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1 # Characterizing the Effects of Glutathione as an Immunoadjuvant in the Treatment of Tuberculosis
2

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33 **Abstract**

34 Mycobacterium *tuberculosis* (*M. tb*) is the etiological agent that is responsible for causing
35 tuberculosis (TB), which continues to affect millions of people worldwide, with an ever-
36 increasing resistance to antibiotics. We tested the synergistic effects of N-acetyl cysteine (NAC,
37 the precursor molecule for the synthesis of glutathione) and individual first-line antibiotics
38 typically given for the treatment of TB such as Isoniazid (INH), Rifampicin (RIF), Ethambutol
39 (EMB) and Pyrazinamide (PZA) to improve the ability of macrophages to control intracellular
40 *M. tb* infection. Glutathione (GSH), a pleiotropic antioxidant molecule has been previously
41 shown to display both antimycobacterial and immune-enhancing effects. Our results indicate that
42 there was not only an increase in beneficial immunomodulatory effects, but a greater reduction in
43 the intracellular viability of *M. tb* when macrophages were treated with the combination of
44 antibiotics (INH/RIF/EMB or PZA) and NAC.

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50 **Introduction**

51 *Mycobacterium tuberculosis* (*M. tb*), is the causative agent of tuberculosis (TB), and a
52 leading cause of death worldwide [1]. According to the World Health Organization (WHO), TB
53 is currently the ninth leading cause of mortality worldwide and the principal cause of death due
54 to a single infectious agent [2]. The WHO reported that 10.4 million people contracted an active
55 TB infection in 2016 alone [2]. *M. tb* infection is acquired via inhalation of respiratory droplets,
56 leading to the seeding of the bacteria within the lungs. More specifically, once *M. tb* enters the
57 lower respiratory tract, it is engulfed by alveolar macrophages and becomes an intracellular
58 pathogen [3]. At this point a competent immune system will mount an attack against the bacteria,
59 either killing it off completely, or more characteristically, sequestering the bacteria within a
60 specialized immune structure known as a granuloma, archetypally localized within the lungs [4].
61 This process of mycobacterial containment within a granuloma is referred to as latent TB and is
62 observed in the majority of TB cases. A granuloma consists of a multitude of immune cells
63 which come together to orcistate this protective immune response, including: macrophages,
64 epithelioid histiocytes, dendritic cells, T cells and natural killer (NK) cells, which isolate the
65 bacteria rendering it latent [5]. The accumulation of these immune cells is mediated by various
66 cytokines, including TNF-α, IL-6, IL-12, IL-2 and IFN-γ[6].

67 However, in an immunocompromised individual *M. tb* granulomas can undergo
68 liquefaction resulting in an active TB infection [7]. Individuals with active TB are not only
69 contagious but are at a serious risk for developing permanent morbidity due to vast cellular
70 damage. Therefore, the Center for Disease Control and Prevention (CDC) recommends that the
71 preferred treatment regimen for an active TB consists of the combined administration of the
72 antibiotics Isoniazid (INH), Rifampicin (RIF), Pyrazinamide (PZA), and Ethambutol (EMB) for

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73 2 months (the intensive phase), followed by the administration of INH and RIF for 4 months (the
74 continuation phase) [8-10]. These antibiotics are primarily used in combination to prevent the
75 bacteria from developing resistance. In lieu of RIF, rifapentine may be used for intermittent
76 dosing, or rifabutin as it has fewer interactions with anti-HIV medications and opioid substitution
77 therapy [11]. However, these alternative medications are more expensive than RIF and are not
78 universally available in all TB programs [12]. Positive prognostic indicators include patients
79 feeling better within a few weeks of beginning treatment and will typically become non-
80 infectious during the intensive phase [12]. The intensive phase is followed by the continuation
81 phase of treatment which consists of four-months of treatment with INH and RIF (the two most
82 powerful first-line antibiotics) [8-10]. A patient is declared cured upon completing this treatment
83 and achieving two negative TB tests. However, TB treatment has been experiencing a rising risk
84 of failure due to the development of multidrug resistant strains of *M. tb*, largely due to
85 noncompliance and inadequate adherence to standard treatment protocols and the necessary
86 cessation of treatment due to the detrimental side effects that can be associated with the use of
87 the aforementioned antibiotics [13]. Side effects, particularly nausea and abdominal pain, are
88 relatively common [14]. Additionally, urine and tears can turn orange, which is harmless but can
89 be disconcerting if patients are not forewarned [15]. More severe side effects, such as joint pain,
90 visual impairment, peripheral neuropathy (nerve damage) and hepatotoxicity leading to liver
91 damage are less common but can be serious when they do occur [16]. In 2012, bedaquiline, used
92 to treat drug resistant-TB (DR-TB), became the first new TB drug from a new class to be
93 approved by the U.S. Food and Drug Administration (FDA) in over 40 years [17]. In 2014,
94 bedaquiline's U.S. accelerated approval was followed by the European Medicines Agency's
95 (EMA's) conditional approval of bedaquiline as well as another new drug, delamanid, for the

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96 treatment of specific forms of DR-TB, emphasizing the urgent need to develop new treatment
97 options for both drug sensitive and drug resistant TB [18].

98 In search of a novel therapeutic agent to augment the treatment of TB, we investigated
99 the synergistic effects of the glutathione (GSH) precursor N-acetyl cysteine (NAC), in
100 conjunction with the individual aforementioned first-line antibiotics in promoting macrophage-
101 mediated killing of *M. tb*. NAC has been widely used for several years to enhance the
102 intracellular levels of GSH [19]. GSH, a tripeptide comprised of glutamate, cysteine and glycine,
103 functions to protect the cells and tissues from oxidative damage thereby restoring redox
104 homeostasis within the body [20]. Further studies have demonstrated that the biological
105 antioxidant GSH has both antimycobacterial effects and immune-modulating properties [21-23].

106 Our laboratory has previously reported that the levels of GSH were significantly
107 decreased in the red blood cells, NK cells, macrophages and T cells derived from the peripheral
108 blood of individuals with HIV infection [24-26]. Furthermore, we have also demonstrated that
109 the levels of GSH were significantly compromised in brain tissue samples derived from the
110 frontal cortex of individuals with HIV infection [21]. The decreased levels of GSH among the
111 HIV infected individuals correlated with the diminished control of *M. tb* infection [21-23].
112 Additional studies have shown that the levels of GSH were significantly compromised in
113 individuals with type 2 diabetes (T2DM) due to diminished levels of enzymes involved in the
114 GSH synthesis [27]. Findings from our clinical trials indicate that supplementation with
115 liposomal glutathione (L-GSH) restored redox homeostasis, induced cytokine balance and
116 improved immune responses against M. *tb* infection [23]. Additionally, NAC has also been
117 shown to have direct mycobactericidal effects [28].

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118 Therefore, we hypothesized that treatment of THP-1 cells with NAC in conjunction with
119 any one of the first line antibiotics: INH, RIF, EMB or PZA will improve the ability of
120 macrophages to effectively control *M. tb* infection. Our study findings indicate a greater
121 reduction in the intracellular viability of *M. tb* when macrophages were treated with the
122 combination of NAC and antibiotics (INH/RIF/EMB or PZA).
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141 **Material and Methods**

142 **THP-1 cell culture**

143 THP-1 cells were maintained RPMI media (Sigma) containing 10% Fetal bovine serum
144 (FBS-Sigma) and 2mM glutamine (Sigma). For the assays, cell suspension was centrifuged at
145 2,000 rpm for 15 minutes, and the pellet was resuspended in RPMI containing 10% FBS. Cell
146 numbers in the suspension were determined by trypan blue dye exclusion staining. THP-1 cells
147 ($2X10^5$ cells/well) were distributed in 24-well plates (Corning), treated with PMA (Phorbol 12-
148 myristate 13-acetate) at 10 ng/ml concentration and incubated overnight at 37°C, 5% CO2 to
149 induce the differentiation to macrophages. Following overnight incubation, media in the wells
150 were replaced with fresh media.

151

152 **Preparation of bacteria for infection assays**

153 The Erdman strain of *M. tb* (was gifted by Dr. Selvakumar Subbian. Rutgers New Jersey
154 Medical School, Biomedical and Health Sciences) was used for all our infection studies. The
155 Erdman strain of M. *tb* (will henceforth be referred to as *M. tb*) has slightly faster doubling time
156 and is more virulent compared to the standard laboratory strain, H37Rv [29]. *M. tb* was cultured
157 in 7H9 media (Middlebrook) supplemented with albumin dextrose complex (ADC) at 37°C until
158 processing. The *M. tb* was processed for infection once the static culture was at the peak
159 logarithmic phase of growth (OD between 0.5-0.8 at A600), and subsequently washed and
160 resuspended in sterile 1X PBS. Bacterial clumps were dispersed by vortexing five times with
161 3mm sterile glass beads at two minutes intervals. The bacterial suspension was then filtered
162 using a 5µm syringe filter (Millipore) to remove any remaining bacterial aggregations. The
163 single cell suspension of processed *M. tb* was serially diluted and plated on 7H11 agar to

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164 determine the bacterial numbers in the processed stock. Aliquots of processed bacterial stocks
165 were frozen at -80°C. At the time of infection, the processed frozen stocks of *M. tb* were thawed
166 and used for the infection. All infection studies and handling of the *M. tb* was done inside a
167 certified biosafety level 3 facility (BSL-3).

168

169 **THP-1 macrophage infection and treatment**

170 Differentiated macrophages were infected with processed *M. tb* at a multiplicity of
171 infection or MOI of 0.1:1 (bacteria to macrophage ratio). Infected macrophages were incubated
172 for 1 hour and then successively washed 3 times with warm 1X PBS to remove the
173 unphagocytozed bacteria. Infected macrophages were then either sham-treated or received a
174 onetime treatment with the MIC of the respective antibiotic with or without NAC addition. NAC
175 (10 mM) was administered at 3 equal intervals throughout the trial. The treatment concentrations
176 administered were as follows: INH (0.125 micrograms/ml), INH (0.125 micrograms/ml) + NAC
177 (10 mM (x3)), RIF (0.125 micrograms/ml), RIF (0.125 micrograms/ml) + NAC (10 mM (x3)),
178 EMB (8.0 micrograms/ml), EMB (8.0 micrograms/ml) + NAC (10 mM (x3)), PZA (50
179 micrograms/ml) and PZA (50 micrograms/ml) + NAC (10 mM (x3)). The Infected cells were
180 maintained at 37°C, 5% CO2 until they were terminated at 1 hour and 12 days post-infection to
181 determine the intracellular survival of M. *tb*.

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183

184 **Termination of macrophages and CFUs assay**

185 Termination of infected macrophages was performed by collecting and storing the cell-
186 free supernatants and lysing THP-1 cells using 250 ul of ice cold, sterile 1X PBS. Cell lysates

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187 collected from the wells were vigorously vortexed and then subjected to freeze/thaw cycles to
188 ensure complete lysis of macrophages. The collected lysates and supernatants were then diluted
189 in sterile 1X PBS and plated on 7H11 medium (Hi Media) enriched with ADC to evaluate M. *tb*
190 survival inside the macrophages by counting the bacterial colonies.
191

192 **Quantification of GSH levels in the cellular lysates**

193 The quantity of total glutathione present was measured by the colorimetric method using
194 an assay kit from Arbor Assay (K006-H1). The macrophages lysates were first thoroughly mixed
195 with an equal volume of cold 5% sulfosalicylic acid (SSA), and then incubated for 10 minutes at
196 4°C, followed by centrifugation at 14000 rpm for 10 minutes. The GSH was measured following
197 the manufacturer's instructions. All measurements were normalized by total protein levels and
198 the results were reported in moles of GSH per gram of protein.
199

200 **Cytokines measurements of culture medium**

201 The effects of *M. tb* infection, antibiotic and NAC treatments in altering the production of
202 TNF-α and IL-10 was determined by quantifying the levels of these cytokines in the macrophage
203 supernatants collected at 12 days post-infection by enzyme-linked immunosorbent assay
204 (ELISA) using assay kits from Affymetrix as per the manufacturer's protocol..
205

206 **Statistical Analysis**

207 Statistical data analysis was performed using GraphPad Prism Software version 7. Levels
208 of cytokines, GSH, MDA and CFUs were compared between untreated control, antibiotic-
209 treatment and treatment with antibiotics in conjunction with NAC using the unpaired t-test with

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210 Welch correction. Reported values are in means. $P<0.05$ was considered significant ($*p<0.05$,
211 $**p<0.005$).
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234 **Results:**

235 **Levels of total GSH in uninfected and *M. tb*-infected THP-1 cells**

236 GSH levels were measured in the lysates of THP-1 cells using an assay kit from Arbor Assays.
237 The levels of total GSH were shown to be significantly diminished in *M. tb* infected
238 macrophages compared to the uninfected control group (Fig. 1A). These results indicate that
239 infection with Erdman strain of *M. tb* can cause a significant two-fold decrease in the
240 intracellular levels of GSH in macrophages.

241

242 **Survival of Erdman strain of *M. tb* inside untreated THP-1 macrophages**

243 There was a statistically significant increase in the intracellular survival of *M. tb* residing inside
244 the untreated macrophages between the initial (1 hour) and final time point of termination (12-
245 days post-infection) [Fig. 1B]. These results signify the ability of Erdman strain of *M. tb* to
246 successfully survive and replicate inside the THP-1 cells.

247

248 **Levels of TNF-α in the supernatants of uninfected and *M. tb*-infected THP-1 cells**

249 Levels of TNF-α, a pro-inflammatory cytokine were quantified in the supernatants collected
250 from the uninfected macrophages and *M. tb*-infected macrophages by "sandwich" ELISA. When
251 compared to the uninfected control category, there was a statistically significant six-fold increase
252 in the levels of TNF-α in *M. tb*-infected macrophages (Fig. 1C). Our results imply that excess
253 production of TNF-α by *M. tb*-infected macrophages result in GSH decrease thereby favoring the
254 intracellular survival of *M. tb.*

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256 **Measurement of GSH, bacterial survival, TNF-α and IL-10 levels in *M. tb*-infected and M.**
257 ***tb*-infected + NAC-treated THP-1 cells**

258 Treatment of *M. tb*-infected macrophages with NAC resulted in a significant two-fold increase in
259 the intracellular levels of GSH when compared to the *M. tb* infected sham control group (Fig.
260 2A). NAC-treatment therefore restores the levels of GSH in *M. tb*-infected macrophages.
261 Restoration in the levels of GSH correlated with significant reduction in the intracellular survival
262 of *M. tb* inside the NAC-treated macrophages (Fig. 2B). GSH-replenishment in *M. tb*-infected
263 macrophages also resulted in a significant 8-fold decrease in levels of TNF-α compared to the *M.*
264 *tb*-infected control group (Fig. 2C). Importantly, NAC-treatment of *M. tb*-infected macrophages
265 resulted in a 4-fold decrease in levels of IL-10 compared to the *M. tb*-infected sham control
266 group (Fig. 2D). These results further confirm that by restoring redox homeostasis and cytokine
267 balance there is improved control of intracellular *M. tb* infection.
268
269
270 **Quantification of GSH levels, *M. tb* survival, TNF-α and IL-10 levels in *M. tb*-infected, M.**
271 ***tb*-infected + INH-treated and *M. tb*-infected with INH + NAC-treated THP-1 cells**

272 INH is the first line antibiotic most commonly used for treatment of *M. tb* infection. *M. tb*-
273 infected macrophages were treated as follows: sham-treatment, treated with INH (0.125
274 micrograms/mL), and treated with combination of INH (0.125 micrograms/mL) +NAC (10mM),
275 and the effects of INH and INH+NAC treatments in altering the levels of GSH and cytokines,
276 and *M. tb* survival was determined. When compared to the infected sham control, there was a
277 significant increase in the levels of GSH in *M. tb*-infected macrophages treated with INH and
278 INH+ NAC (Fig. 3A). In comparison to INH alone category, treatment with INH+NAC resulted

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279 in complete clearance of M. *tb* infection (Fig. 3B). Treatment with either INH or the combination
280 of INH+ NAC resulted in almost 10,000-fold decrease in the viability of *M. tb* when compared to
281 treatment with NAC only (Fig 2B), and roughly 15,000-fold decrease in the viability of *M. tb*
282 compared to sham control group (Fig. 1B). These results further emphasize that reduction in *M.*
283 *tb* burden can restore the intracellular levels of GSH. Treatment of *M. tb*-infected macrophages
284 with INH resulted in a statistically significant decrease in the levels of TNF-α when compared to
285 the infected sham control group. Treatment of *M. tb*-infected macrophages with INH + NAC also
286 resulted in a statistically significant decrease in the levels of TNF-α (Fig. 3C). INH + NAC
287 treatment resulted in further reduction in the levels of TNF-α in comparison to treatment with
288 INH alone. Consistent with our findings from the TNF-α assay, INH treatment of *M. tb*-infected
289 macrophages also resulted in reduction in the levels of IL-10 when compared to the infected
290 sham controls. INH + NAC treatment caused additional decrease in the levels of IL-10 (Fig. 3D).
291 These findings specify the effects of *M. tb* clearance in diminishing the levels of TNF-α and IL-
292 10.
293
294 **Quantification of GSH levels, *M. tb* survival, TNF-α and IL-10 levels in *M. tb*-infected, *M.***
295 ***tb*-infected + RIF-treated and *M. tb*-infected with RIF+ NAC-treated THP-1 cells**
296 RIF is another dominant first-line antibiotic used for the treatment of TB. GSH levels were
297 therefore measured in the *M. tb*-infected sham control macrophages and *M. tb*-infected
298 macrophages treated with either RIF alone (0.125 micrograms/ml) or RIF (0.125 micrograms/ml)
299 in combination with NAC (10mM). The RIF-treatment to infected macrophages resulted in a
300 significant increase in GSH compared to the infected sham control group. Importantly, RIF +
301 NAC treatment resulted in further enhancement in the levels of GSH and a statistically

