International Journal of Nutrition and Metabolism

Volume 4 Number 8 August 2012

ISSN 2141-2340

Academic Journals

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Pitout JDD, Church DL, Gregson DB, Chow BL, McCracken M, Mulvey M, Laupland KB (2007). Molecular epidemiology of CTXM-producing Escherichia coli in the Calgary Health Region: emergence of CTX-M-15-producing isolates. Antimicrob. Agents Chemother. 51: 1281-1286.

Pelczar JR, Harley JP, Klein DA (1993). Microbiology: Concepts and Applications. McGraw-Hill Inc., New York, pp. 591-603.

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International Journal of Nutrition and Metabolism

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International Journal of Nutrition and Metabolism Vol. 4(8), pp. 107 - 113, August 2012
Available online at http://www.academicjournals.org/ijnam
DOI: 10.5897/IJNAM11.057
ISSN 2141-2499 ©2012 Academic Journals

Full Length Research Paper

Problems encountered by breastfeeding mothers in their practice of exclusive breast feeding in tertiary hospitals in Enugu State, South-east Nigeria

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Accepted 31 March, 2012

This study determined the knowledge of mothers about exclusive breast feeding (EBF), identified the difficulties associated with psycho-socio-cultural values and investigated other problems the mothers encountered. Using a convenience sampling technique, 240 women were selected from the study population (800). Researcher's developed questionnaire was used to collect data which were analyzed using descriptive statistics. 50% of the mothers knew that feeding with only breast milk without the addition of water or food for six months was what EBF entailed, 33% mothers believed that giving baby water once in a while also meant EBF, while 8% women expressed no knowledge of EBF. 88% women reported interference from mothers-in-law to give water as a major socio-cultural problem they faced, while 63% agreed that they had never eaten nutritional foods like snail, pork, etc due to cultural myths and superstitions. Psychological problems encountered ranged from worry and stress of feeding at all times even at night (92%), having to breastfeed even in public places (82%), fear that the baby might not be getting enough nutrients (71%), to trauma of expressing breast milk (67%), fear of safety of expressed breast milk (68%) and a feeling that the baby will "dry up" if not given water or other fluids (50%). Other factors identified were: work place not conducive (91.6%), lack of adequate education at ante natal clinics (63%). A thorough health education campaign aimed at educating lactating mothers on the need to practice EBF should address the identified factors.

Key words: Infant nutrition, breastfeeding, exclusive breastfeeding, problems encountered.

INTRODUCTION

Breastfeeding is the feeding of an infant with breast milk directly from female human breasts rather than from a baby bottle or any other container. Breast milk promotes sensory and cognitive development, and protects the infant against infectious and chronic diseases. Breastfeeding contributes to the health and well-being of mothers; it helps to space children, reduces the risk of ovarian and breast cancer, increases family and national resources, is a secure way of feeding and is safe for the environment *(*Kramer et al*.,* 2001*)*. It is absolutely essential for the health and survival of the majority of children in the developing countries (WHO, 2011).

Exclusive breastfeeding, according to the World Health Organization (WHO) and United Nations Children Emergency Fund (UNICEF) is the feeding of the infant with only breast milk for a period of 6 months without any additional food or drink, not even water. Thereafter, infants should receive adequate complementary foods with continued breastfeeding up to 2 years of age or beyond (WHO, 2002a).

Exclusive breast-feeding is internationally the preferred way of feeding infants during the first 6 months of their lives, and is recognized as being one of the most natural and best forms of preventive medicine (WHO, 2002b). Early and exclusive breastfeeding is widely regarded as an important intervention that reduces neonatal, infant, and child mortality, and remains a basis for child survival strategies. Breastfeeding is also associated with improved

maternal recovery post partum and reduced incidence of diabetes and cancers (Tylleskär et al., 2011; Bhutta and Labbok, 2011).

At the fifty-fifth World Health Assembly, the World Health Organization (WHO) recommended that optimal infant nutrition was exclusive breastfeeding for the first six months of life, followed by the introduction of nutritionally adequate and safe complementary feeding with continued breastfeeding for up to the age of two years or beyond (WHO, 2003 in Wong et al., 2007). Promotion of exclusive breastfeeding (EBF) for the first 6 months of life has been estimated to be the most effective preventive strategy for saving the lives of young children in low-income settings and could contribute towards the Millennium Development Goal 4 of reducing child mortality (Jones et al., 2003; Bhutta and Labbok, 2011). In Nigeria, as well as in neighboring countries of West Africa, infant morbidity and mortality have been on the increase despite the efforts of mothers to breast feed their young ones (Anyanwu and Enweonu, 2004). A lot of efforts have been made by Enugu State government to ensure that mothers have the support required for them to be able to breastfeed their infants exclusively. Maternal and child health care centers are instituted at strategic places in the city, functional mobile clinics that go into the remotest parts of the state to enlighten mothers on the need for them to practice EBF as well as aggressive health education campaigns in the cities on the importance and benefits of EBF.

The researcher was perturbed at the level of non compliance to the practice of EBF by mothers who attend pediatric clinics of UNTH and Park lane despite much emphasis that has been placed on the practice of EBF in these two hospitals. Hence, this research is focused on identifying those factors that negate the willingness of mothers attending pediatric clinics in University of Nigeria Teaching Hospital (UNTH) and ESUT Teaching Hospital, Park lane Enugu to practice EBF.

MATERIALS AND METHODS

Descriptive survey design was adopted for this study. Mothers attending the pediatric clinics of these two tertiary hospitals in Enugu State were included in the study. These two hospitals have an extended catchment area for their services, extending to various other states of Nigeria such as Abia, Anambra, Benue, Cross River, Delta, Ebonyi, and Imo State among others. Each of them has training schools for the training of different cadre of health personnel, various specialist units as well as serves as referral centers for primary and secondary level health facilities in Enugu State and its environs. The study population consisted of 800 women who attended the pediatric clinics of these hospitals during the period of study, out of which 240 were recruited through non-probability convenience sampling method using inclusion criteria viz: those nursing babies and attending pediatric clinics at the time, emotional, physical and mental well being, willingness to participate in the study. Convenience sampling was used to select the subjects using inclusion criteria. Those who met the criteria were selected for the study so there was no randomization.

Data were collected by means of a 20-item questionnaire developed by the researcher with guidance from literature search, and interview guide to meet the objectives of the study. Some of the questions were structured (close-ended) while some were unstructured (open-ended). The validity and reliability of the research instruments were tested. Experts in the field of nutrition and a consultant pediatrician evaluated the relevance of the items in the research tool. The questionnaire was pilot tested among twenty mothers attending pediatric clinic at Uwani Health Centre Enugu. After the pilot testing, some question-items in the questionnaire were modified and reframed to ensure validity of the instrument, and facilitate patients' easy understanding. A test re-test method of reliability testing was done and a co-efficient of reliability of 0.85 was computed using Pearson moment correlation coefficient formula. Two research assistants were trained on the use of the instruments. A letter of approval was obtained from the ethical committees of the hospitals studied. Oral permission was then secured from the unit heads of each of the pediatric clinics. An informed consent was signed voluntarily by each of the respondents having understood the total package of the study before the administration of the instrument.

