

# Methylxanthine induced cardiotoxicity and its mechanisms: An experimental study

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## Abstract

**Background:** Methylxanthines like theophylline are re-emerging as effective adjuncts in the treatment of obstructive airway disease and several newer mechanisms have been proposed. In view of its effectiveness, pharmacoeconomic viability, and easy availability, it could rationalize the drug treatment in bronchial asthma and chronic obstructive pulmonary disorders (COPD) – but its narrow therapeutic index and resultant safety concerns need to be aggressively addressed, particularly in developing countries. Cardiotoxicity and neurotoxicity are amongst the most noted adverse effects associated with its clinical use. The present study evaluated the possible mechanisms involved in aminophylline (water-soluble ethylene diamine salt of theophylline) on potential cardiotoxicity parameters in rats. **Methods:** Aminophylline was administered in various doses for seven days to rats of either sex weighing 200-250g. The electrocardiogram and blood pressure were recorded on seventh day. After this the blood was withdrawn to estimate various stress and cardiac markers. **Results:** Aminophylline (50-150 mg/kg for seven days) dose dependently induced tachycardia and elevated mean BP. There were also T-wave inversions and suppressed T-wave areas at higher doses when compared with control group. IBMX (PDE inhibitor) did not influence these parameters per se. Further, pre-treatment with 2-chloroadenosine (adenosine agonist) could not completely attenuate aminophylline effects. The methylxanthine also elevated MDA levels and reduced GSH levels in heart tissue homogenates of aminophylline treated rats. Aminophylline also elevated serum CPK-MBV levels whereas, BNP levels were not much affected. Pre-treatment with the anti-oxidant, alpha-tocopherol (20 and 40 mg/kg) before administration to aminophylline, attenuated the aminophylline induced changes in heart rate, mean BP, and T-wave areas. These cardiac changes after alpha-tocopherol were supported by biochemical findings, wherein antioxidant pre-treatment prevented an increase in MDA and reduced GSH as well as CPK-MB levels. **Conclusion:** The results indicate the possible involvement of free radicals in theophylline induced cardiotoxicity, and suggest that anti-oxidants could help in reducing the safety concerns associated with this drug. **Key words:** microRNA, heme oxygenase, proximal tubule cells.

**Key words:** Theophylline, cardiotoxicity, free radicals, antioxidants

## Introduction

Pharmacotherapy is a crucial component for the treatment of obstructive airway disease like bronchial asthma; anti-inflammatory agents and bronchodilators are essential components of such therapy. Methylxanthines are bronchodilators that have been effectively used therapeutically in

cardiorespiratory disorders for nearly a century. Caffeine and theophylline are common examples of methylxanthines used in therapy and are also found in beverages like tea and coffee, which are commonly consumed. Theophylline (1, 3-dimethylxanthine) is a commonly used bronchodilator which was earlier used effectively, but recently became less preferred due to safety concerns. This is due to its narrow therapeutic index and the resultant propensity of the drug to induce adverse effects. Earlier reported theophylline intoxication include metabolic changes like hyperglycemia, hypokalemia, metabolic acidosis,

