8. CONCLUSIONS & RECOMMENDATIONS

In our study different pattern of responses with different antioxidants were observed with respect to modification of MDA and SOD levels, in patients of Tuberculosis and Hypertension, thereby indicating that the Oxidative stress caused by Tuberculosis and that which is caused by Hypertension are likely to have different mechanisms of manifestation at molecular level.

1. Healthy Volunteers:

In healthy human volunteers MDA and SOD levels were estimated to find out normal range of the parameters, as no standard range for these parameters was available. In present study the normal range for MDA was (16.004 -34.294 nMol/ml) and for SOD was (0.112 -0.140 U/gmHb). The estimated values of MDA and SOD were used for calculation of Protective Index (PI) and Stress Index (SI). The derived ranges for PI (0.003-0.007) and for SI (124-192) were considered as standard ranges for both the indices of healthy human volunteers.

2. Tuberculosis:

2.1. Control:

In the patients suffering from Tuberculosis, the Control group who received only DOT therapy. MDA level increased from 32.528±4.874 nMol/ml (Day-0) to 38.944±4.456 nMol/ml (Day-180) and SOD level reduced from 0.136±0.007 U/gmHb (Day-0) to 0.120±0.016 U/gmHb (Day-180). As a result of this,
reduction in PI from $0.004\pm0.001$ (Day-0) to $0.003\pm0.001$ (Day-180) and progressive increase in SI from $240.655\pm39.019$ (Day-0) to $330.786\pm64.668$ (Day-180) indicated progressive increase in oxidative stress even after completion of course of DOT therapy.

2.2 *Hemidesmus indicus*:

In patients suffering from Tuberculosis and on DOT, with *Hemidesmus indicus*, administration in single and double dose, significant decrease in MDA level associated with statistically significant increase in SOD level was observed as compared with Control group. Statistically significant increase in PI and reduction in SI was also observed. Thus *Hemidesmus indicus* was found to be most effective antioxidant as compared to Vitamin-C, Vitamin-E and their combination.

**Vitamin E:**

In patients suffering from Tuberculosis (T), treated with single antioxidant Vit.E, statistically significant reduction in MDA and increase in SOD was observed with both the doses. As a result increase in PI and reduction in SI with both the doses indicate its effectiveness as antioxidant.

**Vitamin C:**

In patients suffering from Tuberculosis (T) who were administered Vit. C as antioxidant therapy, both the doses moderately reduced MDA and highly significant increase in SOD was observed with single dose of Vit.C (TC1).
Statistically significant increase in PI and reduction in SI was observed with single dose of Vit.C (TC1).

2.5 Combination of Vitamin C and Vitamin E:

MDA was moderately decreased with single dose Combination of Vitamin C and Vitamin E while significant increase in SOD was observed with double dose combination. Statistically significant increase in PI and significant reduction in SI was observed with both doses of combination of Vit.C and Vit. E, indicating antioxidant effect of Combination of Vitamin C and Vitamin E.

2.6 Comparison of single and double doses:

2.6.1 Hemidesmus indicus:

In patients suffering from Tuberculosis on comparison of effects of the single dose and double dose, more effectiveness of double dose of *Hemidesmus indicus* (TH2) was observed. The difference on all parameters i.e. MDA, SOD, PI and SI were statistically significant.

2.6.2 Vitamin E:

In patients of Tuberculosis on comparison of the effects of single dose and double dose of Vit.E, double dose of Vit.E (TE2) was found to be more effective with statistically significant difference.
2.6.3 Vitamin C:

Single dose of Vit. C (TC1) was found to be more effective as compared to its double dose with respect to increase in SOD and favorable modification of PI and SI. All the differences were statistically significant.

2.6.4 Combination of Vitamin C and Vitamin E:

In patients of Tuberculosis comparison of the effects of single dose and double dose of Combination of Vitamin C and Vitamin E, double dose Combination of Vitamin C and Vitamin E (TC2E2) was observed to be more effective with high statistical significance with respect to favorable change in PI and SI both.

3. Patients of Hypertension on Calcium Channel Blockers (CCB):

3.1 Control:

In patients of Hypertension on Calcium Channel Blockers, the Control group receiving only Calcium Channel Blockers, as antihypertensive therapy, MDA level on Day-0 was (24.243±8.791 nMol/ml) which was within normal range, MDA level increased to 41.575±13.158 nMol/ml (Day-180). The SOD level decreased from 0.186±0.048 U/gmHb (Day-0) to 0.130±0.037 U/gmHb (Day-180). At the beginning of the study i.e. on Day-0 the PI was (0.010±0.008) which was found to be significantly increased as compared to Healthy
volunteers. During the course of study, PI (0.003±0.002) gradually decreased and reached normal level at the end of study. The SI (153.842±110.643) was within normal range initially, gradually increased and reached (334.712±139.569) at the end of study. The initial increase in PI and normal SI on Day-0, may be due to Calcium Channel Blockers administration due to their antioxidant effects (240).

