Methionine and vitamin B-complex ameliorate antitubercular drugs-induced toxicity in exposed patients

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Keywords
Antioxidants, biomarkers, drug toxicity, methionine, modulation, tuberculosis

Abstract
Tuberculosis therapy utilizes drugs that while effective cause treatment-related toxicity. Modulation of antitubercular drugs-induced toxicity by methionine and vitamin B-complex in patients was evaluated. 285 treatment-naïve tuberculosis patients at the Chest Clinics of Infectious Diseases Hospital, Yaba and General Hospital, Lagos in Lagos, Nigeria was prospectively recruited and allotted into test (antitubercular medicines, methionine and vitamin B-complex) and control groups (antitubercular medicines). Data on adverse drug reactions and blood samples were collected at initiation, 2 months and 6 months, and then analyzed. Red blood cells and packed cell volume were significantly higher (P < 0.05) in the test group compared to control at 6 months of therapy. At the end of 2 months, results showed a significant decrease (P < 0.001) in aspartate aminotransferase, alkaline phosphatase, alanine aminotransferase, urea, creatinine and total bilirubin in the test group compared to control. Reduced glutathione and superoxide dismutase were significantly increased (P < 0.001) and malondialdehyde significantly decreased (P < 0.001) in the test versus control groups at the end of 2 and 6 months. Adverse drug reactions were significantly lower (P < 0.001) in the test group (32.4%) compared to control group (56.2%), with 1 death. Hepatotoxicity was significantly higher (P = 0.026) in control (6.9%), compared to test group (0%). Alcohol and cigarette smoking were significantly (P = 0.019 and P = 0.027) associated with the occurrence of adverse drug reactions. Methionine and vitamin B-complex modulated hepatic, renal, hematological, antioxidant indices and adverse effects in patients administered antitubercular medicines. Such interventions can enhance compliance and better treatment outcomes in tuberculosis patients.

Abbreviations
DILI, drug-induced liver injury; INH, isoniazid; MDA, malondialdehyde; PZA, pyrazinamide; RIF, rifampin; ROS, reactive oxygen species; RUCAM, Roussel Uclaf Causality assessment method; SAMe, S-adenosyl-L-methionine; TB, tuberculosis.

Introduction
Tuberculosis (TB), an infectious disease caused by Mycobacterium tuberculosis, is a single leading cause of death from any single infectious agent and a major global public health problem worldwide (Mohajan 2015). Isoniazid (INH), rifampin (RIF), pyrazinamide (PZA), and ethambutol (EMB) are first-line drugs used for tuberculosis therapy in adults for an initial 2 months (intensive phase), then a continuation phase of 4 months involving INH and RIF (Huiri et al. 2017). Despite the effectiveness of this treatment regimen, treatment-related toxicity manifesting as hepatotoxicity, skin reactions, and gastrointestinal disturbances have been reported (Tostmann et al. 2008).

Reactive Oxygen Species (ROS) are produced in the presence of diseases or drugs, resulting in lipid peroxidation and oxidative stress (Zhai et al. 2008). Thus, a reduction in lipid peroxidation in tissue and increase in...
superoxide dismutase, catalase and glutathione activities would help to maintain cell integrity and control the increase in markers of oxidative stress (Hamza and Al-Harbi 2015). Elevated hepatic, renal, hematological and antioxidant indices are important biomarkers in confirming toxicity and extent of organ damage.

The liver is implicated in drug-induced toxicity as it plays an important role in the metabolism and excretion of many drugs, including antituberculosis drugs. Drug-induced liver injury (DILI) causes acute and chronic disease, though the link between drugs and preexisting liver disease is complex, more so when symptoms develop in patients with liver disease during treatment (Teschke and Danan 2016). This problem is evident as many physicians attending to chronic liver disease patients are faced with determining if drug therapy is a risk that increases their patient’s chances for drug-induced liver injury (Teschke and Danan 2017). To properly establish cause and effect, it is appropriate to conduct an assessment of causality, using various criteria and methods, such as the Roussel Uclaf Causality Assessment Method (RUCAM) (Teschke and Danan 2016).

Drug-induced toxicity can also cause clinical adverse effects, which can occur either among antituberculosis medicines or between antituberculosis medicines and other medicines (Farazi et al. 2014). This may extend or modify treatment, and cause drug resistance leading to treatment failure (Kaona et al. 2004) and death. Factors like age, sex, race, other drugs, breastfeeding and pregnancy (Alomar 2014) can increase the occurrence of adverse drug reactions. The very young and elderly people are more at risk of developing adverse drug reactions (ADRs) than other age groups (Pretorius et al. 2013), while the female gender has a higher risk of developing adverse drug reactions than males (Rademaker 2001).