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nausea, vomiting and in severe cases, seizures, cardiac arrhythmias, and death.<sup>1</sup> The most serious, among the adverse effects are neurotoxicity and cardiotoxicity, which results in serious morbidity and sometimes mortality in patients. In the cardiovascular system, this methylxanthine bronchodilator has clinical effects on cardiac rate (tachycardia) and rhythm (arrhythmias) at near therapeutic levels and toxic manifestations like anxiety and palpitation are reported. It has positive chronotropic effect that causes a dose dependent increase in heart rate.<sup>2, 3, 4, 5</sup> Cardiac arrhythmias have also been reported during theophylline use in an electrocardiographic study.<sup>6</sup> Combined treatment with beta-agonist and theophylline, which is common during the treatment of bronchial asthma and COPD, showed the potentiating effects on heart rate and mean blood pressure.<sup>7</sup> When administered acutely to clinically stable patients with chronic obstructive lung disease, theophylline generally does not produce arrhythmias. However, others have observed serious arrhythmias in patients with chronic lung disease at serum theophylline concentrations generally accepted as being desirable.<sup>8</sup> The use of theophylline has declined markedly in recent years. This trend can be attributed to factors such as the advent of more effective bronchodilating agents, and the recognition of theophylline's narrow therapeutic index. However, this potent drug continues to have a role in the treatment of neonatal apnea and as prophylactic therapy for asthma or chronic obstructive pulmonary disease.<sup>9</sup> Because of theophylline's continued usage, particularly in economically weaker countries, instances of overdose still occur. Theophylline overdoses generally result from their irregular elimination kinetics (particularly in those at the extremes of age - very young and elderly, irregular use by asthma patients, and administration to patients with hepatic or cardiac abnormalities). The presence of theophylline at in the home can lead to acute intoxication after ingestion by curious toddlers or suicidal adolescent. Errors in drug administration, particularly 10-fold dosing errors are a common cause of acute therapeutic intoxication. The overall incidence of theophylline intoxication is unknown; since 1991, about 27,768 cases of theophylline overdose have been reported to the American Association of Poison Control Centers.<sup>10</sup>

Earlier studies on theophylline pharmacodynamics and toxicodynamics have proposed mechanisms like, phosphodiesterase inhibition, adenosine antagonism, catecholamine release and calcium flux modulation.<sup>11</sup> Studies also showed that toxicity of theophylline depended on plasma concentration by single and also repeated dosing in rats.<sup>12</sup> In our lab, earlier studies showed the possible role of free radicals in theophylline induced neurotoxicity in rats.<sup>13</sup> Low dose theophylline showed antioxidant effects, but at high dose it causes neurotoxicity and cardiotoxicity in rats.<sup>14</sup> CNS stimulant effects of theophylline include seizures, which is a potentially fatal complication of its toxicity.<sup>15</sup> Free radicals produce beneficial as well as harmful effects.<sup>16</sup> Imbalance of pro-oxidant/antioxidant levels result in biochemical and cellular alterations leading to development of various pathophysiological states such as rheumatoid arthritis, septic shock, atherogenesis and diabetes mellitus.<sup>17</sup> The increased endogenous and synthetic catecholamine like isoprenaline also induced acute myocardial infarction (AMI) and subsequent heart failure in rats. The potentiating effects (cardiac toxicity) following the combined use of  $\beta$ -adrenergic bronchodilators and theophylline was shown in experimental animals.<sup>18</sup>

In recent years, there has been resurgence in the interest in theophylline and related methylxanthines, in view of the newly demonstrated anti-inflammatory and immunomodulatory effects, both of which could be beneficial in obstructive airway diseases.<sup>19</sup> Further, low doses (lower than those needed to induce bronchodilator) exert these beneficial effects. The steroid sparing effects of theophylline has also been reported and enhancement of corticosteroid responsiveness in a group of steroid insensitive patients has been reported.<sup>20</sup> Thus, judicious use of theophylline could be of beneficial in obstructive airway disease in developing countries. Further, newer mechanisms are being proposed for theophylline effects and oxidative stress has been indirectly implicated. Though very effective in relieving symptoms, concerns about their adverse effects and reports of sudden death have limited their use. Although the occurrence of cardiac arrhythmias and other associated events with theophylline toxicity

is well recognized, the pharmacological basis of such untoward effects are not clearly defined. Since deleterious adverse effects on the cardiovascular system is the major cause of theophylline induced morbidity and mortality, the present study was designed to evaluate the toxicodynamics of theophylline induced cardiotoxicity in experimental animals.

## Materials and Methods

**Animals:** Wistar rats (200-250 g), of either sex, were used in the study. Rats were housed at a constant temperature of  $22 \pm 2^{\circ}\text{C}$  under a 12 h light: 12 h dark cycle. The animals (n=6) per group had free access to food and water throughout the experiment. Animal care was taken as per guidelines in "Care and use of animals in scientific research", prepared by Indian National Sciences Academy (INSA), New Delhi. The study protocol had the approval of institutional animal ethics committee (IAEC) of VPCI.