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302 significant increase compared to both infected sham control category and also RIF unaided
303 category (Fig. 4A).
304 The notable increase in the levels of GSH in RIF + NAC treatment group was accompanied by
305 significant and 8-fold reduction in the intracellular viability of *M. tb* compared to treatment with
306 RIF alone (Fig. 4B). When comparing the efficacy of INH versus RIF, there was also
307 approximately 70 times more intracellular *M. tb* when macrophages were treated with RIF
308 instead of INH, and 25 times more bacteria when treatment of RIF with NAC was used instead
309 of INH with NAC (Fig. 3B). When compared to sham treatment, RIF treatment reduced bacterial
310 burden by almost 200 times (Fig. 1B) and treatment of RIF+NAC reduced the *M. tb* counts by
311 1,600 times. Treatment of *M. tb*-infected macrophages with combination of RIF+ NAC, also
312 resulted in a statistically significant decrease in the levels of TNF-α compared to both the
313 infected sham control and lone RIF (Fig. 4C). Treatment with RIF caused 50% reduction in the
314 levels of TNF-α compared to sham-treated control (Fig. 4C). In comparison to sham-control,
315 treatment with RIF+NAC resulted in 80-fold reduction in the levels of TNF-α (Fig. 4C). RIF
316 administered alone was not able to lower the levels of TNF-α as much as INH by itself, but the
317 addition of NAC to RIF, lowered the TNF-α levels 8 times more than that of INH and NAC
318 treatment (Fig. 3C). RIF treatment resulted in a decrease in the levels of IL-10 compared to the
319 sham-control group (Fig 4D). Treatment of M. *tb*-infected macrophages with combination of
320 RIF+ NAC, resulted in a further decrease in the levels of IL-10 compared to the control and lone
321 RIF, but neither of these decreases were statistically significant (Fig 4D). IL-10 trends were very
322 similar when comparing RIF and INH treatments. However, INH was twice as effective in
323 lowering the levels of IL-10 in macrophage cultures (Fig. 3D).
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325 **Quantification of GSH levels, *M. tb* survival, TNF-α and IL-10 levels in *M. tb*-infected, *M.*
326 ***tb*-infected + EMB-treated and *M. tb*-infected with EMB+ NAC-treated THP-1 cells**

327 EMB is an antibiotic that is normally given with a myriad of other first line antibiotics for TB
328 treatment. We tested the efficacy of stand-alone EMB (8.0 micrograms/ml), as well as the
329 efficacy of EMB (8.0 micrograms/ml) supplemented with NAC (10mM) in altering the levels of
330 GSH and cytokines, and *M. tb* survival inside the macrophages. GSH levels were increased by 2-
331 fold in the macrophages treated with EMB compared to the infected sham control group,
332 although this increase was not significant. However, when macrophages were treated with
333 EMB+NAC, there was a statistically significant 3-fold increase in the levels of GSH compared to
334 the sham control category (Fig. 5A). Consistent with the increase in the levels of GSH, there
335 was four-fold decrease in the intracellular survival of *M. tb* inside the EMB+NAC-treated
336 macrophages compared to the stand-alone EMB treated macrophages (Fig. 5B). When compared
337 to the infected sham control group, the infected macrophages treated with stand-alone EMB had
338 significantly reduced levels of TNF-α (Fig. 5C). EMB+NAC treatment resulted in further
339 decrease in the levels of TNF-α in comparison to sham-control and EMB-alone groups. EMB
340 and EMB+NAC treatments resulted in more than 50% and 80% decrease respectively, in the
341 levels of TNF-α in comparison to sham-control group (Fig. 5C). Although not significant, a
342 distinct reduction in the levels of IL-10 was observed when *M. tb* infected macrophages were
343 treated with EMB+ NAC (Fig. 5D).

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348 **Quantification of GSH levels, *M. tb* survival, TNF-α and IL-10 levels in M. *tb*-infected, *M.*
349 ***tb*-infected + PZA-treated and *M. tb*-infected with PZA+ NAC-treated THP-1 cells**

350 PZA is an antibiotic that is normally given in combination with all four first-line antibiotics for
351 the curative action against TB. To determine the effects of PZA and PZA+NAC in altering the
352 intracellular survival of *M. tb* and production of GSH and cytokines, *M. tb* infected THP-1 cells
353 were treated with stand-alone PZA (50 micrograms/ml) and the combination of PZA (50
354 micrograms/ml) and NAC (10mM) treatment. When compared to the infected sham control
355 group the levels of GSH in PZA-treated macrophages were significantly elevated (Fig. 6A).
356 There was further enhancement in the levels of GSH when *M. tb* infected macrophages were
357 treated with PZA+ NAC (Fig. 6A). When compared to stand-alone PZA, the combination
358 treatment of PZA+NAC led to a statistically significant decrease in CFUs of *M. tb* (Fig. 6B).
359 PZA was more effective in lowering the bacterial load than both RIF (Fig. 4B) and EMB (Fig.
360 5B). Additionally, the treatment of PZA given in conjunction with NAC was as effective as the
361 treatment of INH alone (Fig. 3B). TNF-α levels were quantified in the supernatants of *M. tb*
362 infected macrophages in presence and absence of PZA sand PZA+NAC. The combined
363 treatment of PZA+NAC exhibited a statistically significant decrease in levels of TNF-α
364 compared to both the infected sham control and the stand-alone PZA categories (Fig. 6C). The
365 levels of TNF-α after treatment with PZA was similar to that of EMB (Fig. 5C) and RIF (Fig.
366 4C). In comparison to sham control group, IL-10 levels were decreased in M. *tb* infected
367 macrophages treated with PZA and PZA+NAC (Fig. 6D). Although not statistically significant,
368 the PZA-treated macrophages showed a marked decrease in IL-10 levels compared to the sham
369 control group. Importantly, treatment with PZA+NAC resulted in a statistically significant

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370 decrease in the levels IL-10, compared to the sham-control group (Fig. 6D). Furthermore, of all
371 the first-line antibiotics tested, PZA treatment resulted in the lowest levels of IL-10 measured.
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393

394 **Discussion:**

395 Immunocompromised individuals, such as HIV positive subjects, are increasingly
396 susceptible to *M. tb* infection. Additionally, the rapid increase in the number of people living
397 with drug resistant TB (DR-TB) and TB/HIV co-infection generates additional challenges for
398 global targets of TB elimination. The standard six-month first-line antibiotic course of treatment
399 consists of two phases: the intensive phase (the first two months) and the continuation phase (the
400 last four months). However, treatment may differ for patients with extrapulmonary TB (TB
401 outside of the lungs). During the intensive phase of standard treatment, patients take a daily
402 combination of four medications: INH, RIF, PZA and EMB. These four drugs are referred to as
403 first-line drugs and are off-patent and relatively inexpensive. However, the treatment of TB can
404 become more complicated due to the development of drug-resistant and multidrug-resistant
405 strains of *M. tb* [30]. Additionally, there are a variety of severe complications and side effects
406 which can occur when taking these medications, especially when consuming the large dosage
407 required to ensure proper elimination of a *M. tb* infection.

408 We previously reported that the virulent laboratory strain of *M. tb*, H37Rv is sensitive to
409 physiological concentrations of GSH (5mM) when grown i*n vitro* (43, 44). We also found that
410 enhancing the levels of GSH in human macrophages by treatment with NAC (10 or 20 mM)
411 resulted in inhibition in the growth of intracellular H37Rv (43-46, 49). Thus, GSH has direct
412 antimycobacterial activity, functioning as an effector molecule in innate defense against *M. tb*
413 infection (43-49). These results unfold a novel and potentially important innate defense
414 mechanism adopted by human macrophages to control *M. tb* infection (43-49). We also reported
415 that GSH in combination with cytokines such as IL-2 and IL-12 enhances the functional activity

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416 of NK cells to inhibit the growth of M. *tb* inside human monocytes (47). We then demonstrated
417 that GSH activates the functions of T lymphocytes to control *M. tb* infection inside human
418 monocytes (48). These results indicate that GSH inhibits the growth of *M. tb* by both direct
419 antimycobacterial effects as well as by enhancing the functions of immune cells (43-47). Finally,
420 we demonstrated that the levels of GSH were significantly compromised in individuals with
421 active pulmonary TB (49).

422 Therefore, we tested the synergistic effects of NAC (as a GSH precursor) and sub-
423 optimal levels of individual first-line anti-TB drugs (INH, RIF, ETMB and PZA) in mediating
424 control of *M. tb* infection inside THP-1 macrophages. The THP-1 cell line is an immortalized
425 monocyte cell line, derived from the blood of a childhood case of acute monocytic leukemia [31,
426 32]. Each antibiotic was administered only once throughout the trial at its MIC. The MIC was
427 administered to ensure that complete bacterial clearance was not achieved by the
428 supplementation of the antibiotics alone.

429 Our results demonstrate that macrophages treated with the combination of suboptimal
430 levels of each of the first-line antibiotics given in conjunction with NAC resulted in a significant
431 reduction in the intracellular survival of *M. tb*, when compared to the administration of
432 unaccompanied suboptimal levels of antibiotics (Fig. 3B, Fig. 4B, Fig. 5B, and Fig. 6B). In fact,
433 complete clearance was observed for the INH category after the additional supplementation of
434 NAC was added (Fig. 3B). These novel findings illustrate the synergistic effects of NAC/GSH
435 and antibiotics in improving macrophages ability to control intracellular *M. tb* and suggests that
436 GSH has suitable potential as an adjunct with the aforementioned first-line antibiotics in clearing
437 a *M. tb* infection and aiding in the cessation of drug resistant strains of *M. tb*.

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438 We observed a significant decrease in the levels of intracellular GSH in *M. tb*-infected
439 macrophages compared to uninfected macrophages (Fig 1A). These results indicate that M. *tb*
440 infection can cause intracellular GSH depletion, which in turn can promote *M. tb* survival and
441 replication inside the host cells (Fig 1C). Furthermore, enhancing the levels of GSH in M. *tb*-
442 infected macrophages by treatment with NAC (Fig 2A) resulted in significant reduction in the
443 intracellular survival of *M. tb* (Fig 2B). Treatment of *M. tb*-infected macrophages with each of
444 the first-line antibiotics resulted in restoration in the levels of GSH and a statistically significant
445 (except of EMB*) increase when compared to the *M. tb*-infected sham control group (Fig. 3A,
446 Fig. 4A, Fig. 5A, and Fig. 6A). These results indicate that use of antibiotics to limit *M. tb* burden
447 in the macrophages enabled the host cells to restore the levels of GSH and improved their ability
448 to combat the infection. Importantly, treatment of *M. tb*-infected macrophages with NAC in
449 conjunction with each of the first-line antibiotics resulted in a statistically significant notable
450 increase in the levels of GSH in all the categories (Fig. 3A, Fig. 4A, Fig. 5A, and Fig. 6A). The
451 levels of GSH detected in macrophages treated with antibiotic in combination with NAC were
452 consistently higher when compared sham control and macrophages treated with antibiotic alone
453 (Fig. 3A, Fig. 4A, Fig. 5A, and Fig. 6A). Antibiotic treatment when given in conjunction with
454 NAC resulted in decreased production of TNF-α and IL-10, by the macrophages. In the context
455 of a *M. tb* infection, TNF-α is an inflammatory cytokine produced by macrophages and is
456 particularly important in promoting the formation and maintenance of a granuloma, whereas IL-
457 10 is an immunosuppressive cytokine which acts as negative regulator to the immune response
458 associated with fighting of the infection [33-35]. TNF-α is a diverse cytokine, which has been
459 shown to functionally aid in the formation and maintenance of a granuloma as well as play a
460 critical role in host defense against *M. tb* in both acute phase and chronic phase of infection [36-

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461 38]. However, at high levels, TNF-α is also implicated as the source of many inflammatory and
462 autoimmune diseases and has been shown to cause severe tissue damage when overexpressed in
463 relation to a *M. tb* infection [39, 40]. When the combination of NAC and each of the first-line
464 antibiotics was administered, the TNF-α levels for each category was significantly reduced from
465 the extremely high levels seen among the infected sham control and presented closer to that of
466 the uninfected group (Fig. 1C, Fig. 3C, Fig. 4C, Fig. 5C, and Fig. 6C). Our results indicate that
467 NAC-treatment can modulate the levels of TNF-α in a manner that it is significant enough to
468 maintain a healthy granuloma but cannot cause cellular damage. Increased levels of the cytokine
469 IL-10 can dampen the effector responses against a *M. tb* infection by inhibiting phagosome-
470 lysosome fusion within macrophages [40, 41, 42]. Although not statistically significant, a notable
471 reduction in the levels of IL-10 was observed for every antibiotic category when administered
472 with NAC (Fig. 3D, Fig. 4D, Fig. 5D, and Fig. 6D). Additionally, when NAC was supplemented
473 unaided, a reduction of roughly double the magnitude of IL-10 was likewise detected (Fig. 2D).
474 This data implies that NAC supplementation supports the immune responses in favor of
475 eliminating a *M. tb* infection by reducing the levels of IL-10, allowing macrophage phagosome
476 maturation and thus enhanced pathogen elimination. Our findings illustrate that in addition to the
477 direct antimycobacterial effects, GSH-enhancement improved the ability of first-line antibiotics
478 to limit intracellular *M. tb* infection and modulated the cytokine production by macrophages.

479 When all four antibiotics were administered together at their MICs, complete
480 mycobacterial clearance was observed with and without the addition of NAC (data not shown).
481 A similar trend was observed as with the supplementation of individual antibiotics, where a
482 significant increase in levels of GSH was detected from the administration of all four antibiotics
483 without NAC and a further increase once NAC was additionally added (data not shown).

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484 Likewise, a reduction in the levels of TNF-a, and IL-10 was observed among the administration
485 of all four antibiotics without NAC supplementation and with statistical significance once NAC
486 was also administered (data not shown).

487 Our findings highlight that GSH exhibits more physiological significance than just
488 intracellular redox homeostasis, advocating that its enhancement aids in cytokine balance as well
489 as augments the ability of first-line anti-TB drugs to clear a *M. tb* infection. Therefore, we
490 believe that including GSH (NAC) in the antibiotic treatment of TB would not only limit cellular
491 damage by means of redox balance, and subsequently reduce potential toxicity to the anti-TB
492 medications but could possibly limit the required dosage necessary to cause complete bacterial
493 clearance as well as help combat further emergences of DR-TB strains.
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530 **References**

531 1. Smith I. *Mycobacterium tuberculosis* Pathogenesis and Molecular Determinants of
532 Virulence. Clinical Microbiology Reviews. 2003;16(3):463-496.
533 2. World Health Organization (2017) Tuberculosis & Diabetes [Fact sheet]
534 3. Guirado E, Schlesinger LS, Kaplan G. Macrophages in Tuberculosis: Friend or Foe.
535 *Seminars in immunopathology*. 2013;35(5):563-583.
536 4. Volkman HE, Clay H, Beery D, Chang JC, Sherman DR, Ramakrishnan L. Tuberculous
537 granuloma formation is enhanced by a mycobacterium virulence determinant. PLoS Biol.
538 2004 Nov;2(11): e367.
539 5. Guirado E, Mbawuike U, Keiser TL, Arcos J, Azad AK, Wang SH, Schlesinger LS:
540 Characterization of host and microbial determinants in individuals with latent
541 tuberculosis infection using a human granuloma model. mBio 2015, 6(1): e02537-02514.
542 6. Domingo-Gonzalez R, Prince O, Cooper A, Khader SA. Cytokines and Chemokines in
543 *Mycobacterium tuberculosis* Infection. Microbiol Spectr. 2016 Oct;4(5).
544 7. Ehlers S, Schaible UE. The Granuloma in Tuberculosis: Dynamics of a Host–Pathogen
545 Collusion. *Frontiers in Immunology*. 2012;3:411.
546 8. Dorman SE, Chaisson RE: From magic bullets back to the magic mountain: the rise of
547 extensively drug-resistant tuberculosis. Nature medicine 2007, 13(3):295-298.
548 9. Ormerod LP: Multidrug-resistant tuberculosis (MDR-TB): epidemiology, prevention and
549 treatment. British Medical Bulletin 2005, 73-74(1):17-24.
550 10. Udwadia ZF. MDR, XDR, TDR tuberculosis: ominous progression. Thorax. 2012 Apr;
551 67(4):286-8.

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552 11. Horne DJ, Spitters C, Narita M. Experience with Rifabutin Replacing Rifampin in the
553 Treatment of Tuberculosis. *The International Journal of Tuberculosis and Lung Disease*.
554 2011;15(11):1485-i.
555 12. Leibert E, Rom WN. New drugs and regimens for treatment of TB. *Expert review of anti-*
556 *infective therapy*. 2010;8(7):801-813.
557 13. Shenoi S, Heysell S, Moll A, Friedland G. Multidrug-resistant and extensively drug-
558 resistant tuberculosis: consequences for the global HIV community. *Current opinion in*
559 *infectious diseases*. 2009;22(1):11-17.
560 14. Arbex MA, Varella Mde C, Siqueira HR, Mello FA. Antituberculosis drugs: drug
561 interactions, adverse effects, and use in special situations. Part 1: first-line
562 drugs. J Bras Pneumol. 2010 Sep-Oct;36(5):626-40. Review.
563 15. Thwaites G, Auckland C, Barlow G, Cunningham R, Davies G, Edgeworth J, Greig J,
564 Hopkins S, Jeyaratnam D, Jenkins N et al: Adjunctive rifampicin to reduce early
565 mortality from Staphylococcus aureus bacteraemia (ARREST): study protocol for a
566 randomised controlled trial. Trials 2012, 13(1):241.
567 16. Ramappa V, Aithal GP. Hepatotoxicity Related to Anti-tuberculosis Drugs: Mechanisms
568 and Management. *Journal of Clinical and Experimental Hepatology*. 2013;3(1):37-49.
569 17. FDA News Release; United States Food and Drug Administration: Silver Spring, MD,
570 dec 31,2012.
571 18. Brigden G, Hewison C, Varaine F. New developments in the treatment of drug-resistant
572 tuberculosis: clinical utility of bedaquiline and delamanid. *Infection and Drug Resistance*.
573 2015;8:367-378.

bioRxiv preprint doi: https://doi.org/10.1101/335323; this version posted May 31, 2018. The copyright holder for this preprint (which was not certified by peer review) is the author/funder. All rights reserved. No reuse allowed without permission.

