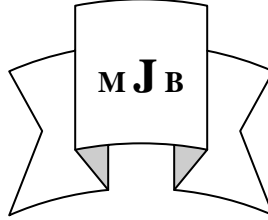


Protective Effect of Aminophylline on Doxorubicin-Induced Cardiotoxicity in Rats

Sabah N. Al-Thamir^(a) Enass Najem Oubaid^(b)

^(a)Dept. of pharmacology and toxicology, Collage of pharmacy, University of Babylon.

^(b)Dept. of pharmacy, Marjan Teaching Hospital, Hilla, Iraq.



Abstract

Background: Doxorubicin is a highly effective antitumor drug, however its usefulness is limited due to its ability to induce cardiotoxicity. Cellular changes leading to this toxicity are suggested to be mediated through a drug-induced increase in free radicals and lipid peroxidation.

Aim of study: to evaluate the protective properties of aminophylline on doxorubicin-induced cardiotoxicity in rats using biochemical approaches.

Materials and methods: Thirty five healthy male Swiss albino rats were used. They were divided randomly into 5 groups (7 animals in each group). All animals supplied with standard food during the experiment with an access of water. They were distributed as follow: first group (normal saline treated group, 1 ml/kg, i.p.) in six equal doses in alternative days over a period of 2 weeks and considered as control group. Second, doxorubicin treated group (2.5 mg/kg, i.p., in six equal doses in alternative days over a period of 2 weeks to make a total cumulative dose of 15 mg/kg, body weight). The third, fourth and fifth groups were treated with aminophylline in doses (10, 20 and 30 mg/kg, i.p.) respectively plus doxorubicin (one hour prior each administered dose of doxorubicin). Blood samples were collected and used to determine the serum levels of cardiac biomarker {Lactate Dehydrogenase (LDH), Creatine Kinase (CK-MB) and troponin I} in addition to oxidative stress parameters {malondialdehyde (MDA) and glutathione (GSH)}.

Results: In aminophylline plus doxorubicin treated groups serum levels of LDH,CK-MB, troponin I and MDA were significantly lower than those of doxorubicin-treated group, While serum GSH was increase. These changes occurred in a dose-dependent manner.

Conclusions: These data show that aminophylline can provide a protective effect against doxorubicin cardiomyopathy. This protective effect of aminophylline may be attributed to its enhancing effect on cellular cyclic nucleotides antioxidant defense on the heart.

الخلاصة

المقدمة: الدوكسوروبيسين هو عقار مضاد للسرطان فعال للغاية، ومع ذلك فإن فائدته محدودة نظراً لتأثيره السمي على القلب. التغيرات الخلوية المؤدية إلى هذه السمية سببها زيادة في تكوين الجذور الحرة وأكسدة الدهون.

الهدف من الدراسة: تقييم التأثير الوقائي للأمينوفيلين على التأثير السمي للدوكسوروبيسين على القلب في الجرذان باستخدام اختبارات كيميائية حيوية.

المواد وطرق العمل: استخدمت في هذه الدراسة خمسة وثلاثون ذكراً بالغاً من الجرذان البيض السويسريه قسمت بشكل عشوائي إلى تسعة مجاميع (سبعة جرذان في كل مجموعة وجميع الحيوانات أعطيت غذاء طبيعي قياسي مع الماء طوال التجربة): الحيوانات في المجموعة الأولى أعطيت ماء ملحي طبيعي (١ مل / كغم) في التجويف البطني (البريتون) على ست جرغ متساوية بين يوم واخر على مدى اسبوعين واعتبرت مجموعة السيطرة الاولى، الحيوانات في المجموعة الثانية أعطيت الدوكسوروبيسين (٢.٥ ملغ / كغم) في التجويف البطني على ست جرغ متساوية بين يوم واخر على مدى اسبوعين لتكون مجموع الجرعة التراكمية (١٥ ملغ/كغم، وزن الجسم)، في حين ان الحيوانات في المجموعات الثالثة والرابعة

والخامسة اعطيت الامينوفيلين بجرعات ١٠، ٢٠، ٣٠ ملغم / كغم في التجويف البطني (البريتون) على التوالي بالإضافة الى الدوكسوروبيسين (قبل ساعة واحدة من كل جرعة معطاة من الدوكسوروبيسين). تم جمع عينات الدم واستخدمت لتحديد مستويات مؤشرات البيولوجية للقلب (LDH و CK-MB و تروبونين ا) ومؤشرات الاكسدة (MDA و GSH) في مصل الدم.