## Drugs and chemicals

The drugs used were aminophylline (ethylenediamine salt of theophylline); 2-chloroadenosine (Adenosine agonist); IBMX (Phosphodiesterase inhibitor); salmeterol (Beta-2 agonist); and alpha-tocopherol (antioxidant). All drugs were procured from Sigma-Aldrich (St. Louis, USA). The drugs were dissolved in appropriate vehicle and injected intraperitoneally (i.p.) or subcutaneously (s.c.) in a volume of 1ml/kg. The ELISA kits for various biomarkers assayed were procured from Weldon Biotec (New Delhi). All other routine chemicals needed for various assays were procured from SRL Labs (New Delhi).

## Studies on Aminophylline induced cardiotoxicity in rats

### a) Effects on Heart Rate, Mean Blood Pressure, and ECG

Aminophylline (ethylene diamine salt of theophylline) was administered daily for 7 days in doses of 50, 100, 150 and 200 mg/kg, i.p.). After this, the rats were anaesthetized by urethane (1.75 gm/kg, i.p.). After anaesthesia, the animals were put on a wooden platform and all four limbs were secured to the board with a thread. The hair on the forelimb and hind limb

area (abdominal region) was shaved with the trimmer and the area was cleaned. The ECG electrodes were fixed – two at forelimbs and two at hind limb regions, and connected to BIOPAC MP-36 system, and the data acquisition system for measuring all parameters.

The BIOPAC MP-36 systems (developed by BIOPACK system, Inc, USA) provided the most complete data acquisition and analysis system for the biological and pharmacological experiments. The MP-36 data acquisition system along with the software presented a flexible, easy to use modular system for data collection and made the analysis simple and efficient.

The ECG was used to measure heart rate, QT interval extraction, myocardial ischemia, and cardiac rhythm in the various treatment groups. Other general physical and behavioural signs as well as mortality were also assessed.

For determination of blood pressure through carotid artery (invasive method), the neck area was cut open to expose the trachea and carotid arteries. Carotid arteries, which lie close to the trachea on either side along with veins and the nerves, were easily recognized by their elastic and pulsating nature. A slit was made in the trachea and a polythene cannula was inserted into trachea, while the other end was connected to an artificial respirator. The right carotid was cleaned/separated from the accompanying structures in the neck region with the help of a blunt dissector scissors. The carotid artery was tied as near the head end as possible, a bulldog clamp was placed about three cm nearer the heart and a thread was tied after the carotid cannulation toward the head region. A 24G cannula was inserted into the carotid artery then connected to the blood pressure transducer and started the BIOPAC MP - 36 for five minutes; care was taken to avoid air bubbles between the cannula and sensor for better results. All parameters like mean blood pressure, systolic and diastolic blood pressure and heart rate were measured by carotid artery cannulation method. After recording cardiovascular parameters,

blood samples and heart were collected from the animals. The blood sample was processed and serum separated. Both blood and heart were stored at -80°C respectively, for estimation of various biochemical parameters.

### Experimental design

Rats were divided into five different groups (n=6 per group).

Group 1- Control (normal saline i.p.), Group 2 - Aminophylline 50 mg/kg, Group 3 - Aminophylline 100 mg/kg, Group 4- Aminophylline 150 mg/kg, and Group 5- Aminophylline 200 mg/kg. Aminophylline is the water soluble, ethylene diamine salt of theophylline. The experimental groups were given different doses of aminophylline daily for seven days by i.p. route. After seven days of drug administration, cardiovascular parameters were recorded by the BIOPAC – MP 36 system as mentioned above. Following this, blood was collected by cardiac puncture, centrifuged at 4°C (3000 rpm) for 10 min and the serum was separated and stored at -80 °C. Biochemical estimation was done using ELISA kits. Heart was removed and stored at -80°C; it was homogenized, supernatant separated and assayed for various biomarkers.