574 19. Bavarsad Shahripour R, Harrigan MR, Alexandrov AV. *N*-acetylcysteine (NAC) in
575 neurological disorders: mechanisms of action and therapeutic opportunities. *Brain and*
576 *Behavior*. 2014;4(2):108-122.
577 20. Forman HJ, Zhang H, Rinna A. Glutathione: Overview of its protective roles,
578 measurement, and biosynthesis. *Molecular aspects of medicine*. 2009;30(1-2):1-12.
579 21. Saing T, Lagman M, Castrillon J, Gutierrez E, Guilford FT, Venketaraman V.
580 Analysis of glutathione levels in the brain tissue samples from HIV-1-positive
581 individuals and subject with Alzheimer's disease and its implication in the
582 pathophysiology of the disease process. BBA Clin. 2016 May 29;6:38-44.
583 22. Allen M, Bailey C, Cahatol I, Dodge L, Yim J, Kassissa C, Luong J, Kasko S,
584 Pandya S, Venketaraman V. Mechanisms of Control of Mycobacterium tuberculosis by
585 NK Cells: Role of Glutathione. Front Immunol. 2015 Oct 5;6:508.
586 23. Ly J, Lagman M, Saing T, Singh MK, Tudela EV, Morris D, Anderson J, Daliva J,
587 Ochoa C, Patel N, Pearce D, Venketaraman V. Liposomal Glutathione Supplementation
588 Restores TH1 Cytokine Response to Mycobacterium tuberculosis Infection in HIV-
589 Infected Individuals. J Interferon Cytokine Res. 2015 Nov;35(11):875-87.
590 24. Morris D, Ly J, Chi PT, Daliva J, Nguyen T, Soofer C, Chen YC, Lagman M,
591 Venketaraman V. Glutathione synthesis is compromised in erythrocytes from
592 individuals with HIV. Front Pharmacol. 2014 Apr 11;5:73.
593 25. Morris D, Nguyen T, Kim J, Kassissa C, Khurasany M, Luong J, Kasko S, Pandya
594 S, Chu M, Chi PT, Ly J, Lagman M, Venketaraman V. An elucidation of neutrophil
595 functions against Mycobacterium tuberculosis infection. Clin Dev Immunol.
596 2013;2013:959650.

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597 26. Morris D, Gonzalez B, Khurasany M, Kassissa C, Luong J, Kasko S, Pandya S, Chu
598 M, Chi PT, Bui S, Guerra C, Chan J, Venketaraman V. Characterization of dendritic
599 cell and regulatory T cell functions against Mycobacterium tuberculosis
600 infection. Biomed Res Int. 2013;2013:402827.

601 27. Lagman M, Ly J, Saing T, Kaur Singh M, Vera Tudela E, Morris D, Chi PT, Ochoa C,
602 Sathananthan A, Venketaraman V: Investigating the causes for decreased levels of
603 glutathione in individuals with type II diabetes. PloS one 2015, 10(3):e0118436.

604 28. Amaral EP, Conceicao EL, Costa DL, Rocha MS, Marinho JM, Cordeiro-Santos M,
605 D'Imperio-Lima MR, Barbosa T, Sher A, Andrade BB: N-acetyl-cysteine exhibits potent
606 anti-mycobacterial activity in addition to its known anti-oxidative functions. BMC
607 microbiology 2016, 16(1):251.

608 29. Daoud AK, Tayyar MA, Fouda IM, Harfeil NA. Effects of diabetes mellitus vs. in
609 vitro hyperglycemia on select immune cell functions. J Immunotoxicol. 2009
610 Mar;6(1):36-41.

611 30. Palomino JC, Martin A. Drug Resistance Mechanisms in *Mycobacterium tuberculosis*.
612 *Antibiotics*. 2014;3(3):317-340.

613 31. Bosshart H, Heinzelmann M. THP-1 cells as a model for human monocytes. *Annals of*
614 *Translational Medicine*. 2016;4(21):438.

615 32. Tsuchiya S, Yamabe M, Yamaguchi Y, et al. Establishment and characterization of a
616 human acute monocytic leukemia cell line (THP-1). Int J Cancer 1980;26:171-6. 10.

617 33. Shrivastava P, Bagchi T. IL-10 modulates in vitro multinucleate giant cell
618 formation in human tuberculosis. PLoS One. 2013 Oct 17;8(10):e77680.

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619 34. Tanaka T, Narazaki M, Kishimoto T. IL-6 in Inflammation, Immunity, and Disease. *Cold*
620 *Spring Harbor Perspectives in Biology*. 2014;6(10):a016295.
621 doi:10.1101/cshperspect.a016295.
622 35. Mootoo A, Stylianou E, Arias MA, Reljic R. TNF-alpha in tuberculosis: a
623 cytokine with a split personality. Inflamm Allergy Drug Targets. 2009
624 Mar;8(1):53-62.
625 36. Chan J, Flynn J. The immunological aspects of latency in tuberculosis. Clin
626 Immunol. 2004 Jan;110(1):2-12. Review.
627 37. Dorhoi A, Kaufmann SH. Tumor necrosis factor alpha in mycobacterial infection.
628 Semin Immunol. 2014 Jun;26(3):203-9. doi: 10.1016/j.smim.2014.04.003. Epub 2014
629 May 10. Review.
630 38. Flynn JL, Chan J. What's good for the host is good for the bug. Trends
631 Microbiol. 2005 Mar;13(3):98-102. Review.
632 39. Dreher D, Nicod LP (2002) Dendritic cells in the mycobacterial granuloma are involved
633 in acquired immunity. Am J Respir Crit Care Med 165: 1577–1578.
634 40. Esposito E, Cuzzocrea S. TNF-alpha as a therapeutic target in inflammatory
635 diseases, ischemia-reperfusion injury and trauma. Curr Med Chem.
636 2009;16(24):3152-67.
637 41. Wolff SP, Dean RT: Glucose autoxidation and protein modification. The potential role of
638 'autoxidative glycosylation' in diabetes. The Biochemical journal 1987, 245(1):243-250.
639 42. O'Leary S, O'Sullivan MP, Keane J. IL-10 blocks phagosome maturation in
640 mycobacterium tuberculosis-infected human macrophages. Am J Respir Cell Mol Biol.
641 2011 Jul;45(1):172-80.

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642 43. 43. Venketaraman V, Dayaram YK, Talaue MT, Connell ND. 2005. Glutathione and
643 nitrosoglutathione in macrophage defense against Mycobacterium tuberculosis. Infect
644 Immun. 73(3):1886-9.
645 44. Dayaram, Y. K., Talaue, M. T., Connell, N. D., and Venketaraman, V. 2006.
646 Characterization of a glutathione metabolic mutant of Mycobacterium tuberculosis and its
647 resistance to glutathione and nitrosoglutathione. J Bacteriology. 188 (4):1364-72.
648 45. Morris D, Guerra C, Khurasany M, Guilford F, Saviola B, Huang Y and
649 Venketaraman V. 2013. Glutathione supplementation improves macrophage functions in
650 HIV. Journal of Interferon and cytokine research. 33 (5): 270-9.
651 46. Morris D, Guerra C, Donohue C, Oh H, Khurasany M, Venketaraman V. 2012.
652 Unveiling the mechanisms for decreased glutathione in individuals with HIV infection.
653 Clin Dev Immunol.2012:734125.
654 47. Guerra C, Johal K, Morris D, Moreno S, Alvarado O, Gray D, Tanzil M, Pearce D,
655 Venketaraman V. 2012. Control of Mycobacterium tuberculosis growth by activated
656 natural killer cells. Clin Exp Immunol. 168(1):142-52.
657 48. Guerra G, Morris D, Gray D, Tanzil M, Sipin A, Kung S, Guilford F, Khasawneh F
658 and Venketaraman V. 2011. Adaptive immune responses against Mycobacterium
659 tuberculosis infection in healthy and HIV infected individuals. PLoS One. 2011. 6(12):
660 e28378.
661 49. Venketaraman, V; Millman, AC; Salman, M; Swaminathan, S; Goetz, M; Lardizabal,
662 A; Hom, D; and Connell, N. D. 2008. Glutathione levels and immune responses in
663 tuberculosis patients. Microbial pathogenesis. 44: 255-261.
664

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665

666 **Author Contribution statement:**

667

668 Ruoqiong Cao – Maintained and Processed macrophages and mycobacteria for experiments,
669 Preformed infection studies, Preformed ELISA assays, Preformed microscopy work.

670

671 Garrett Teskey – Wrote the abstract, methods, and discussion sections, Maintained and
672 Processed macrophages and mycobacteria for experiments, Preformed infection studies,
673 Preformed ELISA assays, Preformed microscopy work.

674

675 Hicret Islamoglu - Maintained and Processed macrophages and mycobacteria for experiments,
676 Preformed infection studies, Preformed ELISA assays, Preformed microscopy work, contributed
677 to writing the results section.

678

679 Rachel Abrahem - Preformed microscopy work, Wrote the results section.

680

681 Karo Gyurjian - Contributed to writing the abstract and introduction sections, Made media for
682 bacterial quantification.

683

684 Li Zhong - Provided funding.

685

686 Vishwanath Venketaraman - Conceived the studies, Prepared figures, interpreted data, provided
687 funding and prepared the manuscript.

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689 **Additional information:**

690 The authors declare that there are **NO** competing interests for this publication.

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711

712 **Figures and Legends**

713 **Fig. 1A: Levels of total GSH in uninfected and M. *tb*-infected THP-1 cells.**

714 GSH assay was performed by colorimetric method using am assay kit from Arbor Assays.
715 Corrections were made to total protein measured by BCA Protein Assay Kit from Thermo
716 Scientific. GSH and total protein levels were measured from the cellular lysates. There was a
717 notable decrease in the levels of GSH when THP-1 cells were infected with the Erdman strain of
718 M. *tb*. Data represent means ±SE from 6 trials. ***p<0.0005 when comparing infected cells
719 with uninfected.

720

721 **Fig. 1B: Survival of Erdman strain of *M. tb* inside untreated THP-1 macrophages.**

722 THP-1 cells were cultured in RPMI containing 10% FBS, and allowed to differentiate into
723 macrophages by the addition of PMA at a concentration of 10 ng/ml. There was a significant
724 increase in bacterial numbers when THP-1 cells were infected with M. *tb*. Data represent means
725 ±SE from 6 trials. ***p<0.0005 when comparing 1 hour with 12-day time point of termination.

726

727 **Fig. 1C: Levels of TNF-α in the supernatants of uninfected and *M. tb*-infected THP-1 cells.**

728 Assay of TNF-α was performed using an ELISA Ready-Set-Go kit from eBioscience. There was
729 a significant increase in the levels of TNF-α when macrophages were infected with M. *tb*. Data
730 represent means ±SE from 6 trials. *p<0.05 when comparing infected samples to uninfected
731 controls.

732

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734

735 **Fig. 2A: Levels of total GSH in *M. tb*-infected and *M. tb*-infected + NAC-treated THP-1**
736 **cells.**

737 GSH assay was performed by colorimetric assay using an assay kit from Arbor Assays.
738 Corrections were made to total protein measured by BCA Protein Assay Kit from Thermo
739 Scientific. GSH and total protein levels were measured from cellular lysates. There was a
740 significant increase in the levels of GSH when *M. tb*-infected samples were treated with NAC.
741 Data represent means ±SE from 6 trials. *$p<0.05$ when comparing *M. tb*-infected samples treated
742 with NAC to infected sham controls.

743

744 **Fig. 2B: Survival of Erdman strain of *M. tb* inside untreated and NAC-treated THP-1**
745 **macrophages.**

746 THP-1 cells were cultured in a medium of RPMI and 10% FBS, and allowed to differentiate into
747 macrophages by addition of PMA at a concentration of 10 ng/ml. There was a significant
748 decrease in bacterial numbers when *M. tb*-infected macrophages were treated with NAC. Data
749 represent means ±SE from 6 trials. *$p<0.05$ when comparing *M. tb*-infected macrophages treated
750 with NAC to infected sham controls.

751

752 **Fig. 2C: Assay of TNF-α in the supernatants from *M. tb*-infected and *M. tb*-infected + NAC-**
753 **treated THP-1 cells.**

754 Assay of TNF-α was performed using an ELISA Ready-Set-Go kit from eBioscience. There was
755 a significant decrease in the levels of TNF-a when macrophages were infected with *M. tb* and

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756 treated with NAC. Data represent means ±SE from 6 trials. *p<0.05 when comparing *M. tb*-
757 infected macrophages treated with NAC to infected sham controls.
758

759 **Fig. 2D: Assay of IL-10 in the supernatants from *M. tb*-infected and *M. tb*-infected + NAC-**
760 **treated THP-1 cells.**

761 IL-10 was measured using an ELISA Ready-Set-Go kit from eBioscience. Although there was a
762 decrease in the levels of IL-10 when M. *tb*-infected macrophages were treated with NAC, this
763 difference was not found to be statistically significant. Data represent means ±SE from 6 trials.
764

765 **Fig. 3A: Levels of total GSH in M. *tb*-infected, *M. tb*-infected + INH-treated and *M. tb*-**
766 **infected + INH + NAC-treated THP-1 cells.**

767 GSH assay was performed using a colorimetric assay kit from Arbor Assays. There was a
768 significant increase in the levels of GSH when M. *tb*-infected macrophages were treated with
769 INH and INH+NAC. Data represent means ±SE from 6 trials. **p<0.005 when comparing
770 infected samples treated with INH to infected sham controls. [#]p<0.05 when comparing infected
771 samples treated with INH+NAC to infected sham controls.
772

773 **Fig. 3B: Survival of Erdman strain of *M. tb* inside INH and INH + NAC-treated THP-1**
774 **macrophages.**

775 THP-1 cells were cultured in a medium of RPMI and 10% FBS and allowed to differentiate into
776 macrophages by addition of PMA at a concentration of 10 ng/ml. INH+NAC treatment resulted
777 in clearance of *M. tb* infection compared to treatment with INH only. Data represent means ±SE
778 from 6 trials.

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780

781 **Fig. 3C: Assay of TNF-α in the supernatants from INH and INH + NAC-treated THP-1**
782 **cells.**

783 Assay of TNF-α was performed using an ELISA Ready-Set-Go kit from eBioscience. There was
784 a significant decrease in the levels of TNF-α when samples were infected with *M. tb* and treated
785 with either INH or INH+NAC. Data represent means ±SE from 6 trials. *p<0.05 when
786 comparing M. *tb*-infected macrophages treated with INH to infected sham controls. $^{\#}$p<0.05
787 when comparing M. *tb*-infected macrophages treated with INH+NAC to infected sham controls.

788

789 **Fig. 3D: Assay of IL-10 in the supernatants from INH and INH + NAC-treated THP-1 cells.**

790 IL-10 levels were measured using an ELISA Ready-Set-Go kit from eBioscience. Although there
791 was a decrease in the levels of IL-10 when samples were infected with *M. tb* and treated with
792 INH or INH+NAC, this difference was not found to be statistically significant. Data represent
793 means ±SE from 6 trials.