النتائج: ان في المجاميع المعاملة بالامينوفيلين بالإضافة الى الدوكسوروبيسين كانت مستويات المؤشرات البيولوجية للقلب (LDH و CK-MB و تروبونين ا) ومؤشرات الاكسدة (MDA) في مصل الدم اقل من مستوياتها بصوره معنوية في المجموعة المعاملة بالدوكسوروبيسين لوحده ، بينما ازداد مستوى GSH في المصل. ان هذه التغيرات حدثت اعتمادا على الجرعة.

الاستنتاج: تظهر النتائج أن الأمينوفيلين يمكن أن يوفر تأثير وقائي ضد التأثير السمي للدوكسوروبيسين على القلب. ويمكن أن يعزى هذا التأثير الوقائي للأمينوفيلين الى تأثيرها على تعزيز الدفاع المضاد للأكسدة للنيكليوتيدات الحلقية على القلب.

Introduction

Doxorubicin is a potent chemotherapeutic drug from the anthracycline antibiotics from the chemical point of view [1]. Anthracyclines have been the corner stone of many cytotoxic regimens treatment since their introduction in the 1960's and remain important in the treatment of several hematogenous and solid human malignancies [2]. However, despite doxorubicin therapeutic efficacy, its clinical usage is limited by the development of cumulative dose-dependent cardiomyopathy [3] which may occur many years after the cessation of doxorubicin treatment [4]. The acute doxorubicin-induced cardiotoxicity is characterized by hypotension, arrhythmia, tachycardia while the chronic effects are manifested as cardiac dysfunction eventually leading to congestive heart failure [5].

The exact pathogenesis of doxorubicin-induced cardiotoxicity is still not entirely clear although a diverse set of mechanisms have been proposed, including oxidative stress [6], intracellular calcium overload [7], mitochondrial DNA damage, inhibition of protein synthesis, disturbance of myocardial adrenergic function, cytokine release, myofibrillar degeneration and cardiomyocyte apoptosis [8]. Among the multiple mechanisms, it is widely accepted that doxorubicin-induced cardiomyocyte apoptosis is primarily due

to the generation of reactive oxygen species (ROS) in the myocardium which triggers intrinsic mitochondria-dependent apoptotic pathway in cardiomyocytes [9,10]. Numerous earlier studies indicate that free radical scavengers and antioxidants may combat doxorubicin-induced cardiotoxicity [11].

On the other hand, Aminophylline is a non-specific phosphodiesterase inhibitor, thereby increasing tissue levels of cyclic AMP [12]. It has been indicated for the treatment of asthma and COPD for more than 50 years [13]. Some previous studies reported that aminophylline exert some antioxidant activity in vitro [14] and others reported that aminophylline has the free radical scavenging effect in lung epithelial tissue. They showed that both aminophylline and theophylline are scavengers of hydroxyl radical (OH•), but they are ineffective against superoxide anion and hydrogen peroxide [15].

Materials and Methods

Animals: A total of 35 adult male Swiss Albino rats aged 10 - 14 weeks with body weight of (190-250g). They were obtained from Animal Resource Center, Collage of Veterinary Medicine, Baghdad University. The animals were apparently healthy and they were housed in individual cages, at a temperature controlled environment (25±5°C) with ambient humidity. Lights were maintained on a 12-h light/dark cycle. The rats received standard chow

diet with water (ad libitum). Rats in the study were maintained and handled in accordance with the Guide for the Care and Use of Laboratory Animals USA, (1996)[16].

Study design:

After 4 weeks acclimatization period, the animals were randomly separated into 5 groups (7 rats in each group):

- i. Normal saline (N.S) treated group:** all rats of this group received normal saline (1 ml/kg, i.p.) in six equal doses in alternative days over a period of 2 weeks
- ii. Doxorubicin treated group:** all rats of this group received doxorubicin (2.5 mg/kg, i.p.) in six equal doses in alternative days over a period of 2 weeks to make a total cumulative dose of 15 mg/kg body weight.
- iii. Aminophylline treated group (10 mg/kg) plus doxorubicin:** all rats of this group received aminophylline (10mg/kg/dose, i.p.) in six equal doses one hour prior each administered dose of doxorubicin over a period of 2 weeks.
- iv. Aminophylline treated group (20 mg/kg) plus doxorubicin:** all rats of this group received aminophylline (20mg/kg, i.p.) in six equal doses one hour prior each administered dose of doxorubicin over a period of 2 weeks.
- v. Aminophylline treated group (30 mg/kg) plus doxorubicin:** all rats of this group received aminophylline (30mg/kg, i.p.) in six equal doses one hour prior each administered dose of doxorubicin over a period of 2 weeks.

After 24hr from the last injection, about 3 ml of blood sample was obtained under

light ether anesthesia from each rat by cardiac puncture using disposable syringe. Each blood sample was placed in a gel tube and left to stand for 15-20 minutes at room temperature and used to obtain serum via centrifugation at 3000 rpm (round per minute) for 15 minutes then preserved at -20 °C until determination the parameters of: serum troponin I, CK-MB, LDH , GSH and MDA.