The severity of antitubercular drug-induced toxicity has led to the idea to use other drugs, which when co-administered may prevent or significantly reduce toxicity. Methionine is an antioxidant that protects glutathione, the major antioxidant in human cells that protect against free radicals and toxic compounds. Vitamin B complex contains Vitamin B1 and B6, which have been reported to possess antioxidative properties (Hellmann and Mooney 2010; Alvarado and Navarro 2016). This study evaluated the modulatory effect of co-administration of methionine and vitamin B-complex on antitubercular drugs-induced toxicity in tuberculosis patients.

**Materials and Methods**

This prospective study was conducted amongst 285 treatment-naïve tuberculosis patients at the Chest clinics of Mainland Hospital, Yaba and General Hospital, Lagos in Lagos, Nigeria, after ethical approval and informed consent were obtained. Participants were allotted into the test (co-administered antituberculosis medicines with methionine and vitamin B-complex) and control groups (administered antituberculosis medicines only). Test group participants were placed on 4 tablets of methionine daily (250 mg each) via the oral route and 2 tablets of vitamin B-complex orally daily for 6 months, representing the period of TB treatment. This was in addition to the combined antitubercular medicine regimen (all patients).

Blood samples were collected at initiation of treatment, at 2 months and 6 months, then hematological, biochemical and antioxidant parameters were analyzed according to standard protocols. Data on patients’ demographics and reported adverse drug reactions was also collected prospectively.

Data entry, coding, cleaning and analysis was done using SPSS version 20.0. Descriptive statistics was summarized using frequency, proportions, measures of central tendency and dispersion. Bivariate analysis such as chi-square test was used to investigate the association between adverse drug reaction and selected variables. Logistic regression was further used to determine the factors that may be significantly associated with adverse drug reactions by the patients. Comparisons between the baseline and follow-up data were performed, using the Mann Whitney U test. All tests were carried out at 5% level of significance.

**Results**

**Demographic characteristics of research participants**

Most of the participants were males (71.28%) versus females (28.72%) in the present study. Participants were aged between 18 to 65 years, with majority (36.84%) aged 31–40 years and 34.39% were aged 18–30. Majority was married (53.19%), singles made up 37.94% of participants, while widows/widowers were the least (3.55%) (Table 1). Study participants with a secondary school certificate were more (50.18%), than those with tertiary education (25.96%).

**Effect of methionine and vitamin B-complex on hepatic parameters in control and test groups at 2 and 6 months of antituberculosis drug treatment**

At the end of 2 months of TB treatment, ALT, AST, ALP and total bilirubin were significantly ($P < 0.001$) lower in test group participants (Median: 8.4, 22.1, 64.7 and 3.1) compared to participants in the control group (Median: 74.95, 56.25, 85.4 and 8.25) (Table 2).

ALT, AST, ALP, and total bilirubin were not significant ($P > 0.05$) in the test group, relative to the control group.
participants at the end of 6 months of tuberculosis therapy (Table 2).

Change in hepatic function parameters from baseline at 2 and 3 months of antituberculosis treatment

A significant difference ($P < 0.001$) was observed in changes in AST, ALT, ALP and total bilirubin from baseline at 2 months between control and test group participants (Table 3). No difference was observed between these same parameters at 6 months compared to baseline. (Table 3).

Effect of methionine and vitamin B-complex on renal parameters between test and control groups at 2 and 6 months of anti-TB therapy

Creatinine and urea levels (Median: 63.13 and 2.6) were significantly ($P < 0.001$) lower and albumin (Median: 37.1) significantly ($P = 0.096$) lower in the test participants, relative to control group participants at the end of 2 months of TB treatment (Table 4). Total protein was not significant ($P > 0.05$) in the test versus control group.

There was no difference in levels of creatinine, urea, total protein and albumin in the test versus control group participants at the end of 6 months (Table 4).

Change in renal function parameters from baseline at 2 and 6 months of antituberculosis treatment

A significant difference was observed in changes in creatinine and urea from baseline at 2 and 6 months between control and test group participants (Table 5). No difference was observed between albumin and total protein in both test and control participants at 2 and 6 months compared to baseline (Table 5).