794

795 **Fig. 4A: Levels of total GSH in M. *tb*-infected, M. *tb*-infected + RIF-treated and *M. tb*-**
796 **infected + RIF + NAC-treated THP-1 cells.**

797 GSH assay was performed using a colorimetric assay kit from Arbor Assays. There was a
798 significant increase in the levels of GSH when *M. tb*-infected macrophages were treated with
799 RIF and RIF+NAC. Data represent means ±SE from 6 trials. ***p<0.0005 when comparing
800 infected macrophages treated with RIF to infected sham controls. $^{\#}$p<0.05 when comparing

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801 infected macrophages treated with RIF+NAC to RIF only. *$p<0.05$ when comparing infected
802 macrophages treated with RIF+NAC to infected sham controls.
803
804 **Fig. 4B: Survival of Erdman strain of *M. tb* inside RIF and RIF + NAC-treated THP-1**
805 **macrophages.**
806 THP-1 cells were cultured in a medium of RPMI and 10% FBS, and allowed to differentiate into
807 macrophages by addition of PMA at a concentration of 10 ng/ml. There was a significant
808 reduction in the bacterial numbers when THP-1 cells were treated with RIF+NAC compared to
809 RIF only. Data represent means ±SE from 6 trials. *$p<0.05$ when comparing infected samples
810 treated with RIF+NAC to RIF only treatment.
811
812 **Fig. 4C: Assay of TNF-α in the supernatants from RIF and RIF + NAC-treated THP-1**
813 **cells.**
814 Assay of TNF-α was performed using an ELISA Ready-Set-Go kit from eBioscience. There was
815 a significant decrease in levels if TNF-α when macrophages were infected with *M. tb* and treated
816 with RIF or RIF+NAC. Data represent means ±SE from 6 trials. *$p<0.05$ when comparing
817 infected macrophages treated with RIF or RIF+NAC to infected controls. $^{\#}p<0.05$ when
818 comparing infected macrophages treated with RIF+NAC to treatment with RIF only.
819
820 **Fig. 4D: Assay of IL-10 in the supernatants from RIF and RIF + NAC-treated THP-1 cells.**
821 IL-10 was measured using an ELISA Ready-Set-Go kit from eBioscience. Although there was a
822 decrease in levels of IL-10 when macrophages were infected with *M. tb* and treated with RIF or

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823 RIF+NAC, this difference was not found to be statistically significant. Data represent means ±SE
824 from 6 trials.
825
826
827
828 **Fig. 5A: Levels of total GSH in *M. tb*-infected, *M. tb*-infected + EMB-treated and *M. tb*-
829 infected + EMB + NAC-treated THP-1 cells.**
830 GSH assay was performed using a colorimetric assay kit from Arbor Assays. There was a
831 significant increase in the levels of GSH when infected macrophages were treated with
832 EMB+NAC. Data represent means ±SE from 6 trials. $^{\#}p<0.05$ when comparing infected samples
833 treated with EMB+NAC to infected controls.
834
835 **Fig. 5B: Survival of Erdman strain of *M. tb* inside EMB and EMB + NAC-treated THP-1
836 macrophages.**
837 THP-1 cells were cultured in a medium of RPMI and 10% FBS, and allowed to differentiate into
838 macrophages by addition of PMA at a concentration of 10 ng/ml. There was a significant
839 reduction in the bacterial numbers when THP-1 cells were treated with EMB+NAC compared to
840 EMB only. Data represent means ±SE from 6 trials. ***$p<0.0005$ when comparing infected
841 macrophages treated with EMB+NAC to EMB lone treatment.
842
843 **Fig. 5C: Assay of TNF-α in the supernatants from EMB and EMB + NAC-treated THP-1
844 cells.**

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845 Assay of TNF-α was performed using an ELISA Ready-Set-Go kit from eBioscience. There was
846 a significant decrease in the levels of TNF-**α** when samples were infected with *M. tb* and treated
847 with EMB or EMB+NAC. Data represent means ±SE from 6 trials. *$p<0.05$ when comparing
848 infected macrophages treated with EMB to infected sham controls. #$p<0.05$ when comparing
849 infected macrophages treated with EMB+NAC to treatment of infected sham controls.
850

851 **Fig. 5D: Assay of IL-10 in the supernatants from EMB and EMB + NAC-treated THP-1**
852 **cells.**

853 IL-10 levels were measured using an ELISA Ready-Set-Go kit from eBioscience. Although there
854 was a decrease in levels of IL-10 when macrophages were infected with *M. tb* and treated with
855 EMB+NAC, and a slight increase when samples were treated with EMB, these differences were
856 not found to be statistically significant. Data represent means ±SE from 6 trials.
857

858 **Fig. 6A: Levels of total GSH in *M. tb*-infected, *M. tb*-infected + PZA-treated and *M. tb*-**
859 **infected + PZA + NAC-treated THP-1 cells.**

860 GSH assay was performed using a colorimetric assay kit from Arbor Assays. There was a
861 significant increase in levels of GSH when infected macrophages were treated with PZA+NAC
862 and PZA only. Data represent means ±SE from 6 trials. #$p<0.05$ when comparing infected
863 macrophages treated with PZA+NAC to infected sham controls. ***$p<0.0005$ when comparing
864 infected macrophages treated with PZA to infected sham controls.
865

866 **Fig. 6B: Survival of Erdman strain of *M. tb* inside PZA and PZA + NAC-treated THP-1**
867 **macrophages.**

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868 THP-1 cells were cultured in a medium of RPMI and 10% FBS, and allowed to differentiate into
869 macrophages by addition of PMA at a concentration of 10 ng/ml. There was a significant
870 reduction of bacterial numbers when THP-1 cells were treated with PZA+NAC compared to
871 PZA only. Data represent means ±SE from 6 trials. **p<0.005 when comparing infected
872 macrophages treated with PZA+NAC to PZA only treatment.

873

874

875

876 **Fig. 6C: Assay of TNF-α in the supernatants from PZA and PZA + NAC-treated THP-1**
877 **cells.**

878 Assay of TNF-α was performed using an ELISA Ready-Set-Go kit from eBioscience. There was
879 a significant decrease in the levels of TNF-α when macrophages were infected with *M. tb* and
880 treated with PZA+NAC. Data represent means ±SE from 6 trials. *p<0.05 when comparing
881 infected macrophages treated with PZA+NAC to infected sham controls. #p<0.05 when
882 comparing infected macrophages treated with PZA+NAC to treatment with PZA only.

883

884 **Fig. 6D: Assay of IL-10 in the supernatants from PZA and PZA + NAC-treated THP-1**
885 **cells.**

886 IL-10 assay was performed using an ELISA Ready-Set-Go kit from eBioscience. There was a
887 significant decrease in the levels of IL-10 when macrophages were infected with *M. tb* and
888 treated with PZA+NAC, and a slight decrease when samples were treated with PZA (not
889 significant). Data represent means ±SE from 6 trials. *p<0.05 when comparing infected
890 macrophages treated with PZA+NAC to infected sham control.

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901 **FIGURES**
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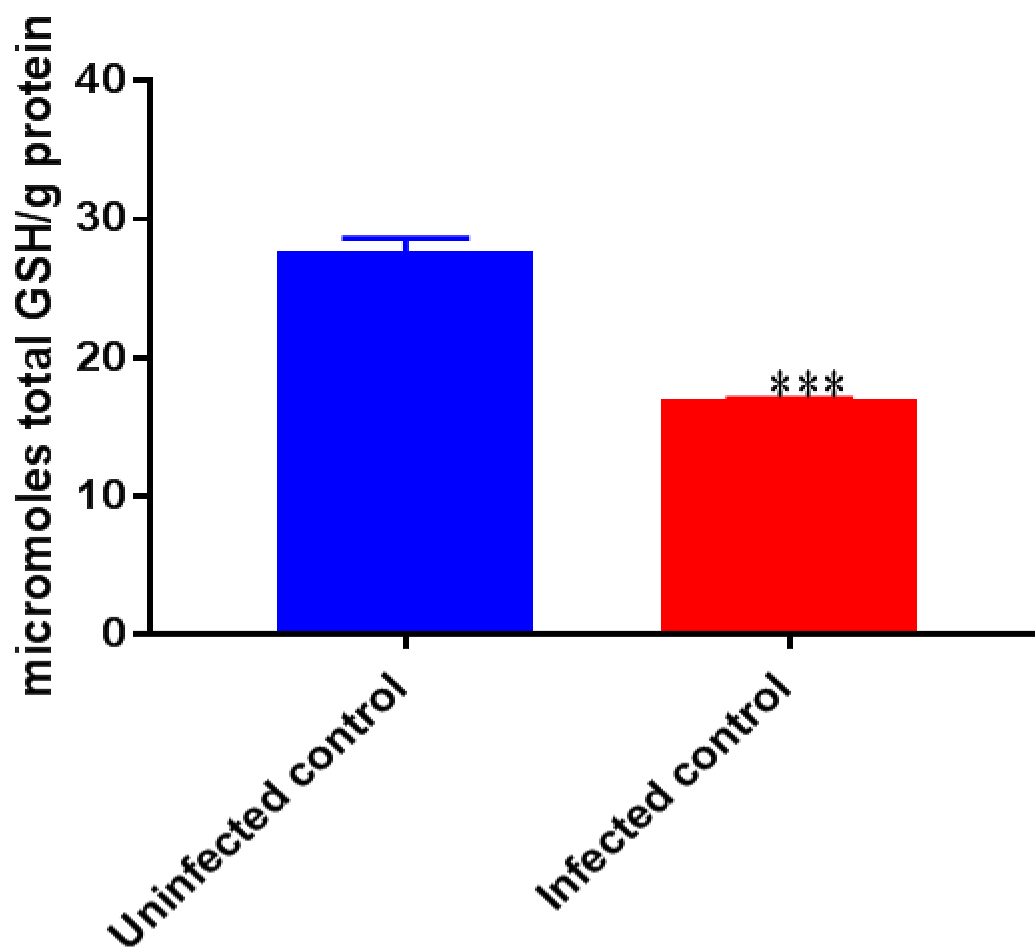

903

904 **Fig. 1A: Levels of total GSH in uninfected and *M. tb*-infected THP-1 cells.**

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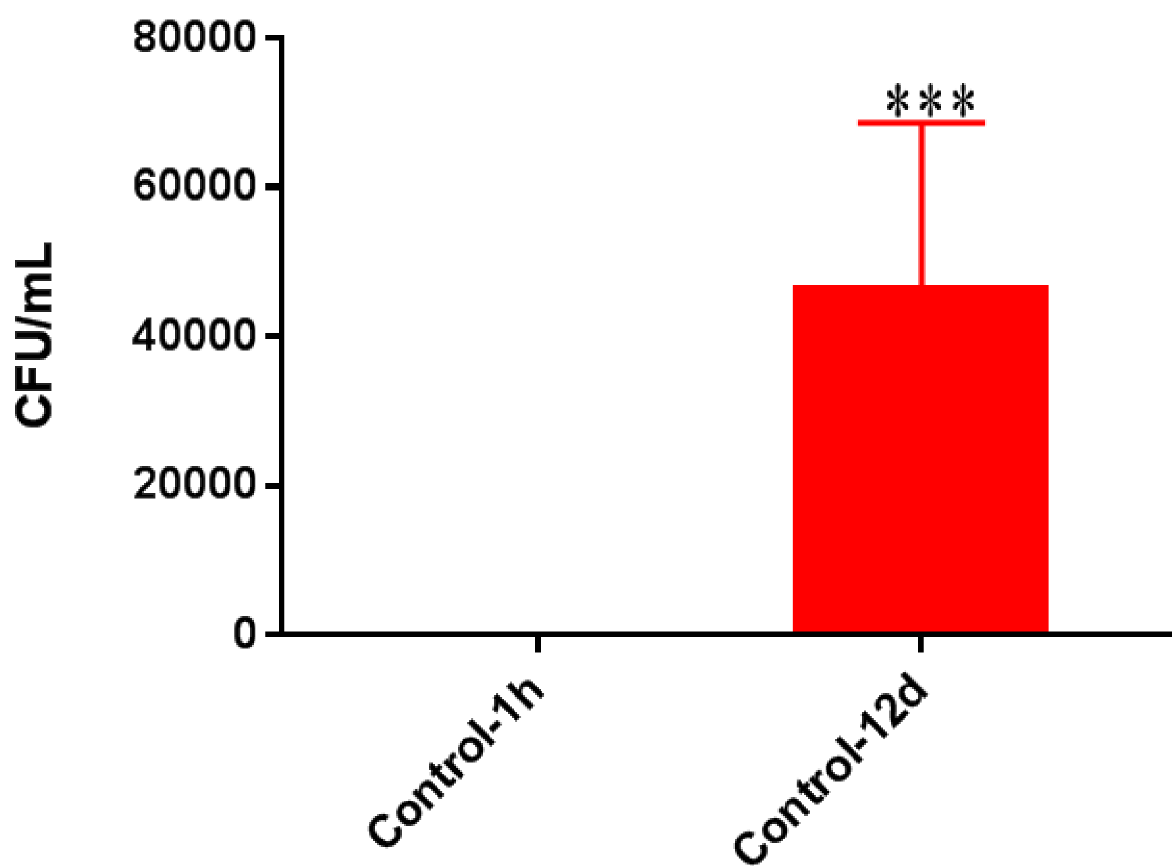

905

906 **Fig. 1B: Survival of Erdman strain of *M. tb* inside untreated THP-1 macrophages.**

907

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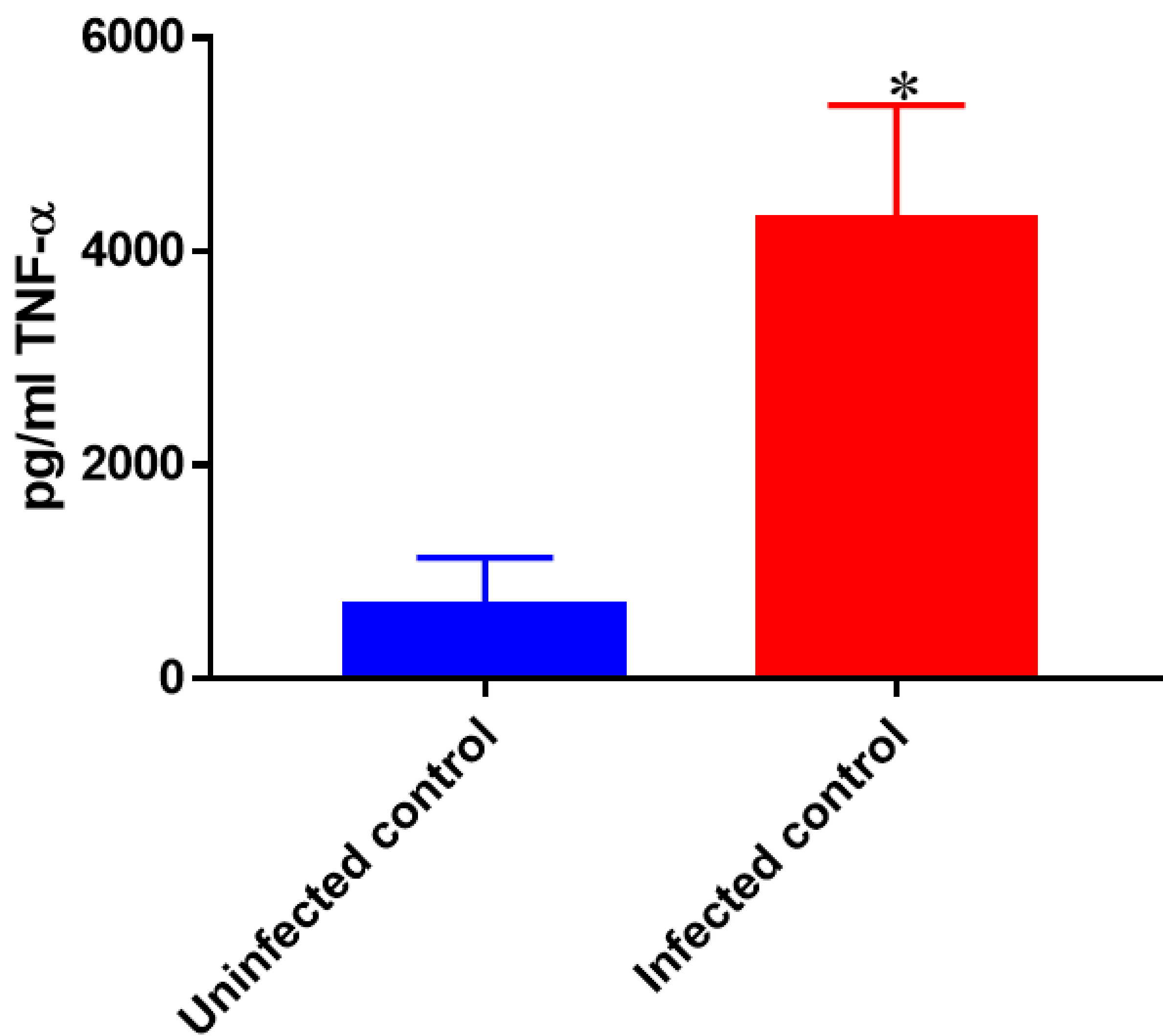

908

909 **Fig. 1C: Levels of TNF-a in the supernatants of uninfected and *M. tb*-infected THP-1 cells.**

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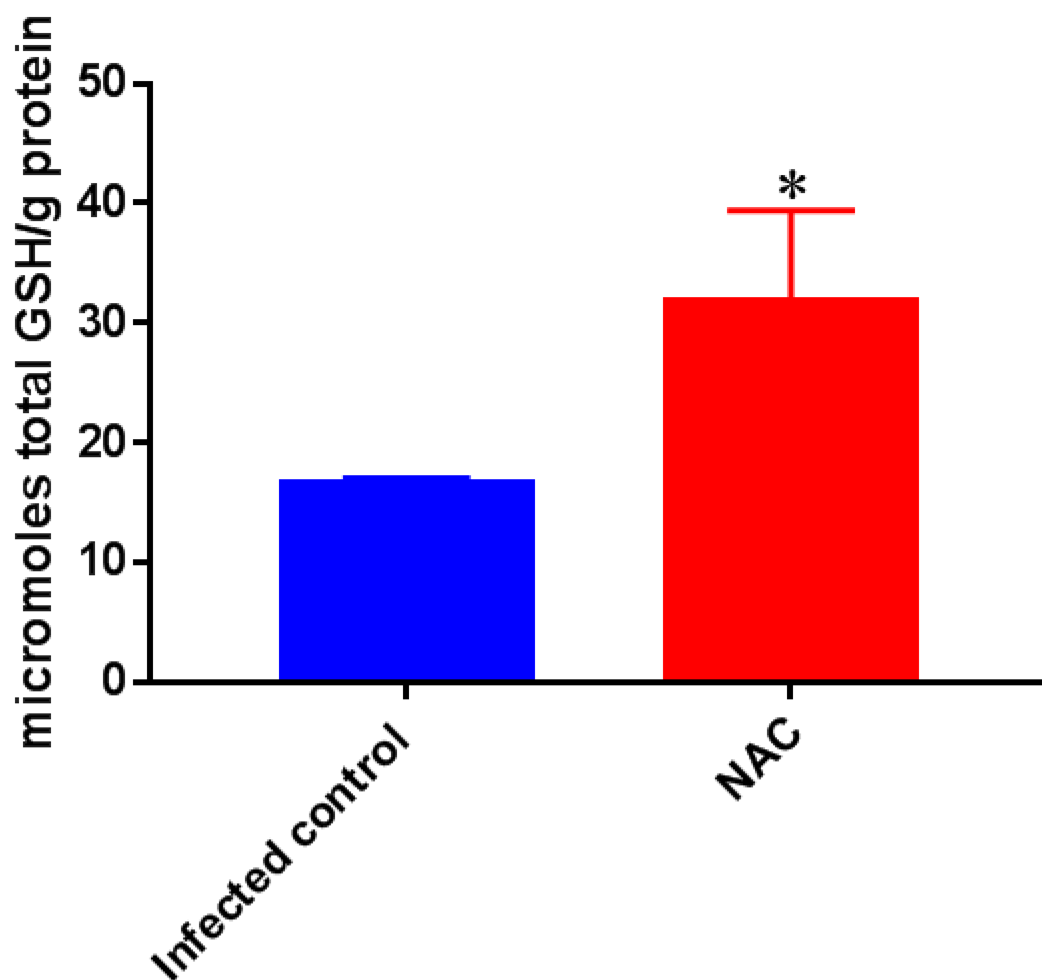