Statistical analysis

Statistical analyses were performed using SPSS version 17 computer program. Data were expressed as means \pm standard deviation. Multiple comparisons were done using t-test as well as one way ANOVA test (LSD). The $p < 0.01$ level of probability was chosen as a criterion for the lowest level of significance [17].

Results

Effect of the administration of doxorubicin and different doses of aminophylline on serum LDH , CK-MB and cardiac troponin I levels:

As shown in figures 1,2,3 serum LDH , CK-MB and cardiac troponin I levels respectively were significantly ($p < 0.01$) higher in doxorubicin treated rats than the controls. The administration of aminophylline (10mg/kg, 20mg/kg and 30mg/kg, i.p.) one hour prior to each administered dose of doxorubicin as scheduled before show a highly significant decrease ($p < 0.01$) in serum LDH , CK-MB and cardiac troponin I levels of treated rats as a dose dependent manner when compared with doxorubicin treated rats.

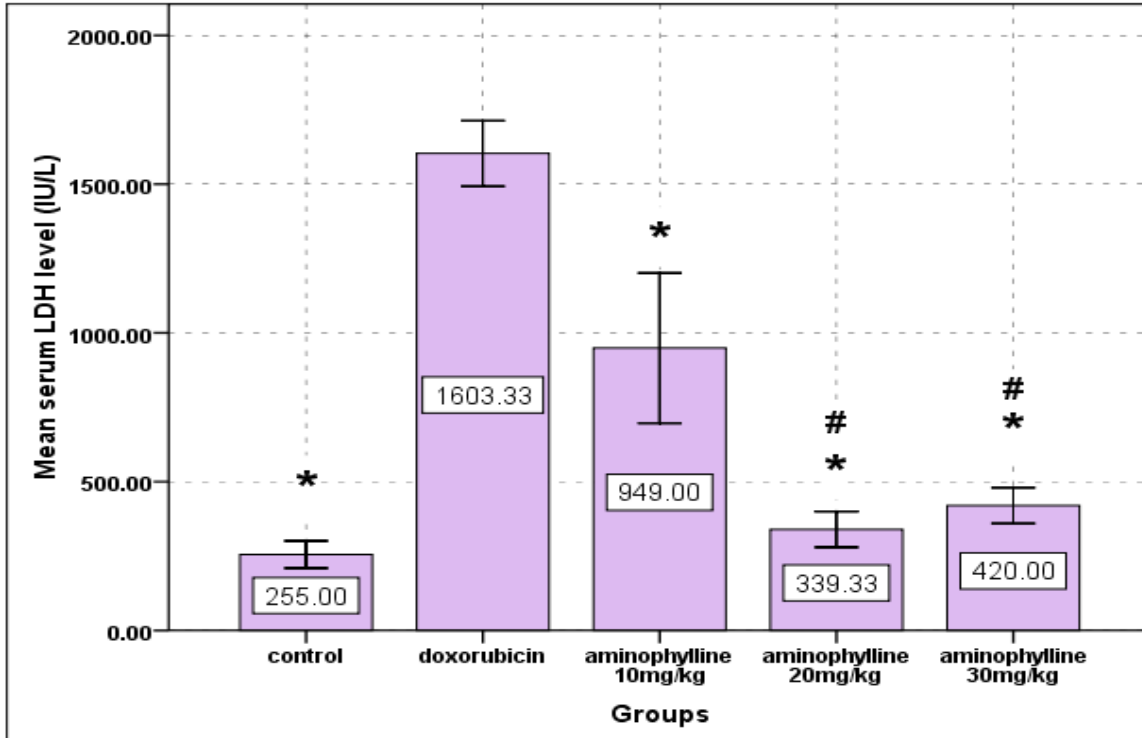


Figure 1 Serum LDH levels of different treated groups. Data was shown as mean \pm S.D. (* = $p < 0.01$ versus doxorubicin treated group) (# = $p < 0.01$ versus aminophylline 10mg/kg treated group).