Data were collected on each clinic day until the required number of respondents was reached. Those who could not speak or read English Language were assisted in filling the questionnaire by the researcher and the two assistants. All copies of the administered questionnaire were retrieved giving a response rate of 100%. Data analysis was by descriptive statistical analysis and converted to frequencies and percentages.

RESULTS

The findings showed that majority (90%) of the respondents were in the age range of 20 and 45, they were mostly married (95%), and some (37%) were illiterate. Most of them (73%) were civil servants and the rest (27%), business women. As shown in Figure 1, 120 (50%) subjects responded to the meaning of EBF as feeding of baby with only breast milk without the addition of water or any food for 6 months. 80 (33.3%) opted for giving baby plain or glucose water once a while. 20 (8.33%) said it meant addition of multivitamin and other drugs to breastfeeding. 13 (5.42%) chose giving baby artificial milk with breastfeeding. 7 (2.92%) said EBF meant the addition of cereals and other nutrients.

On the responses of subjects to various socio-cultural problems they experience as hindering the practice of EBF, 210 (87.5%) opted for interference from mother-in-law to give water. 60 (25%) opined that it was in their culture to give water to babies together with breastfeeding. 80 (33.33%) identified pressure from family and friends as a factor. 150 (62.5%) said it was a misguided information from family, friends and associates on EBF. However, 197 (82.08%) said it was a taboo for them to eat nutritious foods like snail, pork, grass cutter, rein-deer, snake, egg etc while 150 (62.5%) opined that superstition was a factor (Figure 2).

Figure 3 identified the psychological problems the mothers encountered in practice of EBF. It showed that 170 (70.83%) of them had the feeling that their babies might not get enough milk and nourishments. 150 (62.5%) lacked the confidence to breastfeed exclusively. 220 (91.67%) worried about the stresses associated with

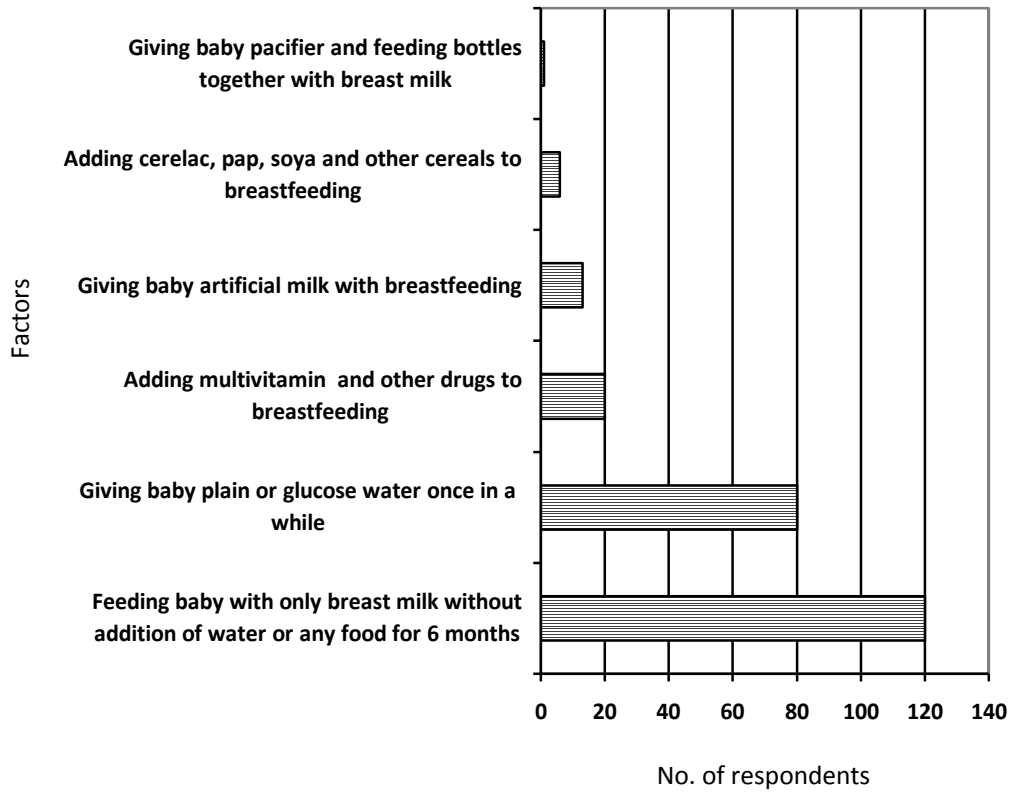

Figure 1. Responses of subjects on what EBF meant. n = 240.

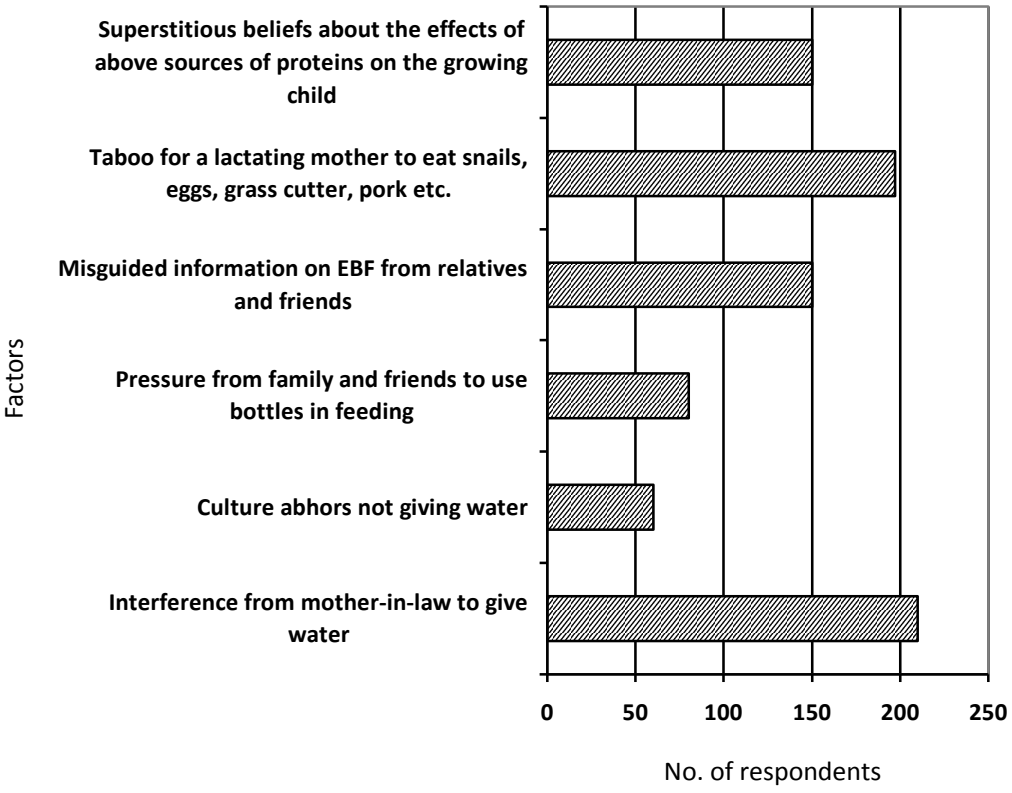

Figure 2. Socio-cultural problems experienced by respondents. n = 240.