911

912 **Fig. 2A: Levels of total GSH in *M. tb*-infected and M. *tb*-infected + NAC-treated THP-1**

913 **cells.**

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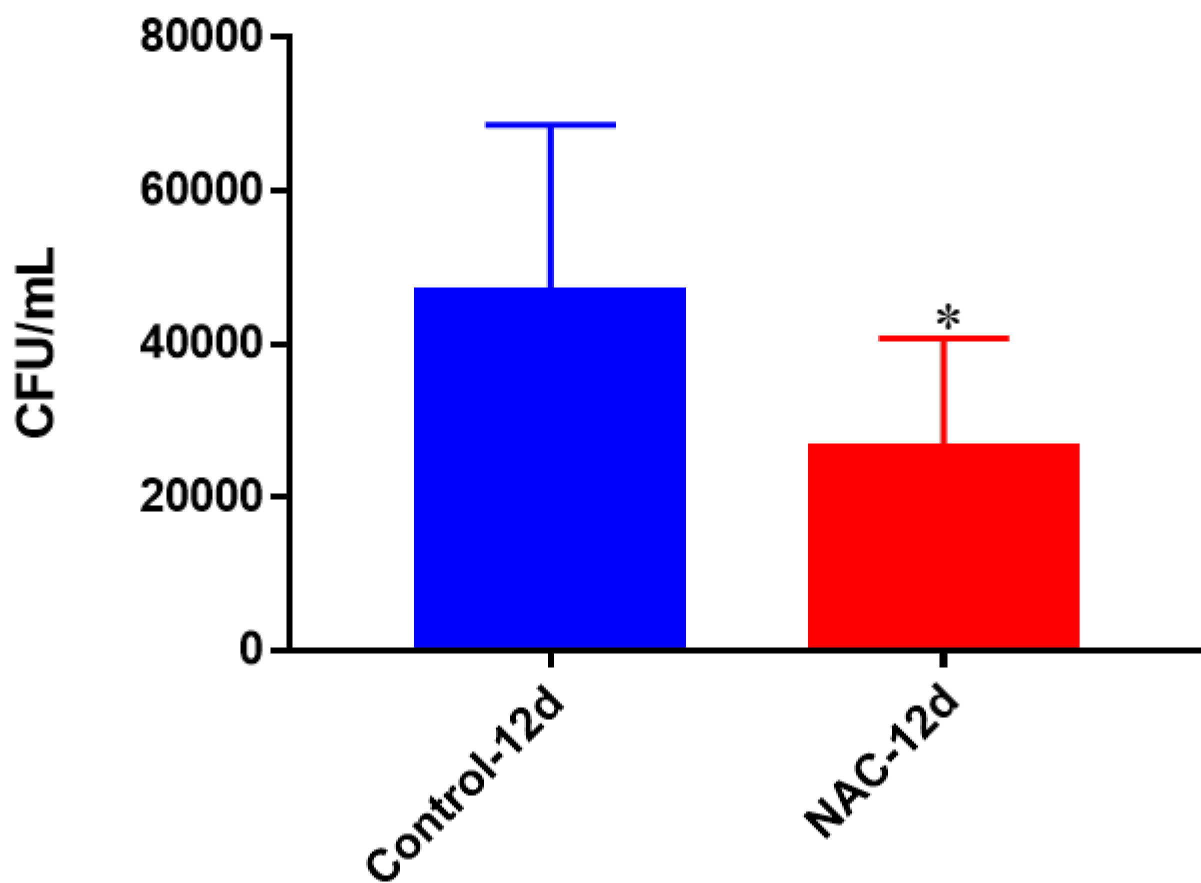

914

915 **Fig. 2B: Survival of Erdman strain of *M. tb* inside untreated and NAC-treated THP-1**

916 **macrophages.**

917

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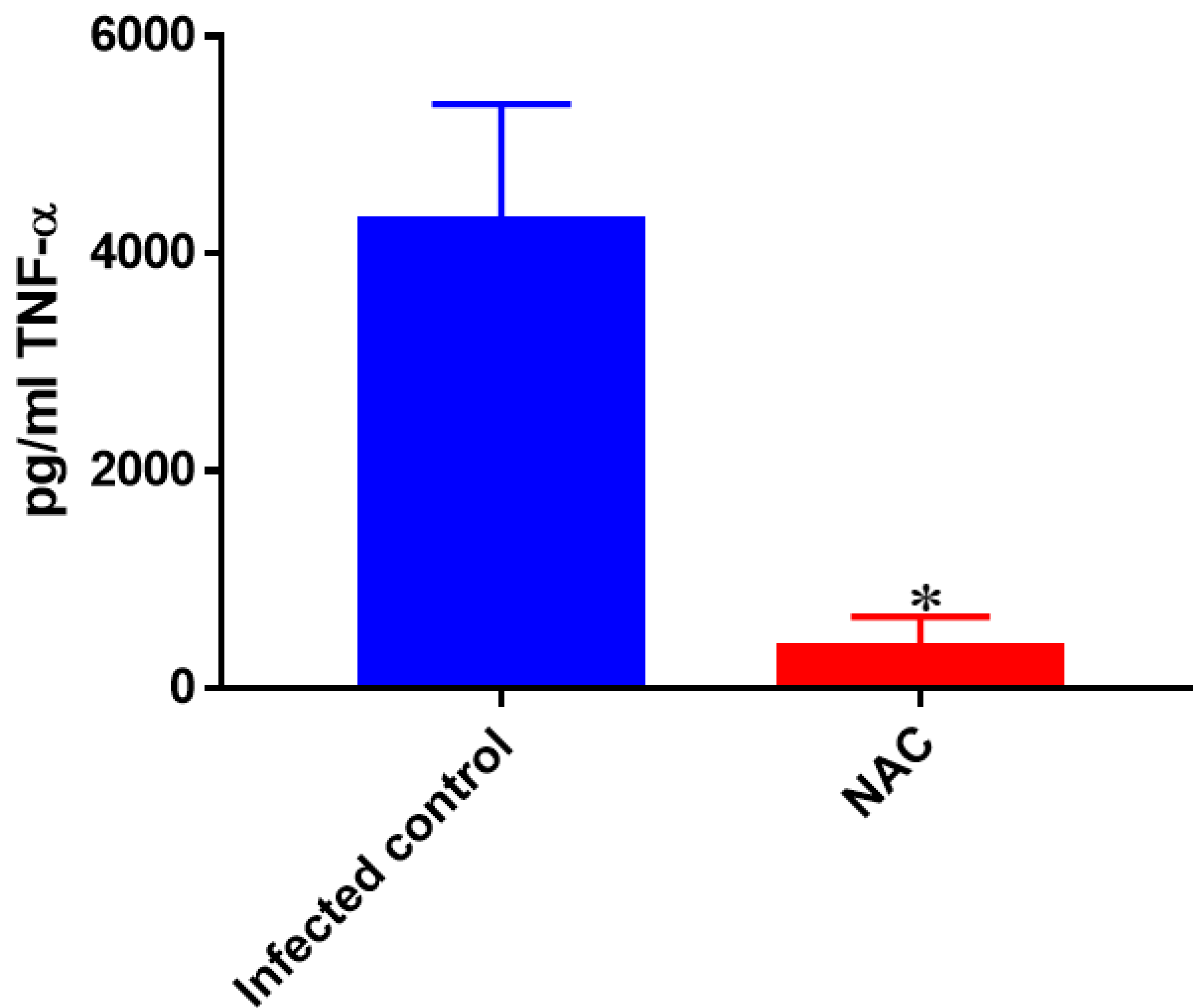

918

919 **Fig. 2C: Assay of TNF-α in the supernatants from *M. tb*-infected and *M. tb*-infected + NAC-**

920 **treated THP-1 cells.**

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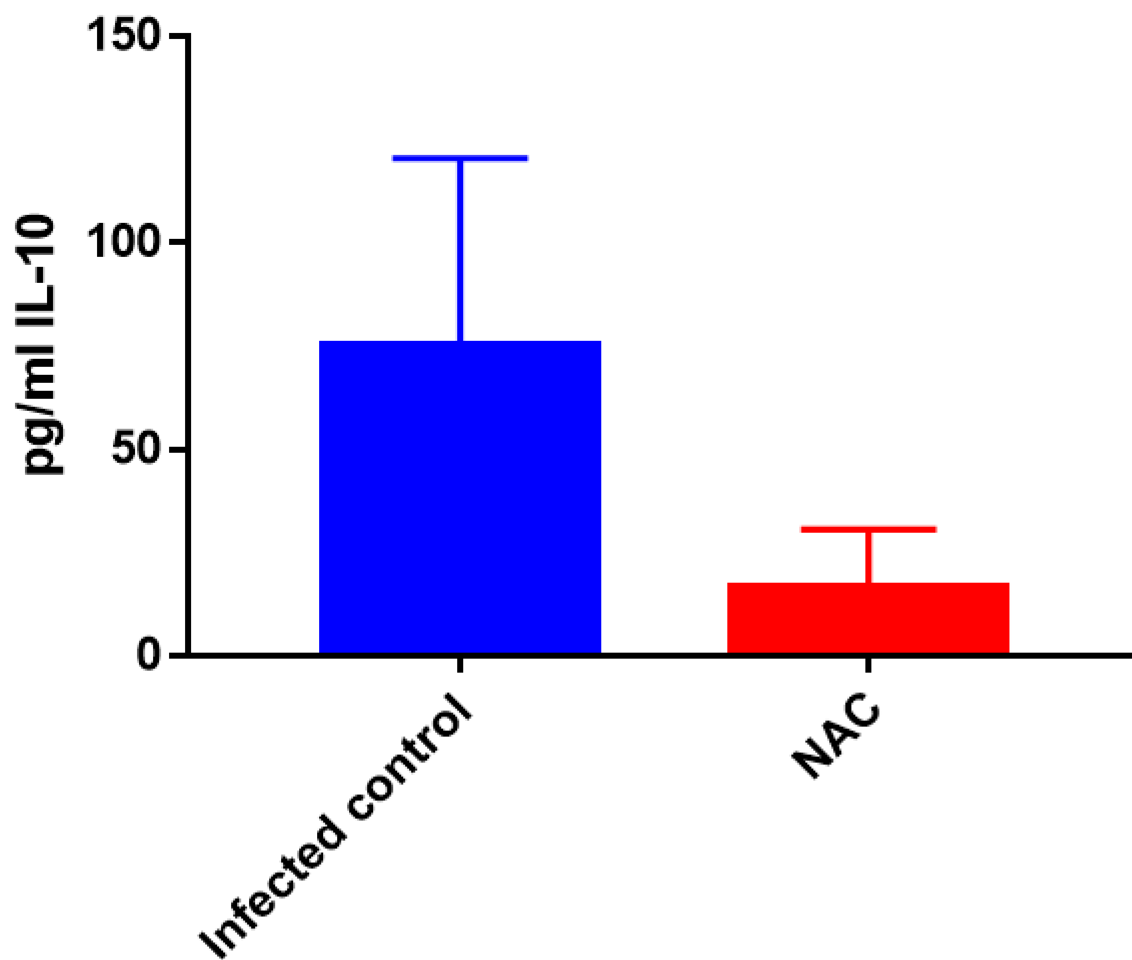

922

923 **Fig. 2D: Assay of IL-10 in the supernatants from *M. tb*-infected and *M. tb*-infected + NAC-**

924 **treated THP-1 cells.**

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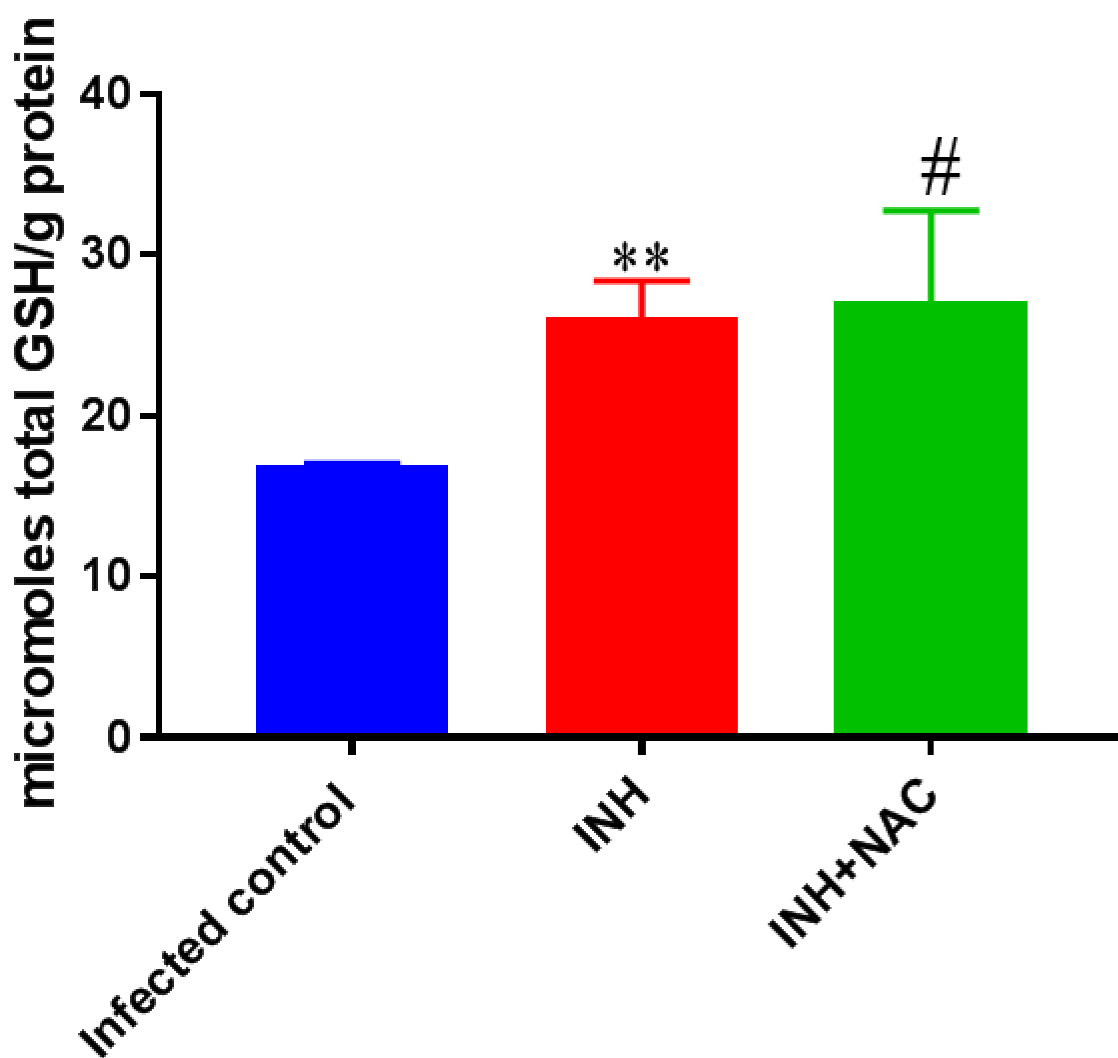

926

927 **Fig. 3A: Levels of total GSH in *M. tb*-infected, *M. tb*-infected + INH-treated and *M. tb*-**