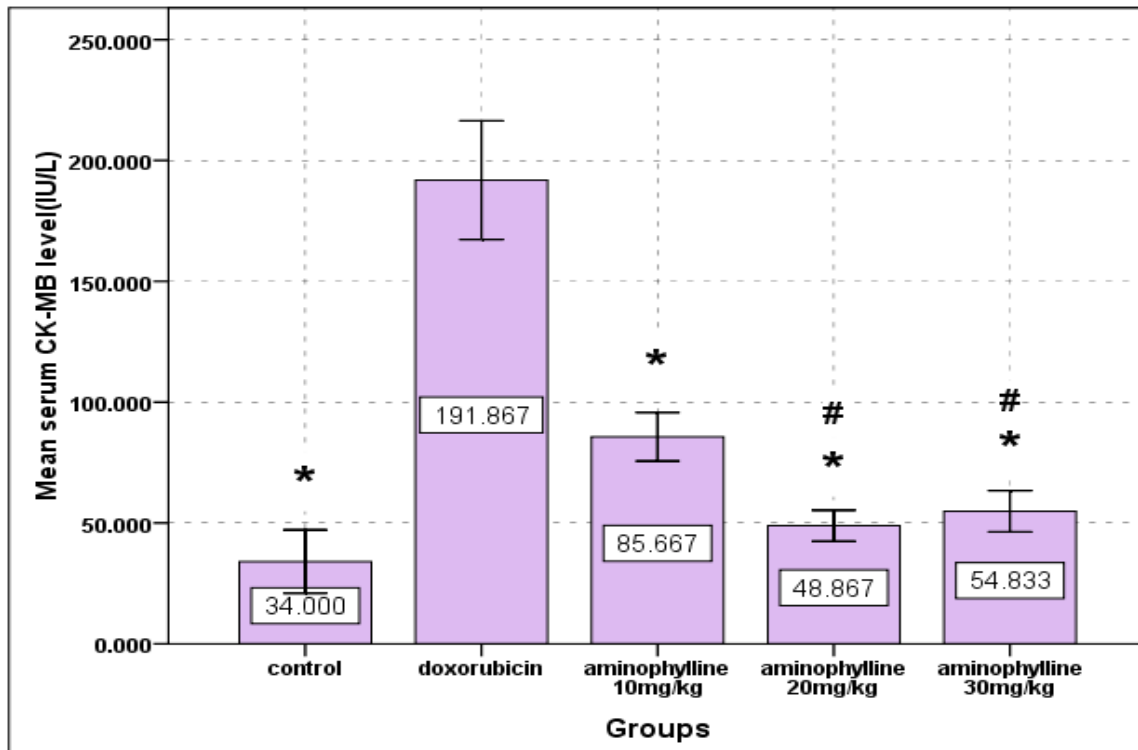


Figure 2 Serum CK-MB levels of different treated groups. Data was shown as mean \pm S.D. (* = $p < 0.01$ versus doxorubicin treated group) (# = $p < 0.01$ versus aminophylline 10mg/kg treated group).

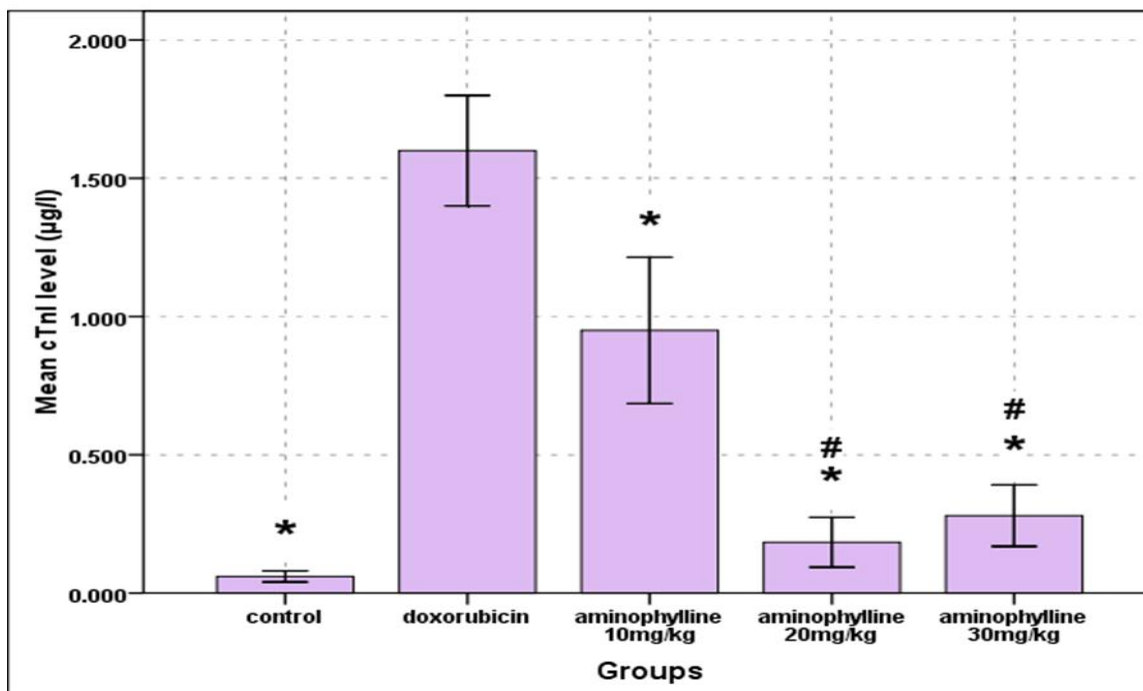


Figure 3 Serum troponin I levels of different treated groups. Data was shown as mean \pm S.D. (* = $p < 0.01$ versus doxorubicin treated group) (# = $p < 0.01$ versus aminophylline 10mg/kg treated group).