Table 1. Demographic characteristics of research subjects.

| Characteristics | Group | All patients n (%) | Control group n (%) | Test group n (%) | P-value |
|-----------------|-------|--------------------|---------------------|------------------|---------|
| Sex             | Male  | 201 (71.28)        | 92 (45.77)          | 109 (54.23)      | 0.137   |
|                 | Female| 81 (28.72)         | 45 (55.66)          | 36 (44.44)       |         |
| Age (years)     | 18–30 | 98 (34.39)         | 49 (50.00)          | 49 (50.00)       | 0.008*  |
|                 | 31–40 | 105 (36.84)        | 39 (37.14)          | 66 (62.86)       |         |
|                 | 41–50 | 44 (15.44)         | 23 (52.77)          | 21 (47.73)       |         |
|                 | 50+   | 38 (13.33)         | 26 (68.42)          | 12 (31.58)       |         |
| Marital status  | Single| 107 (37.94)        | 52 (48.6)           | 55 (51.40)       | 0.582   |
|                 | Married| 150 (53.19)       | 71 (47.33)          | 79 (52.67)       |         |
|                 | Divorced| 15 (5.32)        | 7 (46.67)           | 8 (53.33)        |         |
|                 | Widow/widower| 10 (3.55) | 7 (70.00) | 3 (30.00) |         |
| Highest education| NFE/Primary| 68 (23.86) | 34 (50.00) | 34 (50.00) | 0.810   |
|                 | Secondary| 143 (50.18) | 66 (46.15) | 77 (53.85) |         |
|                 | Tertiary| 74 (25.96)        | 37 (50.00)          | 37 (50.00)       |         |

*Chi-square test (significant). NFE, no formal education.

Table 2. Effect of methionine and vitamin B-complex on hepatic parameters in control and test groups at baseline, 2 and 6 months of antituberculosis drug treatment.

| Parameter | Median (IQR) level Control (a) | Median (IQR) level Test (b) | P-value |
|-----------|---------------------------------|-----------------------------|---------|
| Baseline  |                                 |                             |         |
| AST (U/L) | 22.80 (IQR: 15.25–32.10)        | 35.00 (IQR: 25.25–49.60)    | <0.0001*|
| ALT (U/L) | 9.90 (IQR: 5.7–16.83)           | 14.80 (IQR: 7.4–27.10)      | 0.008*  |
| ALP (U/L) | 65.60 (IQR: 54.85–90.58)        | 82.70 (IQR: 68.80–99.55)    | 0.002*  |
| T. Bilirubin (mmol/L) | 4.75 (IQR: 3.35–6.65) | 5.90 (IQR: 4.50–8.35) | 0.01*   |
| Month 2   |                                 |                             |         |
| AST (U/L) | 56.25 (IQR: 47.20–63.95)        | 22.1 (IQR: 15.10–32.80)     | <0.001* |
| ALT (U/L) | 74.95 (IQR: 65.38–89.03)        | 8.4 (IQR: 4.70–12.30)       | <0.001* |
| ALP (U/L) | 85.4 (IQR: 80.35–102.50)        | 64.7 (IQR: 51.70–88.60)     | <0.001* |
| T. Bilirubin (mmol/L) | 8.25 (IQR: 5.48–15.08) | 3.1 (IQR: 2.50–4.00) | <0.001* |
| Month 6   |                                 |                             |         |
| AST (U/L) | 30.1 (IQR: 19.60–35.50)         | 25.45 (IQR: 16.68–32.73)    | 0.276   |
| ALT (U/L) | 17.2 (IQR: 5.30–70.10)          | 8.45 (IQR: 4.70–30.75)      | 0.183   |
| ALP (U/L) | 71.35 (IQR: 56.28–83.03)        | 68.5 (IQR: 58.30–113.00)    | 0.370   |
| T. Bilirubin (mmol/L) | 4.3 (IQR: 3.25–6.03) | 4.2 (IQR: 3.60–5.30) | 0.910   |

*Mann Whitney U test (Significant); IQR, Interquartile range.
A positive median value of hepatic parameter shows an increase from baseline value, while a negative value indicate a decrease from baseline value.

*Mann Whitney U test (Significant); IQR, Interquartile range.

Table 3. Change in hepatic function parameters from baseline at 2 and 6 months of antituberculosis treatment.

| Parameter       | Control Median (IQR) | Test Median (IQR) | P-value |
|-----------------|----------------------|-------------------|---------|
|                 | (2 or 6 months value Minus baseline value) | (2 or 6 months value Minus baseline value) |         |
| Month 2         |                       |                   |         |
| AST (U/L)       | 33.45 (18.73–42.88)  | −12.6 (−30.2 to 4.7) | <0.001* |
| ALT (U/L)       | 58.75 (48.68–73.98)  | −12.5 (−17.6 to 6.5) | <0.001* |
| ALP (U/L)       | 14.1 (−2.8 to 35.88) | −15.1 (−27.2 to 4.1) | <0.001* |
| T_Bil (mmol/L)  | 3.2 (0–10.1)         | −2.6 (−3.5 to 1.5)  | <0.001* |
| Month 6         |                       |                   |         |
| AST (U/L)       | 4.75 (−11.93–16.33)  | −4.7 (−33.5 to 8.9) | 0.313   |
| ALT (U/L)       | −0.5 (−10.6 to 25.4) | 10.8 (−11.6 to 55.3) | 0.531   |
| ALP (U/L)       | 8.3 (−30.3–25.43)    | −2.2 (−52.6 to 17.9) | 0.454   |
| T_Bil (mmol/L)  | 0.15 (−2.78 to 1.3)  | −1.1 (−3.7 to 0.5)  | 0.918   |