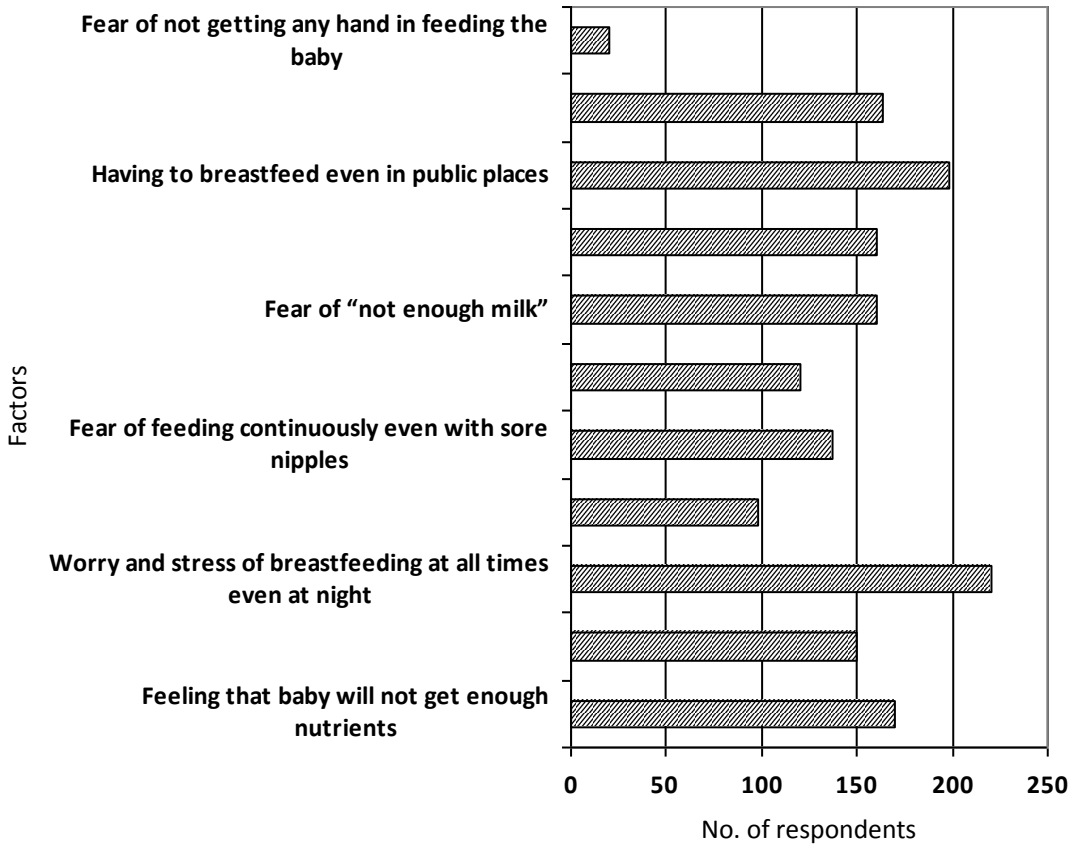

Figure 3. Psychological problems identified by the respondents. n = 240.

breastfeeding exclusively. 98 (40.83%) feared that EBF would crack their nipples and 137 (57.08%) cannot imagine breastfeeding with cracked nipples.

Responses of mothers on other factors that militate against the practice of EBF were shown in Figure 4. It revealed that 220 (91.6%) of the respondents admitted that workplace was not conducive to take baby along. 205 (86%) opted for inconvenience of expressing breast milk. 168 (70 %) said they had no time to breastfeed at work place. 120 (50%) of the respondents agreed that the attitude of health workers towards EBF was a problem. 148 (61.7%) alluded to the fact that lack of adequate education in antenatal clinics on techniques of EBF was the major problem they encountered while 155 (64.6%) responded to poor education on nutrition practices of lactating mothers by health workers as a problem.

DISCUSSION

Respondents' knowledge on exclusive breastfeeding was deficient since they did not have full knowledge of how EBF should be practiced. Not having the full knowledge of EBF is as good as not practicing it since the mother will be unaware of the fact that she is not doing the right thing and continue endangering the life of her baby by giving inadequate nourishment and its attendant consequences; failure to thrive, poor weight gain, compromised immunity status, exposure to childhood infections, etc. The mother also denies her body of the various benefits of exclusive breast feeding; involution of the uterus, prevention of breast and ovarian cancer, lactational amenorrhea etc. Because breastfeeding uses an average of 500 calories a day, it helps the mother lose weight after giving birth (Dewey et al., 1993). Various studies have confirmed the importance of mother's breast-feeding knowledge (Mitra et al., 2004; Avery et al., 2003; Barbara et al., 2007). This finding corresponds with a study carried out in Canada by Bryne et al. (1998), where it was discovered that mother's knowledge about EBF is as good as having the practice done. Similar findings were made by Agampodi et al., (2007) on the knowledge of mothers on EBF which highlighted mother's good knowledge on EBF as the major ingredient in the successful practice of EBF.

In a study carried out in Tanzania by Shirimah et al. (2001), a lot of mothers also expressed harmful cultural practices regarding breastfeeding. Societal influence on natural sources of protein such as snail, grass cutter, reindeer, snakes, eggs, pork, etc, may have a religious or

Figure 4. Other factors that mitigate against the practice of EBF. n = 240.

ethnic connotation, thereby leading to inability of the respondents to eat such highly proteinous food substances. This finding is significant in places where these animals are the only sources of available and affordable first class proteins of high biological value. This is also a problem in a country like Nigeria where breastfeeding is a maternal option that involves a complex interaction of cultural, religious, socio-economic, psychological factors and many more. Extended family system is practiced with much consideration to the culture and antecedents of the predecessors. However, as a socially approved practice, the influence of these social factors should by no means be disregarded (Medlin, 2007). This was also emphasized by Uchendu et al. (2009).

The fact that preventable problems such as nipple cracks and breast engorgement were identified as risk factors for the initiation and sustenance of exclusive breastfeeding during babies' first week of life leaves much to be investigated regarding the health education received by these women during pregnancy and after delivery. Pain in the nipple or breast is linked to incorrect breastfeeding techniques. Failure to latch on is one of the main reasons for ineffective feeding and can lead to infant health concerns. Sore nipple, was also identified as a factor by Judhiastuty et al. (2006) and Giugliani (2004) who also attributed it to improper positioning and latching on techniques. Giugliani (2004) and Lawrence and Lawrence (2005) posited that when the nursing mother is emotionally calm and relaxed, correct breastfeeding technique is enhanced, thus reducing risk of developing sore nipple. Furthermore, relaxed emotional state is related with enhanced work of prolactin and oxytocin, two hormones known to play roles in the production of breast milk.