Effect of the administration of doxorubicin and different doses of aminophylline on serum MDA level:

As shown in figure 4, serum MDA level were significantly ($p < 0.01$) higher in doxorubicin treated rats than the controls. The administration of aminophylline

(10mg/kg, 20mg/kg and 30mg/kg, i.p.) one hour prior to each administered dose of doxorubicin as scheduled before show a highly significant decrease ($p < 0.01$) in serum MDA level of treated rats as a dose dependent manner when compared with doxorubicin treated rats.

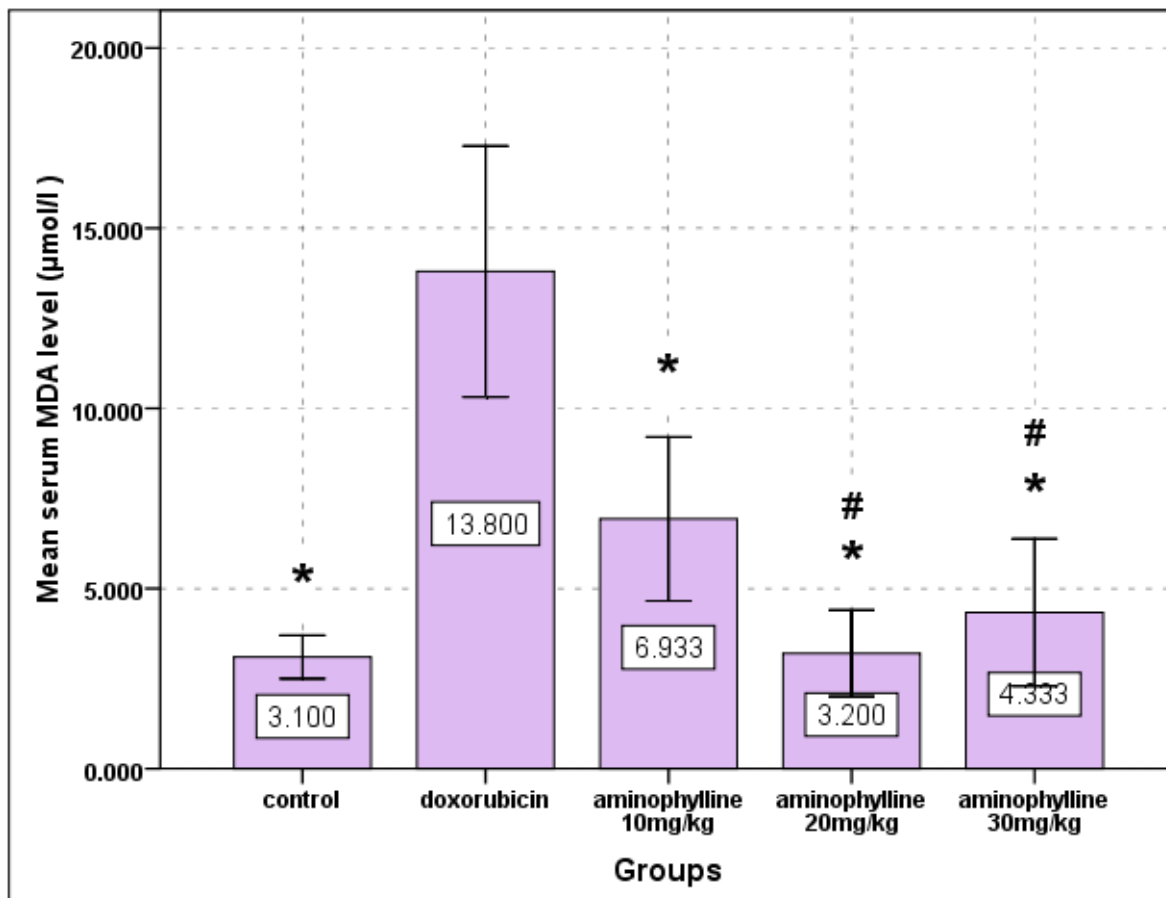


Figure 4 Serum MDA level of different treated groups. Data was shown as mean \pm S.D. (* = $p < 0.01$ versus doxorubicin treated group) (# = $p < 0.01$ versus aminophylline 10mg/kg treated group).