Effect of methionine and vitamin B-complex on antioxidant indices at 2 and 6 months of antitubercular therapy

At the end of 2 and 6 months, GSH level was significantly (P < 0.001) elevated in the test group participants (median: 45.1 and 64.0) compared to the control (median: 34.7 and 20.4, respectively) (Fig. 1). At the end of 2 and 6 months, SOD level was significantly (P < 0.001) elevated in the test group participants (median: 34.7 and 20.4, respectively) compared to the control (median: 37.1 and 29.0, respectively) (Fig. 2). At the end of 2 and 6 months, there was no significant difference in CAT level (P > 0.05) between the test group participants and the control (Fig. 3). A significant (P < 0.001) difference was observed at baseline between control (median: 717.4) and test participants (median: 391.3). At the end of 2 and 6 months, malondialdehyde level was significantly (P < 0.001) lower in the test group participants (median: 2.3 and 1.3, respectively) compared to the control (median: 4.9 and 7.1, respectively) (Fig. 4).

Effect of methionine and vitamin B-complex on hematological indices at 2 and 6 months of antituberculosis treatment

A significant (P < 0.001) difference was observed in ESR between the control and test groups at 2 months of TB treatment (Table 6). WBC count was significantly (P = 0.013) lower in the test group, compared to participants in the control group. Lymphocyte count was significantly (P = 0.009) lower in the test group, compared to the control group participants.
There was no difference in RBC, HGB, PCV, MCV, MCH, and MCHC were higher in test group participants compared to the control group participants. PLT and neutrophil count were not different in the test versus control group participants (Table 6).

Neutrophil count was significantly ($P < 0.001$) and PCV significantly ($P = 0.037$) higher while there was no difference in ESR, HGB, and MCV levels in the test group participants at 6 months of treatment compared to control (Table 7). Lymphocytes (%), RBC and MCHC were significantly lower in the test group participants at 6 months of treatment compared to control, while WBC, PLT, and MCH were not different in test versus control participants (Table 7).

**Prevalence of adverse drug reactions in the different treatment groups**

A significant ($P < 0.001$) difference was observed in the treatment groups, with fewer participants developing ADRs in the test group (32.40%) compared to the control.
Prevalence of different adverse drug effects between test and control groups

Comparing the test versus control, number of participants who developed generalized weakness (14.9% vs. 35%) and tiredness (3.4% vs. 17.5%) were significantly ($P < 0.001$) lower; rash (3.4% vs. 13.9%) and headache (2.7% vs. 12.4%) were significantly ($P = 0.001$ and $P = 0.002$ respectively) lower, while loss of appetite (2.7% vs. 7.3%) was significantly ($P = 0.099$) lower in test participants compared to control (Table 9).

3.4% and 6.1% test group participants developed rashes and abdominal pain, respectively, compared to a higher percentage (13.9% and 12.4%) of control group subjects (Table 7). Headache, fever, and loss of appetite occurred in 2.7%, 2.7%, and 2.7% of test subjects, respectively, compared to 12.4%, 5.8%, and 7.3% in the control group subjects.

13.9% of subjects in the control group reported dark urine as opposed to 9.5% of test group subjects. Death occurred in 1 participant in the control group (Table 9).

Association of risk factors with hepatotoxicity at 2 months of antituberculosis therapy

A significant ($P = 0.026$) difference was observed between the control group participants (6.9%) that developed hepatotoxicity and test group participants (0%) (Table 10). A higher percentage of control (93.1%) and test (100%) did not develop hepatotoxicity. Out of the 4 participants who developed hepatotoxicity, 3 were aged 18–30 and 1 was between 31 and 40; 3 were males and 1 was a female; 1 was a smoker and 3 nonsmokers; 1 used hard drugs while 3 did not (Table 8). These risk factors (age, sex, alcohol, cigarette smoking and substance use) were not significantly associated with the development of ADRs in both control and test group participants (Table 10).

Association between some risk factors and other adverse drug reactions

There was no difference observed between males than females (46.77% vs. 38.27%) who developed ADRs, and between participants in the different age groups who developed ADRs, compared to those who did not. No statistical difference was observed between overweight and obese participants (58.82% and 66.67%, respectively) developed ADRs compared to those who did not (41.18% and 33.33%) (Table 11). There was no statistical difference in weight and ADRs observed in participants who were under weight and normal weight in the test group.

