1990 Feb 15;265(5):2584-7.
12. Qiao H, May JM. Macrophage differentiation increases expression of the ascorbate transporter (SVCT2). Free radical biology & medicine. 2009 Apr 15;46(8):1221-32.
13. Shukla H, Khan SR, Shukla R, Krishnan MY, Akhtar MS, Tripathi T. Alternate pathway to ascorbate induced inhibition of Mycobacterium tuberculosis. Tuberculosis. 2018 Jul;111:161-9.
14. Venketaraman V, Dayaram YK, Talaue MT, Connell ND. Glutathione and nitrosoglutathione in macrophage defense against Mycobacterium tuberculosis. Infection and immunity. 2005 Mar;73(3):1886-9.
15. Lenton KJ, Sane AT, Therriault H, Cantin AM, Payette H, Wagner JR. Vitamin C augments lymphocyte glutathione in subjects with ascorbate deficiency. The American journal of clinical nutrition. 2003 Jan;77(1):189-95.
16. Venketaraman V, Millman A, Salman M, Swaminathan S, Goetz M, Lardizabal A, et al. Glutathione levels and immune responses in tuberculosis patients. Microbial pathogenesis. 2008 Mar;44(3):255-61.
17. Volchegorskii IA, Novoselov PN, Astakhova TV. [The effectiveness of ascorbic acid and emoxipin in treatment of infiltrative pulmonary tuberculosis]. Klinicheskaia meditsina. 2007;85(12):55-8.

18. Jainu M, Mohan KV. Protective role of ascorbic acid isolated from Cissus quadrangularis on NSAID induced toxicity through immunomodulating response and growth factors expression. International immunopharmacology. 2008 Dec 20;8(13-14):1721-7.
19. Teskey G, Cao R, Islamoglu H, Medina A, Prasad C, Prasad R, et al. The Synergistic Effects of the Glutathione Precursor, NAC and First-Line Antibiotics in the Granulomatous Response Against Mycobacterium tuberculosis. Frontiers in immunology. 2018;9:2069. PubMed PMID: 30258443.
20. Hartel C, Strunk T, Bucsky P, Schultz C. Effects of vitamin C on intracytoplasmic cytokine production in human whole blood monocytes and lymphocytes. Cytokine. 2004 Aug 21-Sep 7;27(4-5):101-6.
21. Li-Weber M, Giaisi M, Treiber MK, Krammer PH. Vitamin E inhibits IL-4 gene expression in peripheral blood T cells. European journal of immunology. 2002 Sep;32(9):2401-8.
23. Bulatovic VM, Wengenack NL, Uhl JR, Hall L, Roberts GD, Cockerill FR, 3rd, et al. Oxidative stress increases susceptibility of Mycobacterium tuberculosis to isoniazid. Antimicrobial agents and chemotherapy. 2002 Sep;46(9):2765-71. PubMed PMID: 12183226.