Effect of the administration of doxorubicin and different doses of aminophylline on serum GSH level:

As shown in figure 5, serum GSH level were significantly ($p < 0.01$) lower in doxorubicin treated rats than the controls. The administration of aminophylline

(10mg/kg, 20mg/kg and 30mg/kg, i.p.) one hour prior to each administered dose of doxorubicin as scheduled before show a highly significant increase ($p < 0.01$) in serum MDA level of treated rats as a dose dependent manner when compared with doxorubicin treated rats.

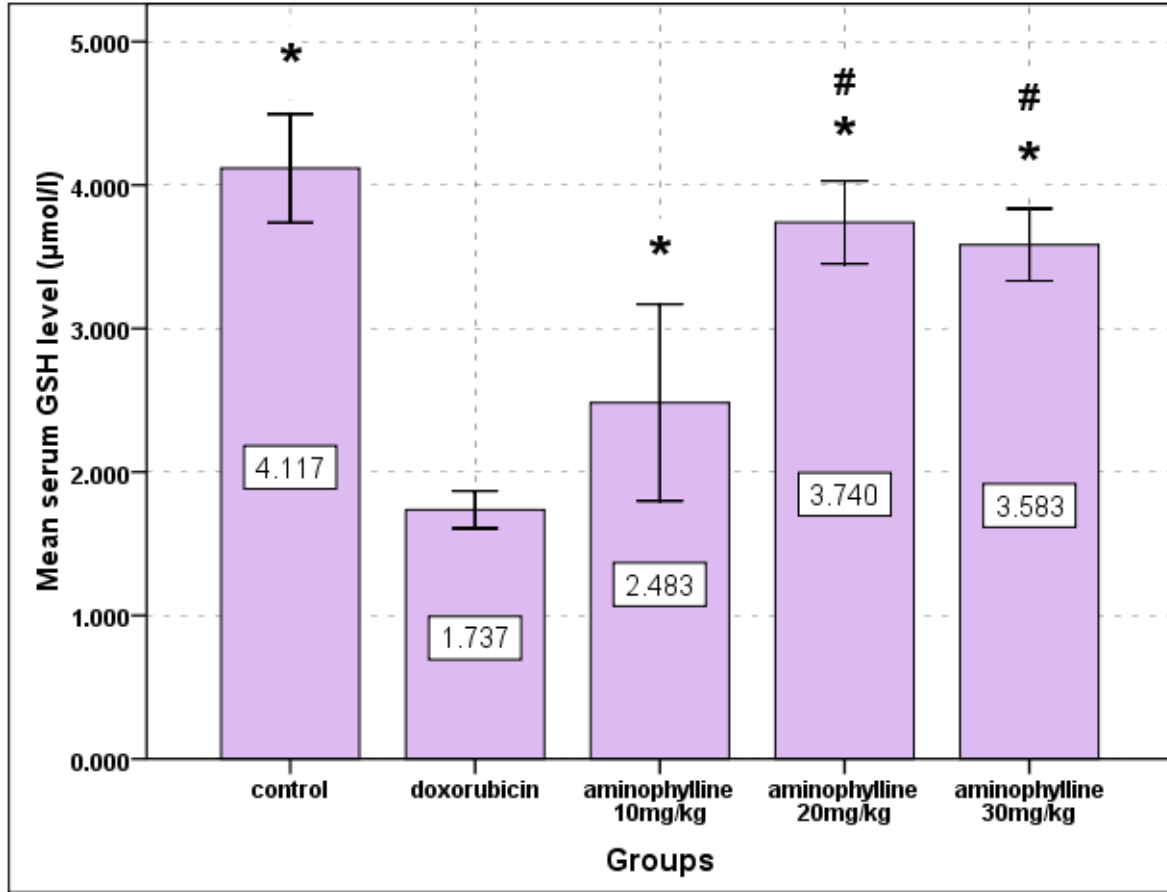


Figure 5 Serum GSH level of different treated groups. Data was shown as mean \pm S.D. (* = $p < 0.01$ versus doxorubicin treated group) (# = $p < 0.01$ versus aminophylline 10mg/kg treated group).

Discussion

Doxorubicin is an effective anti-neoplastic agent. It used widely for treatment of various hematological and solid tumor malignancies including breast cancer, leukemia, and sarcomas. its application of which has been limited due to its cardiotoxic side effects [3]. This study implies the cardioprotective effects of aminophylline on doxorubicin-induced *in vivo* cardiotoxicity in rats.

In the present study, the cumulative dose of doxorubicin (as scheduled by the study) can induced cardiotoxicity as revealed from the increase in serum LDH, CK-MB, cardiac troponin I, MDA levels and decrease serum GSH level. These changes occurred after 24 hours since the

injection of the last dose [19]. These results are consistent with studies in the animal studies [18, 20].