(56.20%) (Table 8). Conversely, more test group participants (67.60%) did not develop ADRs than control group participants (43.80%).
A significant difference was observed amongst participants who consumed alcohol, with more of them (52.63%) developing ADRs compared to those that did not take alcohol (47.37%) (Table 11). Fewer people 38.36% that did not consume alcohol developed ADRs than those who did not develop any ADR. A significant ($P = 0.027$) difference was observed amongst participants who consumed smoked, with more of them (57.63%) developing ADRs compared to those that did not smoke (42.31%) (Table 11).

More participants who reported using hard drugs (58.33%) developed ADRs compared to those that did not use any hard drug (41.67%). 42.86% of participants that did not use any hard drug developed ADRs, compared to those that did not develop any ADR (57.14%) (Table 11).

Logistic regression for factors influencing development of adverse drug reactions

The factors identified to be significantly associated with Adverse Drug Reaction in bivariate analysis (Table 11) were harvested and subjected to multivariate analysis. The
dependent variable in Table 12 above is Adverse Drug Reaction status, a Yes-or-No outcome. Patients who take alcohol were about two times more likely (OR = 1.501, \( P = 0.173 \), 95% CI: 1.02, 2.33) to develop Adverse Drug Reaction than those who do not take alcohol. Also, patients who smoke have about forty percent increase in risk of developing Adverse Drug Reactions (OR = 1.371, \( P = 0.402 \), 95% CI: 0.656, 2.864) compared to those who do not smoke. The model was a good fit as Hosmer–Lemeshow goodness of fit was not significant (\( \chi^2 = 1.220 \), \( P = 0.543 \)) (Table 12).

### Table 9. Prevalence of different adverse drug effects between test and control groups.

| Type of ADR       | Number (% of patients) | \( P \) value a versus b |
|-------------------|-------------------------|--------------------------|
| Weakness          | Control (a) Test (b)    |                          |
| Tiredness         | 48 (35.00) 22 (14.90)   | <0.001*                  |
| Nausea and Vomiting | 42 (30.70) 35 (23.60)   | 0.183                    |
| Dizziness         | 29 (21.20) 21 (14.20)    | 0.122                    |
| Rash              | 19 (13.90) 5 (3.40)      | 0.001*                   |
| Abdominal pain    | 17 (12.40) 9 (6.10)      | 0.064                    |
| Headache          | 17 (12.40) 4 (2.70)      | 0.002*                   |
| Fever             | 8 (5.80) 4 (2.70)        | 0.242                    |
| Loss of appetite  | 10 (7.30) 4 (2.70)       | 0.099*                   |
| Dark urine        | 19 (13.90) 14 (9.50)     | 0.245                    |
| Death             | 1 (0.70) 0 (0.00)        | 0.298                    |

*Chi-square test (significant).

### Table 10. Association of risk factors with hepatotoxicity at 2 months of antituberculosis therapy.

| Risk factor Subgroup | Number (%) of patients | \( P \) value |
|----------------------|------------------------|--------------|
| Treatment Group      | Control (a) Test (b)   |              |
| Age (years)          | 18–30 28 (90.32) 3 (9.68) | 0.210       |
| 31–40                | 39 (97.50) 1 (2.50)     |              |
| 41–50                | 19 (100.00) 0 (0.00)    |              |
| 51–65                | 16 (100.00) 0 (0.00)    |              |
| Sex                  | Male 78 (96.30) 3 (3.70) | 0.917       |
| Alcohol use          | Yes 43 (97.73) 1 (2.27)  | 0.717       |
| No                   | 23 (95.83) 1 (4.17)     |              |
| Smokes               | Yes 19 (95.00) 1 (5.00)  | 0.773       |
| No                   | 80 (96.39) 3 (3.61)     |              |
| Reported substance use | Yes 8 (88.89) 1 (11.11) | 0.245      |
| No                   | 90 (96.77) 3 (3.23)     |              |

*Chi-square test (Significant); ADR, adverse drug reaction; BMI, body mass index.

### Table 11. Association between some risk factors and other adverse drug reactions.

| Risk factor Subgroup | No ADR (n(%)) | ADR (n(%)) | \( P \) value |
|----------------------|---------------|------------|--------------|
| Sex                  | Male 107 (53.23) 94 (46.77) | 0.194       |
| Female               | 50 (61.73) 31 (38.27)     |              |
| Age (years)          | 18–30 56 (57.14) 42 (42.66) | 0.901       |
| 31–40                | 56 (53.33) 49 (46.67)     |              |
| 41–50                | 26 (59.09) 18 (40.91)     |              |
| 50+                  | 22 (57.89) 16 (42.11)     |              |
| BMI (kg/m²)          | <18.5 52 (63.41) 30 (36.59) | 0.293       |
| 18.5–24.99 (normal)  | 68 (57.63) 50 (42.37)     |              |
| 25–29.9 (over weight)| 7 (41.18) 10 (58.82)      |              |

Alcohol intake Yes 54 (47.37) 60 (52.63) \( P = 0.019^* \)
No 98 (61.64) 61 (38.36)

Smokes cigarette Yes 22 (42.31) 30 (57.69) \( P = 0.027^* \)
No 132 (59.19) 91 (40.81)

Reported substance use Yes 10 (41.67) 14 (58.33) \( P = 0.145 \)
No 140 (57.14) 105 (42.86)

*Chi-square test (significant).