### Assay for oxidative stress markers

#### a) Malondialdehyde (MDA) <sup>21</sup>

Malondialdehyde, a marker of lipid peroxidation, was determined as described by Ohkawah et. al. Briefly, the reaction mixture consisted of 0.2 ml of 8.1% sodium lauryl sulphate, 1.5 ml of 20% acetic acid (pH 3.5) and 1.5 ml of 0.8% aqueous solution of thiobarbituric acid and 0.2 ml of heart tissue homogenate. The mixture was made up to four ml with distilled water and heated at 95°C for 60 minutes. After cooling with tap water, five ml of n-butanol and pyridine (15:1 v/v) and one ml of distilled water was added and centrifuged. The organic layer was separated out and its absorbance was measured at 532 nm using a UV-visible spectrophotometer (UV 5740 SS, ECIL) and MDA content was expressed as nmole/mg protein.

#### b) Reduced Glutathione (GSH) <sup>22</sup>

This assay was based on reduction of 5, 5'-dithiobis-(2-nitrobenzoic acid) by SH groups to form 2-Nitro-5-mercaptobenzoic acid. The nitromercaptobenzoic acid anion has an intense yellow colour that was determined spectrophotometrically at 412 nm. Reagents were mixed and the colour developed was read immediately at 412 nm on a Shimadzu spectrophotometer against blank.

Assay for cardiac biomarker

#### a) Creatine phosphokinase (CPK-MB)

CPK-MB, a marker of myocardial ischemia, was determined in blood samples using ELISA KIT (by USCN Life Science Inc.). The microplate well is precoated with specific antibody Creatine Kinase - Muscle/ Brain (CPKMB). The standard or samples were then added to the appropriate microplate wells with a biotin-conjugated antibody specific to CKBB. Next, avidin conjugated to horseradish peroxidase (HRP) was added to each microplate well and incubated for a given time period. After 3, 3', 5, 5'-tetramethylbenzidine (TMB) substrate solution was added, the colour of the solution changed. The enzyme substrate reaction was stopped by adding sulphuric acid solution, and the colour change was measured spectrophotometrically at a wavelength of 450 nm. The concentration of CPKMB in the samples was determined by comparing the optical density (OD) of sample to the standard. The results were expressed in ng/ml.

#### b) Brain natriuretic peptide (BNP)

BNP, a marker for acute congestive heart failure (CHF), was estimated using ELISA kit manufactured by Ray Biotech, Inc. It is an *in vitro* assay for detecting BNP peptide based on the principle of competitive enzyme immunoassay. The microplate well is precoated with anti-rabbit secondary antibody. The sample was added to each well and incubated for 30 minutes at 37°C and bound biotinylated BNP peptide interact with streptavidin-horseradish peroxidase (SA-HRP) which gives colour. Intensity of colour developed is directly proportional to amount

of biotinylated peptide-SA and inversely proportional to BNP peptide in the standard and samples. The colour change was measured spectrophotometrically at a wavelength at 450 nm.

### Statistical analysis

The data were expressed as mean  $\pm$  SEM and analyzed by one-way analysis of variance (ANOVA) followed by post hoc Tukey's multiple comparison tests. A p value  $<0.05$  was considered to be statistically significant.

### Results

Aminophylline (50-150 mg/kg, i.p.) administration induced tachycardia in a dose dependent manner. The heart rate and mean BP increased with graded dose of aminophylline. The results of aminophylline (150 mg/kg) treated group were significantly different from the vehicle control group ( $P<0.05$ , one way ANOVA); maximal effects on heart rate was observed at aminophylline 150 mg/kg as compared to vehicle treated group. The lower doses of aminophylline also induced changes in heart rate and mean BP, but they were not significantly different from control values. The highest dose of the drug (200 mg/kg) exhibited a high mortality rate (80%), hence was not used in further experiments. The PDE inhibitor, IBMX (100 mg/kg), was able to induce only a 10% enhancement in the heart rate and similar nominal increase in mean BP; both these changes were not significantly different from control group values ( $p>0.05$ , in each case). On the other hand, pretreatment of rats with the adenosine agonist, 2-chloroadenosine (2-CADO, 10 mg/kg) only partially attenuated the effects of aminophylline 150 mg/kg on heart rate and mean BP ( $P<0.05$ , vs aminophylline).