Lehman et al. (2006) found that inadequate parental education, incorrect breastfeeding techniques, or both were associated with higher rates of preventable hospital admissions in newborns. In relation to the work of Guyton (2001), psychological problems like having a feeling that the baby will not get enough milk agreed with the findings of this research work. This in turn makes mothers unresponsive to EBF as they tend to be in a bad mood and consider EBF a waste of time and effort.

Work environment and attitude of colleagues have a great role to play in encouraging and supporting the mother in the practice of EBF because she has to go back to work at the expiration of her maternity leave which of course is before 6 months (around 3 months). Returning to work is the most common cited reason for discontinuing breastfeeding as discovered by Galson

(2008). Work by Uchendu et al. (2009) and Bhutta and Labbok (2011) also had similar findings and opined that breastfeeding breaks or extended maternity leave should be instituted for working mothers for the first 6 months after delivery. However, work by Aidam et al. (2005) had a dissimilar finding and posited that mothers breastfeed exclusively irrespective of where they found themselves.

Lack of adequate education in antenatal clinics on techniques of EBF and poor education on nutrition practices for lactating mothers were evident in their responses as some of their fears were unfounded and would have been dispelled by sound health and nutrition talks. Benjamin (2011) opined that practitioners – physicians and nurses have surprising little training in lactation and lactation support. The findings confirm that of Ramachandran (2004), who identified factors like the attitude of health workers towards lactating mothers in their practice of EBF as being unacceptable since some of these health officers lay little or no emphasis on the need for adequate nutrition of the infant till 6 months with only breast milk.

Conclusion

The aim of this study was to identify the various problems encountered by breastfeeding mothers in their practice of EBF in two University hospitals in Enugu (UNTH and ESUTH). The age, occupation, and educational attainments of these women were in conformity with our typical socio-cultural setting and socio-economic background. The levels of education of the women attending the two hospitals were on the average, hence the reason for their knowledge on breastfeeding. From the findings of the study, it is clear that breastfeeding mothers face a lot of problems in their attempt to carry out exclusive breastfeeding. Also one can see that the women have deficient knowledge about EBF. The psycho-socio-cultural factors like the belief system of the people play a pivotal role in discouraging mothers from practicing EBF. Worry and stress of breastfeeding at all times even at nights, fear of cracked and sore nipples, fear that the baby will "dry up" if not given water or other fluids, feeling that baby will not get enough nutrients, trauma" of expressing breast milk and fear of safety of the expressed milk are also identified factors.

Unconducive work environment, poor attitude of colleagues at work as well as lack of education by health personnel on the techniques of breastfeeding and infant nutritional practices during ante natal and post natal services were also noted. Hence the need for enlightenment programme by the concerned authorities to ensure that these women and their spouses are properly educated. While breastfeeding is a natural act, it is also a learned behavior and since breastfeeding exclusively is important preventive health behavior, mothers, caregivers and health practitioners require active support for establishing and sustaining appropriate breastfeeding practices. The health practitioners' knowledge should frequently be updated through workshops and seminars. This will improve the knowledge of both clients and staff thus forestalling the hazardous consequences of non-EBF practice.

ACKNOWLEDGEMENTS

The cooperation of the management of the Hospitals used for the study and especially the willingness to participate in the study and the cooperation and patience of the participants despite their busy schedules is acknowledged.

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International Journal of Nutrition and Metabolism Vol. 4(8), pp. 114-120, August 2012
Available online at http://www.academicjournals.org/IJNAM
DOI: 10.5897/IJNAM12.009
ISSN 2141-2340 ©2012 Academic Journals

Full Length Research Paper

Protective effect of the combination of exercise and curcumin supplementation on cardiac system in rats exposed to lead

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Accepted 22 May, 2012

Effects of exercise training and curcumin supplementation, alone or combined on cardioprotective markers in left ventricle were investigated in rats during chronic exposure to lead acetate. Forty (40) male rats were randomly assigned to; sham (Sh), training + lead (TL), curcumin + lead (CL), training + curcumin + lead (TCL), and lead (L) groups. Heat shock protein (HSP_{72}) and enzyme activities were determined in rat hearts after receiving 20 mg/kg of lead acetate in the TL, TCL, CL and L groups and 30 mg/kg turmeric in the TCL and CL groups for 8 weeks, 3 times a week. In addition, the rats in the TL and TCL groups performed treadmill running at a speed of 15 to 22 m/min for 25 to 64 min, 5 times a week for 8 weeks. One-way analysis of variance (ANOVA) indicated that administration of lead can evoke a significant increase in HSP_{72} and malondialdehyde (MDA) in the left ventricle homogenates and a significant decrease in serum total antioxidant capacity (TAC). However, endurance training and\or curcumin supplementation resulted in a significant decrease in HSP_{72} and MDA levels and significant increase in the level of serum TAC (serum Total Antioxidant Capacity). These results can suggest that concomitant exercise and curcumin supplementation may have a more cardioprotective effect observed by amelioration of lead-induced cardiotoxicity.

Key words: Turmeric, endurance training, lead acetate, stress proteins, antioxidant.

INTRODUCTION

Cardiovascular disease (CVD) has remained the leading cause of morbidity and mortality worldwide, and therefore strategies that aim to improve prevention in people without existing disease (primary prevention) are important for managing the overall burden of disease (Heneghan, 2011). Although regulation of emissions has led to improvements in air quality, epidemiological data indicates clearly that air pollution continues to have widespread effects on human health. Literally, dozens of studies have demonstrated that poor air quality is associated with increased morbidity and mortality due to numerous causes. Air pollution is thought to be responsible for approximately 3 million deaths per year worldwide (Ritz, 2010). Lead is a persistent and common environmental contaminant. Like other commonly found, persistent toxic metals, –mercury, arsenic, and cadmium– lead damages cellular material and alters cellular genetics. The common mechanism of all these toxic

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metals involves oxidative damage. Toxic metals increase production of free radicals and decrease availability of antioxidant reserves to respond to the resultant damage (Patrick, 2006). Recent *in vivo* studies in lead exposed animals and workers showed the generation of reactive oxygen species, stimulation of lipid peroxidation and decreased antioxidant defense system, supporting the role of oxidative stress in lead toxicity (Haleagrahara et al., 2010).