### Table 12. Logistic regression for factors influencing development of adverse drug reactions.

| Variables | Odds ratio SE Wald statistic \( P \) value 95% CI |
|-----------|------------------|-----------------------------|-----------------|-----------------|
| Alcohol intake | Yes 1,501 | 0.298 | 1.856 | 0.173 | (0.837, 2.692) |
| Smoking status | Yes 1,371 | 0.376 | 0.704 | 0.402 | (0.656, 2.864) |

*Reference category.

### Association between some risk factors and ADR by treatment group

More control group participants who consumed alcohol (75.47%) and smoked cigarettes (78.57%) developed ADRs in a significant \( (P = 0.000) \) and significant \( (P = 0.010) \) manner compared to those who did not smoke (51.46%) or consume alcohol (44.16%). Participants in the control group who used hard drugs (73.33%) developed ADRs in an insignificant \( (P > 0.05) \) manner compared to those who did not (Table 13).

More test group participants who consumed alcohol (67.21%), smoked cigarettes (66.67%) and used hard drugs (66.67%) did not develop ADRs in an insignificant
Tuberculosis and the liver are related in many ways, particularly rifampicin (a known nephrotoxic (Manika et al. 2013) and hepatotoxic (Awodele et al. 2011)) agent, are not included in the continuation phase of treatment, as opposed to their use in the intensive phase (along with isoniazid and ethambutol). Thus, the assault on the liver is thus less, therefore improving the ability of the hepatic cells to regenerate. These reasons are likely responsible for recovery of many of the liver and renal function parameters with time in the control group.

Previous studies indicate a strong association between renal injury and oxidative stress in patients treated with antituberculosis drugs (Kwon et al. 2004; Schubert et al. 2010). This causes an elevation in creatinine, urea (Yanardag et al. 2005) and albumin and a decrease in total protein levels (Shabana et al. 2012). This correlates to results from this study when baseline values are compared to values at the end of 2 months of therapy in the treatment groups. Comparing the treatment groups at the end of 2 months showed a significant ($P < 0.001$) decrease in urea and creatinine and a significant ($P = 0.096$) increase in albumin in participants co-administered methionine and vitamin B-complex with the combined antitubercular medicines, indicating an amelioration of the deleterious effects of antitubercular agents. These parameters were similarly affected up to the end of 6 months of therapy but in an insignificant ($P > 0.05$) manner. Albumin has been reported to possess antioxidant properties (Roche et al. 2008), which makes it possible to postulate a potentiation of the antioxidant potential of albumin and methionine/vitamin B-complex for this effect. In the present study, total protein was higher in the control group at the end of 6 months compared to test group participants, possibly due to higher globulin fraction relative to albumin fraction. This corresponds to the result of Shing-dang et al. (2016), which showed higher globulin levels compared to albumin at the end of the continuation phase of TB treatment. Levels of globulin were, however, not evaluated in this present study.

Hematological changes associated with tuberculosis treatments have been reported in many parts of the world (Kassa et al. 2016). Results from this current study

### Table 13. Association between some risk factors and ADR by treatment group.

| Characteristic               | Control No ADR n(%) | Control ADR n(%) | P value | Test No ADR n(%) | Test ADR n(%) | P value |
|-----------------------------|---------------------|------------------|---------|-----------------|---------------|---------|
| Alcohol intake              | Yes                 | 13 (24.53)       | 40 (75.47) | 0.000*          | 41 (67.21)    | 20 (32.79) | 0.945 |
|                             | No                  | 43 (55.84)       | 34 (44.16) |                 | 54 (66.67)    | 27 (33.33) |       |
| Smokes cigarette            | Yes                 | 6 (21.43)        | 22 (78.57) | 0.010*          | 16 (66.67)    | 8 (33.33)  | 0.893 |
|                             | No                  | 50 (48.54)       | 53 (51.46) |                 | 81 (68.07)    | 38 (31.93) |       |
| Reported substance use      | Yes                 | 4 (26.67)        | 11 (73.33) | 0.195           | 6 (66.67)     | 3 (33.33)  | 0.937 |
|                             | No                  | 50 (44.25)       | 63 (55.75) |                 | 89 (67.94)    | 42 (32.06) |       |

*Chi-square test (Significant).