An interesting observation noted was the appearance of inverted T-waves in ECG tracings of the rats in a given time interval after aminophylline with graded effects being seen after 100 and 150 mg/kg doses of the drug, with most consistent and prominent changes in the ECG tracings of the rats treated with aminophylline 150 mg/kg. A number of T-wave inversions were, however, seen at the lowest dose level of 50 mg/kg of the drug. Analysis of the ECG interval extraction data showed that

T-wave area of control (vehicle) group was  $1.19 \pm 0.12$  mV-msec, whereas, the T-wave areas of the aminophylline treated groups were:  $1.05 \pm 0.21$  mV-msec (for 50 mg/kg);  $0.91 \pm 0.42$  mV-msec, (for 100 mg/kg) and  $0.73 \pm 0.27$  mV-msec (for 150 mg/kg in table 1). Thus, the T-wave areas were consistently and dose dependently decreased after aminophylline treatment, when compared to control group, with the data of the 150 mg/kg of aminophylline being statistically significant ( $P<0.05$ ).

Pretreatment of rats with the antioxidant, alpha-tocopherol, at doses of 20 mg/kg and 40 mg/kg, dose dependently attenuated the effects of aminophylline (150 mg/kg) on heart rate and mean BP. The heart rate was reduced to near normal levels after the 40 mg/kg dose of tocopherol, with the lower dose showing lesser reversals in cardiac parameters. Tocopherol pretreatment also reversed the incidence of inverted T-waves to near normalcy and analysis of the T-wave areas showed that the anti-oxidant also attenuated the aminophylline induced reductions in T-wave area by a significant extent. These results are summarized in Table 1.

**Table1. Effects of aminophylline and its modulation by drugs on heart rate, BP and ECG**

Treatment(mg/kg)	Heart Rate (beats/min)	Mean BP (mmHg)	T-Wave area (mv-msec)
Control	400 $\pm$ 9.57	70 $\pm$ 2.30	1.19 $\pm$ 0.12
Aminophylline 50	445 $\pm$ 8.66	80 $\pm$ 2.32	1.05 $\pm$ 0.21
Aminophylline 100	481 $\pm$ 12.22	85 $\pm$ 3.30	0.91 $\pm$ 0.42
Aminophylline 150	550 $\pm$ 11.41*	95 $\pm$ 3.21*	0.73 $\pm$ 0.27*
IMBX 100	445 $\pm$ 7.75	85 $\pm$ 4.30	-
2-CADO10 +Aminophylline150	480 $\pm$ 8.86#	70 $\pm$ 1.31#	-
$\alpha$ -TP 20 + Aminophylline 150	420 $\pm$ 10.80#	80 $\pm$ 5.24	0.99 $\pm$ 0.33
$\alpha$ -TP 40 + Aminophylline 150	405 $\pm$ 9.10#	70 $\pm$ 2.11#	1.11 $\pm$ 0.29#

All value expressed as Mean  $\pm$  SEM; one way ANOVA followed by post hoc Tukey's multiple comparison test, (n=6), \* $P<0.05$ vs control, # $P<0.05$  vs aminophylline 150 mg/kg considered as significance

Biochemical assay data showed that graded doses of aminophylline (50, 100 and 150 mg/kg) induced

dose related changes in oxidative stress and cardiac biomarkers when compared to control values, with 150 mg/kg of the drug showing most consistent effects on most parameters tested. There was a near 300% increase in the MDA levels and a 66% reduction in the GSH levels in heart tissue homogenates after aminophylline 150 mg/kg as compared to controls ( $p < 0.05$ ). Pretreatment with alpha tocopherol (20 and 40 mg/kg) attenuated the aminophylline induced increases in MDA, also reversed the suppressed GSH to near baseline levels. Aminophylline (150 mg/kg) also markedly increased the CPK-MB levels in serum, whereas, serum BNP levels were only marginally elevated. Pretreatment with tocopherol attenuated both these biochemical derangements in a dose related manner, with the effects of the higher dose of the anti-oxidant being more consistent. Another interesting observation was the elevation in serum CPK-MB levels, which were markedly raised after aminophylline 150 mg/kg. These changes in aminophylline induced rise in CPK-MB levels were attenuated after tocopherol pretreatment with the effects of 40 mg/kg of the antioxidant being most effective. On the other hand, only marginal elevations were seen in serum BNP levels after aminophylline administration which was not statistically significant as compared to controls ( $p > 0.05$ ). These results are summarized in Table 2.