928 **infected + INH + NAC-treated THP-1 cells.**

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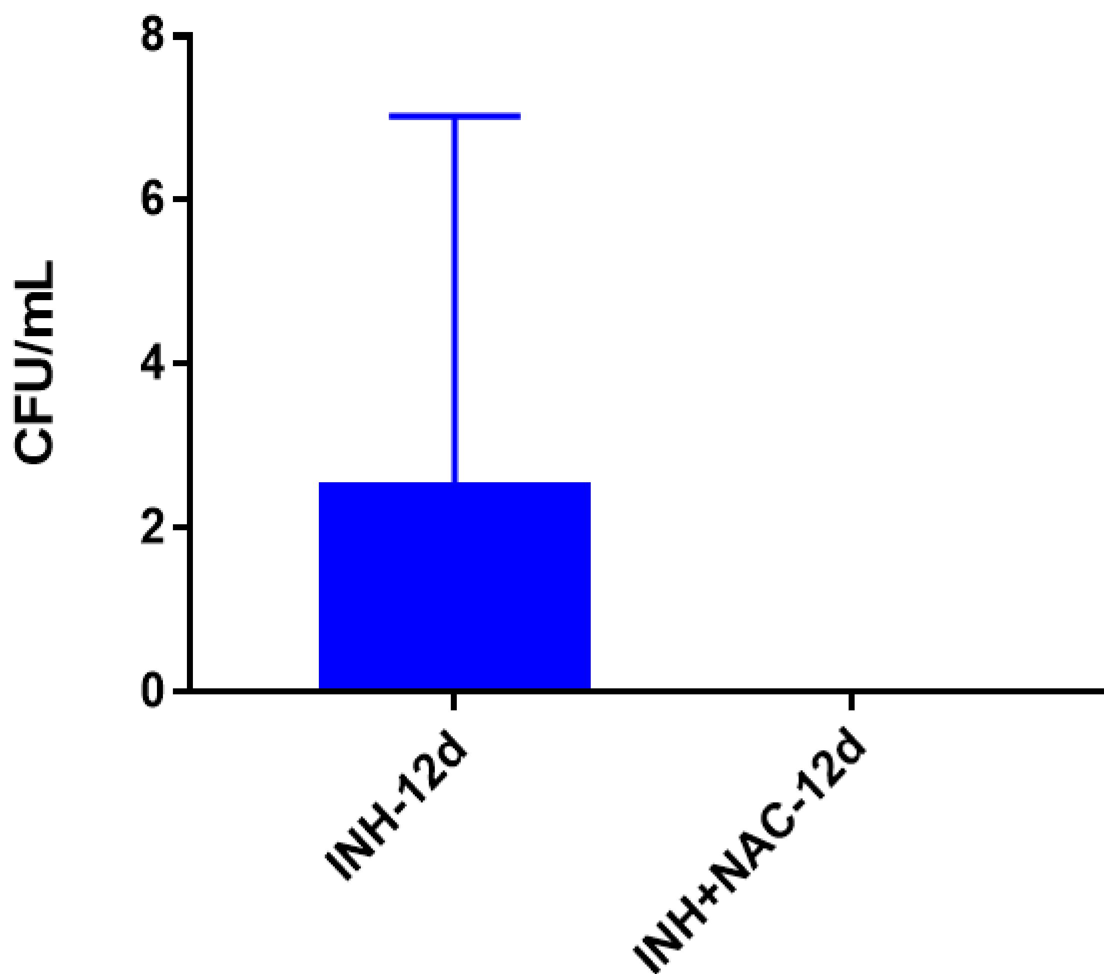

929

930 **Fig. 3B: Survival of Erdman strain of *M. tb* inside INH and INH + NAC-treated THP-1**

931 **macrophages.**

932

933

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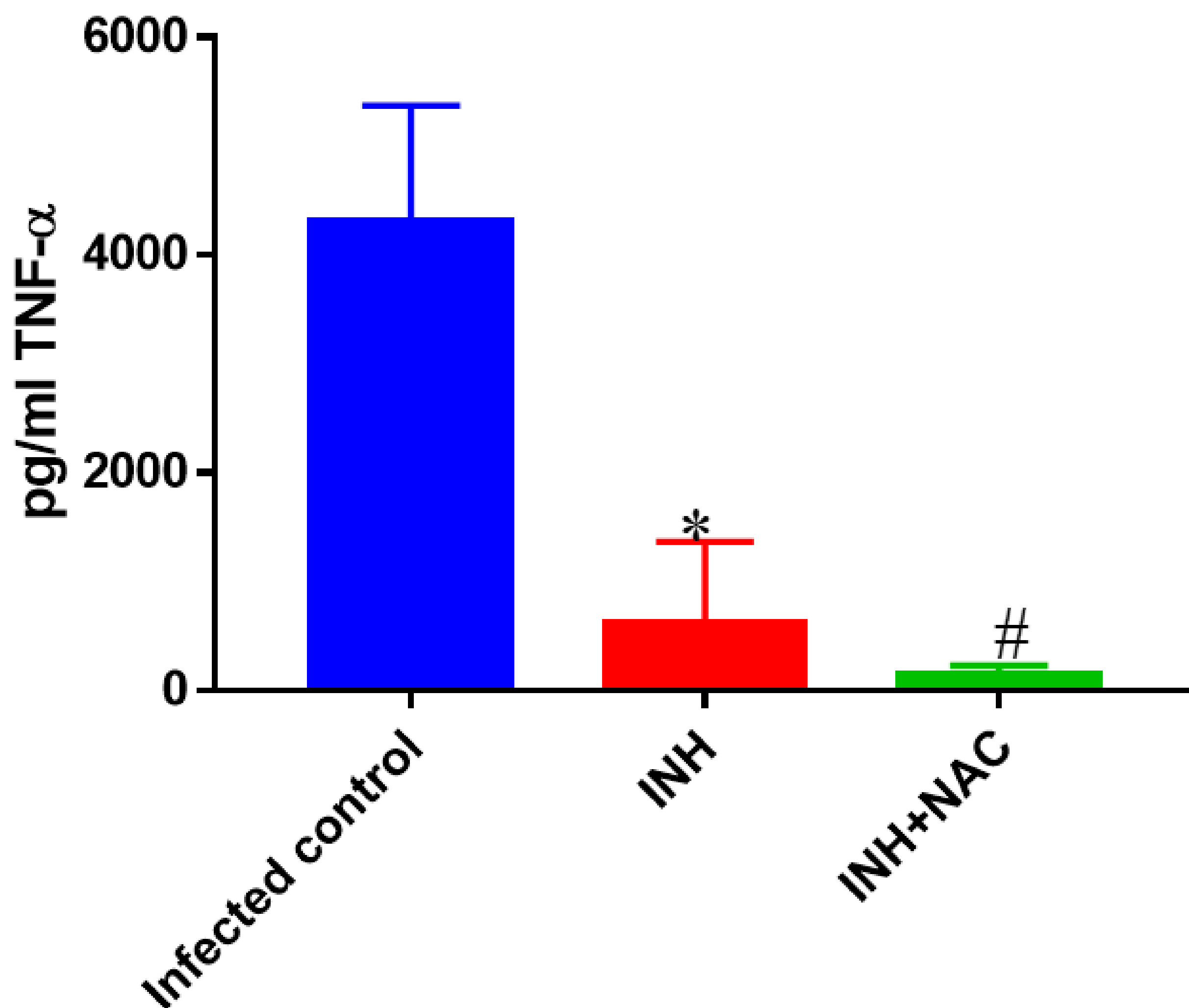

935

936 **Fig. 3C: Assay of TNF-α in the supernatants from INH and INH + NAC-treated THP-1**

937 **cells.**

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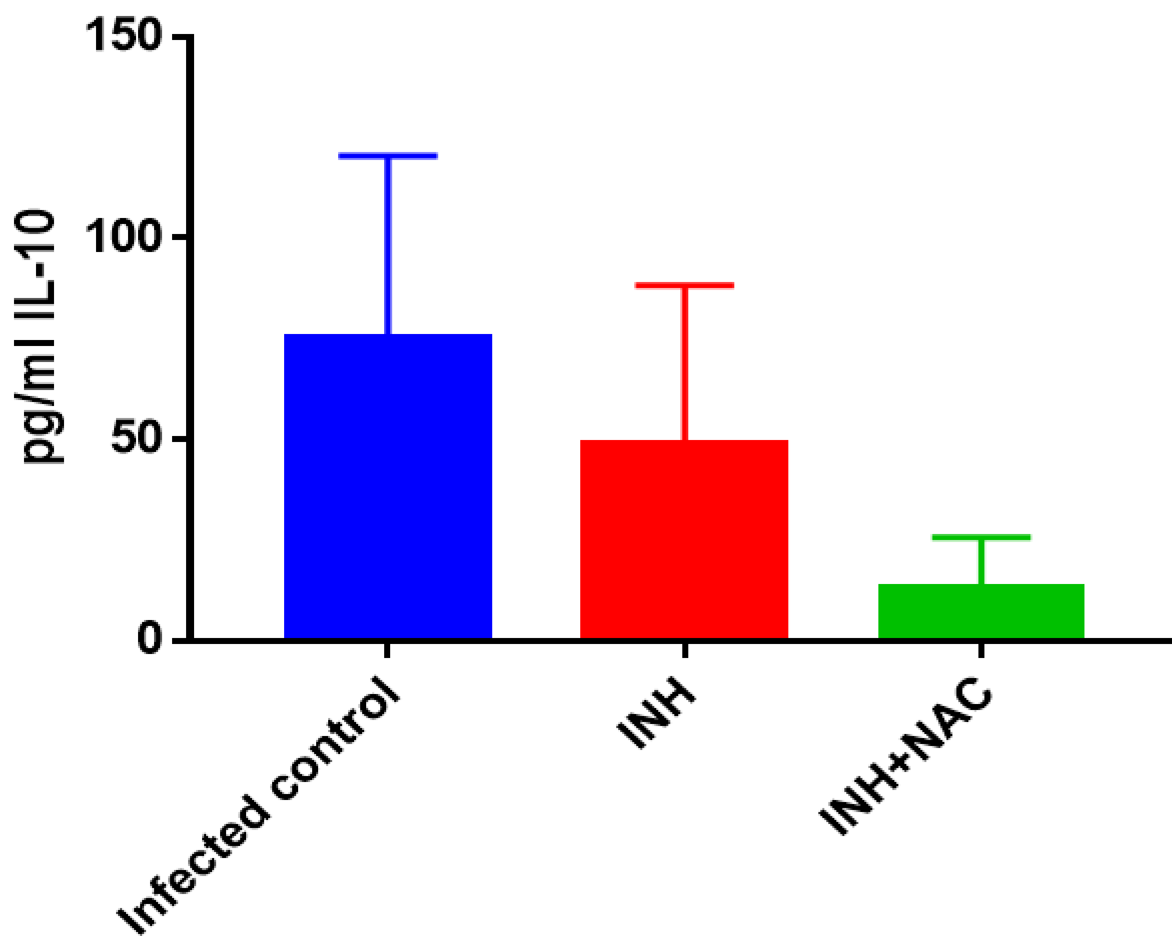

938

939 **Fig. 3D: Assay of IL-10 in the supernatants from INH and INH + NAC-treated THP-1 cells.**

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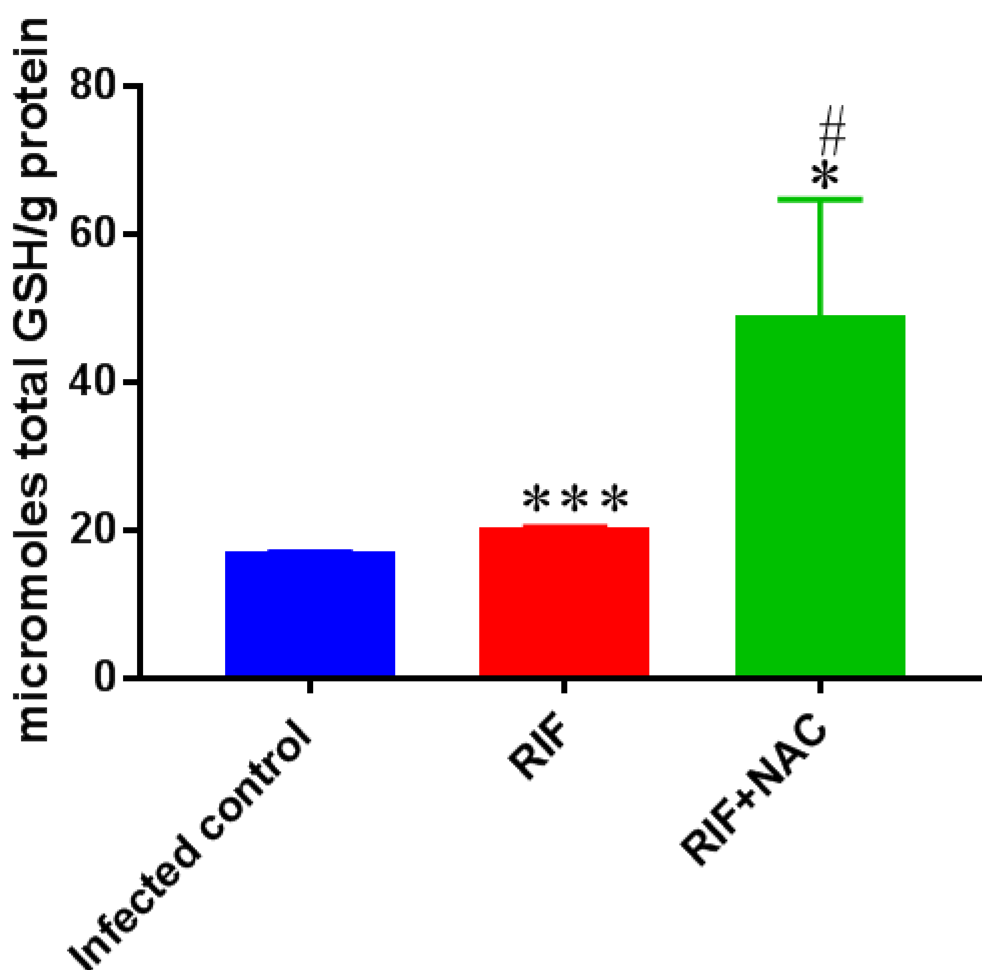

942

943 **Fig. 4A: Levels of total GSH in *M. tb*-infected, M. *tb*-infected + RIF-treated and *M. tb*-**

944 **infected + RIF + NAC-treated THP-1 cells.**

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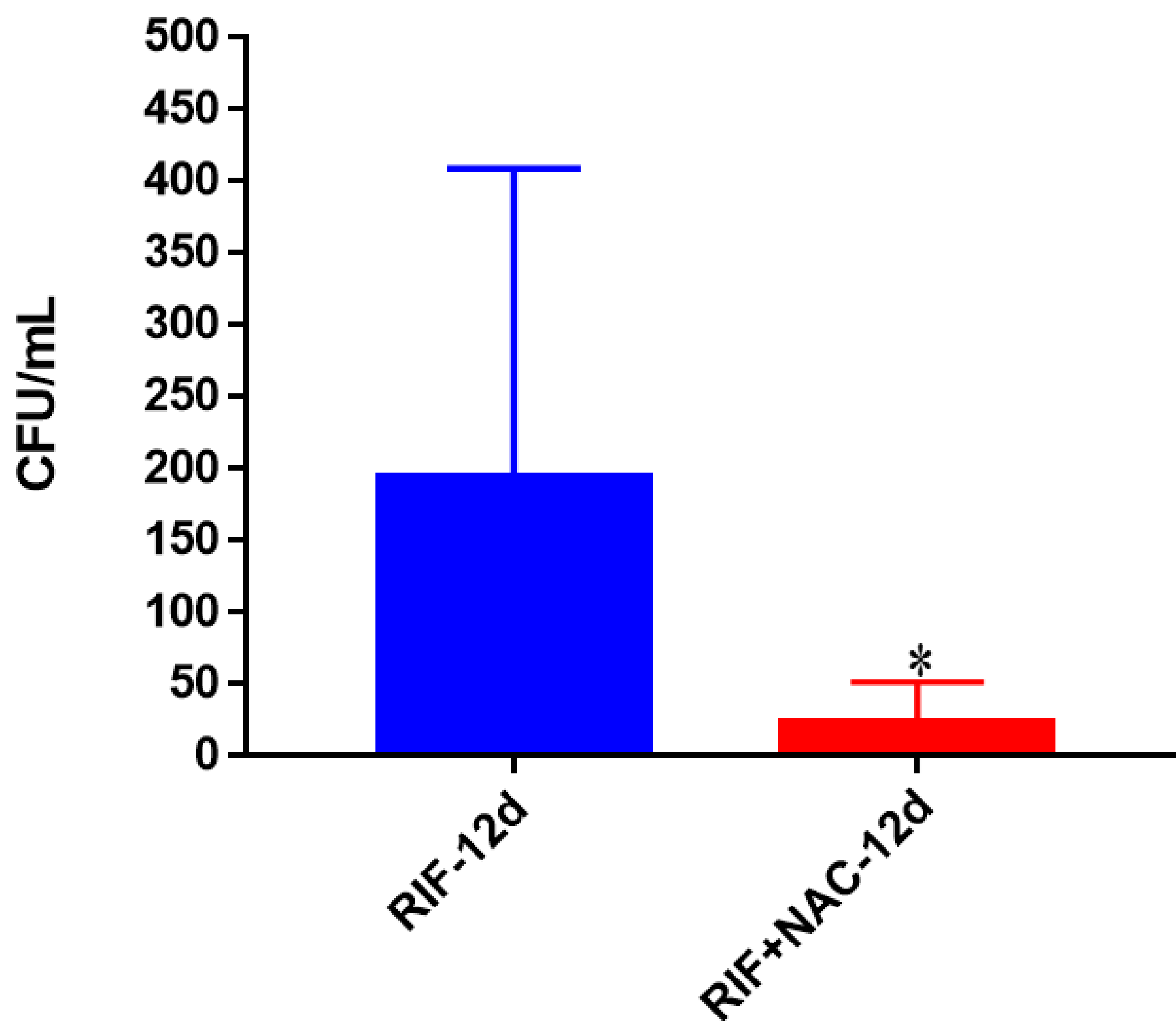

945

946 **Fig. 4B: Survival of Erdman strain of *M. tb* inside RIF and RIF + NAC-treated THP-1**