($P > 0.05$) manner compared to those who developed ADRs (32.79%, 33.33% and 33.33%, respectively) (Table 13).

## Discussion

Hepatic transaminases, renal, hematological and antioxidant indices are mostly affected by deleterious antitubercular drug-induced toxicity in patients undergoing treatment. An elevation in AST and the liver-specific ALT would, therefore, indicate leakage from injured tissues (Ozer et al. 2008), while an increase in ALP level occurs due to overproduction and release in blood (Ramaiah 2007). Supplementation with agents capable of modulating the harmful effects of these antitubercular medicines would be of immense benefit to patients undergoing treatment for tuberculosis. Achieving this would reduce often severe adverse effects, enhance compliance and ultimately improve treatment outcomes. Compared to participants on only the antitubercular medicines, administration of methionine and vitamin B-complex (in the presence of antitubercular medicines) showed a significant ($P < 0.001$) decrease in total bilirubin and the liver enzymes-ALT, AST, and ALP at the end of the intensive phase of treatment. This decrease can be attributed to the reported antioxidant activity of methionine and B-complex, while the higher levels in the control group support the knowledge that antitubercular medicines, particularly rifampicin, induce hepatocellular injury and hyperbilirubinemia (Singh et al. 2016).

Tuberculosis and the liver are related in many ways, one of which is the direct hepatic involvement by the disease itself that can impair hepatic functions (Essop et al. 1984) and elevate indices like ALT, AST, and ALP. It is thus possible to infer that as the bacterial load reduced in the course of treatment (from the 2nd to the 6th month of treatment), the toxic effect of the infection reduced, allowing the hepatic and renal cells to recover and reduce elevated parameters. This is important, considering that the liver is a regenerative organ. According to the standard protocol of tuberculosis treatment in Nigeria, pyrazinamide and rifampicin (Manika et al. 2013) and hepatotoxic (Awodele et al. 2011) agent, are not included in the continuation phase of treatment, as opposed to their use in the intensive phase (along with isoniazid and ethambutol). Thus, the assault on the liver is thus less, therefore improving the ability of the hepatic cells to regenerate. These reasons are likely responsible for recovery of many of the liver and renal function parameters with time in the control group.

Average values at the end of 2 months showed a significant ($P < 0.001$) decrease in total protein compared to baseline values and a significant ($P < 0.001$) decrease in the total bilirubin and liver enzymes-ALT, AST, and ALP at the end of the intensive phase of treatment. This decrease can be attributed to the reported antioxidant activity of methionine and B-complex, while the higher levels in the control group support the knowledge that antitubercular medicines, particularly rifampicin, induce hepatocellular injury and hyperbilirubinemia (Singh et al. 2016).

The standard protocol of tuberculosis treatment in Nigeria, pyrazinamide and rifampicin (Manika et al. 2013) and hepatotoxic (Awodele et al. 2011) agent, are not included in the continuation phase of treatment, as opposed to their use in the intensive phase (along with isoniazid and ethambutol). Thus, the assault on the liver is thus less, therefore improving the ability of the hepatic cells to regenerate. These reasons are likely responsible for recovery of many of the liver and renal function parameters with time in the control group. Antioxidants have been reported to possess antioxidant properties (Roche et al. 2008), which makes it possible to postulate a potentiation of the antioxidant potential of albumin and methionine/vitamin B-complex for this effect. In the present study, total protein was higher in the control group at the end of 6 months of therapy compared to test group participants, possibly due to higher globulin fraction relative to albumin fraction. This corresponds to the result of Shing-dang et al. (2016), which showed higher globulin levels compared to albumin at the end of the continuation phase of TB treatment. Levels of globulin were, however, not evaluated in this present study.

Hematological changes associated with tuberculosis treatments have been reported in many parts of the world (Kassa et al. 2016). Results from this current study
showed lower levels of RBC, HGB, PCV, and PLT in control group subjects at the end of the intensive phase of treatment, relative to test subjects. This suggests antitubercular drug-induced deleterious effects on these indices, for example, isoniazid, which has been reported to cause a decrease in HGB synthesis (Ghosh et al. 2017). Methionine and vitamin B-complex are therefore able to inhibit this effect by promoting HGB synthesis, hence its increase in test group subjects. PCV was observed in the present study to be higher in patients on combined antitubercular medicines, methionine and vitamin B-complex, relative to those on antitubercular medicines alone. This is similar to the results of Bharti et al. (2017), who reported that propolis, an antioxidant, elevated both HGB and PCV following exposure to combined antitubercular medicines. It is reasonable to, therefore, state that methionine, which has been reported to have free radical scavenging activity, would produce a similar effect.