3.2 *Hemidesmus indicus:*

In patients on Calcium Channel Blockers treated with *Hemidesmus indicus*, change in MDA level was statistically significant with it’s both the doses. While, moderate increase in SOD was observed with double dose of *Hemidesmus indicus* (CCBH2). However single dose of Hemidesmus indicus (CCBH1) produced statistically significant rise in SOD levels as compared to Control group. Consequently PI increased with single dose (CCBH1) from 0.006±0.002 (Day-0) to 0.008±0.003 (Day-180) while with double dose (CCBH2) progressive reduction in SI from 0.014±0.004 (Day-0) to 0.009±0.004 (Day-180) was observed. As far as SI is concerned, single and double dose of *Hemidesmus indicus* were effective in reducing the SI as compared to Control group.

3.3 *Vitamin E:*

Hypertensive patients on Calcium Channel Blockers (CCB) and treated with Vit.E, statistically significant decrease in MDA and moderate increase in SOD
was observed as compared to Control group, with both single and double doses of Vit. E. Resultantly statistically significant increase in PI and reduction in SI with both the doses was observed.

3.4 Vitamin C:

With both single and double doses of Vit.C statistically significantly reduction in MDA levels in hypertensive patients using Calcium Channel Blockers (CCB) was observed. Statistically significantly increase in SOD was observed with single dose of Vit.C (CCBC1). Although PI was not increased significantly but SI was significantly reduced with both the doses as compared to control group.

3.5 Combination of Vitamin C and Vitamin E:

Single and double dose Combination of Vitamin C and Vitamin E moderately reduced MDA and increased SOD which were not statistically significant. Combination of Vitamin C and Vitamin E did not show statistically significant effect on PI. However SI was statistically significantly reduced with double dose combination of Vitamin C and Vitamin E in hypertensive patients on Calcium Channel Blockers (CCB).
3.6 Comparison of Antioxidants (Single and Double dose):

3.6.1 *Hemidesmus indicus*:

In patients of Hypertension and using Calcium Channel Blockers (CCB) with single dose of *Hemidesmus indicus* (CCBH1) least C.V. value were observed for all specific parameters and their indices. Reason for the rise in SOD brought about by *Hemidesmus indicus* may be due to free radical scavanging property of *Hemidesmus indicus* root powder. As reported by Ravishankara et al (2002) *Hemidesmus indicus* root is effective in several free radical mediated disease conditions. \((65,293)\). The lesser effectiveness of double dose of *Hemidesmus indicus* as compared to it’s single dose could be due to the phenomenon of optimum concentration as observed by Gilberto Perez \((294)\).

3.6.2 Vitamin E:

In hypertensive patients on Calcium Channel Blockers (CCB), double dose of Vit. E (CCBE2) has least C.V. value for MDA and SOD along with correspondingly statistically significant change in PI and SI. This observation again suggests complex inter-antioxidant interaction and antioxidant-disease interaction.

3.6.3 Vitamin C:

On comparison of the effects of single (CCBC1) and double (CCBC2) doses of Vitamin C in hypertensive patients on Calcium Channel Blockers
(CCB), it was observed that single dose of Vit. C (CCBC1) was more effective as reflected by statistically significant and greater ‘t’-value for MDA and SI. The lesser efficacy of double dose of Vit.C as compared to it’s single dose could be due to the fact that some substances can act as either antioxidants, or pro-oxidants, depending on the specific set of conditions. Some of the conditions that are important in this respect include the concentration of the chemicals, presence of oxygen or transitional metals, as reported by various researchers (255).

3.6.4 Combination of Vitamin C and Vitamin E:

In hypertensive patients treated with Calcium Channel Blockers (CCB), comparison of single dose and double dose Combination of Vitamin C and Vitamin E, moderate reduction in SI with single dose and statistically significant reduction in SI with double dose was observed.

4. Patients of Hypertension on Antihypertensive drugs Other than Calcium Channel Blockers (OA):

4.1 Control :

In Hypertensive patients treated with Antihypertensive drugs Other than Calcium Channel Blockers (OA), the Control group which received only Other Antihypertensive drugs, it was observed that increase in MDA from \(25.225 \pm 9.591\) nMol/ml (Day-0) to \(38.121 \pm 8.579\) nMol/ml (Day-180) occurred. This increase was statistically significant. Reduction in SOD was from \(0.132 \pm 0.032\) U/gmHb (Day-0) to \(0.116 \pm 0.017\) U/gmHb (Day-180). The PI was
maintained within normal range throughout the study. The SI was found to be increased progressively from $208.960 \pm 103.630$ (Day-0) to $339.048 \pm 104.218$ (Day-180), in spite of administration of Other Antihypertensive drugs than Calcium Channel Blockers.

4.2 Hemidesmus indicus:

In patients on Antihypertensive drugs Other than Calcium Channel Blockers (OA), *Hemidesmus indicus* administration reduced MDA level and increased SOD levels as compared to Control group. These differences were statistically significant. Single dose of *Hemidesmus indicus* (OAH1) produced moderate increase while double dose of *Hemidesmus indicus* (OAH2) brought about highly significant increase in PI from $0.006 \pm 0.003$ (Day-0) to $0.015 \pm 0.004$ (Day-180). Statistically significant decreased in SI was observed with both single and double doses of *Hemidesmus indicus*. Statistically significant effects of *Hemidesmus indicus* on PI and SI indicated *Hemidesmus indicus* to be effective antioxidant in patients taking Antihypertensive drugs Other than Calcium Channel Blockers (OA).