Recent emphasis on the use of natural and complementary medicines has drawn the attention of the scientific community for the potential of these treatment options (Daniel et al., 2004; Kalpana et al., 2007). The lifestyle modification approach focuses on dietary control, regular exercise, weight reduction and stress management, aiming toward normalization of CVD risk factors (Srimahachota et al., 2010). Curcumin, a hydrophobic polyphenol, is the yellow pigment in the Indian spice turmeric derived from the rhizome of the herb *Curcuma longa*. Curcumin is also known as diferuloylmethane and chemically is a bis-α, β-unsaturated β-diketone. Differing in methoxy substitutions on the aromatic ring, turmeric contains three natural analogues, the so-called curcuminoids, with curcumin being the most abundant (77%) and the less common demethoxycurcumin (17%) and bisdemethoxycurcumin (3%). It has a long history as an herbal remedy for a variety of diseases. It has been ascribed a multitude of therapeutic activities and has been associated with suppression of inflammation, angiogenesis, tumorigenesis, and diabetes, and with therapeutic effects in diseases of the cardiovascular, pulmonary and neurological systems and of skin and liver. In general, most of these effects can be attributed to the antioxidant, anti-inflammatory and anti-cancer activities of curcumin. Curcumin is an effective scavenger of reactive oxygen species and reactive nitrogen species (Rajasekaran, 2011).

Epidemiological studies have shown that physical activity reduces the risk of various common diseases such as CVD, diabetes, and cancer; it also helps in reducing visceral adipose tissue (Aoi et al., 2011). A partial list of proposed mechanisms for exercise-induced cardioprotection include induction of heat shock proteins (HSP_{72}), increase in cardiac antioxidant capacity, expression of endoplasmic reticulum stress proteins, anatomical and physiological changes in the coronary arteries, changes in nitric oxide production, adaptational changes in cardiac mitochondria, increased autophagy, and improved function of sarcolemmal and/or mitochondrial ATP-sensitive potassium channels (Golbidi and Laher, 2011). Overall, more scientific evidence will undoubtedly encourage the widespread advocacy of the clinical benefits of exercise therapy in the prevention and treatment of CVD (Yung et al., 2009).

Despite the knowledge that lead can induce oxidative stress, studies have identified favorable effects of exercise training and/or antioxidants on certain cardiovascular biomarkers after acute exposure to air pollution (Kalpana et al., 2007). However, there are few data available with respect to concomitant effects of regular aerobic training and curcumin supplementation, particularly the oxidant/antioxidant equilibrium during chronic exposure to lead acetate. Therefore, the aim of the current study was to determine the effects of aerobic exercise with and/or without curcumin supplementation on cardio-protection related markers in left ventricle, including HSP_{72} and serum total antioxidant capacity (TAC). In addition, changes in malondialdehyde (MDA) level were studied in rats chronically exposed to lead acetate. It was hypothesized that these results would provide novel insights into the myocardial ameliorative potential of turmeric antioxidant and exercise training during chronic exposure to lead acetate.

MATERIALS AND METHODS

Forty (40) male Wistar rats, 8 weeks of age with body weight range of 240 ± 20 g were obtained from the Laboratory of Animal Bearing and Multiplying at the Pasture Institute of Iran. The animals were housed to a polycarbonate cage (20 × 15 × 15 cm), made at Pasture Institute of Iran. All rats were weighed on a weekly during the exercise training phase. Rats were provided with food, a standard rat chow provided by Pars Institute for animals and poultry with a daily regimen of 10 g/ 100 g body weight. Water was available *ad libitum*. The animals were maintained under standard conditions of temperature at 22 ± 2°C and 50 ± 5% humidity with an alternating 12 h light/dark cycle. The pollutant standard index (PSI) was in the acceptable range as determined by the Iranian Meteorological Organization.

The experimental protocol was approved by Department of Physiology, University of Mazandaran and was performed according to guiding procedures in the Care and Use of Animals, prepared by the Council of the American Physiological Society. Rats were acclimatized to ambient rearing conditions for 4 to 5 days (four rats per cage) and were familiarized with endurance running training on the treadmill, that designed to increase oxygen consumption and improve functioning of the cardiovascular and respiratory systems). A mild shock (0.75 mA, 500 ms duration, 0.5 Hz rate) was delivered through these loops to motivate the rats to continuously walk on the moving belt and thus avoid foot shock. The wire loops were activated during all exercise sessions, and an experimenter monitored all treadmill sessions. Rats quickly learned to stay on the belt and avoid shock, except for one rat, which would not stay on the moving belt, and thus, was quickly removed from the exercise group. They were randomly assigned to five experimental groups of 8 rats each. The groups were defined as follows:

Group 1: lead acetate (L), the animals received lead acetate at a concentration of 20 mg/kg in a water solution (for intra peritoneal injection, i.p.), 3 days in a week for 8 weeks.
Group 2: Endurance training and lead acetate (TL) - the rats in this group received lead acetate similar to that in Group 1, and in addition, they performed progressive running exercise of 15 to 22 m/min for 25 to 64 min, 5 times a week. The running speed and distance was gradually increased.
Group 3: Curcumin and lead acetate (CL) similarly received lead acetate, as well as curcumin 30 mg/kg 5 days weekly for 8 weeks (i.p.).
Group 4: Endurance training and curcumin and lead acetate (TCL);

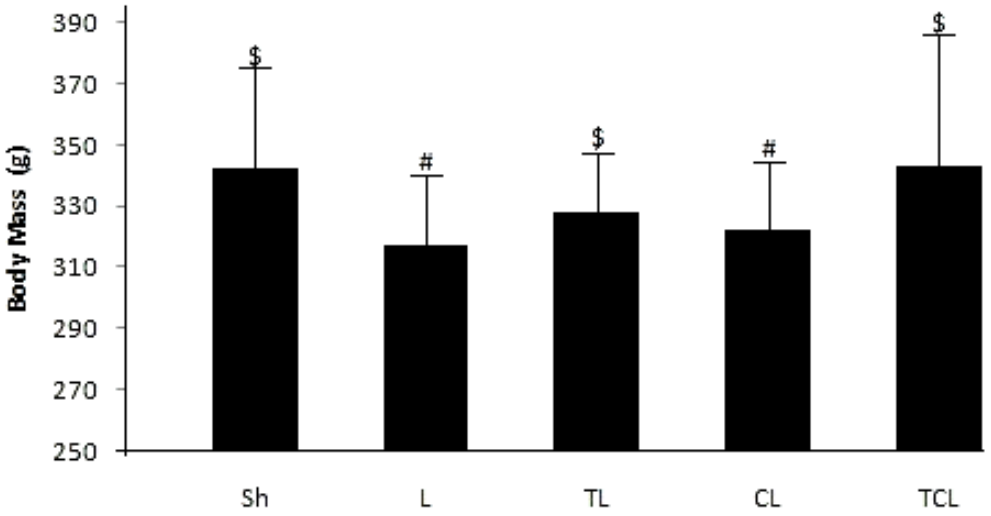

Figure 1. Body mass changes in rats during chronic exposure to lead acetate. Sh, Curcumin resolvent or ethyl oleate; TL, training + lead; TCL, training + curcumin + lead; L, lead acetate; CL, curcumin+ lead. Data are presented as mean ± SD; $, significantly different from lead group ($P < 0.001$); #, significantly different between combination (training + lead + curcumin) group with training and/or curcumin groups ($P < 0.05$).

the rats in this group performed an endurance training protocol similar to that in Group 2, and in addition, similarly received curcumin supplement and lead acetate, similar to that in Group 3.