Oxidative stress has long been, and remains, the most studied and widely accepted cause of cardiotoxicity, although evidence also exists for several non-oxidative mechanisms. Reactive oxygen species (ROS) are formed when the quinine moiety of doxorubicin is reduced to semiquinone, initiating a cascade of free radical formation that leads to many deleterious effects on cells, cell membranes and subcellular apparatuses [9]. Ultimately, these changes can lead to cell death and organ damage. The importance of cardiac mitochondria as key mediators of the cardiotoxicity of

doxorubicin has been increasingly observed [21]. Doxorubicin impair mitochondrial calcium homeostasis, causing loss of stability of the mitochondrial membrane and ultimately, cell death [22].

The results of this study showed a cardioprotective effects of aminophylline in a dose related manner as revealed from the use of three aminophylline doses. In fact, this effect is reported here for the first time. The cardioprotective effects of aminophylline were reflected on the decreased levels of the serum LDH, CK-MB, troponin I and MDA levels in a dose related manner. While, serum GSH was increased but also in a dose related manner. These results are in agreement with [23, 24].

The mechanisms by which aminophylline cause myocardial protection are not clear because of multiple pharmacological effects of this agents [24]. It is well known that the primary pharmacological effect of aminophylline is to increase intracellular cAMP concentration by non-selectively inhibiting phosphodiesterase activity [25]. The increased intracellular cAMP phosphorylates sarcolemmal calcium channels [26]. Furthermore, Shahid and Rodger (1991), reported that cAMP was one of the essential factors of Ca²⁺ handling at reperfusion, and it inhibits Ca²⁺ overload [27]. Also Several lines of evidence indicate that an abnormal calcium handling of myocardial cells may explain, at least in part, the cardiac dysfunction seen in doxorubicin-induced cardiomyopathy [7]. In the same context, aminophylline also has some of antioxidant activity as this drug, it was found to be as scavenger of hydroxyl radical (OH•) [15].

Conclusions

Aminophylline is able to protect the heart against doxorubicin-induced cardiomyopathy in a dose related manner. Usefulness of aminophylline as adjunct to doxorubicin therapy, there need for further studies including human trials.

References

- 1- Ronaldo, V.T.S.; Miguel, L.B.J.; Érico, C.C. and Luis, F.B.P., C.R., (2007): Chronic supplementation of creatine and vitamins C and E increases survival and improves biochemical parameters after doxorubicin treatment in rats. CEPP; 34(12):1294-1299.
- 2- Smith, L.A.; Cornelius, V.R.; Plummer, C.J.; Levitt, G.; Verrill, M.; Canney, P. and Jones, A., (2010): Cardiotoxicity of anthracycline agents for the treatment of cancer: systematic review and meta-analysis of randomised controlled trials. BMC Cancer; 10:337.
- 3- Chen, Y.; Wan, Y.; Zhang, H. and Jiao, Z., (2011): Anticancer efficacy enhancement and attenuation of side effects of doxorubicin with titanium dioxide nanoparticles: International Journal of Nano medicine; 6 :2321–2326.
- 4- Minotti, G.; Menna, P.; Salvatorelli, E.; Cairo, G. and Gianni, L., (2004): Anthracyclines: molecular advances and pharmacologic developments in antitumor activity cardiotoxicity. Pharmacol Rev; 56: 185-229.
- 5- Ryberg, M.; Nielsen, D.; Cortese, G.; Nielsen, G.; Skovsgaard, T. and Andersen, P. K., (2008): New insight into epirubicin cardiotoxicity: competing risks analysis of 1097 breast cancer patients. Journal of the National Cancer Institute; 100(15): 1058-1067.
- 6- Raschi, E.; Vasina, V.; Ursino, M.G.; Boriani, G.; Martoni, A. and de Ponti, F., (2010): Anticancer drugs and cardiotoxicity: insights and perspectives in