4.3 Vitamin E:

In Hypertensive patients on Antihypertensive drugs Other than Calcium Channel Blockers (OA) administration of Vit.E, reduced MDA and increased in SOD levels. These changes were statistically significant. Double dose of Vit.E (OAE2) was the most effective as indicated by greater ‘t’-value. There was
marginal increase in PI. However SI reduced significantly with double dose of Vit.E (OAE2).

4.4 Vitamin C:

Vit.C alone administered in Patients of Hypertension who were on Antihypertensive drugs Other than Calcium Channel Blockers (OA). Reduction in MDA and increase in SOD was observed. These changes were statistically significant. The increase in PI was not statistically significant with either single or double dose. SI was reduced significantly as compared with Control group to statistically significant level.

4.5 Combination of Vitamin C and Vitamin E:

Reduction in MDA in Hypertensive patients on Antihypertensive drugs Other than Calcium Channel Blockers (OA) with single and double Combination of Vitamin C and Vitamin E was not statistically significant. Although both doses of combination increased SOD significantly, however the increase in PI was not statistically significant, statistically significant reduction in SI was observed.

4.6 Comparison of Single and Double dose.

4.6.1 Hemidesmus indicus:

In patients of Hypertension treated with Antihypertensive drugs Other than Calcium Channel Blockers (OA) double dose of Hemidesmus indicus
(OAH2) was found to be highest effective as judged by greater ‘t’-value of all the treatment groups, in terms of MDA, SOD, PI and SI.

4.6.2 Vitamin E:

In patients suffering from hypertension and using Antihypertensive drugs Other than Calcium Channel Blockers (OA) on comparison of single dose and double dose of Vit.E, double dose of Vit.E (TE2) was found to be more effective.

4.6.3 Vitamin C:

On comparison of single dose and double dose of Vit.C in patients on Antihypertensive drugs, Other than Calcium Channel Blockers (OA), Confidence Interval for OAC2 (0.005–0.006) was observed with more consistency of the results and thereby the double dose was considered more effective, in lowering MDA and SI with rise in PI in patients of hypertension treated with Antihypertensive drugs Other than Calcium Channel Blockers (OA).

4.6.4 Combination of Vitamin C and Vitamin E:

In patients of hypertension on Antihypertensive drugs Other than Calcium Channel Blockers (OA), double dose of Combination of Vitamin C and Vitamin E (OAC2E2) with significant ‘t’-value with respect to changes in SOD and both the indices i.e.PI and SI was found to be more effective as compared to single dose Combination of Vitamin C and Vitamin E (OAC1E1).
5. Comparison of selected doses:

i. Double dose of *Hemidesmus indicus* (TH2) was found to be the most effective dose out of all antioxidant treatments in patients suffering from Tuberculosis.

ii. Single dose of *Hemidesmus indicus* (CCBH1) was found to be the most effective dose in patients on Calcium Channel Blockers (CCB) with respect to favorable alteration of all the parameters.

iii. In patients on Antihypertensive drugs Other than Calcium Channel Blockers (OA), double dose of *Hemidesmus indicus* (OAH2) was found to be the most effective out of all treatments in reducing oxidative stress as judged by MDA, SOD, PI and SI.

### Table-7.1 Comparative cost of treatment of the three antioxidant therapies:

<table>
<thead>
<tr>
<th>Dose</th>
<th>Hemidesmus indicus</th>
<th>Vit. E</th>
<th>Vit.C</th>
<th>Vit.C + Vit. E</th>
</tr>
</thead>
<tbody>
<tr>
<td>Single dose</td>
<td>135/-</td>
<td>261/-</td>
<td>345.60-/</td>
<td>606.60/-</td>
</tr>
<tr>
<td>Double dose</td>
<td>270/-</td>
<td>522/-</td>
<td>691.20-/</td>
<td>1213.20/-</td>
</tr>
</tbody>
</table>

On comparison of the three antioxidant treatments, treatment cost for 180 days as on 30/04/2010, as per MRP of the drugs, the *Hemidesmus indicus* comes out to be the cheapest and most effective amongst the three antioxidant drugs used in this study.
**Conclusion and Recommendation**

**Recommendations:**

1. Antioxidants should form the regular part of prescription in Tuberculosis and Hypertension as supportive therapy.

2. Our study is suggestive of antioxidant specificity and dose specificity, further studies are indicated to prove or rule out the hypothesis.

3. The study also points out towards “Window Effect” of antioxidants although confirmation is required.

4. Disease selectivity of antioxidants, their combinations and complexity of inter-oxidants interaction and disease-antioxidant interaction needs further objective specific clinical studies for optimization of beneficial clinical effects.

5. Optimal doses of various antioxidants in different forms of oxidative stress required to be determined.