Group 5: The sham-operate (sham); these rats received water and ethyl oleate in the same manner and for the same duration of time.

Lead acetate (Sigma) was solubilized in Milli-Q water, and curcumin was solubilized in 50% ethanol. In order to perform intra peritoneal (i.p) injections, curcumin was solubilized in ethyl oleate and was injected at a dose of 30 mg/kg. Turmeric was protected from light throughout the experiment (Daniel et al., 2004). We replicated a previously-reported lead dosing regimen that caused oxidative stress such that the doses of turmeric and lead acetate were 30 and 20 mg/kg, respectively (Asali et al., 2011; Daniel et al., 2004; Roshan et al., 2011).

Animals were decapitated after 12 to 14 h overnight fasting after anesthesia with ketamine and xaylozine. The chest cavity was then opened and the heart was rapidly removed from the aortic root. Heart tissues were weighed and placed into Petri dishes containing cold isolation medium (0.1 M K_2HPO_4, 0.15 M NaCl, pH = 7.4) to remove the blood. Left ventricular (free wall) tissue then was separated and were frozen immediately in liquid nitrogen and stored at -80°C for subsequent analysis of oxidant/antioxidant biomarkers. Frozen samples of heart tissue were thawed and homogenized in ice-cold 10 mM Tris-HC1, pH 8.2, containing 0.25 M sucrose, 2 mM 2-mercaptoethanol, 10 mM sodium azide, and 0.1 mM phenylmethylsulfonyl fluoride with a Polytron (4 vol/wt), and centrifuged at 50,000 g (20 min, 4°C). The homogenates were diluted with cold 20 mM Tris-HCl and centrifuged (10 min at 5°C, 3,000 g). The enzyme-linked immunosorbent assay (ELISA) method was used to determine the HSP_{72} content of the heart tissue homogenates, as described by Salo et al., (1991).

Lipid peroxidation (MDA) level in the left ventricular tissue was measured with the thiobarbituric-acid reaction using the method of Asali et al. (2011) and Roshan et al. (2011). The quantification of thiobarbituric acid reactive substances was determined at 532 nm by comparing the absorption to a standard curve of MDA equivalents generated by acid catalyzed hydrolysis of 1, 1, 3, 3 tetramethoxypropane. The value of MDA in left ventricle was expressed as nmol/mg tissue. Furthermore, serum TAC was measured using a commercially available kit (Randox Laboratories, Crumlin, UK) as previously described by Dabidi et al. (2011) and Asali et al. (2011). In this method, the most potent radical, hydroxyl radical, is produced. First, a ferrous ion solution is mixed with hydrogen peroxide. The sequentially produced radicals such as the brown colored dianisidinyl radical cations, produced by the hydroxyl radical, are potent radicals. The antioxidative effect of the sample against the potent free radical reactions is then measured. The assay has excellent precision values, which are lower than 3%. The results are expressed in µmol/ml. In accordance with the protocols of Asali et al. (2011), Daniel et al. (2004) and Roshan et al. (2011), we analyzed the lead acetate concentration using a spectrophotometer method only in the lead acetate group.

Statistical analysis was performed using a commercial software package (SPSS version 16.0 for Windows). Results are expressed as means $\pm$ SD. Data for HSP_{72} and oxidative stress-related biomarkers were normally distributed after log-transformation. A one-way analysis of variance (ANOVA) was used to detect statistical differences between groups. A post-hoc test (Tukey test) was performed to determine differences in the various markers between groups. The differences were considered significant at $P < 0.01$.

RESULTS

Data in Figures 1, 2 and 3 are shown, levels of body mass, heart mass, and heart- body mass ratio for the five groups, respectively. Lead acetate administration (20 mg/kg) caused significant decreases in body mass and heart mass were detected as compared to the other groups ($p< 0.01$) (Figures 1 and 2). Moreover, endurance training and curcumin supplementation protocols during chronic exposure to lead acetate caused preservation in body mass. However, aerobic training, but not curcumin supplementation significantly increased heart mass and

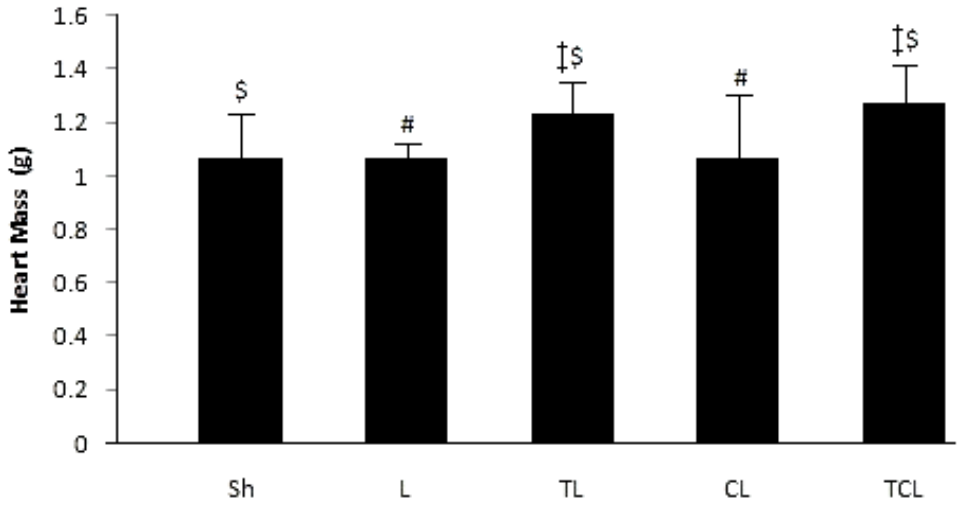

Figure 2. Heart mass changes in rats during chronic exposure to lead acetate. Sh, Curcumin resolvent or ethyl oleate; TL, training + lead; TCL, training + curcumin + lead; L, lead acetate; CL, Curcumin+ lead. Data are presented as mean ± SD; ‡significantly different from sham group ($P < 0.001$); $, significantly different from lead group ($P < 0.001$); #, significantly different between combination (training + lead + curcumin) group with training and/ or curcumin groups ($P < 0.05$).