**Table 2: Effects of aminophylline and its modulation by drugs on biochemical markers**

Treatment (mg/kg)	MDA(nmol/mg protein)	GSH( $\mu$ mol/mg protein)	CPKMB(ng/mL)	BNP (pg/ml)
Control	4.5 $\pm$ 0.06	5.2 $\pm$ 0.23	4.5 $\pm$ 2.17	22.52 $\pm$ 0.17
Aminophylline 50	6.2 $\pm$ 0.14	3.4 $\pm$ 0.33	4.9 $\pm$ 1.29	24.31 $\pm$ 0.19
Aminophylline 100	9.1 $\pm$ 0.90	2.1 $\pm$ 0.53	6.2 $\pm$ 1.22	24.81 $\pm$ 0.12
Aminophylline 150	12.6 $\pm$ 1.01*	1.7 $\pm$ 0.66*	15.5 $\pm$ 1.32*	25.21 $\pm$ 0.85
$\alpha$ -TP 20 + Amino 150	6.2 $\pm$ 1.02#	4.5 $\pm$ 0.22#	5.6 $\pm$ 2.10#	23.30 $\pm$ 0.72
$\alpha$ -TP 40 + Amino 150	5.2 $\pm$ 3.03#	5.0 $\pm$ 0.12#	4.1 $\pm$ 2.62#	24.41 $\pm$ 0.06

All value expressed as Mean  $\pm$  SEM; one way ANOVA followed by post hoc Tukey's multiple comparison test, (n=6), \*P<0.05vs control, #P<0.05 vs aminophylline 150 mg/kg

## Discussion

Methylxanthines are bronchodilators that have been effectively used therapeutically in cardiorespiratory disorders for nearly a century. Caffeine and theophylline are common examples of methylxanthines used in therapy and are also found in tea and coffee, commonly consumed beverages. Theophylline (1,3-dimethylxanthine) has been a commonly used bronchodilator in respiratory disorders like bronchial asthma and COPD, particularly in developing countries, due to its easy availability and low cost. It had, however, become less popular due to its continuing safety concerns and availability of better and safer agents. However, in recent years there has been resurgence in the interest with the use of theophylline in respiratory disorders due to some newly proposed mechanisms of action.<sup>23</sup> The present study was designed to investigate the possible mechanisms involved in theophylline induced cardiotoxicity in order to propose and devise possible strategies to counteract this very relevant clinical problem associated with the this effective agent.

The present results showed that aminophylline (50-150 mg/kg) induced dose dependent increases in heart rate of anaesthetized rats. The maximal effect being seen with the highest dose of the drug tested as compared to control values. The tachycardia seen after aminophylline was associated with a rise in the mean BP, which again was most prominent at the higher dose of 150 mg/kg of the drug. When a still higher dose of the drug was given (200 mg/kg), there was marked increase in mortality (80%) which was not seen with the lower doses. This suggests that the highest dose of aminophylline could have resulted in cardiac arrest due to ventricular tachycardia and fibrillations – a finding that is also reported in patients.<sup>18</sup> Theophylline and related methylxanthines are commonly known to act by two mechanisms – phosphodiesterase (PDE) inhibition and adenosine antagonism.<sup>21</sup> In order to evaluate the possible mechanism involved in such theophylline effects, the involvement of PDE inhibition and adenosine antagonism in aminophylline effects were assessed. Our experiments showed that the nonspecific PDE inhibitor, IBMX, only marginally