947 **macrophages.**

948

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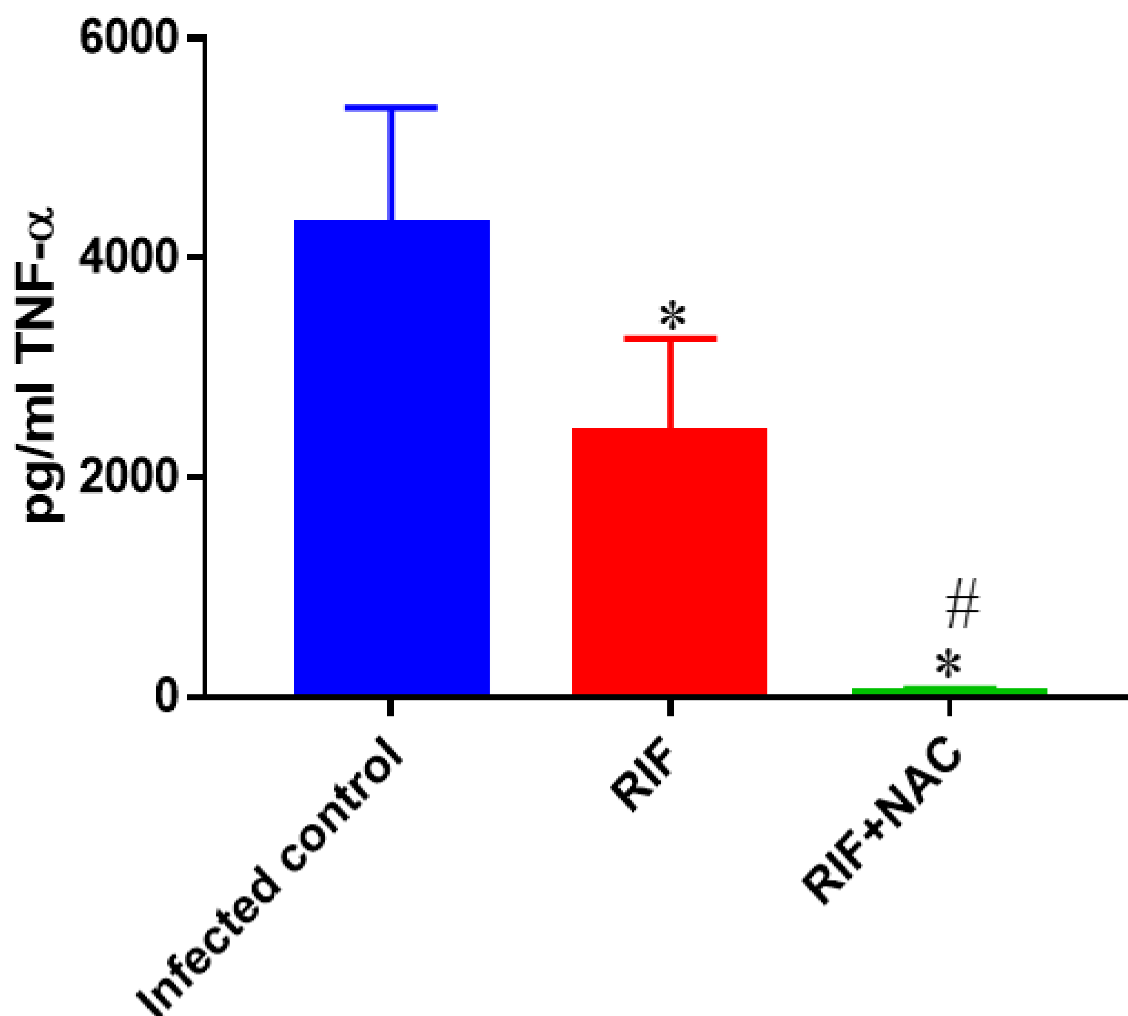

949

950 **Fig. 4C: Assay of TNF-α in the supernatants from RIF and RIF + NAC-treated THP-1**

951 **cells.**

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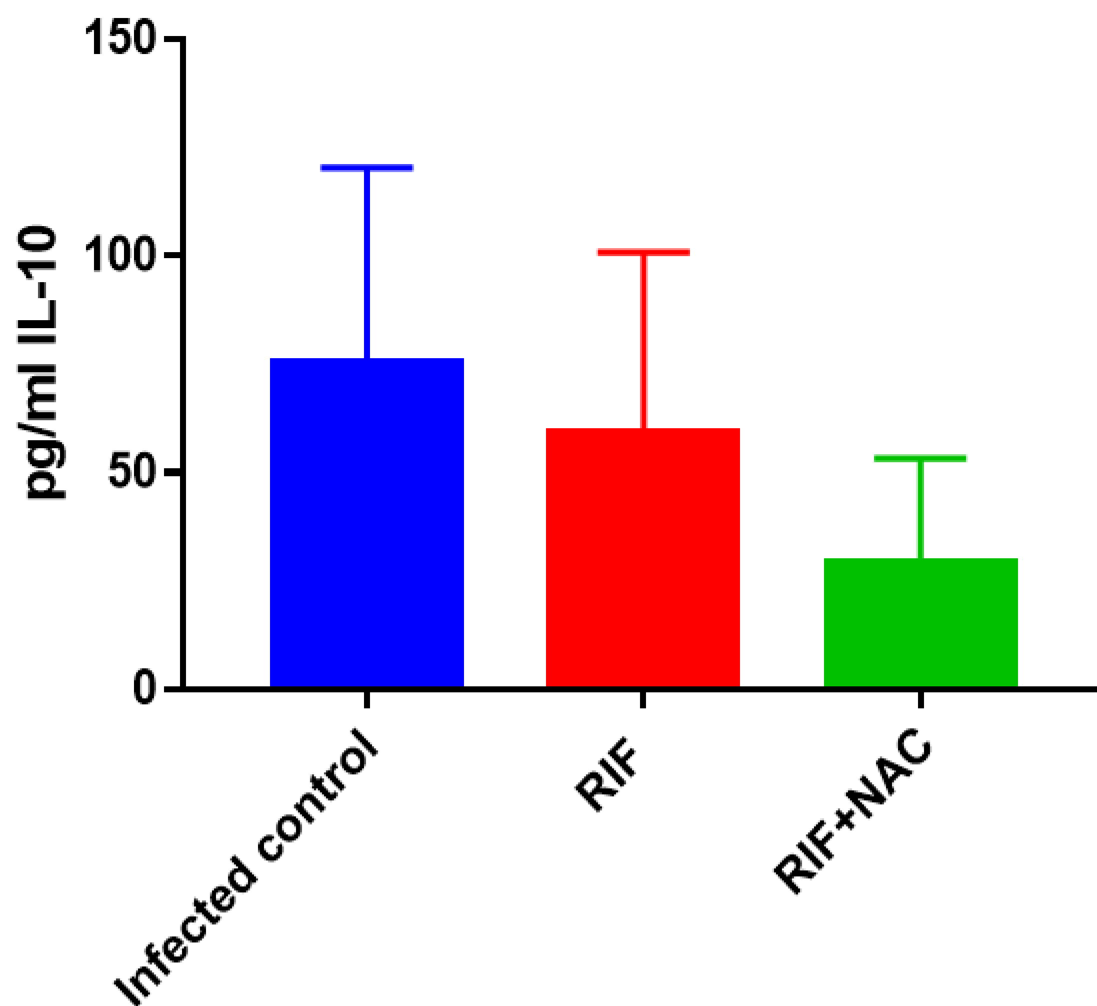

952

953 **Fig. 4D: Assay of IL-10 in the supernatants from RIF and RIF + NAC-treated THP-1 cells.**

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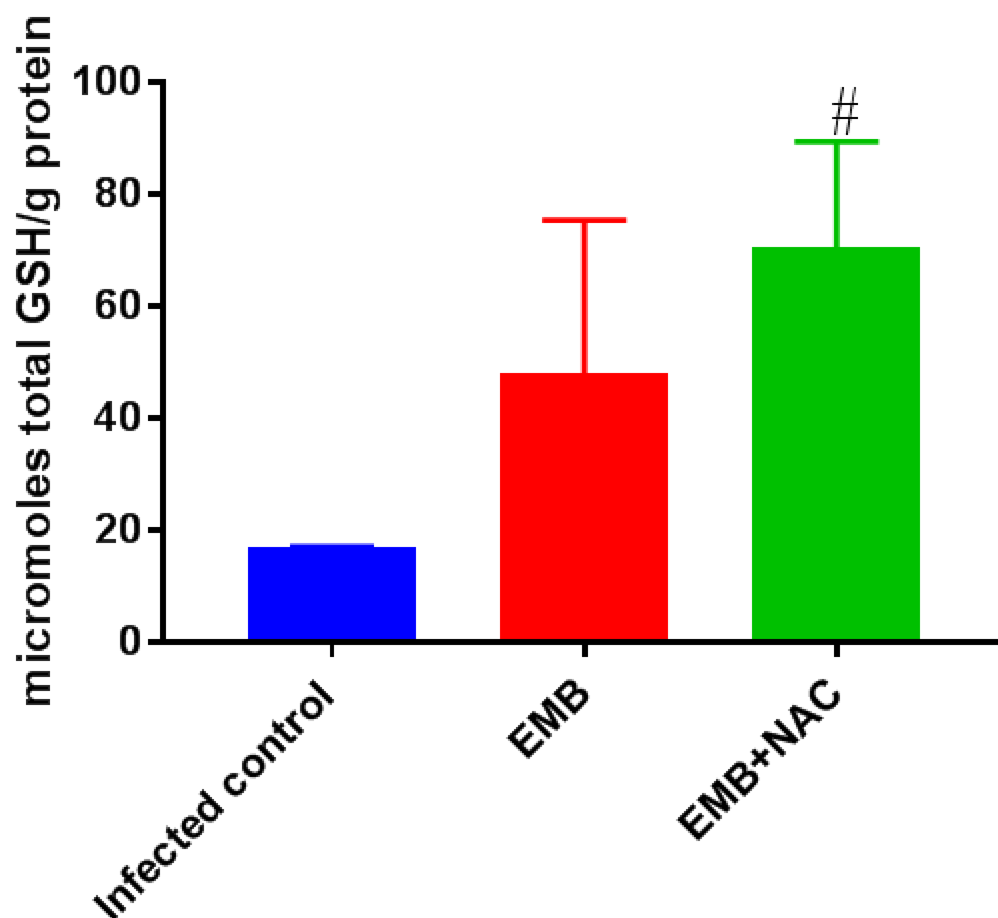

956

957 **Fig. 5A: Levels of total GSH in *M. tb*-infected, *M. tb*-infected + EMB-treated and *M. tb*-**

958 **infected + EMB + NAC-treated THP-1 cells.**

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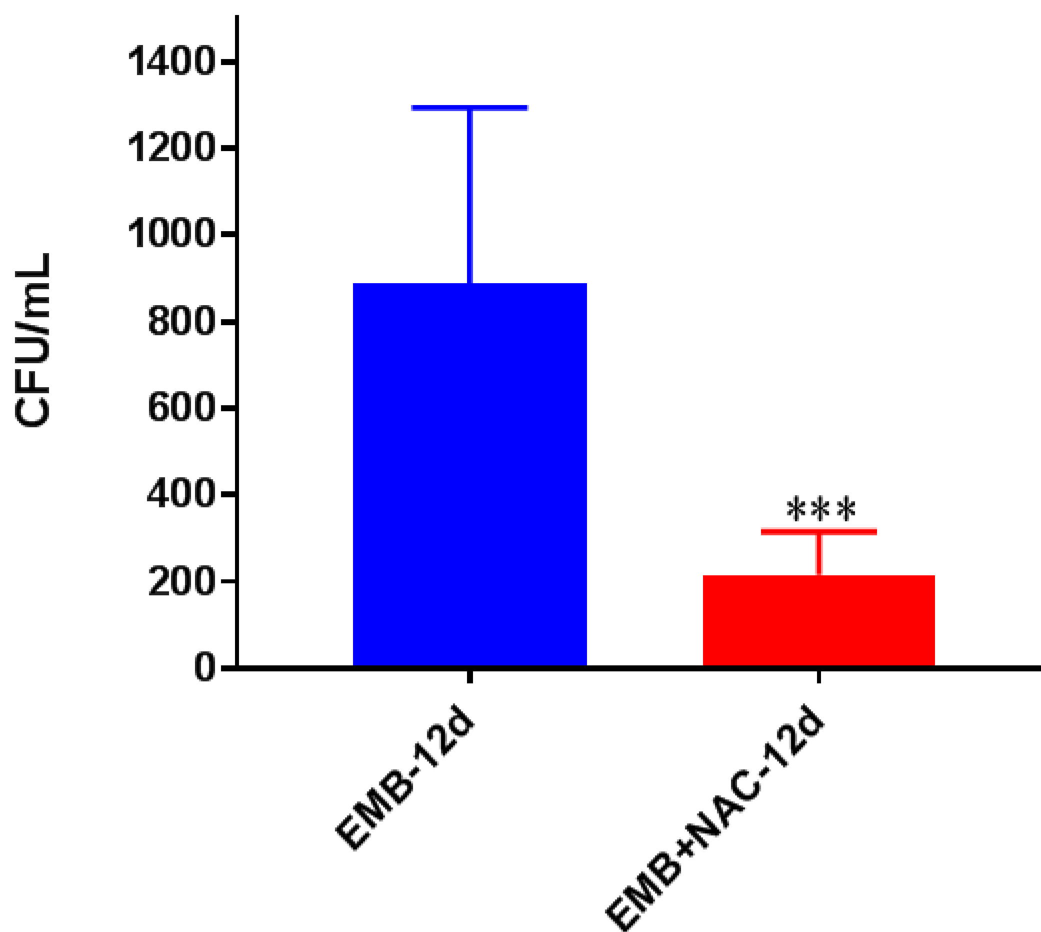

959

960 **Fig. 5B: Survival of Erdman strain of *M. tb* inside EMB and EMB + NAC-treated THP-1**

961 **macrophages.**

962

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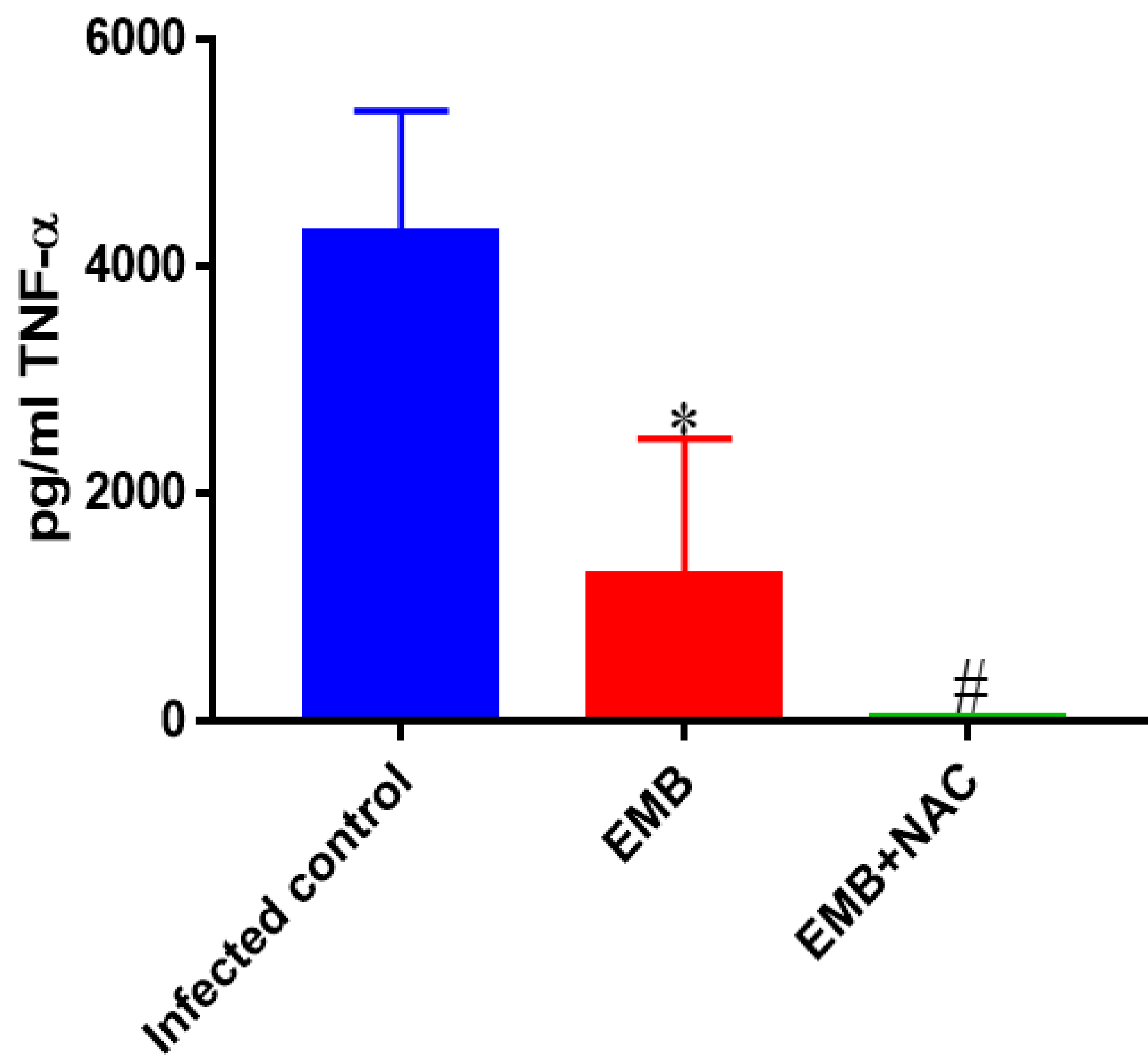

964

965 **Fig. 5C: Assay of TNF-α in the supernatants from EMB and EMB + NAC-treated THP-1**