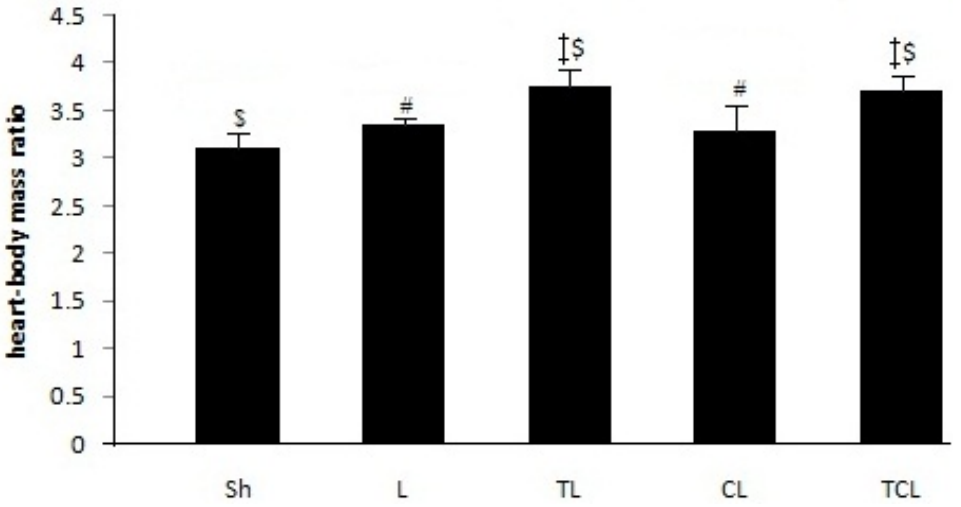

Figure 3. Heart mass and heart-to-body mass ratio in rats during chronic exposure to lead acetate. Sh, Curcumin resolvent or ethyl oleate;TL, training + lead; TCL, training + curcumin + lead; L, lead acetate; CL, curcumin+ lead. Data are presented as mean ± SD; ,‡ significantly different from sham group ($P < 0.001$); $, significantly different from lead group ($P < 0.001$); #, significantly different between combination (training + lead + curcumin) group with training and/or curcumin groups ($P < 0.05$).

heart-to-body mass ratio as compared to rats in the lead acetate group ($P < 0.01$) (Figure 3).

Data in Figures 4, 5 and 6 show changes in left ventricular HSP_{72} level, and oxidative stress-related biomarkers (MDA and TAC) in the rats exposed to lead acetate, respectively. Intra-peritoneal administration of lead acetate (20 mg/kg) caused a significant increase in the levels of left ventricle HSP_{72} and MDA by 15 and 70%, respectively, as compared to the sham (Sh) group ($P < 0.01$ and $p < 0.001$, respectively) (Figures 4 and 5). Furthermore, the administration of lead acetate for 8 weeks resulted in a decrease in TAC levels by 27%, in comparison to the Sh group ($P < 0.01$) (Figure 6). In contrast, curcumin supplementation, exercise training, and in particular their combination ($P < 0.001$) reversed MDA level, significantly. In other words, curcumin +

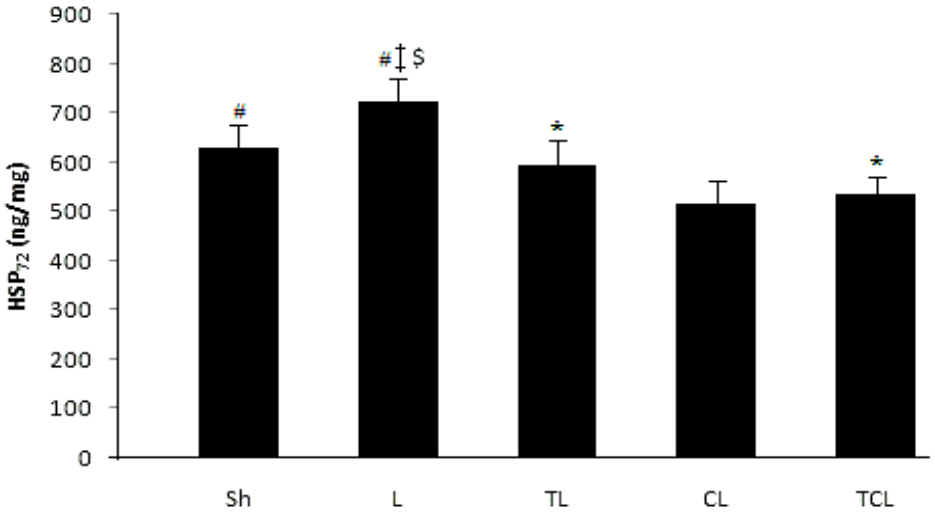

Figure 4. Left ventricle heat shock protein (HSP_{72}) levels in rats during chronic exposure to lead acetate. Sh, Curcumin resolvent or ethyl oleate; TL, training + lead; TCL, training + curcumin + Lead; L, lead acetate; CL, curcumin+ lead. Data are presented as mean ± SD; ‡, significantly different from sham group ($P < 0.001$);*, significantly different from Lead group ($P < 0.01$); $, significantly different from TL (training + lead) group ($P < 0.001$); #, significantly different between combination (training + lead + curcumin) group with training and/or curcumin groups ($P < 0.05$).

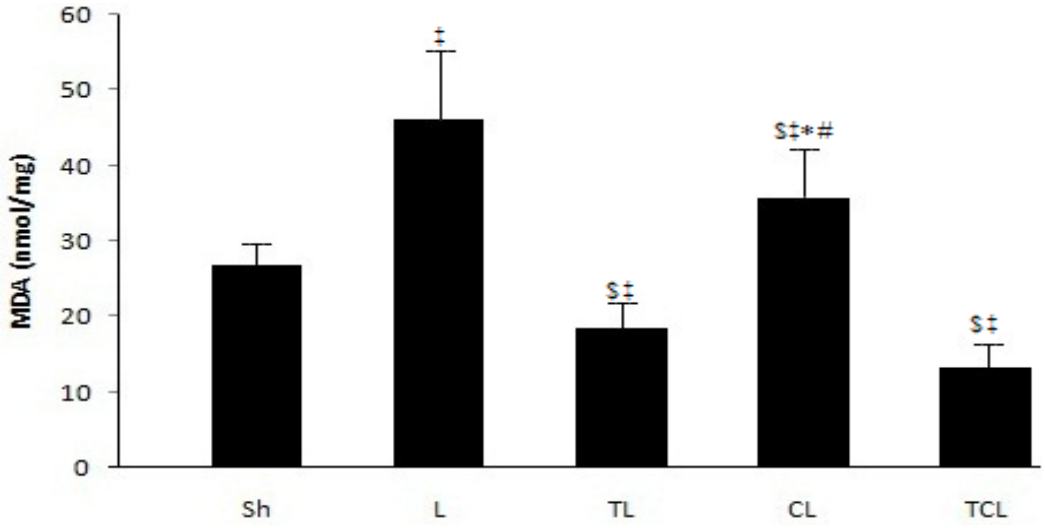

Figure 5. Left ventricle MDA levels in rats during chronic exposure to lead acetate. Sh, Curcumin resolvent or ethyl oleate; TL, training + lead; TCL, training + curcumin + lead; L, lead acetate; CL,(Curcumin+ lead. Data are presented as mean ± SD; ‡, significantly different from sham group ($P < 0.001$); $, significantly different from lead group ($P < 0.001$); *, significantly different from training group ($P < 0.01$); #, significantly different between combination (training + lead + curcumin) group with training and/or curcumin groups ($P < 0.05$).

training + lead treatment was more effective than curcumin + lead or training + lead alone treatments.