influenced the heart rate and mean BP as compared to what was seen after aminophylline. This indicates that PDE inhibition was possibly not involved in the aminophylline induced cardiotoxic effects. Adenosine is a well-documented neuromodulator substance and is known to have regulatory effects on cardiovascular pathophysiology. The anti-arrhythmic effects of adenosine in supraventricular tachycardia are also known.<sup>24</sup> The possibility of adenosinergic involvement in aminophylline induced cardiotoxic effects were also evaluated in our experiments. Pretreatment with the adenosine agonist, 2-chloroadenosine (2-CADO) only partially influenced the aminophylline induced cardiovascular changes on heart rate and mean BP. This suggests that the adenosine antagonism component of aminophylline could not totally explain the observed cardiotoxic effects of the drug. Thus, it was apparent that the observed cardiac effects aminophylline could not be explained based on either PDE inhibition or adenosine antagonism, and it was likely that other alternative mechanisms could be involved in these cardiotoxic effects. Earlier studies on aminophylline-induced neurotoxicity had also shown similar results in that the role of PDE inhibition and adenosine antagonism could not explain aminophylline induced neurotoxicity.<sup>25</sup>

Another very interesting aspect of our findings was the detection of frequent inverted T-waves in the ECG recordings of these rats particularly at the higher doses of aminophylline (150 mg/kg). These T-waves were seen at very regular intervals and any stretch of ECG recording and was not seen in either control group of rats or with lower doses of the methylxanthine (50 mg/kg). T-wave areas, as calculated by ECG interval extractions, are a crucial indicator of the extent of T-wave inversions and is used a standard measure to assess cardiac ischemia. Extraction of ECG interval data showed that T-wave areas in aminophylline (150 mg/kg) treated rats were much lower than that of the control group. Interestingly, none of the other ECG interval extraction data viz. PR interval, RR interval, and QTc interval showed any appreciable change after aminophylline administration as compared to control values. An inverted T-wave in the ECG and reduced

T-wave area are both suggestive of cardiac ischemia and it is quite possible that such ischemia could have been associated with or lead to the observed cardiac effects of aminophylline.

Oxidative stress results an imbalance between prooxidant and anti-oxidants. It plays an important role in health and disease—several pathophysiological conditions have been attributed to deleterious effects of free radicals in the body.<sup>26, 27, 28</sup> The involvement of oxidative stress in cardiovascular regulation is well known and the anti-oxidants have been reported to have beneficial effects.<sup>29</sup> Recently, free radicals have been concern in many drug and chemical induced toxicities.<sup>30</sup> Thus, the involvement of free radicals during administration of theophylline induced seizures was reported. The xanthine-xanthine oxidase system is an important pathway of generation of ROS/RNS in biological systems,<sup>31</sup> and one of the major metabolites of theophylline is a substrate for xanthine oxidase.<sup>32</sup> It is, therefore, possible that the increased production of ROS during metabolism of high doses of theophylline could result in oxidant/antioxidant imbalance and accordingly precipitate cardiotoxicity.

Our studies showed that pretreatment with the potent antioxidant, alpha-tocopherol (20 and 40 mg/kg) attenuated the aminophylline induced tachycardia in a dose related manner. Antioxidants are known to neutralize the effects of free radical induced oxidative stress and our study showed that alpha-tocopherol reversed aminophylline induced changes in heart rate and mean BP towards control levels – suggesting the involvement of oxidative stress. To support our pharmacological observations, we measured the levels of oxidative stress markers in serum and heart tissue in aminophylline treated and drug interacted rats. Our results showed that aminophylline induced tachycardia and raised mean BP was associated with suppressions in GSH in heart tissue homogenates. GSH is an effective marker of anti-oxidant status of the organism and the lowered levels suggest the weakening of anti-oxidant defense mechanisms during aminophylline induced cardiotoxicity. MDA, which is an index of lipid peroxidation, was also markedly elevated after aminophylline as compared to controls. The

anti-oxidant, alpha-tocopherol clearly reversed the changes in GSH and MDA levels towards normalcy, further confirming the depletion of antioxidant defense systems during such aminophylline effects. The imbalance between pro and antioxidant forces resulted in the dominance of oxidants, which resulted in the oxidative stress. Taken together, aminophylline induced changes in heart rate and mean BP were probably due to the increased oxidative stress – our pharmacological and biochemical data support this hypothesis. Our findings are also along the lines of some earlier studies which showed that aminophylline induced neurobehavioural toxicity was mediated by free radicals and antioxidants were effective in reversing such pathophysiological states.<sup>33</sup>