A previous study had reported that INH-induced oxidative stress in red blood cells (RBCs) (Yilmaz et al. 2008), and also inhibits hem biosynthesis (Huang and Benz 2001). This corresponds to results from this present study, where the RBC counts were lower in participants on antitubercular medicines compared to those on methionine and vitamin B-complex (in the presence of antiTB medicines). This modulatory role can be explained by the link between phosphatidylcholine, methionine and vitamin B12. Phosphatidylcholine, a major component of red blood cell membranes (Cooper and Hausman 2015), is synthesized from the metabolite of methionine, S-Adenosyl-L-Methionine (SAMe). Vitamin B12 is used by methionine synthase to convert homocysteine into S-Adenosyl-L-Methionine (SAMe). Vitamin B12 is synthesized from the metabolite of methionine, S-Adenosyl-L-Methionine (SAMe). Vitamin B12 is used by methionine synthase to convert homocysteine into S-Adenosyl-L-Methionine (SAMe). Vitamin B12 is synthesized from the metabolite of methionine, S-Adenosyl-L-Methionine (SAMe). Vitamin B12 is used by methionine synthase to convert homocysteine into S-Adenosyl-L-Methionine (SAMe). Vitamin B12 is synthesized from the metabolite of methionine, S-Adenosyl-L-Methionine (SAMe). Vitamin B12 is used by methionine synthase to convert homocysteine into S-Adenosyl-L-Methionine (SAMe). Vitamin B12 is synthesized from the metabolite of methionine, S-Adenosyl-L-Methionine (SAMe). Vitamin B12 is used by methionine synthase to convert homocysteine into S-Adenosyl-L-Methionine (SAMe). Vitamin B12 is synthesized from the metabolite of methionine, S-Adenosyl-L-Methionine (SAMe). Vitamin B12 is used by methionine synthase to convert homocysteine into S-Adenosyl-L-Methionine (SAMe). Vitamin B12 is synthesized from the metabolite of methionine, S-

Noxious Effects of Antitubercular Drugs Modulated

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Toxicity due to antitubercular medicines can manifest clinically as adverse effects (Blumberg et al. 2003). A significant \( P < 0.001 \) decrease in the prevalence of ADRs in test group participants (56.2%) was observed in the present research, compared to control (32.4%). Rash was reported in 3.4% of patients co-administered antitubercular medicines with methionine and vitamin B-complex, compared to 13.9% on only antitubercular medicines. INH, RIF, PZA, and EMB have been reported to cause this adverse effect in tuberculosis patients (Kaswala 2013).

Males tend to engage more in smoking, use of alcohol consumption than females; all these are risk factors that increase their susceptibility to tuberculosis (Jeong et al. 2015). This could also be responsible for the observation in the present research where more males (46.77%) developed adverse drug reactions (ADRs) than females (38.27%). A significant interaction occurred between smoking \( (P = 0.027) \), alcohol use \( (P = 0.019) \) and adverse drug reactions in the present study, with smokers having about forty-percent increase in risk of developing adverse drug reactions, compared to those who do not smoke, while patients who take alcohol regularly were about two times more likely to develop ADRs than those who do not take alcohol. This link between smoking, alcohol and adverse drug effects was similarly reported by (Chung-Delgado et al. 2011).

In this present research, hepatotoxicity was observed in 6.9% of control group participants on only the antitubercular medicines, lower than 18.2% reported by Isa et al. (2016). Hepatotoxicity is caused by isoniazid and rifampicin in patients (Devarbhavi et al. 2010). One death was reported in the present study due to complications of tuberculosis and possibly, drug-induced hepatotoxicity as a secondary cause.

**Conclusion**

Toxicity to hepatic, renal, and hematological indices and the antioxidant system, as well as adverse effects observed in patients exposed to antitubercular medicines during the 6-month period of treatment was modulated by the combination of methionine and vitamin B-complex tablets. This clearly indicates that such interventions could form part of new treatment strategies aimed at reducing adverse effects due to the antitubercular medicines and improve treatment outcomes in tuberculosis patients.

**Acknowledgements**

The authors acknowledge the support of Lagos State Health Services Commission, Mainland Hospital, Yaba and General Hospital, Lagos, Nigeria, for enabling access to patients, hospital records, personnel and office/workspace. Special thanks to Pharm. Isaac Abah of Jos University Teaching Hospital, Jos, Nigeria and Mr. O.J. Akinsola of Department of Community Health and Primary care, University of Lagos, Nigeria, both of whom performed the statistical analysis. The immense contribution of our research assistants- Elizabeth Kuponiyi Oluwafunmi and Sobande Tolulope Oluwatosin is also acknowledged.

**Disclosure**

The authors declare that no conflict of interest exists in the course of conducting and funding this research. All authors had final decision regarding the manuscript and the decision to submit the findings for publication.

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