DISCUSSION

We investigated effects of endurance training and curcumin supplementation, alone and combined on HSP_{72} and serum TAC and MDA changes in male rats during chronic exposure to lead acetate. The present data showed intra-peritoneal administration of lead acetate (20 mg/kg) caused an increase in the levels of HSP_{72} and MDA in left ventricle and a decrease in the serum TAC concentration, by 15, 70 and 27%,

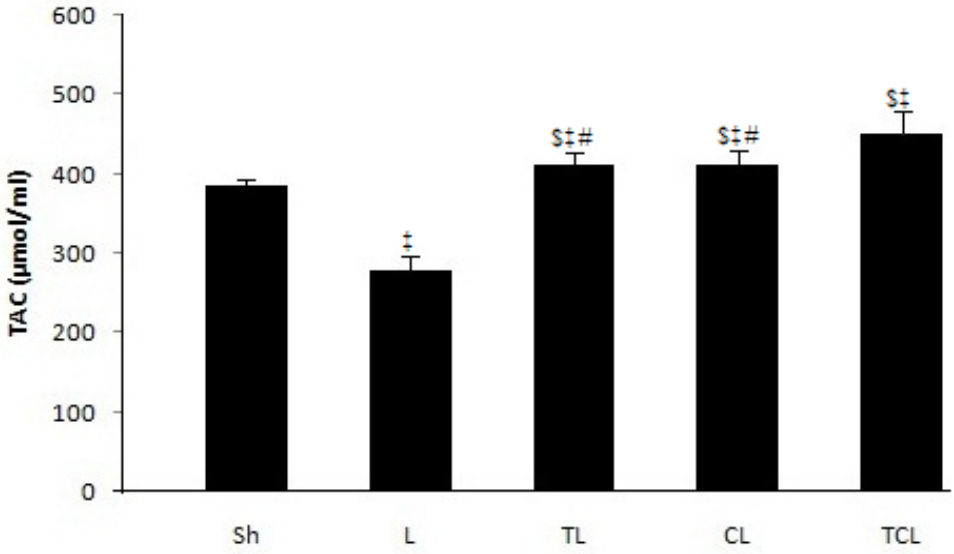

Figure 6. Serum TAC levels in rats during chronic exposure to lead acetate. Sh, Curcumin resolvent or ethyl oleate; TL, training + lead; TCL, training + curcumin + lead; L, lead acetate; CL, curcumin + lead. Data are presented as the mean ± SD for 8 rats; ‡, significantly different from sham group ($P < 0.001$); $, significantly different from lead ($P < 0.001$); #, significantly different between combination (training + lead + curcumin) group with training and or curcumin groups ($P < 0.05$).

respectively, as compared to the Sh group.

Lead is capable of inducing oxidative damage to brain, heart, kidneys, and reproductive organs and the results of this study support the hypothesis; lead acetate toxicity appears to affect organs with low antioxidant defenses such as the heart (Daniel et al., 2004; Gholam-Hosseini et al., 2009; Gurer and Ercal, 2000; Patrick, 2006), since our data stated that oxidative stress biomarker (MDA) levels in lead acetate group were higher than in the other treatment groups. Increased MDA and decreased antioxidant defense biomarkers in the lead acetate group suggests an increased myocardial lipid peroxidation. The mechanisms for lead-induced oxidative stress include the effects of lead on membranes, DNA, and antioxidant defense systems of cells (Ahamed and Siddiqui, 2007; Ashry et al., 2010; Somashekaraiah et al.,1992). In addition, one of the mechanisms by which lead can exert some of its toxic effects is through the disruption of the delicate proxidant/antioxidant balance that exists within mammalian cells (Gholam-Hosseini et al., 2009; Jackie et al., 2011). *In vivo* and *in vitro* studies suggest that lipid metabolism is altered both in acute and chronic exposure to lead. Lead inhibits antioxidant enzyme activity, and also, increasing lipid peroxidation. The HSP_{72} and serum TAC protect cellular membranes from peroxidative damage (El-Tohamy and El-Nattat, 2010). In contrast, endurance training and curcumin supplementation, particularly, their combination resulted in a significant increase in TAC, and a significant decrease in HSP_{72} and MDA, as compared to lead acetate and Sh groups. Training alone decreased the level of MDA in myocardial homogenates in rats exposed to lead acetate. Furthermore, training and curcumin together decreased the myocardial HSP_{72} and MDA levels, and actually restored lead-induced myocardial damage and tended to normalize these biomarkers suggesting a reversal of lead-induced cardiotoxicity and confirms the free radical scavenging property of curcumin.

Curcumin's protective function against peroxidative damage of biomembranes, known to be a free-radical-mediated chain reaction, has mainly been attributed to the scavenging of the reactive free radicals involved in peroxidation. These scavenging properties of curcumin have also been considered to be responsible for its protective role against oxidative damage of DNA and proteins, believed to be associated with a variety of chronic diseases such as cancer, atherosclerosis, neurodegenerative diseases and aging. In addition to its direct antioxidant activity, curcumin may function indirectly as an antioxidant by inhibiting the activity of inflammatory enzymes or by enhancing the synthesis of glutathione. The anti-inflammatory activity of curcumin seems to be comparable to steroidal drugs and non-steroidal drugs such as indomethacin and phenyl-butazone (Rajasekaran, 2011). When administered in the current study, curcumin effectively increased serum TAC levels during lead exposure. The result of present study is consistent with the hypothesis that a prolonged exercise and curcumin supplementation protocol is effective in preventing lead-induced myocardial oxidative stress and increases myocardial antioxidant defensive properties (Muhammad et al., 2011; Roshan et al., 2011;

Srivastava and Mehta, 2009).

Since activity of antioxidant enzymes depends on various essential trace elements for proper molecular structure and enzymatic activity, it is a potential target for lead toxicity (Patrick, 2006). The presence of the free -SH group is necessary for the proper action of antioxidant enzymes. Researchers have shown a decrease in the concentration of the free -SH groups in the blood as well as in the urine of rats exposed to lead (Tandon et al., 2003). The increase in the HSP_{72} and MDA in the heart and the decrease in TAC concentration in the serum of rats exposed to lead can be a result of this heavy metal-induced depletion in the free -SH groups noted in these animals. However, a second possibility that must be considered is that the rats with long-term exposure to lead acetate without treatment interventions were more prone to oxidative stress in organs with low antioxidant defense, which in turn increased the need for improved antioxidant defenses.

In conclusion, the present study demonstrates that chronic lead acetate administration can induce an imbalance in myocardial antioxidant defenses. In addition, we observed that training + curcumin + lead treatment was more effective than curcumin + lead and/or training + lead alone, that in turn, suggests that exercise training in concomitant with curcumin can potentially be more effective for inhibiting myocardial damage caused by lead in rats. These results suggest that exercise and curcumin supplementation may have a more cardioprotective effect by ameliorating lead-induced cardiotoxicity.

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UPCOMING CONFERENCES

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