The present study showed that aminophylline induced clear changes in the ECG wherein inversions of T-waves were seen very consistently, particularly at the higher dose (150 mg/kg) of the drug. Extraction of intervals from ECG tracings showed that the T-wave areas in the aminophylline treated rats were much lower as compared to those of controls. These two observations provided both qualitative and quantitative evidence that was strongly suggestive of cardiac ischemia. It is possible that the rapid heart rate could have resulted in dysregulation of oxygen homeostasis and increased oxygen demand in the heart muscle, which in turn resulted in cardiac ischemia. To confirm this aspect, we assessed cardiac biomarkers relevant to ischemia in the serum of these rats. Interestingly, the serum levels of CPK-MB were markedly higher in aminophylline (150 mg/kg) treated rats as compared to controls and the lower doses of this drug. Elevated levels of these cardiac biomarkers are a hallmark of diagnosis of cardiac ischemia in the clinical setting. Pretreatment with the antioxidant reversed both ischemic changes seen in the ECG as well as the blood biochemical marker in aminophylline treated rats. Collectively, these findings strongly suggest that aminophylline induced tachycardia, elevated mean BP and resultant cardiac ischemia could have been due to increased oxidative stress, and treatment with the anti-oxidant, alpha-tocopherol reversed both cardiac and biochemical changes associated with these phenomenon.

Increased heart rate and cardiac ischemia may lead to heart failure and to ascertain this we evaluated the effects of aminophylline on cardiac biomarkers related to this pathophysiology. BNP is a well-known marker for heart disease and failure. The ventricles generate BNP in response to increased mechanical load and wall stretch. It protects the heart from untoward effects of overload by enhancing diuresis, relaxing vascular smooth muscles, inhibiting the renin-angiotensin system and preventing cardiac hypertrophy and fibrosis.<sup>31</sup> Our results showed that aminophylline did not influence the levels of BNP to any significant extent as compared to that seen in the control group of rats. Further, alpha-tocopherol was able to influence the BNP levels in both normal as well as aminophylline treated groups. This suggests that aminophylline induced tachycardia of the present experimental set up was not adequate to induce overt heart failure in these rats. It is probable that a longer duration of aminophylline therapy (more than the present seven-day treatment schedule) would have sufficed to induce frank heart failure and the resultant change in this biomarker. However, it can also be argued that BNP is a relatively non-specific marker of heart failure and it can only compliment the other characteristic features of the decompensated /compensated ventricular dysfunction.

In conclusion, the results of the present study indicate that aminophylline induced cardiotoxicity manifested as tachycardia, raised mean BP, and cardiac ischemia. The tachycardia seen after aminophylline was associated with elevations in mean BP. Most interestingly, there was dose dependent effect on cardiac ischemia and this was evident by the consistently observed T-wave inversions in the ECG and reductions in the T-wave area after ECG interval extraction. These changes in cardiac parameters associated with alterations in oxidative stress markers (GSH and MDA) in heart tissue were strongly suggestive of free radical involvement. Ischemic changes were observed in ECG tracing after treatment with aminophylline 150 mg/kg. Pretreatment with anti-oxidant, alpha-tocopherol attenuated the effects of aminophylline on (a) cardiac parameters, (b)

oxidative stress markers, and (c) cardiac biomarkers. It is inferred that oxidative stress plays a crucial role in theophylline-induced cardiotoxicity. This study is of immense translational significance, in view of the re-emergence of theophylline for the treatment of obstructive airway diseases like bronchial asthma and COPD and it could help in devising strategies for the effective management of theophylline-induced cardiotoxicity.

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