- the era of targeted therapy. *Pharmacol Ther*; 125(2):196-218.
- 7- Arai, M.; Yoguchi, A.; Takizawa, T.; Yokoyama, T.; Kanda, T.; Kurabayashi, M. and Nagai, R., (2000): Mechanism of doxorubicin-induced inhibition of sarcoplasmic reticulum Ca^{2+} -ATPase gene transcription. *Circulation Research* ;86(1):8-14.
- 8- Sawyer, D.B. ; Peng, X.; Chen, B.; Pentassuglia, L. and Lim, C.C. (2010): Mechanisms of anthracycline cardiac injury: can we identify strategies for cardioprotection? *Prog Cardiovasc Dis* ; 53(2): 105-113.
- 9- Simunek, T.; Sterba, M.; Popelová, O.; Adamcová, M.; Hrdina, R. and Gersl, V.,(2009): Anthracycline-induced cardiotoxicity: overview of studies examining the roles of oxidative stress and free cellular iron. *Pharmacological Reports*; 61(1):154-171.
- 10- Zhang, Y.W.; Shi, J.; Li, Y.J. and Wei, L., (2009): Cardiomyocyte death in doxorubicin-induced cardiotoxicity. *Arch Immunol Ther Exp (Warsz)* ;57(6):435-445.
- 11- Kumar, D.; Kirshenbaum, L.A.; Li, T.; Danelisen, I. and Singal, P.K.(2001): Apoptosis in adriamycin cardiomyopathy and its modulation by probucol. *Antioxid Redox Signal* ;3:135-145.
- 12- Szczypka, M. and Obmińska-Mrukowicz, B., (2010): Modulating effects of nonselective and selective phosphodiesterase inhibitors on lymphocyte subsets and humoral immune response in mice. *Pharmacological Reports* ;62:1148-1158.
- 13- Barr, R.G.; Rowe, B.H. and Camargo, C.A. Jr.(2003): Methylxanthines for exacerbations of chronic obstructive pulmonary disease: meta-analysis of randomized trials. *BMJ* ; 327:643-646.
- 14- Kang, M.Y.; Tsuchiya, M.; Packer, L. and Manabe, M., (1998): In vitro study on antioxidant potential of various drugs used in the peri operative period. *Acta Anaesthesiol Scand*; 42(1):4-12.
- 15- Lapenna, D.; De Gioia, S.; Mezzetti, A.; Ciofani, G.; Festi, D. and Cuccurullo, F., (1995): Aminophylline: could it act as an antioxidant in vivo?. *Eur Clin invest* ;25: 464-470.
- 16- Guide for the Care and Use of Laboratory Animals. National Academy Press, Washington, D.C. 1996.
- 17- Daniel, w., (1999): Biostatistics: A Foundation for Analysis in the Health Sciences; Seventh Edition: by Wiley Medical, New York :83-123.
- 18- Abd Elbaky, N.A.; Ali, A.A. and Ahmed, R.A., (2010): Cardioprotective effect of simvastatin on doxorubicin - induced oxidative cardiotoxicity in rats. *Journal of Basic and Applied Sciences*; 6(1): 29-38.
- 19- Elberry, A.A.; Abdel-Naim, A.B.; Abdel-Sattar, E.A.; Nagy, A.A.; Mosli, H.A.; Mohamadin, A.M. and Ashour, O.M. (2010): Cranberry (Vaccinium macrocarpon) protects against doxorubicin-induced cardiotoxicity in rats. *Food Chem Toxicol.* ;48(5):1178-1184.
- 20- Xin, Y.; Zhang, S.; Gu, L.; Liu, S.; Gao, H.; You, Z.; Zhou, G.; Wen, L.; Yu, J. and Xuan, Y. (2011): Electrocardiographic and biochemical evidence for the cardioprotective effect of antioxidants in acute doxorubicin-induced cardiotoxicity in the beagle dogs. *Biol Pharm Bull* ;34(10):1523-1526.
- 21- Wallace, K. B. (2007): Adriamycin-induced interference with cardiac mitochondrial calcium homeostasis. *Cardiovasc Toxicol* ;7(2):101-107.
- 22- Zhou, S.; Palmeira, C.M. and Wallace, K.B. (2001): Doxorubicin-induced persistent oxidative stress to cardiac myocytes. *Toxicol Lett* ;121:151-157.
- 23- Milani, E.; Nikfar, S.; Khorasani, R.; Zamani, M.J. and Abdollahi, M.(2005): Reduction of diabetes-induced oxidative

stress by phosphodiesterase inhibitors in rats. *Comp Biochem Physiol C Toxicol Pharmacol* ;140(2):251-255.

24- Abdel-Aal, F.A.; Attia, A.M.A.; Youssef, H. A.; Mohareb, A.M.; Abdel Azeem, H.G. and Abdel Rahman, K. A. (2010): The effect of aminophylline versus milrinone on ischemia -reperfusion myocardial injury during open heart surgery. *EJCTA*; 4(1):22-34.

25- Barnes, P.J.(2003): Theophylline: new perspectives for an old drug. *Am J Respir Crit Care Med*; 167:813-818.

26- Endoh, M.; Yamashita, S. and Taira, N.(1982): Positive inotropic effect of amrinone in relation to cyclic nucleotide metabolism in the canine ventricular muscle. *J Pharmacol Exp Ther* ; 221:775-783.

27- Shahid, M. and Rodger, I.W.(1991): Enhancement of amrinone induced positive inotropy in rabbit papillary muscle with depressed contractile function: effects on cyclic nucleotide levels and phosphodiesterase isoenzymes. *J Pharm Pharmacol* ; 43: 88-94.