966 **cells.**

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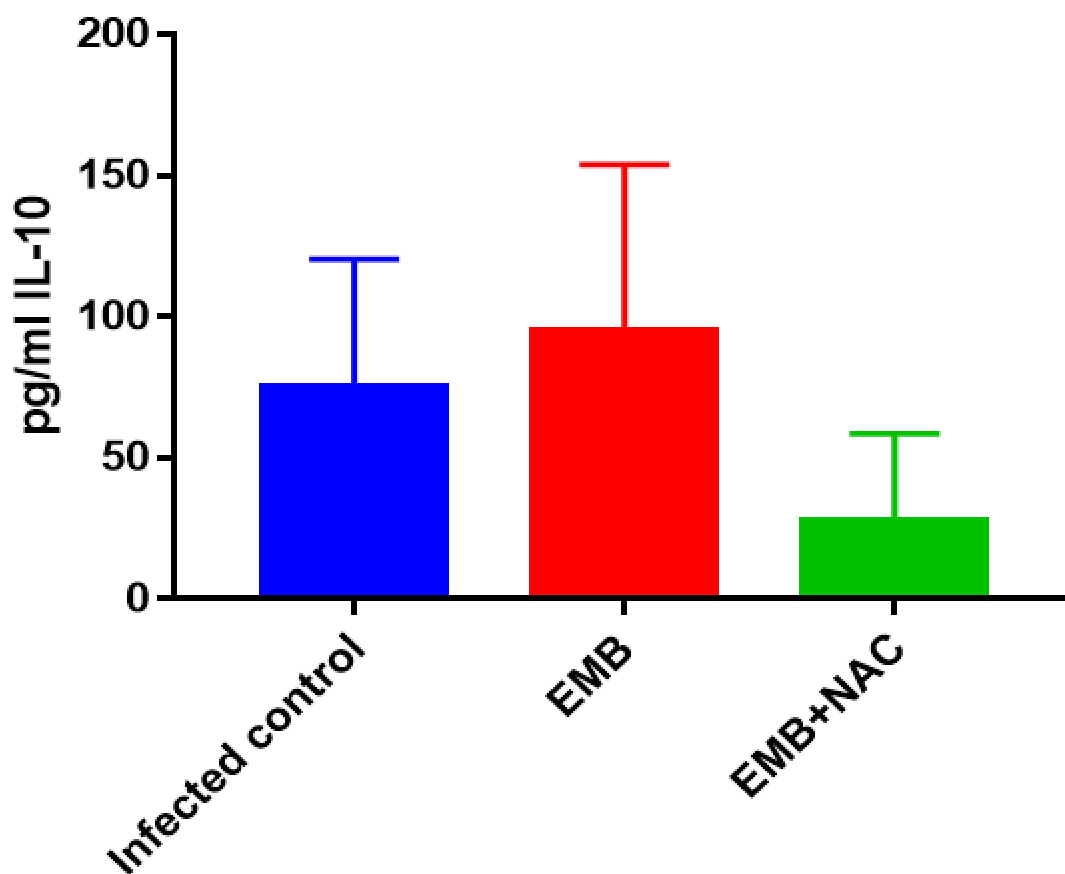

968

969 **Fig. 5D: Assay of IL-10 in the supernatants from EMB and EMB + NAC-treated THP-1**

970 **cells.**

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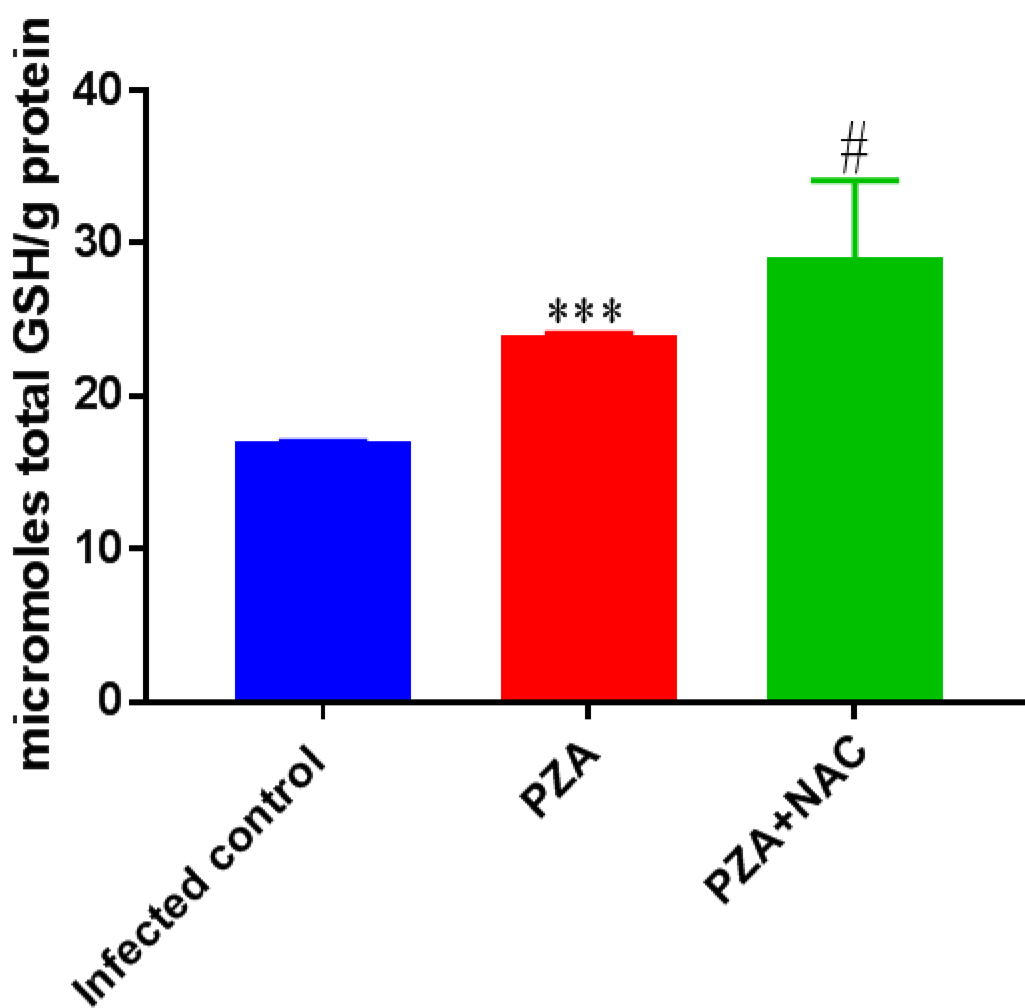

974

975 **Fig. 6A: Levels of total GSH in *M. tb*-infected, *M. tb*-infected + PZA-treated and *M. tb*-**

976 **infected + PZA + NAC-treated THP-1 cells.**

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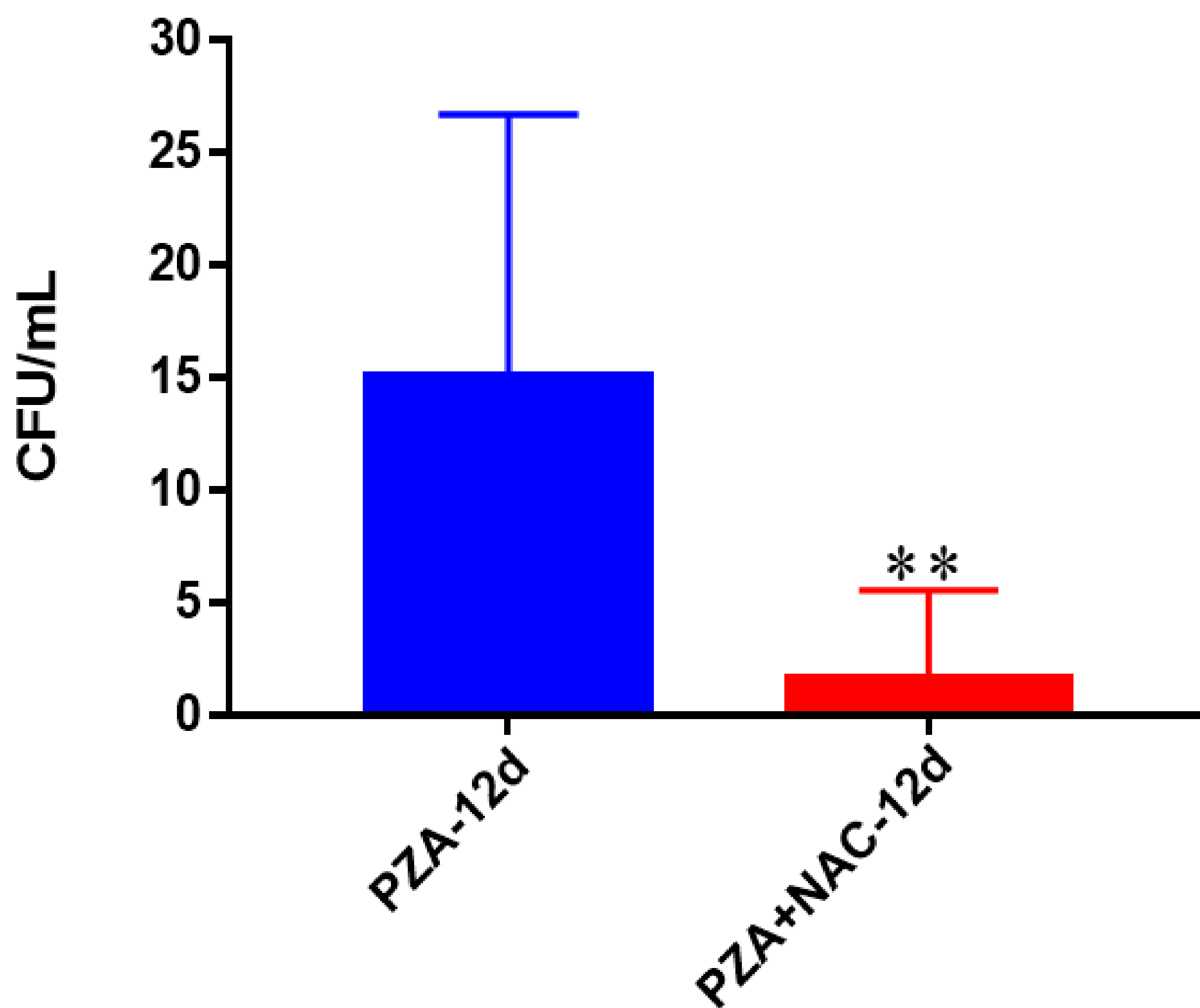

977

978 **Fig. 6B: Survival of Erdman strain of *M. tb* inside PZA and PZA + NAC-treated THP-1**

979 **macrophages.**

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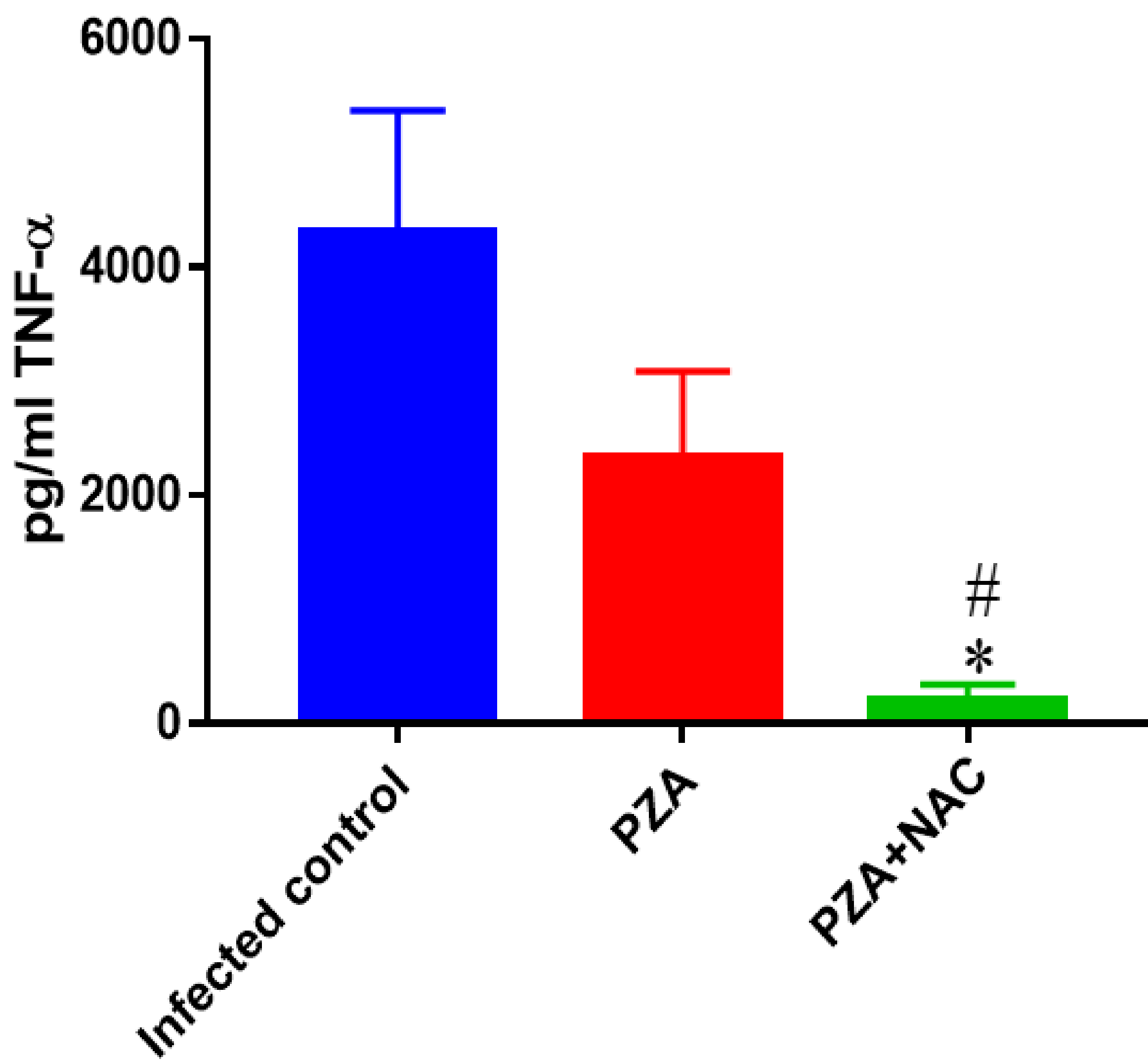

980

981 **Fig. 6C: Assay of TNF-α in the supernatants from PZA and PZA + NAC-treated THP-1**

982 **cells.**

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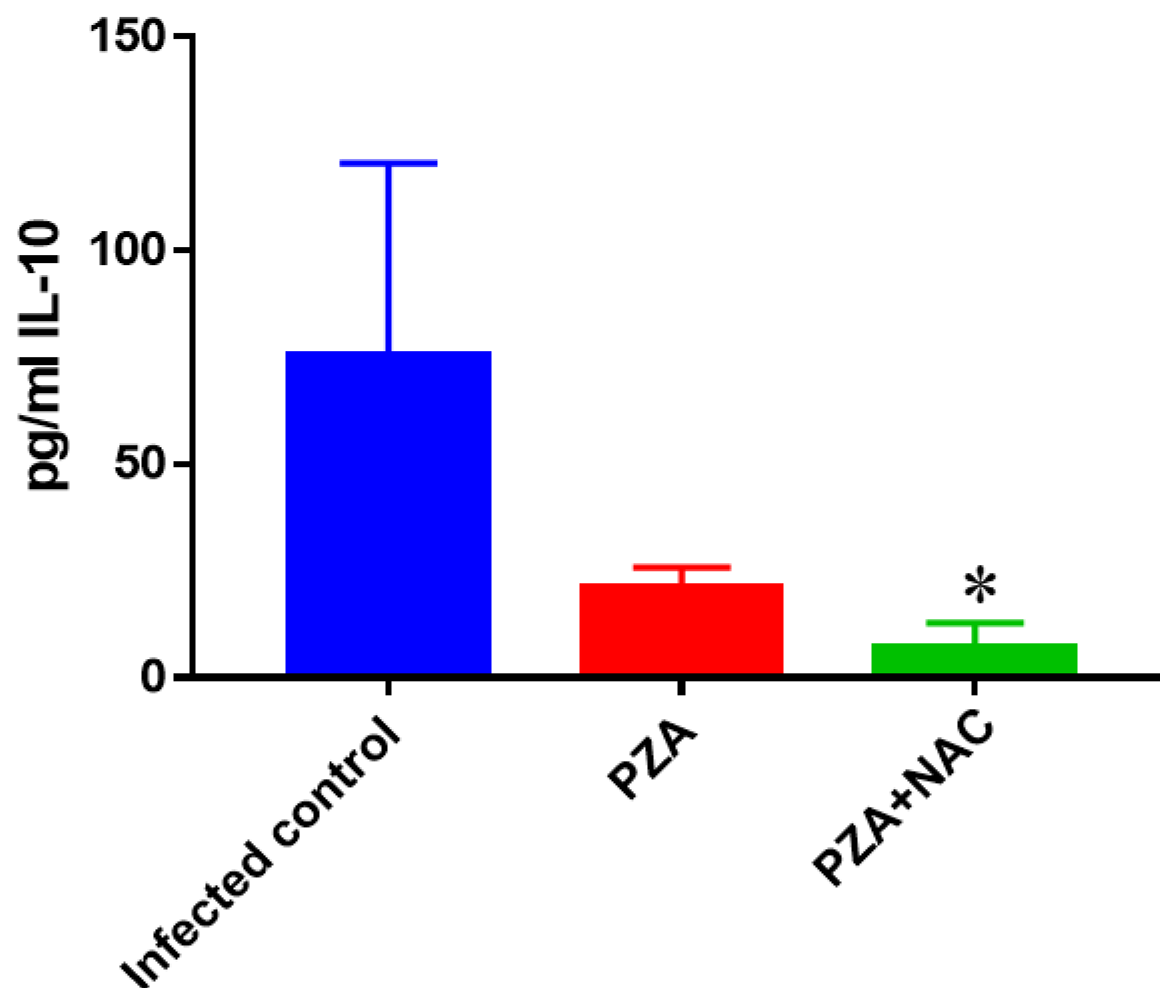

984

985 **Fig. 6D: Assay of IL-10 in the supernatants from PZA and PZA + NAC-treated THP-1**

986 **cells.**

987

988